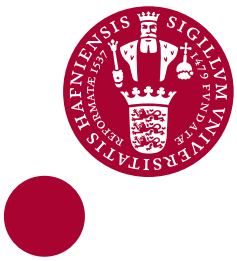

Structure-Activity Study of alpha-Peptoid/alpha-Peptide Peptidomimetics: Influence of Hydrophobicity on Antimicrobial Activity and Cytotoxicity

Frederiksen, Nicki; Bjorkling, Fredrik; Nielsen, Peter; Franzyk, Henrik

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26th-31st August 2018 | Dublin City University, Ireland

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Journal of PeptideScience

Aims & Scope

The official Journal of the *European Peptide Society EPS*

The *Journal of Peptide Science* is a cooperative venture of John Wiley & Sons, Ltd and the European Peptide Society, undertaken for the advancement of international peptide science by the publication of original research results and reviews. The *Journal of Peptide Science* publishes four types of articles: Research Articles, Rapid Communications, Reviews and Protocols.

The scope of the Journal embraces the whole range of peptide chemistry and biology: the isolation, characterisation, synthesis properties (chemical, physical, conformational, pharmacological, endocrine and immunological) and applications of natural peptides; studies of their analogues, including peptidomimetics; peptide antibiotics and other peptide-derived complex natural products; peptide and peptide-related drug design and development; peptide materials and nanomaterials science; combinatorial peptide research; the chemical synthesis of proteins; and methodological advances in all these areas. The spectrum of interests is well illustrated by the published proceedings of the regular international Symposia of the European, American, Japanese, Australian, Chinese and Indian Peptide Societies.

The *Journal of Peptide Science* publishes the bi-annual EPS Newsletter and sponsors the EPS Website http://www.eurpepsoc.com

TIME	26-SUNDAY	27-MONDAY	28-TUESDAY		29-WEDNESDAY	30-THURSDAY	31-FRIDAY
		Biologically active peptides 1	Amino acids, proteins and synthesis 1		Peptide mimetics	Peptides in chemical biology and therapeutics 2	
08.30-09.10		David R. Spring	Marion de Jong		Knud J. Jensen	Dek Woolfson	
09.10-09.25		Nermina Malanovic	Chuanliu Wu		Jung-Mo Ahn	Anne Conibear	
09.25-09.40		Steven Ballet	Tsubasa Inokuma		Jean-Alain Fehrentz	Ana Salomé Veiga	
09.40-09.55		Norelle Daly	Muhammad Jbara		Stefan Roesner	Timothy Reichart	
09.55-10.10		Sarah Jones	William Lubell		Carles Mas Moruno	Paul Alewood	
10.10-10.25		Marta De Zotti	Kirtikumar B Jadhav		Steven L Cobb	Roland Hellinger	
10.25-11.00		COFFEE	COFFEE		COFFEE	COFFEE	
		Glycopeptides, lipopeptides and macromolecular assemblies	Biologically active peptides 2		Proteomics, bioinformatics, Structural and conformational studies	Peptides in chemical biology and therapeutics 3	Excursions and social activities
11.00-11.30		Finbarr O'Harte	Meritxell Teixidó		Beate Koksch	Wilfred van der Donk	
11.30-11.45		Andy Wilson	Evelien Wynendaele		Jose Martins	Youhei Sohma	
11.45-12.00		Claudia Bello	Hidehito Mukai		Andrew Jamieson	Minying Cai	
12.00-12.15		Oliver Zerbe	Thibaut Thery		Daniela Marasco	Sónia Henriques	
12.15-12.30		Veronica Dodero	Shai Rahimipour		Quentin Kaas	Jane Aldrich	
12.30-12.45		Eoin Scanlan	Marina Rautenbach		Zbigniew Szewczuk	Maayan Gal	
12.45-13.45		LUNCH	LUNCH		LUNCH	LUNCH	
13.45-14.15		EXHIBITON	EXHIBITON		EXHIBITON	EXHIBITON	
				ESF-Mixer		Peptides in chemical biology and therapeutics 4	
14.15-14.30	Registration					Glenn F. King	
14.30-14.45							
14.45-15.00		POSTER	POSTER		POSTER	Christina Schroeder	
15.00-15.15						Lydia Behring	
15.15-15.30						Tamis Darbre	
15.30-15.45						Garry Laverty	
15.45-16.15		TEA	TEA		TEA	TEA	
			Amino acids, proteins and synthesis 2		Peptides in chemical biology and therapeutics 1	Amino acids, proteins and synthesis 3	
16.15-16.30		Jakob Gaar	Robert Zitterbart		Andrew White	Abhishek Iyer	
16.30-16.45	Opening	Lea Albert	Christian Becker		Dorien Van Lysebetten	Hironobu Hojo	
16.45-17.00		Chun Yuen Chow	Yoshio Hayashi		Piotr A Mroz	Hannes Ludewig	
17.00-17.15		Varsha Thombare	Vincent Aucagne		Benjamin Liet	Juergen Machielse	
17.15-17.30	Awards	Karlijn Hollanders	Sira Defaus		Nir Qvit		
17.30-17.45		Daria Roshchupkina	Gerbrand van der Heden van Noort		Victor J Hruby		
	James P Tam, Christian Hackenberger, Norman Metanis	YIMS	Prodrugs, conjugates, targeting and uptake		Nanotechnology, imaging and analytical techniques	Closing	
17.45-18.00		Tyler Lalonde	Ming-Hsin Li		Friedrich Bialas		
18.00-18.15		Elise Naudin	Gilles Subra		Konstantin Kuhne		
18.15-18.30		Sabine Schuster	Ikuhiko Nakase		Neil O'Brien-Simpson	BACHEM Reception	
18.30-18.45		Esben Matzen Bech	Adina Borbély		Dorian J Mikolajczak		
18.45-19.00		Clara Pérez-Peinado	Conan Wang		Ramon Subiros-Funosas		
19.00-19.30							
19.30-20.00	Reception						
20.00-21.00						Dinner	
21.00-22.00							

Welcome to the 35th EPS in Dublin

Dear friends, partners and fellow colleagues

On behalf of the organising committee, it is our pleasure to invite you to join in the 35th European Peptide Symposium, 35EPS, on 26-31 August 2018 at the Dublin City University, Dublin, Ireland. Ireland has been known as the land of Saints and Scholars. The capital city of Dublin lies at the mouth of the river Liffey embracing the Irish Sea. There is evidence of a community living in Dublin since the fifth century. It is a city rich in historical and cultural attractions including the unique Book of Kells, the famous Guinness brewery, the neighbouring hills of Tara and the monastic village of Glendalough.

The European Peptide Society is a non-profit association established for the public benefit to promote in the advancement of education and scientific study of the chemistry, biochemistry, biology and pharmacology of peptides in Europe and in neighbouring countries. In co-operation with John Wiley & Sons, the EPS publishes the Journal of Peptide Science and the EPS Newsletter. The EPS supports the organisation of the European Peptide Symposium, sponsors local and specialised meetings and workshops, and supports and promotes young scientists with travel grants and dedicated seminars.

The European Peptide Symposium is a well-established biennial peptide conference that has grown substantially. It has been well attended by Nobel Laureates, world-class scientists, leading scientific investigators, world-renowned commercial partners, publishers and leading companies in the area of peptide research and its applications. This symposium includes sessions covering every aspect of the research, education and innovation related to the peptide science.

The 35EPS will take place at the Helix, a purpose built conference venue associated with the Dublin City University. The Helix is located approximately 5 km north of Dublin city centre short distance away from the Dublin International Airport. The scientific sessions, poster sessions and exhibition will take place in the Helix and its adjacent rooms. Dublin City University has excellent on-site accommodation, restaurants and cafes which will cater for symposium delegates.

The 35EPS symposium will start on 26th of August with award and prize giving lectures and a welcome reception. In addition to plenary and invited speaker presentations, there will be poster presentations and a mini symposium for young scientists for covering every aspect of peptide science. The symposium gala dinner will take place on the 30th of August at the Crowne Plaza Hotel and the excursions are planned on Friday for sampling the history and culture of Dublin and its surroundings towns.

The executive council, the scientific advisory board, and the program committee hope to make the 35EPS symposium an exciting and memorable scientific event. You will also have the opportunity to visit the hospitable and attractive city of Dublin with many cultural attractions. It is our pleasure to welcome you to Dublin, and we hope that you enjoy the symposium and your visit.

35 EPS Organising Committee
Chandralal Hewage, University College Dublin (Chairman)

Dear colleagues and friends in the worldwide peptide community

The 35th edition of the European Peptide Society symposium, 35EPS, is well on the move! We are happy that this symposium signals the expansion of our society's sphere of influence into a so far unvisited EPS member nation. With a rich cultural heritage, a forward-looking society and, more to the point, a dynamic biotech sector, Ireland is an ideal location for talking shop on peptides in the 21st century. In continuation of the highly successful 34EPS in Leipzig, to be followed by equally enticing calls in Barcelona (2020) and Florence (2022), this upcoming 35EPS promises to be a unique opportunity to learn about the ever-growing impact of peptide research in a multitude of scientific areas, to share your own results and interests, and to build productive professional ties with like-minded scientists on a worldwide scale. Assisted by capable scientific and program committees, Prof. Chandralal Hewage, the symposium chair, is planning not only a first-rate scientific event but has also arranged for top-notch conference facilities, including affordable accommodation, and for an attractive social program.

On behalf of the European Peptide Society, I look forward to greeting you personally in Dublin, August 26-31, 2018!

With best personal regards,
David Andreu
President, European Peptide Society

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General Information

Registration desk and conference office

The registration desk and conference office are located in the Helix and will be open Sunday the 19th August and remain open until the end of the symposium. The registration desk opening hours are as follows:

Sunday 26th	14:15 - 19:00
Monday 27th	08:00 - 18:30
Tuesday 28th	08:00 - 16:00
Wednesday 29th	08:00 - 16:00
Thursday 30th	08:00 - 16:00

Locations of sessions

The poster sessions will be held in the Helix. Odd numbered posters will be presented on Monday and Tuesday, and even numbered posters will be presented on Tuesday and Wednesday.

Name Badges & Regulations

Name badges are required when in the Helix, for all of the symposium sessions, including the poster session and hospitality suites, and all other symposium functions and related activities. No smoking is permitted on the DCU campus grounds. Mobile phones must be turned off or silenced during the symposium. No photography or recording is permitted in any session, including the poster session.

Speakers

Stewards will be available to assist speakers in transferring their presentations to provided computers or setting up personal computers. Speakers are kindly asked to be present in the lecture hall 30 minutes before the session starts.

Speaker Rooms

The speaker ready room will be available for speakers only; the room is located on the ground floor of the Helix, close to the main auditorium. Please note computers are not provided in these rooms.

Poster Presentations

Categories

Amino acids and peptide synthesis	P1	-	P46
Protein synthesis and semi-synthesis	P47	-	P57
Peptide-based materials and catalysis	P58	-	P74
Nanotechnology, imaging and analytical techniques	P75	-	P86
Macromolecular assemblies	P87	-	P89
Glycopeptides and lipopeptides	P90	-	P95
Peptide mimetics	P96	-	P112
Structural and conformational studies	P113	-	P140
Biochemistry and biology	P141	-	P147
Biologically active peptides	P148	-	P186
Peptides in chemical biology and therapeutics	P187	-	P225
Prodrugs, conjugates, targeting and update	P226	-	P248
Peptides in proteomics and bioinformatics	P249	-	P250

Set-up and removal

Authors are kindly asked to have their posters put up before 14:00 on Monday the 27th of August, and leave them on display for the duration of the symposium.

Poster areas are separated according to category and all boards will be labelled with the individual abstract code which can be found in this book in the list of abstracts.

Posters should be removed before 17:30 on Thursday the 30th or 10:00 on Friday the 31st of August; any unclaimed posters will be discarded.

Sessions

There are three poster sessions, from Monday the 27th of August to Wednesday the 29th of August each day between 14:15 and 15:45. Authors are kindly asked to be present at their posters on the day they have been allocated. Poster session allocations are as follows:
Odd Numbers: Monday and Tuesday
Even Numbers: Tuesday and Wednesday

Lunch, Tea/Coffee, and Water

Tea/Coffee will be provided in the Helix conference centre.
Lunches will be held in the DCU Restaurant.

Welcome mixer, farewell reception and conference banquet

The welcome reception will be held in the Helix on Sunday, August 26th at 19:00 and we invite you to join us for some refreshments.

The closing reception will be held by BACHEM in the Helix on Thursday, August 30th at 18:00.

The conference banquet will be held at the Crowne Plaza Dublin Airport Hotel, Santry, Dublin 9 on Thursday at 20:00. This is a ticketed dinner. Coach transport is arranged for travel to the venue.

Public transportation

DCU accommodation is located on campus; however several bus routes serve the university.

A bus stop located near the Helix is serviced by bus number 44, which departs approximately every hour.

A bus stop located on the Ballymun Road, near the university, is serviced by bus numbers 4, 9, 11 and 13.

A single ticket from DCU to St Stephen's Green (central location) costs €2.15 (Leap Card) or €2.85 (cash, coins only, no change given). Visitor Leap Cards are available for purchase at Dublin Airport.

Additional information, including real time passenger information for the above routes, can be found at www.dublinbus.ie

Internet

There is a free WiFi service available throughout the Helix. The username for DCU Guest WiFi is 'wifiguest'. No password is required.

Conference Tours

The following tours will be available for symposium participants:

National Stud and Japanese Gardens Tour
Dublin City and Guinness Storehouse Tour
Coastal Scenic Tour with Malahide Castle and Gardens Tour
Dublin Tour with Trinity College & Book of Kells

For further information contact the registration desk.

Next EPS Symposia

The 36 EPS will be held in Barcelona, Spain on August 30 - September 4, 2020. The 37 EPS will be held in Florence, Italy in 2022.

Awards

Miklós Bodanszky Award

The Miklós Bodanszky Award is presented in commemoration of his outstanding contributions to peptide science. The award is given to the scientist who made significant contributions to peptide-based drug research in the period of ten years after having obtained the PhD degree. The Award is presented during the EPS Symposium and is sponsored by BCN PEPTIDES. The 2018 award will be announced at the 35th EPS opening ceremony.

Awardee: **Norman Metanis**; The Hebrew University of Jerusalem, Israel

Leonidas Zervas Award

The Leonidas Zervas Award is presented in commemoration of his outstanding contributions to peptide science. The award is given to the scientist who has made the most outstanding contributions to the chemistry, biochemistry and/or biology of peptides in the five years preceding the date of selection, and is sponsored by the donation of Dr. Rao Makineni. The 2018 award will be announced at the 35th EPS opening ceremony.

Awardee: **Christian Hackenberger**; FMP Berlin, Germany

Josef Rudinger Memorial Award

The Josef Rudinger Memorial Lecture Award is presented "in commemoration of Josef Rudinger's role in the foundation of the European Peptide Symposia and of diverse contributions he made to peptide chemistry". The Award is presented during the EPS Symposium and is sponsored by PolyPeptide Group. The 2018 award will be announced at the 35th EPS opening ceremony.

Awardee: **James P. Tam**; Nanyang Technological University, Singapore

ESCOM Science Foundation (ESF) Dr. Bert L. Schram Symposium and Awards

On behalf of the ESCOM Science Foundation (ESF), 35EPS is pleased to announce the Dr. Bert L. Schram Young Investigators' Mini Symposium (YIMS) for speakers under 35 years of age. Additionally, there will be oral presentation and poster presentation awards and a networking mixer.

The Dr. Bert L. Schram Oral Presentation Awards

There will be two oral presentation awards, €750 for the winner and of €500 for the runner-up. They will also receive a signed certificate in the joint names of EPS and ESF. Awards will be presented by ESF representatives at the symposium banquet.

The Dr. Bert L. Schram Poster Presentation Awards

There will be six poster presentation awards. The top two winners will receive each a cash amount of €500 and the next four winners a cash amount of €250 each. They will also receive a signed certificate in the joint names of EPS and ESF. Awards will be presented by ESF representatives at the symposium banquet.

The Dr. Bert L. Schram Networking Mixer

The Networking Mixer is a buffet lunch bringing together EPS young scientists and international senior scientists from academia and corporate research. This is an exclusive event and the attendees will gain invaluable insights on current research trends, and orientation on career planning and professional opportunities. Attendees will have ample opportunity to network and meet and greet EPS officers and ESF representatives.

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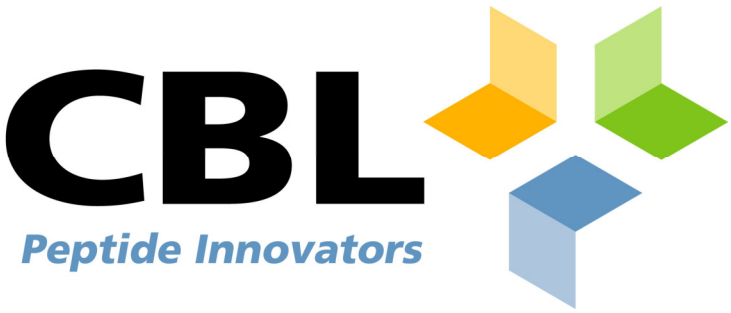
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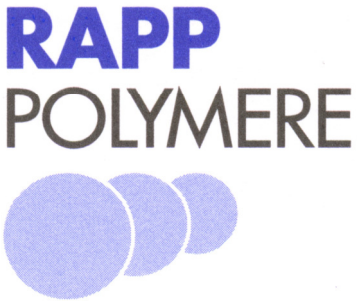
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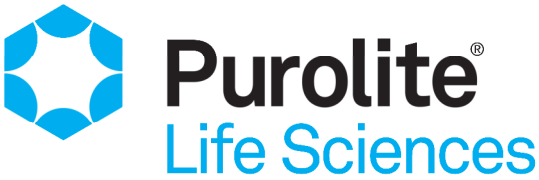
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Programme

	August 26 Sunday PM
14.00-17.00	**REGISTRATION**
16.30-19.00	**OPENING CEREMONY**
	AWARDS
	Miklós Bodanszky Award
	AL1 - Norman Metanis, The Hebrew University of Jerusalem, Israel Lessons from Selenium Chemistry to Study Protein Science
	Leonidas Zervas Award
	AL2 - Christian Hackenberger, FMP Berlin, Germany The power of chemoselectivity: Functional peptide- and protein-conjugates for extra- and intracellular targeting
	Josef Rudinger Memorial Award
	AL3 - James P Tam, Nanyang Technological University, Singapore Advances in Site-specific and Linkage-specific Ligation

	August 27 Monday AM
	Chairs: David Andreu
08.30-09.10	**IL1 - David R Spring** Adventures in Drugging Undruggable Targets
	Chairs: Tia Keyes, Glenn King
	Biologically Active Peptides 1
09.10-09.25	**OP1 - Nermina Malanovic** Lipid domain formation induced by antimicrobial peptides OP-145 and SAAP-148 results in efficient killing of Escherichia coli
09.25-09.40	**OP2 - Steven Ballet** Engineering Hybrid Peptidomimetics for Improved Pain Treatments
09.40-09.55	**OP3 - Norelle Daly** An engineered cyclic peptide alleviates symptoms of inflammation in a murine model of inflammatory bowel disease
09.55-10.10	**OP4 - Sarah Jones** Penetrating the Impenetrable: Progress towards a Non-hormonal Male Contraceptive
10.10-10.25	**OP5 - Marta De Zotti** Peptide-Based Biopesticides
	Chair: Marc Devocelle
11.00-11.30	**IL2 - Finbarr O'Harte** The design and assessment of the therapeutic potential of apelin-13 peptide mimetics for alleviating metabolic dysfunction in diabetes and obesity
	Chairs: Ekaterina Kolesanova, David Spring
	Glycopeptides, Lipopeptides and Macromolecular Assemblies
11.30-11.45	**OP6 - Andy Wilson** Development and Exploitation of Photo-Crosslinking Methodology to Study Protein-Protein Interactions
11.45-12.00	**OP7 - Claudia Bello** Toward homogeneous glycoproteins via auxiliary-assisted sequential glycosylation and ligation of peptides
12.00-12.15	**OP8 - Oliver Zerbe** Peptide-Guided Assembly of Armadillo Repeat Protein Fragments
12.15-12.30	**OP9 - Veronica Dodero** Are gluten-related disorders a new protein aggregation disease?
12.30-12.45	**OP10 - Eoin Scanlan** Chemical Synthesis of Glycopeptides and Glycoproteins Using Acyl-thiol-ene Mediated 'Click' Ligation

	August 27 Monday PM
	Chairs: Jane Aldrich, David Craik
	Young Investigator Mini Symposium
16.15-16.30	**MS1 - Jakob Gaar**
	Generation and characterisation of novel antibodies from selectively Advanced Glycation Endproduct (AGE) modified Collagen Model Peptides (CMPs)
16.30-16.45	**MS2 - Lea Albert**
	Light-controlled inhibition of MLL1 methyltransferase by azo-containing peptides: towards optoepigenetic leukemia regulation
16.45-17.00	**MS3 - Chun Yuen Chow**
	Development of NaV-Selective Agonists with Potential for Treatment of Dravet Syndrome Epilepsy
17.00-17.15	**MS4 - Varsha Thombare**
	Exploiting thioamide reactivity in peptide synthesis
17.15-17.30	**MS5 - Karlijn Hollanders**
	Zn-catalyzed tert-butyl nicotinate-directed amide cleavage for applications in peptide synthesis and peptidomimetic design
17.30-17.45	**MS6 - Daria Roshchupkina**
	Synthesis of peptides glycated at Lys residues
17.45-18.00	**MS7 - Tyler Lalonde**
	Targeting Ghrelin Receptor Homodimer: Bivalent Ligands with Exceptional Binding Affinity and Potency
18.00-18.15	**MS8 - Elise Naudin**
	De novo designed proteins catalyzing amide bond forming reactions
18.15-18.30	**MS9 - Sabine Schuster**
	Synthesis and biochemical evaluation of GnRH-III-drug conjugates
18.30-18.45	**MS10 - Esben Matzen Bech**
	Directing PYY3-36 internalization through half-life extenders
18.45-19.00	**MS11 - Clara Pérez-Peinado**
	Mechanisms of bacterial membrane permeabilization of snake venom-derived peptides crotalicidin (Ctn) and Ctn[15-34]

	August 28 Tuesday AM
	Chair: Paula Gomes
08.30-09.10	**IL3 - Marion de Jong** Theranostic radiopeptides
	Chairs: Zbigniew Szewczuk, John Wade
	Amino Acids, Proteins and Synthesis 1
09.10-09.25	**OP11 - Chuanliu Wu** Multicyclic peptides constrained through noncanonical disulfide bonds and thioether crosslinks
09.25-09.40	**OP12 - Tsubasa Inokuma** Novel methodology for the synthesis of α-indolyl-glycine containing peptide via direct asymmetric Friedel–Crafts reaction to peptidyl imine
09.40-09.55	**OP13 - Muhammad Jbara** Palladium Prompted On-Demand Cysteine Chemistry for the Synthesis of Challenging Proteins
09.55-10.10	**OP14 - William Lubell** Synthesis and Biomedical Applications of Substituted Amino-Lactam and Amino-Imididazolone Constraints
10.10-10.25	**OP15 - Kirtikumar B Jadhav** Recombinant synthesis and purification of hTFF2 protein in S. Cerevisiae
	Chair: Anna Maria Papini
11.00-11.30	**IL4 - Meritxell Teixidó** Gate2Brain Shuttle Peptides, From Discovery to Applications
	Chairs: Marion de Jong, Finbarr O'Harte
	Biologically Active Peptides 2
11.30-11.45	**OP16 - Evelien Wynendaele** Do Quorum Sensing Peptides Play a Role in Psychiatric Disorders?
11.45-12.00	**OP17 - Hidehito Mukai** Mitocryptides: First Demonstration of Pathophysiological Involvements in Inflammatory Diseases
12.00-12.15	**OP18 - Thibaut Thery** Antifungal activity and immunomodulation of a de novo synthetic peptide
12.15-12.30	**OP19 - Shai Rahimipour** Targeting Amyloidogenic Proteins with Self-assembled Cyclic D,L-alpha-Peptides
12.30-12.45	**OP20 - Marina Rautenbach** Tyrocidines and Gramicidin S: Glorified cyclodecapeptide detergents or precise chemical scalpels

	August 28 Tuesday PM
	Chairs: Florine Cavelier, Fintan Kelleher
	Amino Acids, Proteins and Synthesis 2
16.15-16.30	**OP21 - Robert Zitterbart**
	Enabling Parallel Peptide Purification by a Novel Traceless Purification Linker
16.30-16.45	**OP22 - Christian Becker**
	Effects of non-enzymatic posttranslational modifications on protein function
16.45-17.00	**OP23 - Yoshio Hayashi**
	A New Aspect of Npys-based Solid Phase Disulfide Peptide Synthesis
17.00-17.15	**OP24 - Vincent Aucagne**
	N-2-hydroxybenzyl-cysteine peptide crypto-thioesters for native chemical ligation
17.15-17.30	**OP25 - Sira Defaus**
	Expanding the potential and multivalency of the B2T synthetic peptide vaccine against foot-and-mouth disease virus
17.30-17.45	**OP26 - Gerbrand van der Heden van Noort**
	A general method towards ADPr ribosylated peptides and proteins
	Chairs: Meritxell Teixidó, John Howl
	Prodrugs, Conjugates, Targeting and Uptake
17.45-18.00	**OP27 - Ming-Hsin Li**
	A Novel Evans Blue Organic Compound-appended PSMA-617 Peptide as SPECT Molecule Imaging Agent for Human Prostate LNCaP Animal Model
18.00-18.15	**OP28 - Gilles Subra**
	Sol-gel and peptides: an attractive route to unprecedented biomaterials
18.15-18.30	**OP29 - Ikuhiko Nakase**
	Arginine-rich cell-penetrating peptide-modified exosomes for macropinocytosis induction and effective cellular uptake
18.30-18.45	**OP30 - Adina Borbély**
	Novel Cryptophycin-based Conjugates for Tumor Targeting
18.45-19.00	**OP31 - Conan Wang**
	A Journey Alongside Cyclosporin on the Road to Peptide Oral Bioavailability

	August 29 Wednesday AM
	Chair: Paul Alewood
08.30-09.10	**IL5 - Knud Jensen**
	Selective N-terminal acylation of peptides and proteins with an optimized His-sequence
	Chairs: Tamara Paypanova, William Lubell
	Peptide Mimetics
09.10-09.25	**OP32 - Jung-Mo Ahn**
	Structure-Based Design of Alpha-Helix Mimetics to Target and Disrupt Estrogen Receptor-Coregulator Interactions in Breast Cancer
09.25-09.40	**OP33 - Jean-Alain Fehrentz**
	Ghrelin receptor ligands: from the bench to the drug on the market
09.40-09.55	**OP34 - Stefan Roesner**
	Macrocyclization of Small Peptides Enabled by Oxetane Incorporation
09.55-10.10	**OP35 - Carles Mas Moruno**
	The use of RGD peptidomimetics on biomaterials: new advances via selective integrin-subtype targeting
10.10-10.25	**OP36 - Steven L Cobb**
	Stabilising Peptoid Helices Using Non-Chiral Fluoro-Alkyl Monomers
	Chair: Norbert Sewald
11.00-11.30	**IL6 - Beate Koksch**
	Fluorine in Peptide and Protein Engineering
	Chairs: Ernest Giralt, Dick FitzGerald
	Proteomics, Bioinformatics, Structural and Conformational Studies
11.30-11.45	**OP37 - Jose Martins**
	Concerted biophysical and biological evaluation of Pseudomonas lipopeptides as a premise to unlock their application potential
11.45-12.00	**OP38 - Andrew Jamieson**
	Rationally Designed Peptidomimetics
12.00-12.15	**OP39 - Daniela Marasco**
	Amyloidogenicity of regions of Nucleophosmin 1: a direct link between protein misfolding and Acute Myeloid Leukemia
12.15-12.30	**OP40 - Quentin Kaas**
	Inhibition of nicotinic acetylcholine receptor subtypes by the ribbon isomers of α-conotoxins.
12.30-12.45	**OP41 - Zbigniew Szewczuk**
	Quaternary Ammonium Isobaric Labeling for a Relative and Absolute Quantification of Peptides

	August 28 Wednesday PM
	Chairs: Maria Luisa Mangoni, Wilfred van der Donk
	Peptides in Chemical Biology and Therapeutics 1
16.15-16.30	**OP42 - Andrew White**
	Development of serine protease inhibitors using the 1,2,3-triazole motif as a disulfide mimetic in the cyclic peptide sunflower trypsin inhibitor-1
16.30-16.45	**OP43 - Dorien Van Lysebetten**
	Peptide-Bile acid Cyclisation as a Tool for the Development of a Universal Vaccine Against Influenza A
16.45-17.00	**OP44 - Piotr A Mroz**
	Stereochemically modified glucagon with improved biophysical parameters
17.00-17.15	**OP45 - Benjamin Liet**
	Design, synthesis and study of multimeric peptidic conjugates for a new approach of anti-tumoral immunotherapy
17.15-17.30	**OP46 - Nir Qvit**
	Engineered protein-protein interaction regulators for therapeutic applications
17.30-17.45	**OP47 - Victor J Hruby**
	Utilizing Combinations of New Approaches to Peptide and Peptidomimetic Design for G-Protein Coupled Receptors
	Chairs: Nuno Santos, Kund Jensen
	Nanotechnology, Imaging and Analytical Techniques
17.45-18.00	**OP48 - Friedrich Bialas**
	Immobilising integral membrane proteins on silica
18.00-18.15	**OP49 - Konstantin Kuhne**
	Cathepsin B-Activatable Cell-Penetrating Peptides for Imaging Cancer-related Cathepsin B
18.15-18.30	**OP50 - Neil O'Brien-Simpson**
	Designing antibiotic peptide polymer adjuvants for multidrug resistant bacteria
18.30-18.45	**OP51 - Dorian J Mikolajczak**
	Tuning the Catalytic Activity and Substrate Specificity of Peptide-Nanoparticle Conjugates
18.45-19.00	**OP52 - Ramon Subiros-Funosas**
	Lighting up programmed cell death in real-time: apoTRACKER as an in vivo compatible apoptosis diagnostic tool

	August 30 Thursday AM
	Chair: Luis Morodor
08.30-09.10	**IL7 - Dek Woolfson** Peptide design and assembly: from the test tube to inside cells
	Chairs: Alethea Tabor, Gábor Mező
	Peptides in Chemical Biology and Therapeutics 2
09.10-09.25	**OP53 - Anne Conibear** Synthetic integrin-binding immune stimulators target cancer cells and prevent tumor formation.
09.25-09.40	**OP54 - Ana Salomé Veiga** Activity of the viral-derived peptide pepR against Staphylococcus aureus biofilms and insights into the mechanism of action
09.40-09.55	**OP55 - Timothy Reichart** Development of Mirror-Image Monobodies for Targeted Cancer Therapies
09.55-10.10	**OP56 - Paul Alewood** Subtle modifications to oxytocin produce ligands that retain potency and improved selectivity across species
10.10-10.25	**OP57 - Roland Hellinger** Cyclotides as novel inhibitors of the human prolyl oligopeptidase
	Chair: Norbert Sewald
11.00-11.30	**IL8 - Wilfred van der Donk** Posttranslational Modifications during Cyclic Peptide Biosynthesis
	Chairs: Rosario Gonzalez-Muñiz, Christian Gruber
	Peptides in Chemical Biology and Therapeutics 3
11.30-11.45	**OP58 - Youhei Sohma** Near-infrared photoactivatable oxygenation catalyst of amyloid peptide
11.45-12.00	**OP59 - Minying Cai** Development of the MC1R Selective Ligands for the Melanoma Prevention
12.00-12.15	**OP60 - Sónia Henriques** Is the mirror image a true reflection? Lipid chirality in the activity of the prototypic cyclotide kalata B1
12.15-12.30	**OP61 - Jane Aldrich** Substitution of Aromatic Residues in the Macrocyclic Opioid Peptide [D-Trp]CJ-15,208 Alters the Opioid Activity Profile in vivo
12.30-12.45	**OP62 - Maayan Gal** Developing novel bi-specific peptide as an immunosuppressant using directed-evolution optimization

	August 30 Thursday PM
	Chair: Maurice Manning
14.15-14.45	**Glenn F King**
	From pain to pain-killers: using spider-venom peptides to help us understand and treat chronic pain
	Chairs: Beate Koksch, Ale Närvänen
	Peptides in Chemical Biology and Therapeutics 4
14.45-15.00	**Christina Schroeder**
	The use of peptide-membrane interactions in the design of selective and potent sodium channel inhibitors
15.00-15.15	**Lydia Behring**
	Dipeptide-derived Alkynes as Novel Irreversible Inhibitors of Cathepsin B
15.15-15.30	**Tamis Darbre**
	Potent Antimicrobial Peptide Dendrimers against Multi-Drug Resistant Pseudomonas aeruginosa and Acinobacter baumanii
15.30-15.45	**Garry Laverty**
	Modifying short phenylalanine-phenylalanine peptide sequences to create multifunctional nanomaterials with biomaterial and drug delivery applications
	Chairs: Norman Metanis, Steven Ballet
	Amino Acids, Proteins and Synthesis 3
16.15-16.30	**Abhishek Iyer**
	Reviving Old Protecting Group Chemistry for Site-Selective Peptide-Protein Conjugation
16.30-16.45	**Hironobu Hojo**
	Use of selenoester for the efficient synthesis of protein
16.45-17.00	**Hannes Ludewig**
	Eukaryotic macrocyclases enhance biocatalytic production of cyclic peptides
17.00-17.15	**Juergen Machielse**
	Cost Effective peptide Purification via ZEOsphere DRP Mixed-Mode Chromatography
17.15-18.00	**CLOSING CEREMONY**
18.00-20.00	**BACHEM RECEPTION**
20.00-22.00	**CONFERENCE BANQUET**

Award Lectures

AL1/A228

Lessons from Selenium Chemistry to Study Protein Science

Norman Metanis

HUJI, Israel

Selenium occurs rarely in natural proteins, but is becoming a commonly used element in unnatural contexts to aid in the study of protein structure and function. In its natural context, selenium's role remains uncovered in half of the 25 human selenoproteins. With the aid of chemical protein synthesis, a full characterization of many of these proteins looms close on the horizon. In unnatural contexts, selenium serves as a traceless handle in native chemical ligations and as a folding chaperone. New amino acids containing selenium allow previously unfavorable protein syntheses to occur with good yield. These lessons also allow us to study protein chemistry without the use of selenium. We will discuss selenium's contributions to protein chemistry thus far, as well as its potential in future applications.

AL2/A235

The power of chemoselectivity: Functional peptide- and protein-conjugates for extra- and intracellular targeting

Christian Hackenberger

FMP and HU Berlin, Germany

Our lab aims to identify new bioorthogonal reactions for the synthesis and modification of functional peptides and proteins. We apply these highly selective organic reactions1 to study functional consequences of naturally occurring posttranslational protein modifications (PTMs), in particular phosphorylated Lys- and Cystein-peptides,2 as well as to generate novel peptide- and protein-conjugates for pharmaceutical and medicinal applications. In this presentation l will focus on the chemical modification of functional proteins as well as their cellular delivery. Thereby, we employ cyclic cell penetrating peptides (cCPPs) to transport a functional full length protein to the cytosol of living cells as recently demonstrated by the direct delivery of GFP-conjugates.3 For protein modification we use a combined approach of intein expression as well as recently developed bioorthogonal reactions and enzymatic ligations, for instance the so-called Tub-tag labeling.4 This concept is finally applied to generate new antibody-drug conjugates, multivalent protein-scaffolds as well as cell-permeable nanobodies, i.e. small antigen binding proteins that remain active within the reductive milieu inside living cells, to interfere with intracellular targets.5

References 1. D. Schumacher, C.P.R. Hackenberger, Curr. Opin. Chem. Biol. 2014, 22, 62-69. 2. a) J. Bertran-Vicente, R.A. Serwa, M. Schümann, P. Schmieder, E. Krause, C.P.R. Hackenberger, J. Am Chem. Soc. 2014, 136(39), 13622-13628; b) J. Bertran-Vicente, M. Penkert, O. Nieto, M. Schümann, P. Schmieder, E. Krause, C.P.R. Hackenberger, Nature Comm. 2016, 7,12703. 3. N. Nischan, H.D. Herce, F. Natale, N. Bohlke, N. Budisa, M.C. Cardoso, C.P.R. Hackenberger, Angew. Chem. Int. Ed. 2015, 54(6), 1950-1953. 4. a) D. Schumacher, J. Helma, F.A. Mann, G. Pichler, F. Natale, E. Krause, M.C. Cardoso, C.P.R. Hackenberger, H. Leonhardt, Angew. Chem. Int. Ed. 2015, 54(46), 13787-13791, b) D. Schumacher, O. Lemke, J. Helma, L. Gerszonowicz, V. Waller, T. Stoschek, P.M. Durkin, N. Budisa, H. Leonhardt, B.G. Keller, C.P.R. Hackenberger, Chem. Sci. 2017, 8, 3471-3478 5. H. Herce, D. Schumacher, F.A. Mann, A. Ludwig, A. Schneider, M. Filies, S. Reinke, C. Cardoso, C.P.R. Hackenberger, Nature Chem. 2017, 9, 762-771.

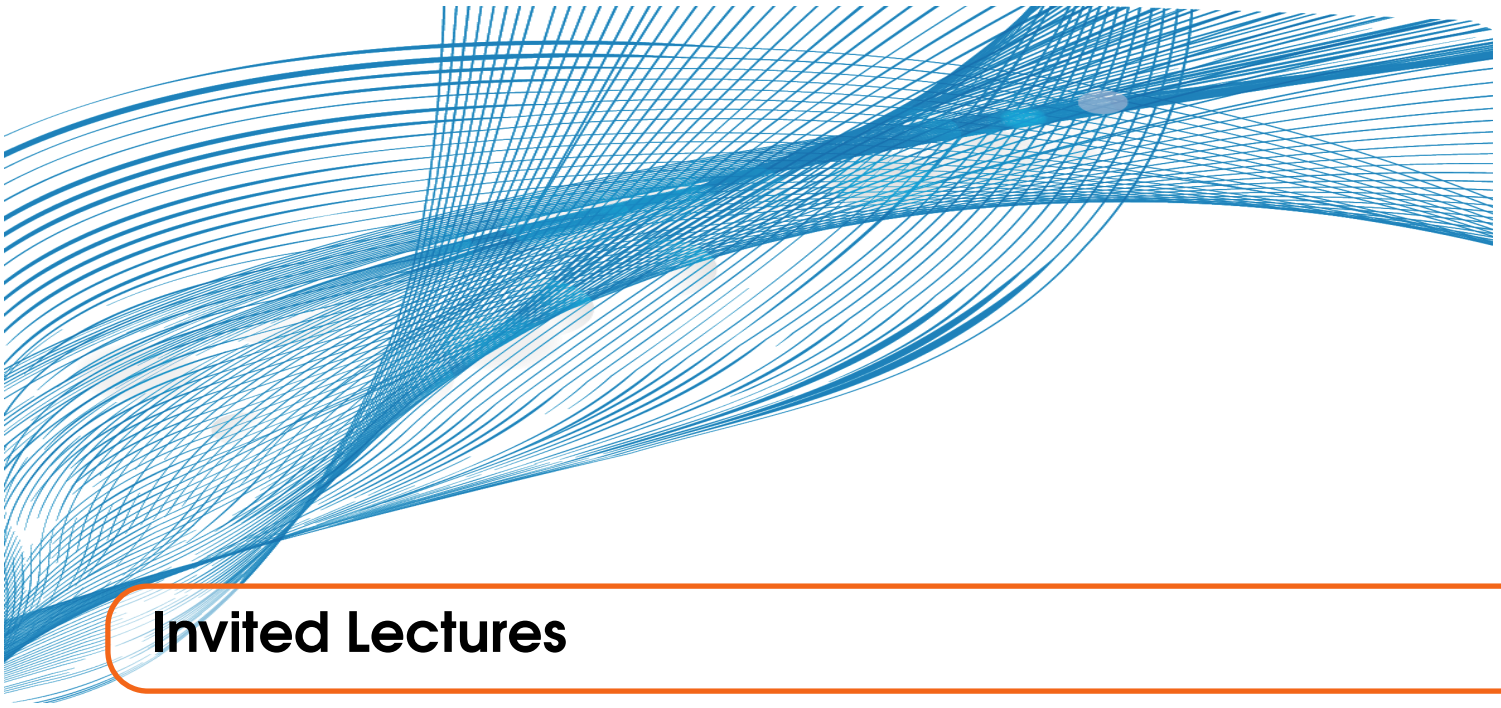
AL3/A247

Advances in Site-specific and Linkage-specific Ligation

James Tam

School of Biological Sciences, Synzymes and Natural Products Center, Nanyang Technological University, Singapore

Over the past 25 years, major advances are ligation chemistries to form peptide or non-peptide bonds between molecules. These advances are enabled by the development of novel chemical ligation methods and expansion of genetic condons as well as the discovery of inteins and peptide ligases. Of particular interest to our laboratory are methods to bond specifically between peptides, peptide-to-protein, and protein-to-protein, without protecting groups, activating agents and under aqueous conditions. Here, I will present our work in ligation chemistry to enable site-specific bonding of chemicals, polymers, peptides and proteins to form new compounds under physiological conditions. In particular, I will discuss our work on the Asx-specific peptide ligases such as the Butelases which act as peptide stapler or superglue for labeling proteins and live cells as well as precision biomanufacturing industrial enzymes and therapeutics.



Invited Lectures

IL1

Adventures in Drugging Undruggable Targets

David Spring

University of Cambridge, United Kingdom

The pharmaceutical industry is an enormously successful business sector. However, the current challenges facing the industry are unprecedented. A high attrition rate of clinical candidates (ca. 93-96%) is prevalent throughout the industry. My talk aims to highlight some of the underlying factors of this. Namely, (1) the relative lack of structural diversity and (2) the relative lack of targets exploited in drug discovery. The talk will include recent research within my laboratory aimed at addressing these issues, especially our work on conformationally constrained peptides.

IL2

The design and assessment of the therapeutic potential of apelin-13 peptide mimetics for alleviating metabolic dysfunction in diabetes and obesity

Finbarr O'Harte

Ulster University, United Kingdom

Peptides involved in cross-talk between tissues could hold multiple beneficial metabolic effects which in turn could be harnessed for drug development. With the success of glucagon-like peptide-1 (GLP-1) mimetics for treating Type 2 diabetes over the past two decades, interest in the potential of peptide therapeutics for managing metabolic disorders has increased. Peptides have the ability to target specific receptors in multiple tissues and improve various metabolic functions. This work will focus on stable peptide mimetics of the adipokine apelin-13, which we have shown has important anti-diabetic and anti-obesity actions. Preproapelin processing in adipose tissue leads to the production of multiple molecular isoforms derived from the C-terminal region of the 58 amino acid precursor, including apelin-36, -17, -13 and pGlu-apelin-13. Apelin-13 remains the smallest bioactive form of the peptide which binds to the cognate APJ receptor. Apelin is already well recognised for its benefits in cardiovascular physiology. Here we have produced multiple stable mimetics of apelin-13 which show very promising metabolic benefits in various mouse models of obesity and diabetes. The work of designing, testing their efficacy in vitro using cell models, assessing their stability in mouse plasma and their acute and chronic metabolic actions in vivo will be covered here. Selected stable apelin-13 analogues were tested in comparative studies against established GLP-1 mimetics such as exendin-4 and liraglutide, in high fat fed diet induced obese diabetic mice, as well as diabetic db/db mice. Chronic treatment with acylated apelin-13 analogues show similar or enhanced therapeutic benefit compared with incretin mimetics in diabetic mice. In conclusion, these analogues show promising anti-diabetic and anti-obesity actions which could be further exploited to target these common metabolic diseases.

IL3

Theranostic radiopeptides

Marion De Jong

Erasmus MC Rotterdam, Netherlands

One of the research programs of the department of Radiology at the Erasmus MC in Rotterdam focuses on peptide receptor-targeted imaging (PRI), applying positron emission tomography (PET) and single photon emission computed tomography (SPECT), and peptide receptor-targeted radionuclide therapy (PRRT) of receptor-positive cancers with radiolabelled peptides. Selective receptor-targeting radiopeptides have emerged as a very important class of radiopharmaceuticals for molecular imaging and therapy of tumours that overexpress peptide receptors on the cell membrane. After such peptides labeled with gamma-emitting radionuclides bind to their receptors, they allow visualization of receptor-expressing tumors non-invasively. Peptides labeled with beta- or alpha-particle emitters can eradicate receptor-expressing tumors. In the clinic we imaged thousands patients using radiolabelled somatostatin analogues, whereas over 2000 treatments were given. Our efforts now concentrate on widening the therapeutic window by combination therapies, increasing the tumour radiation dose, and/or decreasing the dose to the normal, healthy, organs. Finally we aim at curing these now incurable metastasized cancers. We also design and evaluate analogues of other peptides, including bombesin, CCK, and neurotensin analogues, that bind to their receptors in a variety of different tumours, to expand the panel of tumours to be imaged and treated.

IL4

Gate2Brain Shuttle Peptides, From Discovery to Applications.

Meritxell Teixidó

IRB Barcelona, Spain

Gate2Brain shuttle peptides represent salvage for new or previously rejected CNS drug candidates by providing a way to cross the blood-brain barrier (BBB).

Gate2Brain technology consist on a toolbox of peptides able to cross the BBB and carry compounds covalently attached (including small molecules, peptides, proteins, antibodies, plasmids, siRNA or mRNA loaded nanoparticles, etc…) that cannot cross this barrier unaided. They have proofed to carry these cargoes in vitro and in vivo. These peptide shuttles use the existing transport mechanisms at the BBB without affecting the normal functioning of these mechanisms and preserving brain homeostasis.

By improving the delivery of therapeutic candidate to the CNS, we will ensure immediate impact in many CNS diseases patients. In addition, in a broader perspective, Gate2Brain technology may help to repurpose existing therapies previously rejected because of difficulty to reach the brain, accelerating the translation towards clinical development. Gate2Brain will also result in the application of lower concentrations of therapeutic agent, thereby significantly lowering systemic side effects and reducing the cost of the treatment.

Gate2Brain peptides combine protease resistance, capacity to carry a wide range of cargoes thanks to their versatility, low production costs, and low immunogenic risk. They provide a non-invasive, non-antigenic, permeable, stable, soluble and receptor-specific way to transport drugs across the BBB and into the CNS.

References:

1 B. Oller-Salvia et al. Chem. Soc. Rev. (2016), 45, 4690-4707. 2. M. Sánchez-Navarro et al. Curr. Opin. Chem. Biol. (2017), 38, 134-140. 3.

Invited

M. Sánchez-Navarro et al. Acc. Chem. Res. (2017), 50, 1847-1854. 4. R. Prades et al. Angew. Chem. Int. Ed. (2015), 54, 3967-3972. 4. B. Oller-Salvia et al. Angew. Chem. Int. Ed. (2016), 55, 572-575.

IL5

Selective N-terminal acylation of peptides and proteins with an optimized His-sequence

Knud J. Jensen

University of Copenhagen, Denmark

Site-selective modification of proteins is highly desirable for the controlled introduction of bioorthogonal handles, small probes like biotin, or larger moieties such as PEG. Due to their general applicability, chemical methods targeting the N-terminus are highly attractive. We set out to develop a new, flexible and mild method for selective N-terminal acylation of proteins. The starting point was a side-reaction found to occur during protein expression, where an N-terminal His-tag reacts with gluconolactone. First, we optimized the N-terminal end to promote its selective acylation. We discovered that Gly-His3-6 promoted near-quantitative N-terminal acylation. Next, we fine-tuned the acylating agents, with 4-methoxyphenyl esters providing excellent selectivity. Finally, the method was successfully applied to three model proteins. Maltose-binding protein, a small ubiquitin-like modifier, and enhanced green-fluorescent protein were selectively modified with azido and biotin moieties. We believe that this novel and highly selective method could find general application for the chemical modification of expressed proteins.

IL6

Fluorine in Peptide and Protein Engineering

Beate Koksch

Freie Universität Berlin, Institute for Chemistry and Biochemistry, Germany

In a league of its own, fluorine has the potential to enable us to engineer biopolymers with highly desirable properties. However, the particular challenge in using it as a tool lies in our ability to juggle the interplay between the specific properties of the fluorinated building block and its responsiveness to the environment it is exposed to. Fluorine has been shown to impart often favorable but seldom predictable properties to peptides and proteins, a phenomenon that is caused by the nature of the fluorine atom and properties of the C-F bond. Up until about two decades ago, the outcomes of fluorine modification of peptides and proteins were largely left to chance. Driven by the motivation to extend the application of the unique properties of the element fluorine from medicinal and agro chemistry to peptide and protein engineering we have established multiple peptide and protein models with different properties for the purpose of studying the consequences of fluorine substitution in the context of a protein environment. In general, the consequences of incorporating the C-F bond into a biopolymer can be attributed to two distinct yet related phenomena: 1) the fluorine substituent can directly engage in intermolecular interactions with its environment and/or 2) the other functional groups present in the molecule can be influenced by the electron withdrawing nature of this element (intramolecular) and in turn interact differently with their immediate environment (intermolecular). Also, we could show that not only the nature of the side chain but also its immediate environment determine the outcome of this substitution. We have especially been fascinated to observe that the difference of as subtle as one fluorine atom in the side chain of an amino acid can dramatically influence the key properties of peptides and proteins such as hydrophobicity, polarity and secondary structure propensity. These properties are crucial factors in peptide and protein engineering as they direct properties as important as proteolytic stability and folding, and thus affect protein function. One of our current projects studies the way in which not just biomolecules in the laboratory, but whole living organisms accommodate fluorine and this talk will introduce our first results of this endeavor.

IL7

Peptide design and assembly: from the test tube to inside cells

Dek Woolfson

Schools of Chemistry and Biochemistry & Bristol BioDesign Institute, University of Bristol, United Kingdom

Protein design—that is, the construction of entirely new protein sequences that fold into prescribed structures—has come of age. It is now possible to design proteins de novo using simple rules of thumb or computational design methods. The designs can be made rapidly via peptide synthesis or the expression of synthetic genes; and the resulting proteins can usually be characterised all the way through to high-resolution X-ray crystal structures. Contemporary questions in the protein-design field include: What do with these new-found skills? What protein structures and functions do we target? How far can we move past the confines of natural protein structures and functions? And, how can we take protein design from an exercise largely done in silico and in vitro into a truly synthetic biology and take it in vivo?

This talk will address these questions with reference to simple through to complex and functional peptide designs and assemblies that we have explored over the past 5 – 10 years. These use a straightforward protein structure, called the α-helical coiled coil, which are bundles of 2 or more α helices found in many protein-protein interactions. Coiled coils provide an excellent basis for building proteins from the bottom up.

The vast majority of coiled-coil designs have been based on simple rules of thumb learnt from natural proteins or derived empirically through experiment.1 These rules relate sequence to structure to guide the specification of coiled-coil oligomerization state, strand orientation, partner selection, and, to some extent, stability. This has been extremely informative and productive, and design and engineering is probably more advanced for coiled coils than for any other protein structure.2 However, to move past the low-hanging fruit of coiled-coil design, and into the so-called dark matter of protein structures, we will all have to learn new tricks. To address this we have begun to tackle coiled-coil design parametrically using computational methods.3 We have developed easy-to-use computational modelling tools4 and a more-sophisticated suite of programs called ISAMBARD5 that allow the rapid generation and optimisation of protein designs in silico.

References De novo protein design: how do we expand into the universe of possible protein structures? Curr Opin Struct Biol 33, 16-26 (2015)

Engineered synthetic scaffolds for organizing proteins within the bacterial cytoplasm. Nature Chem Biol 14, 142-147 (2018)

IL8

Posttranslational Modifications during Cyclic Peptide Biosynthesis

Wilfred van der Donk

Department of Chemistry and Howard Hughes Medical Institute, University of Illinois at Urbana-Champaign, United States

The genome sequencing efforts of the first decade of the 21st century have revealed that ribosomally synthesized and post-translationally modified peptides (RiPPs) constitute a very large class of peptide natural products. These molecules are produced in all three domains of life, their biosynthetic genes are ubiquitous in the currently sequenced genomes, and their structural diversity is vast. Lanthionine-containing peptides (lanthipeptides) are examples of this growing class and many members are highly effective antimicrobial agents that display nanomolar minimal inhibitory concentrations (MICs) against pathogenic bacteria (lantibiotics). These peptides are post-translationally modified to install multiple thioether crosslinks. During their biosynthesis, a single enzyme typically breaks 8-16 chemical bonds and forms 6-10 new bonds with high control over regio- and chemoselectivity. This presentation will discuss investigations of the mechanisms of these remarkable catalysts as well as their use for the generation of non-natural cyclic peptide libraries.

IL9

From pain to pain-killers: using spider-venom peptides to help us understand and treat chronic pain

Glenn King

The University of Queensland, Australia

Most venoms have evolved to facilitate defense against predators, either as the sole purpose of the venom (e.g., bees and caterpillars) or as a function that is secondary to its role in predation (e.g., centipedes, cone snails, snakes, and spiders). In most cases, the primary method of predator deterrence is the injection of venom peptides that cause immediate, intense pain. We are taking advantage of the ability of venoms to robustly activate nociceptive pathways in mammalian neurons to interrogate novel pain signaling mechanisms and identify new analgesic drug targets. Moreover, since the venoms of arthropod predators such as spiders are dominated by disulfide-rich peptides that target a wide variety of ion channels, we are screening these natural combinatorial libraries to identify peptides that target newly identified ion channel drug targets.

We used this approach to show, for the first time, that the voltage-gated sodium channel Nav1.1 is involved in transduction of mechanical pain, such as the abdominal pain that occurs in inflammatory bowel syndrome (IBS). This was made possible by isolation of a spider-venom peptide (Hm1a) that selectively inhibits inactivation of Nav1.1, and to a lesser extent Nav1.3, without affecting any of the other seven human sodium channel subtypes [1]. We show that Hm1a inhibits channel inactivation by impeding movement of the domain IV voltage sensor, which is linked to the channel inactivation gate. By screening a large panel of spider venoms we isolated several disulfide-rich peptides that selectively inhibit activation of Nav1.1 and the well known analgesic target Nav1.7. We have used these peptides to show that dual targeting of these channels is an effective strategy for abrogating gut pain in a mouse model of IBS.

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Oral Presentations

OP1

Lipid domain formation induced by antimicrobial peptides OP-145 and SAAP-148 results in efficient killing of Escherichia coli

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OP-1451 and SAAP-1482, two amphipathic antimicrobial peptides developed from the human cathelicidin LL-37, kill Gram-positive bacteria such as Staphylococcus aureus by permeabilizing the cytoplasmic membrane interacting preferentially with their major lipid constituent, the negatively charged phosphatidylglycerol. Both peptides also kill Gram-negative bacteria at similar concentrations. Here, we show that the killing of Escherichia coli does not fully correlate with membrane permeabilization of live bacteria, although both peptides neutralize the surface charge of Escherichia coli as shown by Zeta potential measurements. Whereas both peptides fully permeabilize membranes composed of phosphatidylglycerol, they exhibit limited permeabilization of membranes composed of either lipids extracted from Escherichia coli or mixtures of synthetic phosphatidylglycerol/phosphatidylethanolamine/cardiolipin, which mimic the inner membrane of Gram-negative bacteria. Nevertheless, differential scanning calorimetry studies clearly indicate lipid domain formation and a disordering of the lipid chain packing. Formation of lipid domains is also observed in staining experiments on live Escherichia coli using the fluidity sensitive dye Nile Red. Interestingly, the concentration-dependent domain formation is in accordance to the killing rate of Escherichia coli.
[1] Malanovic et al, Biochim Biophys Acta. 2015.; [2] de Breij et al, Sci Transl Med. 2018

OP2

Engineering Hybrid Peptidomimetics for Improved Pain Treatments

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To address the different types of pain, different classes of medications, mainly non-steroidal anti-inflammatory drugs and narcotics (opioids), are used. The alleviation or treatment of moderate to severe pain states, in particular, commonly invokes the use of opioids. Unfortunately, their chronic administration induces various undesirable side effects. One strategy to overcome these major side effects and to prolong the antinociceptive efficiency of the applied drugs involves the creation of multifunctional compounds which contain hybridized structures. Combination of opioid agonist and antagonist pharmacophores in a single chemical entity has been considered and extensively investigated, but opioids have also been combined with non-opioid bioactive neurotransmitters and peptide hormones that are involved in pain perception (e.g. substance P, neurotensin, etc.).[1] Such novel chimeras (also called designed multiple ligands or DMLs), may interact independently with their respective receptors and potentially result in more effective antinociceptive properties. The designed multiple ligands presented in this work include peptide-based opioid-non-opioid dimer analogs, such as for example opioid-neurokinin 1 receptor,[2,3] opioid-nociceptin [4] and opioid-neuropeptide FF DMLs.[5] Some of the prepared ligands demonstrated to be dually effective in both acute and neuropathic pain models. Gratifyingly, compounds with reduced analgesic (cross-)tolerance (with morphine), physical dependence, and respiratory depression were unraveled. The synthesis and in vitro and in vivo pharmacological evaluation will be discussed. Additionally, another type of hybridization will be presented: a fusion between opioid pharmacophores and hydrogel-forming hexapeptide sequences. While the above opioid pharmacophores induce analgesic effects up to 8 hours post injection, fusion with self-assembling hexapeptide sequences allowed formation of injectable hydrogels, which slowly degrade subcutaneously after injection. This innovative controlled-drug delivery method gave antinociceptive effects of 72-96h.[6] The generic nature of this methodology indicates that this system could be broadly applicable to other biologically active peptides as well.

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OP3

An engineered cyclic peptide alleviates symptoms of inflammation in a murine model of inflammatory bowel disease

Claudia Cobos Caceres, Paramjit Bansal, Severine Navarro, David Wilson, Laurianne Don, Paul Giacomin, Alex Loukas, Norelle Daly

James Cook University, Australia

Inflammatory bowel diseases (IBDs) are a set of complex and debilitating diseases, for which there is no satisfactory treatment. Recent studies have shown that small peptides show promise for reducing inflammation in models of IBD. However, these small peptides are likely to be unstable and rapidly cleared from the circulation, and therefore, if not modified for better stability, represent non-viable drug leads. We hypothesized that improving the stability of these peptides by grafting them into a stable cyclic peptide scaffold may enhance their therapeutic potential. Using this approach, we have designed a novel cyclic peptide, which comprises a small bioactive peptide from the annexin A1 protein grafted into a sunflower trypsin inhibitor cyclic scaffold. We used native chemical ligation to synthesize the grafted cyclic peptide. This engineered cyclic peptide maintained the overall fold of the naturally occurring cyclic peptide, was more effective at reducing inflammation in a mouse model of acute colitis than the bioactive peptide alone, and showed enhanced stability in human serum. Our findings suggest that the use of cyclic peptides as structural backbones offers a promising approach for the treatment of IBD and potentially other chronic inflammatory conditions.

OP4

Penetrating the Impenetrable: Progress towards a Non-hormonal Male Contraceptive

Sarah Jones, John Howl

University of Wolverhampton, United Kingdom

The intracellular delivery of bioactive agents and subsequent therapeutic manipulation of sperm physiology poses a significant challenge. The sperm plasma membrane presents a static physical barrier and mechanisms such as endocytosis, which facilitate the movement of macromolecules into the majority of eukaryotic cells, are severely restricted in mature sper-

matozoa. This limitation is further compounded by the fact that at the time mammalian sperm are released into the seminiferous tubules, the processes of transcription and translation have largely been silenced thus limiting therapeutic targets to protein-protein interactions and post-translational modifications. We have previously established that cell penetrating peptides (CPPs) rapidly and efficiently enter bovine and human spermatozoa whilst being compatible with sperm physiology. Moreover, a range of structurally diverse CPPs demonstrate a propensity to accumulate in different sperm compartments, thus propounding their utility for the delivery of bioactive cargoes to specific intracellular loci [1,2]. The development and application of bioportides, bioactive cell permeable peptides that mainly act by a dominant-negative mode of action, is a unique approach to the regulation of sperm physiology. Identified by QSAR prediction algorithms designed to recognize CPPs within entire proteins or sites of protein-protein interactions, bioportides possess the dual features of cellular penetration and biological activity and are therefore distinct from the more commonly used inert vectors such as tat and penetratin [3]. As an initial proof of concept we utilized bioportide technology to modulate Ca2+ signalling in human spermatozoa, which governs fundamental processes such as hyperactivation, capacitation and the acrosome reaction. Synthesis and evaluation of STIM371-392, a candidate bioportide corresponding to the cationic region the STIM1-Orai1-activating domain of STIM1, an endoplasmic reticulum calcium sensor and regulator of [Ca2+]i, sustained progesterone-induced [Ca2+]i transients in human spermatozoa[2]. Our recent endeavours have focused on bioportides that control male fertility. Sperm motility, a prognostic parameter of human fertilization capacity, is regulated by the activity of a male gamete-specific isoform of phosphoprotein phosphatase 1 (PPP1), phosphoprotein phosphatase 1 catalytic subunit gamma 2 (PP1gamma2) during epididymal transit. Bioportides designed to target the PP1gamma2 interactome, discretely and rapidly inhibit human sperm motility and have proven unmatched potential for validation as a non-hormonal male contraceptive [4]. In conjunction with Margarida Fardilha at the University of Aviero, our strategy has been to selectively target post-testicular events with these peptide therapeutics and in doing so, avoid the many side effects associated with hormonal-based male contraception. Our current objective is to further develop these bioportides towards clinical utility as a potential contraceptive option.

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OP5

Peptide-Based Biopesticides

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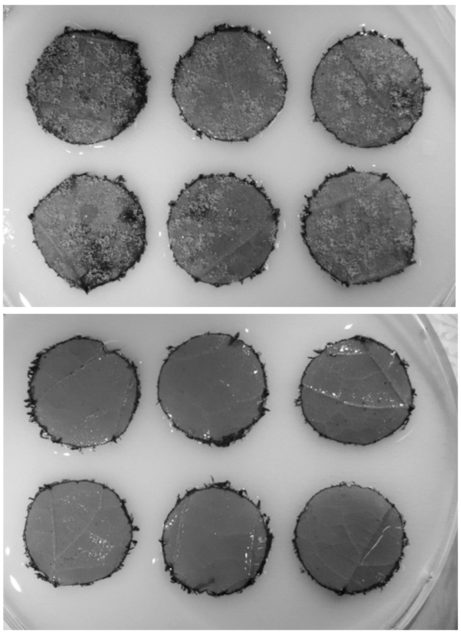
In this presentation we describe the great potential of peptides as biopesticides. Currently Europe is greatly encouraging research in sustainable pest-management. Finding eco-friendly, effective alternatives to synthetic pesticides is of paramount importance, especially against the so-called priority pests of fruits and vegetables. For some of these pests, such as botrytis cinerea and peronospora viticola, no effective bio-alternatives to small organic molecules are available so far. Fungi belonging to the genus Trichoderma are distributed worldwide and have been used successfully in field trials against many crop pathogens. They produce peptaibols, a peculiar family of peptides, as part of their defense system against other microorganisms. Such secondary metabolites are known for their plant-protection properties: they (i) possess antimicrobial activity, (ii) act as stimulants of plant defences and growth (iii) elicit plant production of volatiles to attract natural enemies of herbivorous insects. By means of a versatile SPPS strategy, we produced several analogs of such naturally occurring peptides. With such compounds, we can circumvent both the health hazards and the unreliable effectiveness in open field connected with the use of antagonistic microorganisms as biological control agents, while keeping the biomolecules responsible for their beneficial effects. Our peptides have been tested (alone or in combination) both in vitro and in vivo against a variety of priority pests, such as the fungi Botrytis cinerea and Penicillum italicum, and the bacterium Pectobacterium carotovorum. We identified several peptaibol analogs with a broad-spectrum activity as biopesticides, able to completely inhibit the growth of B. cinerea and many other pathogens for over a week at low micromolar concentrations.



OP6

Development and Exploitation of Photo-Crosslinking Methodology to Study Protein-Protein Interactions

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A key problem in life-sciences research is to understand protein-protein interactions (PPI) with molecular and temporal resolution – this would allow the identification of the transient intermediates that play key roles in the function of biomacromolecular machines, signalling, translocation and folding to illuminate our understanding of disease development e.g. aberrant intracellular cell signalling in cancer and aggregation in amyloid disease. In this presentation we will describe our latest developments in the area of photo-activated peptide/protein labelling and cross-linking chemistry. In the first section we will discuss the development of a suite of diazirine-based cross-linking reagents; these reagents could transform structural proteomics methodology. Diazirines are ideal cross-linking groups because upon excitation with UV light, they generate highly reactive carbenes capable of indiscriminate insertion into proximal bonds.[1] We are interested in applying these reagents to study β-sheet mediated PPIs specifically assembly of peptides into amyloid fibrils[2] and will illustrate how, in combination with state-of the art mass-spectrometry, cross-linking encodes non-covalent structure in cross-linked peptides.[3] In the second part of the presentation, focusing on the helix mediated NOXA-B/MCL-1 PPI[4] we will describe efforts to develop peptides conjugated to ruthenium (II) (tris)chelates as reagents that can perform photo-activated traceless protein-labelling reactions[5] – a necessary first step in being able to label proteins in live cells without abrogating their normal function

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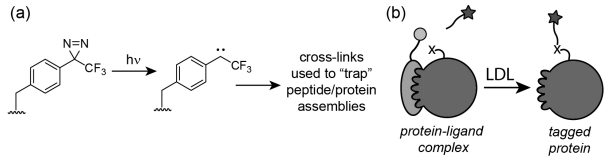

***Figure 1**. Protein Labelling by PIC (a) UV activated carbene formation (b)schematic depicting the use of photo-activated probes to tag proteins*

OP7

Toward homogeneous glycoproteins via auxiliary-assisted sequential glycosylation and ligation of peptides

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Having access to homogeneous proteins carrying complex posttranslational modifications (PTMs) is essential for studying the role of the PTMs in protein function and misfunction.

We established a strategy for the synthesis of peptides carrying multiple and complex PTMs, focusing at first on the preparation of homogenously glycosylated peptides. The tumor marker MUC1, a protein abundantly O-glycosylated in his extracellular domain, was chosen as synthesis target. Chemically synthesized mucin peptides are conjugated to a photocleavable ligation auxiliary [1], obtained via multistep synthesis, that supports native chemical ligation (NCL) and carries a PEG polymer. This facilitates effective enzymatic glycosylation and recovery of the resulting glycopeptides without the need for chromatographic steps [2]. It also gives access to glycosylated peptide α-thioesters that are otherwise inaccessible [3]. The conjugates are linked to each other via auxiliary-mediated NCL and the ligation products are recovered as unprotected glycopeptides after UV irradiation.

Currently a library of mucin polypeptides with multiple glycosylations is assembled that will be used in proteomic studies to provide new insights into the role of glycosylation in mucin functions and cancer progression. At the same time we are expanding the approach to sequential ligation [4] in combination with sequential glycosylation, to gain access to larger, more complex homogenous glycoproteins.

These developments also require extension of this method towards ligation at sites different from glycine and to other PTMs, in order to broaden it toward the synthesis of any kind of homogeneously posttranslationally modified protein.

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OP8

Peptide-Guided Assembly of Armadillo Repeat Protein Fragments

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We have recently discovered that it is possible to reconstitute a designed Armadillo repeat protein (ArmRP) from complementary fragments [1]. The two fragments, when mixed, will form a complex with nanomolar affinity. The structure of the complex is almost identical to the structure of the entire (single-chain) protein. ArmRP can be split at several places, within repeats as well as between repeats. The affinity of the fragments varies between nanomolar and micromolar Kd's, depending on the exact location of the split site. We have further shown that an ArmRP binder for a peptide can be split into two fragments, and that the reconstituted complex again recognizes the peptide. Moreover, it is possible to split the ArmRP such that the two fragments only interact in the presence of a peptide ligand (conditional assembly). We have then created a small library consisting of one (unlabeled) N-terminal plus a number of amino-acid selectively isotope-labeled C-terminal fragments. We could demonstrate using heteronuclear NMR that addition of a peptide shifts the equilibrium of formed complexes such that the complex that represents the best binder for that peptide is significantly enriched [2], even when the differences in binding affinities of the corresponding proteins complexes are small. I present spectroscopic (NMR) and other biophysical data to characterize the underlying proteins and protein complexes. We feel we have discovered a novel interesting property of ArmRP, in that they can be reconstituted from a large number of different complementary fragments, and that interesting biochemical applications may emerge from these systems. We feel that the methodology has distinct advantages when maturing new peptide binders over existing methods in that the split system has superior discriminatory power when compared to the usual single-chain approaches.

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OP9

Are gluten-related disorders a new protein aggregation disease?

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Gliadin, an immunogenic protein present in wheat, is not fully degraded by humans and after the normal gastric and pancreatic digestion, the immunodominant 33-mer gliadin peptide remains unprocessed. This 33 amino acid fragment is directed involved in celiac disease and probably in other immune pathologies associated to gliadin, with high prevalence such as non-celiac gluten sensitivity (7%). Although, the immunological response observed in the chronic phase of the celiac disease is well characterized; the cause and the initiation of the inflammatory events are still obscure (1). From the molecular point of view, the 33-mer behaves as an amphiphile forming nano-aggregates. The morphology of the nano-aggregates depend

strongly on concentration, and the secondary structure ranges from random, and PPII to parallel beta structure (2, 3). Recently, we reported that only large structures of 33-mer (>200 nm) induce an innate immune response in macrophages which is mediated by Toll-like receptor (TLR) 4 activation in humans. (4) Our investigations showed that if the 33-mer peptide aggregates, can trigger a specific innate immune response. Herein, I will present new insights into 33-mer oligomerization and its behavior in the cellular context by super-resolution optical microscopy. Our findings open a new understanding of the early stages of the disease and connect these disorder in the context of protein aggregation disease.

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OP10

Chemical Synthesis of Glycopeptides and Glycoproteins Using Acyl-thiol-ene Mediated 'Click' Ligation

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Trinity College Dublin, Ireland

Thiol-ene ligation has emerged as a highly efficient 'click' process with diverse applications in peptide synthesis and chemical biology. The high yield, mild reaction conditions and complete regioselectivity have enabled site specific elaboration of peptides and proteins with a range of biomolecules including carbohydrates. The methodology is fast and is compatible with oxygen, buffers and an aqueous environment. We have developed a novel thiol-ene mediated ligation strategy involving thio-acyl radicals (Acyl-Thiol-ene) for the rapid chemical synthesis of N-linked glycopeptides and glycoproteins. Using an sequential, one-pot, ligation/acyl-transfer/desulfurisation approach we have prepared a range of glycopeptides and glycoproteins with native linkages. The Acyl-Thiol-ene ligation is suitable for both peptide ligation and late stage N-glycosylation, rendering it an extremely useful methodology for glycoprotein synthesis. This approach represents a highly efficient strategy for accessing homogeneous peptides and proteins suitable for biological evaluation.

OP11

Multicyclic peptides constrained through noncanonical disulfide bonds and thioether crosslinks

Chuanliu Wu

Xiamen University, China

Multicyclic peptides, lying between larger biologics and small molecules in size and presumably combining the advantages of both, have been a treasured chemical space for drug development. Among the diverse peptide scaffolds constrained through covalent crosslinks, disulfide-rich peptides are most extensively explored. However, the synthesis and reengineering of these disulfide-constrained peptides are challenging, owing to the complexity of the oxidative folding process involving a number of diverse isomeric structures. In my group, we developed orthogonal disulfide pairing technologies to direct the oxidative folding of peptides into specific isomers, which involve synergistic manipulations of CXC motifs and cysteine analogs such as penicillamine.[1,2] By taking advantage of our orthogonal disulfide pairing technologies, a great diversity of multicyclic peptides with different disulfide patterns or connectivities have been designed and synthesized.[3,4,5] More importantly, these multicyclic scaffolds are extremely tolerant to sequence manipulations and disulfide isomerizations, thus are of particular interest for further designing or screening peptides with new structures and functions. Considering that disulfide-rich peptides might be not stable in biologically reducing environments, we also developed novel crosslink strategies to design and synthesize multicyclic peptides constrained with stable thioether bonds.[6,7] Benefiting from the recent development of these crosslink strategies, many new multicyclic scaffolds and bioactive peptides were de novo designed and synthesized in our lab. This lecture will describe the latest progress on the design and synthesis of multicyclic peptides constrained through disulfide or thioether crosslinks in our group.

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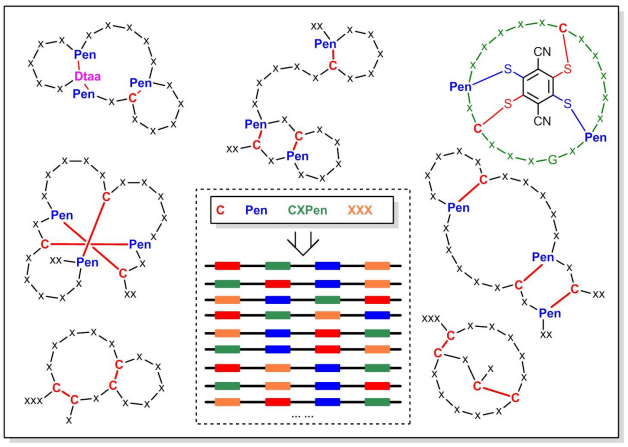

OP12

Novel methodology for the synthesis of α-indolyl-glycine containing peptide via direct asymmetric Friedel–Crafts reaction to peptidyl imine

Tsubasa Inokuma, Kodai Nishida, Akira Shigenaga, Ken-Ichi Yamada, Akira Otaka

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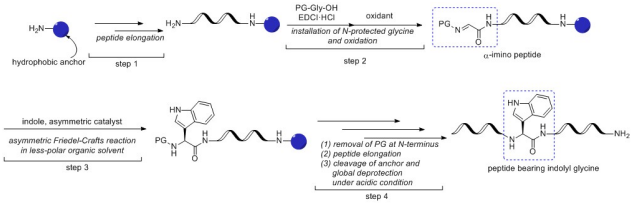
Peptides containing unnatural amino acids have received considerable attention as novel drug candidates because they often exhibit better biological activity and/or proteolytic stability than those of the peptides consisting of proteinogenic amino acids. The conventional strategy for the preparation of unnatural amino acid-containing peptides relied on (1) asymmetric construction of unnatural amino acids and (2) installation of those parts into peptide synthesis. As a method for the synthesis of unnatural amino acids, 1,2-addition to α-imino esters is a powerful tool because it can access diverse derivatives by changing only the nucleophiles. However, multi-step reactions are required for the preparation of protected amino acids suitable for peptide synthesis, which has hampered the diversity-oriented synthesis of peptides bearing unnatural amino acids. To solve this problem, we envisioned a novel strategy which employs an asymmetric construction of unnatural amino acid units by asymmetric 1,2-addition in a growing peptide chain. If an α-imino amide moiety could be installed onto the N-terminus of an elongating peptide, asymmetric 1,2-addition of the nucleophiles into the imino moiety would yield a peptide possessing an unnatural amino acid in its N-terminus. In this protocol, change of nucleophiles allows for the easy access to a diverse range of unnatural amino acid-containing peptides. We planned to incorporate a hydrophobic anchor that makes the peptidic substrate soluble in less-polar solvents because peptidic compounds are basically insoluble in less-polar organic solvents, such as toluene, chloroform, and dichloromethane, which are often most suitable for asymmetric catalytic reactions. As an unnatural unit of the target peptide, we chose α-indolyl glycine, which can be used as an analogue for proteinogenic aromatic amino acids. The first step is peptide elongation on the amine moiety of a hydrophobic anchor using liquid-phase peptide synthesis. Next, condensation of N-protected glycine on the growing peptide chain followed by oxidation of the N-terminal residue to the corresponding imine provides the site at which a wide range of unnatural amino acid residues can be incorporated. Next, asymmetric 1,2-addition of indole on the resulting imine is performed. Subsequent removal of the protecting group of N-terminus followed by the usual manipulations for peptide elongation, cleavage of hydrophobic anchor, and global deprotection by acidic treatment afford the desired peptides bearing α-indolyl glycine. In this presentation, we will discuss (1) optimization of the reaction condition of the asymmetric Friedel–Crafts reaction, (2) substrate scope of the Friedel–Ctafts reaction, (3) model study for deprotection and peptide elongation of α-indolyl glycine which is constructed by our reaction system, and (4) application to the synthesis of the α-indolyl glycine-containing peptide.



OP13

Palladium Prompted On-Demand Cysteine Chemistry for the Synthesis of Challenging Proteins

Muhammad Jbara[1], Shay Laps[1], Michael Morgan[2], Guy Kamnesky[1], Guy Mann[1], Cynthia Wolberger[2], Ashraf Brik[1]

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Thiazolidine (Thz) and Acetamidomethyl (Acm) protecting groups (PGs) are widely utilized in peptide and protein synthesis, however, the reaction time and the harsh conditions of their removal limit their utilities. Developing optimal removal conditions for theses PGs remains a challenge. We recently reported that palladium complexes can remove multiple Cys PGs within minutes in a fully aqueous medium,[2,3] which also could be coupled in-situ with native chemical ligation conditions to provide excellent yields of the desired products.[4,5,6] Although these new conditions have resulted in important advances in chemical protein synthesis, the utility of these approaches is limited by the lack of chemoselectivity of the palladium complexes when applying more than one PG in the synthesis. Here, we demonstrate unprecedented palladium chemoselectivity in a fully aqueous medium for on-demand orthogonal deprotection of Thz and Acm protections. We also show for the first time the efficient removal of Cys t-butyl PG using palladium in a fully aqueous medium and demonstrate its stability under the removal conditions of the Thz and Acm protections. The new chemistries reported here were applied for one-pot and site-specific modification of peptides containing multiple Cys residues, as well as for the total chemical synthesis of the CSP-1 protein, which contains 13 Cys residues. We also show the first total chemical synthesis of an activity-based probe of ubiquitinated histone H2A at Lys119 for the preparation of ubiquitinated nucleosome core particle probes. This probe exhibited reactivity with the with Calypso/ASX heterodimer deubiquitinase to form a stable covalent nucleosome-enzyme complex.

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OP14

Synthesis and Biomedical Applications of Substituted Amino-Lactam and Amino-Imididazolone Constraints

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Employing structural constraints to identify active peptide geometry and enhance therapeutic potential, our laboratory has pursued the synthesis of alpha-amino-gamma-lactam (Agl) and N-aminoimidazalone (Nai) residues [1]. The Agl and Nai residues can favor peptide backbone beta-turn geometry, as first shown for the former by Freidinger and Veber [2]. Extending their utility to study both side chain and backbone geometry, methods have been explored to add substituents respectively at the beta- and 5-positions of Agl and Nai residues [3]. Our presentation will describe the synthetic methods as well as application of these heterocycles to study

the conformation of peptide allosteric modulators of the interleukin-1 receptor for the treatment of preterm labour and related complications, such as retinopathy of prematurity [4,5].

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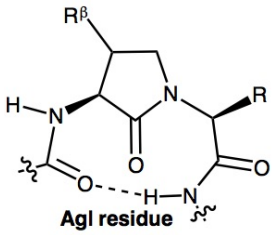

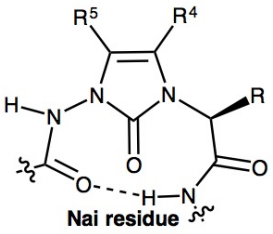

OP15

Recombinant synthesis and purification of hTFF2 protein in S. Cerevisiae

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Human trefoil factor family protein 2 (hTFF2) belongs to a family of peptides containing one or more characteristic trefoil domains —a distinctive three-leaved structure formed and stabilized by three disulfide bonds.[1] hTFF2 contains 106 amino acid residues and two trefoil domains formed by 6 disulfide bonds and these domains are interconnected with a 7th disulfide bond.[1b] Because of these disulfide bonds, they are stable secretory proteins expressed in gastrointestinal mucosa. Their physiological functions are not defined,[3] but they may protect the mucosa from insults, stabilize the mucus layer and affect healing of the epithelium.[2,4] Their size and cysteine-rich character are the main reason why these peptides have never been successfully synthesized. Only very limited amounts of hTFF2 can be prepared from human tissue extraction. Here, we describe a yeast expression system designed for the production of sufficient amounts of hTFF2 for physiological and biochemical studies. To obtain natively folded hTFF2 protein, we chose to express it in S. cerervisiae. We designed the hTFF2 gene encoding a fusion protein consisting of a hybrid leader sequence and the hTFF2 sequence. Recombinant plasmids were constructed. The leader sequence served to direct the fusion protein into the secretory pathway of cell and to expose it to the Kex 2 processing enzyme system. We optimized conditions for protein expression. The secreted hTFF2 was found in a glycosylated and an non-glycosylated form.[5] The two forms of hTFF2 were purified from the yeast fermentation broth by a combination of ultrafiltration, ion-exchange chromatography & preparative HPLC. The hTFF2 and glycosylated hTFF2 were analyzed by HR-MS.[5] Subsequently, we have synthesized N15 enriched analogue of hTFF2 protein and currently, we are analyzing its three dimensional structure by NMR. Moreover, this recombinant hTFF2 would serve to identify its receptor by using LRC-TRICEPs method and elucidate its role in intestinal wound healing.

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OP16

Do Quorum Sensing Peptides Play a Role in Psychiatric Disorders?

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The human microbiota is known to influence health. However, a changing human (gut) microbiota has also been linked to several diseases, including several psychiatric manifestations and neuro-developmental/-degenerative diseases like increased anxiety, depression, autism spectrum disorders, alterations in social behavior, and memory loss disorders. The mechanisms by which the gut microbiota influence these health conditions are however not yet fully understood. Currently, evidence indicates that these pathologies can be influenced by different bacterial metabolites, such as short chain fatty acids (SCFAs). In vitro studies from our group have now shown that quorum sensing peptides, i.e. bacterial metabolites which are constitutively produced and used by bacteria as inter-bacterial communication molecules, are also able to functionally modify neuronal brain cells [1]. Initial kinetic studies further indicated that these peptides are able to permeate the intestinal wall (in vitro), as well as the blood-brain barrier (in vivo) [2,3]. One of these quorum sensing peptides is PapRIV, a heptapeptide originating from Bacillus species and demonstrating pro-inflammatory effects on BV-2 microglia cells: significantly increased levels of IL-6 and TNF-alpha, together with an increased expression of NFkB both demonstrate a clear effect of PapRIV on these cells, which are crucial in physiological brain function. These results thus indicate a possible causal relationship between microbially produced quorum sensing peptides and mental disorders, which need to be confirmed by on-going in vivo mice studies.

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OP17

Mitocryptides: First Demonstration of Pathophysiological Involvements in Inflammatory Diseases

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Mitocryptides are a novel family of endogenous bioactive peptides that induce neutrophilic migration and activation. Namely we have purified and identified novel neutrophil-activating cryptides, mitocryptide-1 and mitocryptide-2, which were derived from mitochondrial cytochrome c oxidase subunit VIII, and cytochrome b, respectively, from healthy porcine hearts [1-3]. We also identified more than 40 neutrophil-activating cryptides derived from various mitochondrial proteins in silico, and found that many of mitochondrial transit signal sequences that nuclear-encoded mitochondrial proteins have efficiently activate neutrophils after the cleavage of mitochondrial transit signal sequences from their parent proteins by mitochondrial processing peptidases. Recently, mitochondrial damage-associated molecular patterns (mtDAMPs) are intensively studied as proinflammatory factors for non-infective inflammatory diseases. It has been proposed that damaged cells possibly release endogenous mtDAMPs that stimulate innate immune responses associating excessive neutrophilic functions [4, 5]. However, it is obscure what molecules are responsible for those functions. Mitocryptides are promising candidates for those unknown activating factors in mtDAMPs since they are derived from mitochondrial proteins and efficiently activate neutrophils at nano-molar concentrations. In this presentation, we firstly demonstrate that mitocryptides involve the onset of non-infective inflammatory diseases including hepatic injury as "mtDAMPs factors". We also discuss about various physiological regulations by mitocryptides.

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OP18

Antifungal activity and immunomodulation of a de novo synthetic peptide

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The reduction of ingredients and food waste due to fungal contamination is one of the global major challenges, from both a health and an economic point of view. The research of novel solutions to fight microbial food contaminants rests upon two pillars, which are the development of resistant strains and consumer's demand for less chemical preservatives, which suffer from bad reputation. Natural antimicrobial peptides possess the qualities to overcome these issues but their tedious purification is a major drawback to their use. De novo design of novel antifungal compounds is a major progress that the identification of parameters involved in the antimicrobial activity has allowed. We designed a 14-residue peptide named KK14, with the sequence KKFFRAWWAPRFLK-NH2. This peptide inhibited conidial germination and fungal growth of food contaminants within the range 6.25-50 μg/ml and 50-100 μg/ml, respectively. The study of three analogues of the peptide highlighted the role of some residues in the structural conformation of the peptide and its antifungal activity. The substitution of a Pro residue with Arg increased the helical content of the peptide, its antifungal activity but also its cytotoxicity. Despite a more prominent side chain, the insertion of unnatural bulky residue β-diphenylalanine did not improve the antifungal activity or the stability of KK14. A full d-enantiomerization appeared as the best modification to increase the antifungal potency. The four peptides showed similar behaviour towards salt increase, heat treatment and pH decrease. Interestingly, only the d-enantiomer remains the most active at high pH and after proteolytic digestion. The four peptides did not present haemolytic activity up to 200 μg/ml but different behaviours of cytotoxicity and immunomodulation. These differences could be crucial for potential application as pharmaceutical or food preservative. So far, in contrast to natural extracts, the use of these synthetics peptides remains a concept due the cost of synthesis and the difficulties to get authorizations. However, as observed for the antibacterial peptide nisin, the decrease of the price and the easy of upscaling make conceivable their use in food products.

OP19

Targeting Amyloidogenic Proteins with Self-assembled Cyclic D,L-alpha-Peptides

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Protein misfolding and aggregation is the fundamental cause of more than 20 amyloidogenic diseases affecting either the central nervous system or a variety of peripheral tissues. Although peptides and proteins of various sequences can self-assemble into toxic amyloid structures, they share common three-dimensional features that may promote their cross-reaction. Given the significant structural and biochemical similarities between amyloids and the architecture of self-assembled cyclic D,L-alpha-peptides, we screened an unbiased library of six-residue cyclic D,L-alpha-peptides and optimized the activity of a lead peptide to discover cyclic D,L-alpha-peptide (CP-2) that interacts strongly with Alzheimer's disease associated Abeta and inhibits its aggregation and toxicity. Further studies including Thioflavin T assays, electron microscopy, circular dichroism spectroscopy and NMR, collectively suggested that CP-2 could also effectively cross-interact with Parkinson's disease associated alpha-syn and tau-derived PHF6 peptide, prevent their aggregation, and remodel their fibrils to non-toxic amorphous species, through an 'off pathway' mechanism. NMR studies show that CP-2 interacts with the N-terminal and the non-Abeta component region of alpha-syn, which are responsible for its membrane interactions and self-assembly, and so changes its conformation. Dot-blot and cell survival assays suggest that CP-2 reduces the amount of toxic alpha-syn oligomers and protects PC-12 and SH-SY5Y cells from alpha-syn-induced toxicity. Moreover, CP-2 permeates cells through endosomes/lysosomes, co-localizes with intracellular alpha-syn and reduces its accumulation and toxicity in neuronal cells overexpressing α-syn. CP-2 also prevents alpha-syn- and PHF6-dirved membrane damage as shown by liposome dye release experiments. Our studies suggest that targeting the common structural conformation of amyloids may be a promising approach for developing new therapeutics for amyloidogenic diseases.

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OP20

Tyrocidines and Gramicidin S: Glorified cyclodecapeptide detergents or precise chemical scalpels

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The dogma that cationic antimicrobial peptides primarily target membranes rich in anionic phospholipids still prevails nearly 30 years after the frog peptide magainin was discovered. Pore formation via barrel-stave and toroidal models, and membrane damage via detergent action or via the carpet model are still is used to explain the physiological phenomena of membrane disfunction. As the models were generated using only model membranes, the question must be asked how an AMP truly works in under in vivo conditions where membranes consists of multiple types of phospholipids, functional regions such as rafts containing specific membrane proteins, specific areas of high fluidity associated with certain peripheral proteins, as well as highly organized positive and negative curvature strain areas within the membrane architecture to maintain the cell shape. We investigated the mode of action of homologous cyclic β-sheet decapeptides from the tyrocidine group and gramicidin S by (1) model membranes utilizing biophysical techniques and (2) a bacterial cytological profiling approach using Bacillus subtilis as in vivo target. The model membrane studies revealed that the tyrocidine exposure led to very fast leakage kinetics by forming defined long-life ion-conducting pores without inducing lipid distortion. Gramicidin S also lead to leakage, but at a much slower rate without the formation of defined pores. Gramicidin S and the tyrocidines all led to changes in phase transition temperature of model membranes. In vivo the tyrocidines induced lipid phase separation, as well reducing membrane fluidity. This resulted in delocalization of a broad range of peripheral and integral membrane proteins. Gramicidin S had a much subtler effect and only caused mild lipid de-mixing with minor effects on membrane fluidity and permeability, despite that gramicidin S share 50 % sequence identity with tyrocidines. Gramicidin S also delocalized peripheral membrane proteins, particularly those involved in cell division and cell envelope synthesis. We observed that the tyrocidines directly or indirectly also caused DNA damage and interfered with DNA-binding proteins, while gramicidin S did not affect integral membrane proteins or DNA. The tyrocidines and gramicidin S are highly lytic and membrane active molecules at high concentrations. However, we have shown at concentrations where the cell remains intact these peptides still have a major effect on the membrane architecture and in particular on membrane proteins that are crucial for cell growth and survival. Our in vivo profiling study highlighted the multifaceted antibacterial mechanisms of these small antimicrobial peptides. The fact that the peptides do not have localized affect on the cell membrane, but that they globally disrupt the membrane architecture and membrane proteins could explain why resistance to them is virtually non-existent.

OP21

Enabling Parallel Peptide Purification by a Novel Traceless Purification Linker

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Solid-phase peptide synthesis (SPPS) is a powerful and flexible tool providing facile access to peptides required in drug research, development and production. However, purification of peptides via preparative HPLC is a bottleneck, especially regarding higher throughput missions. Furthermore, purification by preparative HPLC is a formidable challenge when difficult peptides have low solubility, a tendency to aggregate or when truncations co-elute with the full-length peptide. Herein, we report the facilitated purification by covalent catch (of crude peptides) and reduction-triggered release (of pure peptides) that allows purification of standard and difficult peptides in parallel formats. Our method is based on a new purification linker (PL) that is coupled to the N-terminal end of the peptide in the last step of solid-phase synthesis. The PL contains an alkoxyamine group connected to the peptide via a reductively cleavable azido unit. Owing to capping, truncations do not carry the PL. Thereby, only the full-length peptide obtained after solid-phase synthesis is immobilized onto an aldehyde-modified agarose resin by means of oxime ligation. This bio-orthogonal reaction is complete in less than one hour. Immobilization can be performed directly from the cleavage cocktail, avoiding laborious ether precipitation prior to purification and enabling the purification of strongly hydrophobic peptide. If precipitation is desired, denaturing agents such 7 M urea, TFA or organic solvents can be used to facilitate solubilization of aggregation-prone peptides. After immobilization, the truncated, acetylated peptide sequences are removed by simple washing. The PL is designed to allow liberation of the native peptide by a reduction-triggered 1,6-elimination, which yields the desired native peptide in pure form without occurrence of side reactions. By using catch & release based purification with the new PL, the peptide can be efficiently isolated from complex mixtures and especially potentially co-eluting truncations. The feasibility of the method has been tested with many peptides, including very hydrophobic amyloid ß (1-20), rationally designed peptides with co-eluting truncations and base-sensitive sequences. Peptides were purified in parallel set-ups using vacuum manifolds to prove practicability. The tolerance of our catch & release process to TFA or denaturing agents and the ability to liberate peptides of a high purity by reductive cleavage renders the method universal, paving the way for a true alternative to HPLC.

OP22

Effects of non-enzymatic posttranslational modifications on protein function

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At least 50% of all human proteins are predicted to experience one or more posttranslational modification (PTM) during their life cycle [1]. These PTMs can result from enzymatic or non-enzymatic reactions and enzymatic PTMs are well-known for being involved in regulating many cellular events such as gene expression, intracellular and extracellular signal transduction, protein-protein as well as cell-cell interactions [2, 3]. Non-enzymatic posttranslational modifications (nPTMs) are increasingly recognized to affect such events as well, with a special emphasis on age-related,

metabolic and neurodegenerative diseases [4].

Here two examples that involve different semisynthesis routes to access site-specific, non-enzymatic enzymatic PTMs within the N-, C- and middle domains of proteins with 205 to 441 amino acids will be discussed. One example is the small heat shock protein Hsp27 that has been shown to carry argpyrimidine modifications in certain cancers and for which we can demonstrate that a single argpyrimidine modification significantly influences Hsp27 function [5]. The second example deals with the incorporation of a carboxymethylated lysine residue within the microtubule binding region of Tau4, a non-enzymatic PTM that significantly influences tubulin polymerization.

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OP23

A New Aspect of Npys-based Solid Phase Disulfide Peptide Synthesis

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3-Nitro-2-pyridinesulfenyl (Npys) is a unique functional group for protecting a thiol (SH) group [1]. The "-Cys(Npys)-" readily reacts with an unprotected SH group to form a disulfide bond [2]. Many applications using this chemistry have already been reported to particularly be effective for the regioselective disulfide bond formation in peptide synthesis [3]. In the presentation, we would like to discuss the new aspect of Npys-based peptide chemistry with an emphasis on our very recent studies. Using a newly synthesized "Npys chloride resin", we developed a "solid-phase-assisted disulfide ligation method" to prepare a disulfide peptide from two kinds of peptide fragments with cysteine residues [4]. Application of this new Npys solid-phase strategy realizes a unique and innovative "disulfide-led synthetic methodology" for disulfide-containing cyclic peptides. Oxytocin and Endothelin with one and two disulfide bonds, respectively, were efficiently synthesized as a fundamental model of this method for more complex cyclic peptides. This strategy also realizes the solid-phase synthesis of tandemly arranged multi-disulfide-peptides for example disulfide-bridged oligo RGD peptide, and moreover provides an efficient method for chemical conjugation between hydrophilic and hydrophobic components with solubility problem, particularly useful for peptide-drug conjugation [5,6]. As another new aspect of Npys chemistry, we will discuss a new solid- or no-solid-supported Npys reagent (Npys-sulfenate, Npys-OR) [7,8]. This reagent enhances intra-molecular disulfide bond formation between two cysteine residues for the effective synthesis of cyclic-disulfide peptides. The use of this moderate oxidizing agent successfully affords synthetic hANP and α-conotoxin without oligomer formation under mildly acidic conditions (but not basic conditions) even applying a relatively higher peptide concentration.

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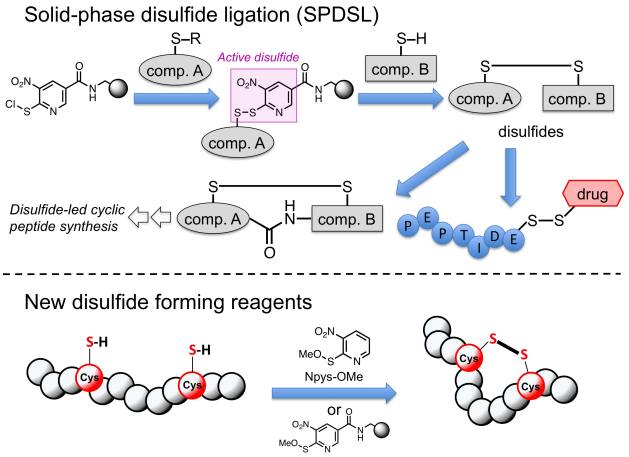

OP24

N-2-hydroxybenzyl-cysteine peptide crypto-thioesters for native chemical ligation

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Recent advances in Fmoc-based solid phase synthesis of peptide α-thioesters for the convergent synthesis of proteins via native chemical ligation (NCL) significantly contributed to push up the boundaries of the field. In particular, promising beta-mercapto amide-based thioesterification devices have emerged. If most systems require an acid catalysis, a few of them can undergo an N $\rightarrow$ S acyl shift under NCL conditions (neutral pH): peptide bearing such devices are called crypto-thioesters.1 We recently reported an N-(2-hydroxy-4-nitrobenzyl)cysteine-based device (N-Hnb-Cys),2-5 which allows routine automated synthesis of crypto-thioesters from inexpensive building blocks. Conveniently, no post-SPPS steps are required, and these thioester surrogates are perfectly stable to handling, storage and purification. This strategy is thus easily and readily usable by non-specialists. We illustrated the potential of this methodology for the synthesis of long,2,3,5 cyclic4 and N-terminal Cys-containing3 disulfide-rich peptides. To assess the scope and limitations of the method, we undertook systematic kinetic studies that showed that NCL reactions using N-Hnb-Cys-based crypto-thioesters are only 4-to-5 fold slower than those using a benchmark preformed alkyl thioester. These relatively fast kinetics are thought to arise from a bio-inspired design, aimed at mimicking intein-like intramolecular catalysis with a well-positioned phenol group.2 In order to rationally design further optimized second-generation devices, we aimed at finely understand the molecular basis underlying the fast rearrangement. These efforts gratifyingly led to a promising second generation device with a 2 fold increase of the NCL kinetics.

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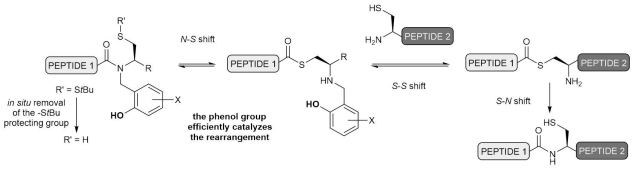

OP25

Expanding the potential and multivalency of the B2T synthetic peptide vaccine against foot-and-mouth disease virus

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Foot-and-mouth disease virus (FMDV) causes a highly transmissible infection of pigs and other animals, recognized as the animal disease with the direst economic effects worldwide. High incidence rates in large areas of Eurasia, Africa or Latin America stress the need for effective ways to control FMD, among which safe, marker vaccines (i.e., allowing to tell infected from vaccinated animals) are viewed as the most sensible option, with peptide-based vaccines receiving growing attention in this regard. In 2016 we reported the full protection of swine against FMD by vaccination with B2T, a platform displaying two and one copies, respectively, of FMDV B- and T-cell peptide epitopes in a branched fashion. To the known advantages of peptide vaccines (safety, marker nature, fine-tuning to various strains, easy shipping and storing), B2T adds highly efficient synthesis by thiol-ene conjugation of prepurified modules, confering it fast adaptability in emergency responses to new outbreaks. We are now exploring the potential of the B2T design to provide enhanced performance in terms of, e.g., lower dosage, longer-lasting protection and/or multivalency. To this end, strategies such as tandem display of >1 T-cell epitope, or back-to-back fusion of homologous or heterologous B2T units by chemoselective reactions (e.g., CuAAC) are being studied. Results from trial vaccinations of Swiss mice and swine will be discussed.

OP26

A general method towards ADPr ribosylated peptides and proteins

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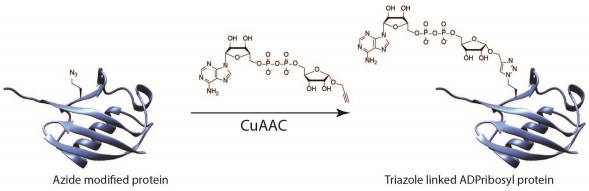
Ubiquitination of target proteins is a post-translational modification involved in almost all aspects of eukaryotic biology including the regulation of immune responses, cell proliferation and cell survival. The attachment of the 76 amino acid long protein Ubiquitin (Ub) to a nucleophilic functionality in the amino acid side chain of proteins alters the fate of the modified protein. The distinct signals that are brought about by this modification are mostly invoked by poly ubiquitination. Ub has the ability to form polymers by connecting one of eight amines of the first Ub molecule and the C-terminal carboxylic acid of a second Ub molecule etc. This will lead to eight differently linked homotypic poly Ub chains. All of these linkages have been detected in cells and their abundance changes during specific cellular events, indicative of their various functions. Extensive investigations of Ub led to the discovery of proteins able to assemble, recognize and break down Ub chain types specifically. In general Ub is activated and ligated to its target protein by a cascade of E1-, E2-, and E3-enzymes. This process is reversed by a large group of proteases called deubiquitinating enzymes (DUBs) that break down the ubiquitin modification, liberating the substrate protein and recycling Ub. The basis of the Ub system thus is fairly simple, but complexity comes from the large amounts of possible combinations of E2/E3 enzymes that dictate substrate specificity and formation of the appropriate poly Ub linkage type. We thought we understood the system, but do we actually? Recently it has been discovered that Ub can be regulated on a totally new level via modification with adenosine diphosphate ribose (ADPr), a modification that is found to play crucial roles in bacterial infection and DNA damage response pathways. Enzymes responsible for this are able to activate the hosts' Ub pool and catalyze the ubiquitination of substrates proteins independent of the regular E1–E2-E3 ubiquitination machinery and without the use of energy source ATP. Synthesis of ADPr-peptides is tricky business due to the incompatibility of the nucleotide and pyrophosphate regions in the ADPr part with strong acidic conditions commonly used in peptide chemistry. Reversely the alkaline conditions commonly used in nucleotide chemistry are incompatible with peptide integrity. To circumvent such issues a general strategy to produce ADPr-peptides or more specific ADPribosyl-ubiquitin conjugates based on a copper catalyzed click reaction between an alkyne carrying ADPribosyl building block and a site selective azide mutant peptide or protein to generate a triazole linked ADPr-protein analogue was developed. This method gives easy access to the first example of ADPribosylated peptides and proteins. Such post-translationaly modified proteins retain their biologic activity and can be applied in the studies of this highly irregular pathogenic pathway.



OP27

A Novel Evans Blue Organic Compound-appended PSMA-617 Peptide as SPECT Molecule Imaging Agent for Human Prostate LNCaP Animal Model

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The PSMA-617 is a high binding affinity ligand for prostate-specific membrane antigen (PSMA) which is strongly expressed in prostate cancer and up-regulated in poorly differentiated, metastatic and hormone-refractory carcinoma compared with health tissue. To conjugate with albumin is a new method for improving the pharmacokinetic profile of peptides. We used a new R&D strategy that changed the linking site of tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid in PSMA-617 and modified with truncated Evans blue which was a good human serum albumin binder. The modified PSMA-617 was abbreviated to DOTA-EB-PSMA-617. The DOTA-EB-PSMA-617 were prepared by linking a trun-

cated Evans blue to the lysine attaching the DOTA chelator to the α-amine, attaching a maleimide to the ε-amine, and finally, attaching thiolated PSMA-617 peptide to the maleimide. To determine radiolabeling parameters, the DOTA-EB-PSMA-617 (peptide amount 10-25μg) was labeled with 6mCi indium-111 chloride (from INER, Institute of Nuclear Energy Research in Taiwan) in sodium acetate (NaOAc) with pH 6.0 at 95°C for different incubation time (5 to 20mins). The radiochemical purity (RCP) of In-111-DOTA-EB-PSMA-617 was qualified by radio-TLC with citric acid/ sodium citrate buffer. The solvent front was free indium-111 and the origin spot was In-111-DOTA-EB-PSMA-617. Then we used prostate carcinoma cells LNCaP (PSMA +) and PC-3 (PSMA-) to determine the in vitro bio-activity of radiolabeled products by cell binding assay. And we used LNCaP tumor bearing mice to determine the in vivo bio-distribution of In-111-DOTA-EB-PSMA-617 via nanoSPECT/CT. The radiochemical purity of In-111-DOTA-EB-PSMA-617 was greater than 95%. In cell binding assay, the peptide concentration of In-111-DOTA-EB-PSMA-617 was 2.5nM to 250nM and incubated for 45mins at 37°C. With the amount of treated peptide increase, there had been strong and higher radio-counts in LNCaP cells than PC-3 cells analyzed by γ-counter. And we found that radio-activity accumulated in the LNCaP tumor site at 48 hours post-injection of 18.5MBq In-111-DOTA-EB-PSMA-617. There has longer circulating time of In-111-DOTA-EB-PSMA-617 compared with In-111-PSMA-617 on literature. In conclusion, we altered the pharmacokinetic/pharmacodynamic (PK/PD) profile of PSMA-617 successfully by conjugating PSMA-617 with EB. And PSMA-617 modified with EB motif has potential to be a theranostics agent to change the therapeutic cycle of PSMA-617 in shorter time.

OP28

Sol-gel and peptides: an attractive route to unprecedented biomaterials

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Affording biological properties to a material is one of the challenges the chemists have to tackle, to improve the efficiency of existing devices and also to propose new biomaterials. Most of synthetic approaches to obtain functional materials rely on non-covalent coatings or post-grafting realized by multistep ligation chemistry.

In this context, we envisioned a bottom-up strategy to introduce bioactive peptides in polymers or materials. The approach relies on the synthesis of hybrid blocks displaying one or several hydroxysilane groups. The condensation of such moieties proceeds chemoselectively, at room temperature and is compatible with the handling of biomolecules. Interestingly, any type of peptides but also biopolymers and dyes can be silylated and thus can participate to the covalent formation of the material.

Grafting of wound-healing or antibacterial peptides on glass, silicone catheter[1] and dressings[2], silica and titanium[3] surfaces was performed but direct synthesis of hybrid materials can be also operated[4]. Comb-like and silicone linear polymers were prepared.[5,6]

We recently focus our attention on 3D printing of cell-containing hydrogels using collagen-inspired hybrid peptides[7–9], and multi-ligands nanoparticules for cancer imaging.[10,11]

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OP29

Arginine-rich cell-penetrating peptide-modified exosomes for macropinocytosis induction and effective cellular uptake

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Exosomes (extracellular vesicles, EVs) are small secretory vesicles (30 200 nm in diameter) released from various cell types and contain bioactive molecules [1]. Exosomes are highly expected to be next-generation biological tools for delivering therapeutic molecules because of their pharmaceutical advantages, including controlled immunogenicity, utilization of cell-to-cell communication pathway, modification and encapsulation of therapeutic molecules. In this presentation, we will discuss novel techniques to enhance cellular exosome uptake by chemical modification of arginine-rich cell-penetrating peptides (CPPs) [2] that can actively induce macropinocytosis [3, 4]. We recently found that macropinocytosis (accompanied by actin reorganization, ruffling of the plasma membrane, and engulfment of large volumes of extracellular fluid) induced by cancer-receptor activation (e.g. EGFR) and oncogenic K-Ras expression is an important cellular uptake route of exosomes [5]. Therefore, we modified exosomes to actively induce macropinocytosis by utilizing oligoarginines, which are CPPs and induce macropinocytosis via the proteoglycan on plasma membranes [3, 4]. Oligoarginine (Rn; n = 4–16)-modified exosomes were prepared by cross-linking the peptides and exosomes using a N-ε-maleimidocaproyl-oxysulfosuccinimide ester linker. When we examined the effects of the peptide modification on cellular exosome uptake, the number of arginine residues in the peptide sequence was found to significantly affect cellular uptake via macropinocytosis induction. In addition, we artificially encapsulated the ribosome-inactivating protein, saporin (SAP), in exosomes (SAP-exosomes) modified with oligoarginines. Modification of hexadeca-arginine (R16) peptide on the SAP-exosomes resulted in effectively attained anti-cancer activity, suggesting efficient cytosolic release of the SAP [4]. Our findings may contribute to the development of intracellular exosome-based delivery systems with advanced exosomal functionality.

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OP30

Novel Cryptophycin-based Conjugates for Tumor Targeting

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The lack of tumor preference in traditional chemotherapeutics causes severe side effects. Indeed, some promising highly cytotoxic agents (e.g. cryptophycins) can not be currently used due to the narrow therapeutic window. For this reason, targeted therapy has recently come to the forefront in pharmacotherapy for cancer treatment pursuing the "magic bullet" early vision of Paul Ehrlich [1]. Cryptophycins are very potent antimitotic agents that interrupt cell division by inhibiting the assembly of tubulin into microtubules. Their activity is from 100- to 1000- fold greater than other agents such as paclitaxel or Vinca alkaloids. This activity is not impaired by multidrug resistance mechanisms, making cryptophycins very promising agents for targeted therapy [2]. These interesting properties attracted the attention of different companies that developed novel cryptophycin analogues containing an addressable functional group for the formation of antibody-drug conjugates. However, they faced a major problem concerning the stability of the macrocycle that halted the clinical development [3, 4]. Among many, cryptophycin-55 glycinate, a synthetic analogue containing a suitable functional group for conjugation is of particular interest. It is known to display high cytotoxicity and the stability of the macrocycle is greatly enhanced, according to our findings. These characteristics provide solid evidence to use it as payload in a directed therapy approach. The construction of small-molecule drug conjugates using the above mentioned payload with the homing device octreotide, an octapeptide targeting the somatostatin receptors, will be reported. The stability of the conjugates was highly depending on the linker used and one showed good stability in mouse and human plasma. The cytotoxic effects of the novel conjugates were evaluated on a diverse panel of tumor cell lines revealing high activity of these compounds. In order to prove the selective delivery of the conjugate to tumor, a fluorescently labeled analogue was prepared for internalization and uptake studies, which showed selectivity for somatostatin receptors, as it was envisioned.

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OP31

A Journey Alongside Cyclosporin on the Road to Peptide Oral Bioavailability

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The path of translation from peptide lead to drug is too often blocked by the challenge of poor oral bioavailability. What is it about cyclosporin then that allows it to defy the odds to achieve high membrane permeability? And, can we use these 'secrets' in peptide design? To address these questions, let me take you on a journey that investigates its structural features - i.e., its hydrogen bonding network, lipophilicity, and flexibility - and their effects on cell and membrane permeability of peptides. Let me begin with our initial work probing the hydrogen bond network of peptides using NMR temperature coefficients to select amides for N-methylation, resulting in designed peptides with high in vitro permeability, including one with an oral bioavailability of 30% in a rat model. Let me then walk you through subsequent work on the permeability of a library of 62 cyclic hexapeptides, establishing the importance of lipophilicity and solubility. Beyond these structural features is one that is perhaps less well understood - flexibility - because of the difficulty in studying it using conventional chemical means. We have used extended molecular dynamics simulations to observe and manipulate the conformational behavior of cyclosporin (as well as other cyclic peptides) in explicit water, chloroform, water/chloroform mixture, cyclohexane, and in the presence of a POPC lipid bilayer. The simulations were validated against NMR experiments, measuring conformational exchange, nuclear spin relaxation, and structures in matched solutions, such as DPC micelles, to mimic the lipid environment. We have found that conformational dynamics is a key determinant of the membrane permeability of cyclic peptides, and affects all stages of passage across membranes. Not only does dynamics enable cyclic peptides to transition into conformations that have physicochemical properties that favor membrane insertion but also enable them to wiggle their way through the dynamic polymer-like network of the membrane core. At the end of this talk, I will show how the structural features discussed so far are in fact interrelated using a model of permeation. By understanding the features that limit or enable the permeability of peptides, the hope is to venture towards a future of oral peptide therapeutics.

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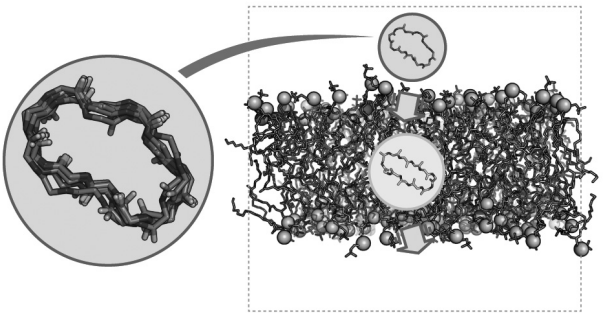

OP32

Structure-Based Design of Alpha-Helix Mimetics to Target and Disrupt Estrogen Receptor-Coregulator Interactions in Breast Cancer

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Protein-protein interactions (PPIs) are fundamental biochemical processes that regulate numerous cellular pathways, and often found to be mediated by key secondary structures like alpha-helices. Whereas short peptides

derived from proteins have been used to inhibit such PPIs, peptides in general may suffer from drawbacks that severely compromise their effective in vivo use, such as rapid degradation, poor bioavailability, and low cell permeability. Thus, small molecules that mimic functions of helical peptides would be of great interest in targeting and disrupting PPIs that take place inside cells. We have designed oligo-benzamides as rigid templates to emulate protein helical surfaces. A tris-benazmide scaffold can place 3 functional groups to match the side chains found at the i, i+4, and i+7 positions in a helix. And, it can accommodate two additional functional groups at its N- and C-termini to render higher target interaction, selectivity, and improved physicochemical properties. In addition, tris-benzamides are easy to be assembled by following high-yielding and iterative steps in solution- and solid-phase. As estrogen receptor (ER) recognizes a helical and consensus LXXLL motif to recruit its coregulators, we have designed and synthesized a series of tris-benzamides based structural design. A leading compound TK11 disrupts both ER dimerization and interaction with several coregulators, and effectively blocks ER-mediated oncogenic signaling. It showed potent growth inhibition on therapy-sensitive and -resistant breast cancer cells, in both preclinical mouse xenograft models and patient-derived tumor explant models. It is orally bioavailable with no identifiable toxicity, showing a new mechanism of action to treat breast cancer.

OP33

Ghrelin receptor ligands: from the bench to the drug on the market

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Ghrelin receptor or GHS-R1a for Growth Hormone Secretagogue Receptor type 1a is a G protein-coupled receptor (GPCR) that mediates ghrelin-induced growth hormone (GH) secretion, food intake, and reward-seeking behaviors. Because of its possible implication in several physiological disorders such as obesity and drugs/alcohol addictions, GHS-R1a represents a major target for the development of therapeutic small molecules. We previously described a potent Growth Hormone Secretagogue (GHS), the pseudopeptide compound JMV 1843. This compound is a selective agonist of GHS-R1a able to mimick ghrelin and to act by oral route in man. Acting as a GHS, it is able to elicit a large secretion of GH which can be dosed in the blood. It has completed its clinical phases and was approved by the FDA for GH deficiency diagnosis in aldults. This compound will be commercialized by Æterna-Zentaris in 2018 under the trademark of Macrilen®. It is also in clinical phase II for the treatment of cachexia. The implication of GHS-R1a in food intake focused the attention on ghrelin receptor antagonists for the treatment of obesity. However for an efficient anti-obesity activity, a GHS-R1a antagonist should counteract the orexigenic effect of ghrelin but not the GH secretagogue effect, since GH deficiency is frequently associated with increased adiposity. Starting from JMV 1843, we achieved to design and prepare a series of novel small molecules, based on the 1,2,4-triazole scaffold, able to antagonize ghrelin-induced GHS-R1a signaling. This peptidomimetic series have been investigated on one hand for their ability to compete for ghrelin binding and to activate or to inhibit calcium uptake in cells transiently expressing GHS-R1a and on the other hand for their effects on food intake and GH secretion in animal models. Our results showed that several non-peptide compounds characterized in vitro as antagonist or partial agonist of GHS- R1a inhibited food intake without altering GH secretion in vivo. We found that the dissociated effect of these ligands on food intake and GH secretion was correlated with their functional selectivity toward signaling pathways of GHS-R1a, making these ligands "biased". Thus this study supports the feasibility of a specific pharmacological modulation of the ghrelin effect on appetite. The preclinical results obtained with some of our best compounds (such the JMV 2959) will be presented. Finally, as GHS-R1a displays a very high constitutive activity (more than 50% of its maximal activity) it is thought that inverse agonists could potentially decrease food intake and body weight. Starting from JMV 2959, we recently developed a series of inverse agonists based on our 1,2,4-triazole scaffold. In vitro results of these compounds will also be presented.

OP34

Macrocyclization of Small Peptides Enabled by Oxetane Incorporation

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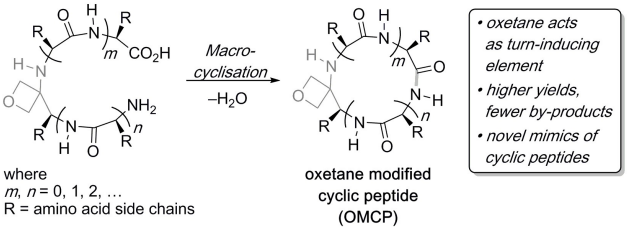
Compared to their linear counterparts, cyclic peptides benefit from enhanced cell permeability, increased target affinity, and higher resistance to proteolytic degradation. Moreover, they are capable of acting as inhibitors against some of the most challenging targets, including protein-protein interactions. One major obstacle to the discovery and development of new cyclic peptide drugs relates to the difficulties associated with their synthesis. The head-to-tail cyclization of short peptides containing seven or less amino acids is especially challenging. Common problems encountered during cyclization are C-terminal epimerization, cyclooligomerization and the appearance of side products arising from polymerization. Consequently, there is a pressing need to discover new macrocyclization strategies that can provide easy access to a variety of small cyclic peptide scaffolds.

A new type of turn-inducing element for peptide macrocyclization in which one of the backbone C=O bonds is replaced with an oxetane ring is reported. The cyclization precursors are conveniently made in solution or by solid-phase peptide synthesis. The subsequent macrocyclizations are demonstrated to be general, enabling a variety of difficult ring closures including those producing head-to-tail and side-chain to side-chain linkages. The method tolerates variation in the size of the macrocyclic ring and the location of the turn-inducing 3-amino-oxetane element with respect to the amide bond being formed. Additional data reveal that oxetane modified cyclic peptides are excellent bioisosteres of conventional cyclic peptides and as such this new method offers considerable potential in drug discovery programs.



OP35

The use of RGD peptidomimetics on biomaterials: new advances via selective integrin-subtype targeting

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Engineering biomaterials with integrin-binding activity is a very powerful approach to promote cell adhesion, modulate cell behavior, and induce specific biological responses at the surface level. However, most strategies in this field have focused on using RGD-containing peptides or proteins with relatively low integrin-binding potential and receptor selectivity.

In this work, I will present the recent development of highly active and selective $\alpha v\beta 3$- or $\alpha 5\beta 1$-binding peptidomimetics derived from the RGD peptide sequence (Figure 1), and their use to modify material surfaces and generate cell instructive and bioactive biomaterials (1).

In detail, I will focus on 3 recent examples: i) the capacity of peptidomimetic ligands to promote osteoblast adhesion, proliferation and mineralization on implant materials (2); ii) their potential to support stem cell adhesion and osteo-differentiation on highly nanostructured surfaces (3); and finally iii) their capability to instruct stem cell response in an integrin-selective manner in vitro and enhance bone formation in vivo (4).

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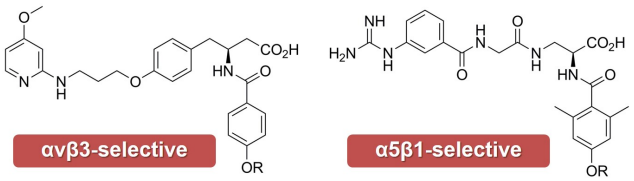

OP36

Stabilising Peptoid Helices Using Non-Chiral Fluoro-Alkyl Monomers

Steven Cobb

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Peptoids are a class of synthetic foldamers that are being developed as potential therapeutics, biomaterials, and organocatalysts. In particular, peptoids represent an attractive platform for pharmaceutical applications, as they are highly resistant to protease degradation. However, given their tertiary amide backbone, peptoids lack the capacity to form hydrogen bonds so their secondary structures are dominated by relatively weak interactions. Considerable efforts have been made to try and understand the relationships between a peptoid primary sequence and its folded structure. Cis/trans isomerization of the tertiary amide bond is the major cause of conformational heterogeneity in peptoid oligomers. Despite this, Zuckermann and Barron have demonstrated that α-chiral aromatic monomers, like NSpe can stabilize the cis configuration of the peptoid amide bond largely through steric effects. Gorske and Blackwell, found that the synergistic application of steric and non-covalent $n \rightarrow \pi^*$ interactions (NCIs) in aromatic systems could also be used to design stable cis-amide peptoid monomers (e.g. Ns1npe). However, it is not possible to use NCIs to stabilize the cis-amide conformation of alkyl peptoid monomers and this has meant that the design of stable peptoid helices remains dominated by the use of chiral aromatic residues. Recently, Faure and Taillefumier exploited steric effects in the design of a non-chiral tBu alkyl monomer, which has a clear cis-amide preference (NtBu). While NtBu offers one route to control peptoid structure that avoids the use of aromatic building blocks the design of non-chiral but stable cis-amide alkyl monomers is an area that is still highly underdeveloped. We sought to explore the application of fluorine as a tool to modulate the conformational preferences of alkyl peptoid monomers. Through NMR and X-ray analysis we demonstrate that simple non-chiral fluoro-alkyl monomers can be used to influence the key Kcis/trans equilibria of a peptoid amide bond in model systems and induce a remarkable degree of cis-amide preference. The cis-isomer preference displayed is highly unprecedented given the fact that neither chirality nor charge is being used to control the peptoid amide conformation. Our data supports that inductive effects imparted by the fluorine atom(s) and not fluorine gauche effects underpin the cis-isomer stabilization observed. We have also used the novel fluoro-alkyl monomers to prepare a series of peptoid oligomers that exhibit stable helical structures despite only having one chiral aromatic residue. The application of fluorine in the design of alkyl monomers offers a new approach to control amide bond isomerism in peptoid sequences, overcoming the current need for high levels of chiral side chains. Given the lack of alternatives available the fluoro-alkyl monomers developed offer exciting new tools to design structurally stable peptoid systems with applications in a range of areas including medicine and biomaterials.

OP37

Concerted biophysical and biological evaluation of Pseudomonas lipopeptides as a premise to unlock their application potential

José Martins, Yentl Verleysen, Benjamin Kovacs, Vic De Roo, Christophe Ampe, Annemieke Madder, Marleen Van Troys, Niels Geudens

Ghent University, Belgium

Cyclic lipodepsipeptides (CLPs) are secondary metabolites with a broad array of biological functions, mostly produced by Bacillus and Pseudomonas. First recognized for their antimicrobial action, their involvement as natural agents in plant protection has led to their application in the context of plant biocontrol and crop protection. More recently, scattered literature reports have indicated their anticancer activity as well. Given their appearance as promising tools for biomedical and agricultural research or applications, studies that aim to elucidate their mechanism of action, and structure-activity relationship studies in particular, should be high on the agenda. However, the study of these compounds quite often follows an approach where following their initial isolation and MS characterization, a short-cut is taken to directly assess their biological activity, thereby altogether bypassing their chemical and biophysical characterization. Even then, with more than 100 CLPs from Pseudomonas (Ps-CLPs) currently reported, a detailed investigation of biological activity reports for 51 Ps-CLPs indicates that, beyond some generalisations, little tangible information with respect to structure-activity relationships may be extracted from these, given the diversity of assays and assay conditions used. As a result, we have advocated that a concerted research action should be undertaken if the full potential of the different classes of CLPs is to be realized.[https://www.future-science.com/doi/abs/10.4155/fmc-2017-0315] We propose the results of our first steps towards this goal. Despite the large diversity in amino acid sequence we show that a novel and simplified nomenclature scheme can be introduced to organize the large diversity of CLPs from Pseudomonas. Using subsequent sequence alignment, we show that all currently known Ps-CLPs can be assigned to up to 15 different groups, each containing multiple members with varying degrees of homology. Next, using the solution structure determination by NMR of representative structures from 6 different groups (bananamides, viscosin, orfamides, amphisins, xantholysins and one newly uncovered group) and 2 available from literature (tolaasin and syringomycin) we propose that all Ps-CLPs adopt one of three folds: a stapled helix fold, a golf-club fold and a tennis-ball seam fold. All these are characterized by an amphipathic character that lies at the core of the variety of biological activities. Newly isolated Ps-CLPs can now easily be classified according to group and fold family provided their sequence is determined. Also, the added level of organization – 3 fold families rather than 15 Ps-CLPs groups, provides critical guidance in selecting Ps-CLPs for further biophysical and biological evaluation. By screening a selection of 8 Ps-CLPs belonging to the stapled-helix fold or the golf-club fold against a variety of cancer cell lines, the importance of the fold is illustrated while simultaneously, a link between trends in activity and overall amphipathicity appears to emerge.

OP38

Rationally Designed Peptidomimetics

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Peptides are well known to act as potent and selective ligands for a variety of bimolecular targets. Although there are a many peptide drugs in clinical use, their potential has been limited by unfavorable physicochemical properties including limited plasma stability and a lack of cell permeability. Peptidomimetics are molecules that can be rationally designed to overcome many of the issues associated with peptides and provide tool molecules for chemical biology and lead compounds for drug discovery. In this presentation I will discuss the Jamieson Group's efforts in the development of new chemical technologies including girder peptides and a disulfide bond mimetic. The application of these new conformational constraints to conotoxins will also be discussed.

OP39

Amyloidogenicity of regions of Nucleophosmin 1: a direct link between protein misfolding and Acute Myeloid Leukemia

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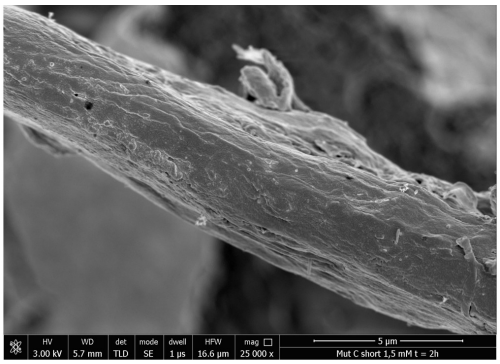
Nucleophosmin (NPM1) is a multidomain protein involved in a variety of biological processes and identified as the most frequently mutated gene in Acute Myeloid Leukemia (AML). Its C-terminal domain (CTD) is endowed with a three helix bundle tertiary structure: H1 (243-259), H2 (264-277) and H3 (280-294) helices fold through a compact transition state and unfold keeping a residual secondary structure at the interface between H2 and H3 helices (Grummitt, et al. 2008). Our studies outline that the neighbouring regions H2 (Di Natale, al. 2015) (Russo, et al. 2017) and H3 in AML mutated variants (Scognamiglio, et al. 2016) form amyloid-like assemblies endowed with fibrillar morphology and b-sheet structure proved to be toxic in cell viability assays. These regions are able to interact with membranes with different mechanisms and the presence of cholesterol revealed of crucial importance. Noticeably, wt CTD and Cterm mutA (the most common AML mutation) directly bind to membrane models in vitro albeit with different mechanisms (De Santis, et al. 2018). A short stretch (nine residues) deriving from the aromatic core of the helical bundle Solution and fiber state experiments related on demonstrated able to self-aggregate and that helical intermediates have a clear prompting role in the aggregation process: derived nanostructures revealed flat with twisted profiles at higher concentrations and typical cross-β structures (La Manna S, submitted). These investigations led to speculate that the destabilization of the a-helical bundle by AML-associated mutations predispose it to the formation of toxic aggregates causing the exposure of the H2 and H3 regions. More recently we found that all AML mutations, from A to F, without both or one Trp of the NoLS (nucleolar localization signal) induce amyloid-like aggregation and cytotoxicity. These findings are crucial to unveil the AML molecular mechanisms and leukemogenic potentials of AML mutations.



OP40

Inhibition of nicotinic acetylcholine receptor subtypes by the ribbon isomers of α-conotoxins.

Quentin Kaas[1], Xiaosa Wu[1], Han-Shen Tae[2], Yen-Hua Huang[1], David J Adams[2], David Craik[1]

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α-Conotoxins are a large family of toxins extracted from the venom of carnivorous marine cone snails and inhibiting with exquisite selectivity some subtypes of nicotinic acetylcholine receptors (nAChRs), which are involved in cognition, memory and learning as well as related neuronal diseases. α-Conotoxins display two disulfide bond cross-links, which can form three distinct connectivities: ribbon, bead and globular, with the latter displayed by wild-type toxins. We have previously discovered that the non-native ribbon isomer of α-conotoxin AuIB inhibits a specific stoichiometry of the $\alpha3\beta4$ nAChR subtype and with greater potency than the wild-type toxin, providing a high-precision molecular probe for neuroscience studies. We here present a complete structure-activity relationship study of ribbon AuIB, and we determined the binding mode of the toxin on the $\alpha3\beta4$ nAChR using a combination of peptide synthesis, molecular dynamics simulations, nuclear magnetic resonance spectroscopy and electrophysiology experiments. We compare the binding mode of the well-studied α-conotoxin globular isomers with that of the ribbon isoforms, highlighting differences that could be exploited to design probes of nAChR subtypes that have been hitherto difficult to target selectively. We also identified positions of the ribbon scaffold that could influence its folding, dynamics and therefore activity. This study lays the groundwork for utilising the ribbon connectivity of α-conotoxins as an alternative to the globular isoform to design selective nAChR inhibitors.

OP41

Quaternary Ammonium Isobaric Labeling for a Relative and Absolute Quantification of Peptides

Mateusz Waliczek, Bartosz Setner, Monika Kijewska, Remigiusz Bąchor, Dorota Gąszczyk, Piotr Stefanowicz, Zbigniew Szewczuk

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Isobaric tags for relative and absolute quantitation (iTRAQ) is a labeling method used in quantitative analysis of peptides by tandem mass spec-

trometry to determine the amount of peptides in complex mixtures in a single experiment. Although the isobaric labeling quantification has become a widely used method in proteomics studies, the currently available isobaric tags are very expensive and do not offer significant improvement of sensitivity of detection during MS experiment. Tagging of peptides with quaternary ammonium (QA) salts has been widely used to increase sensitivity of detection by ESI-MS[1]. Previously we have proposed linear[2] and bicyclic[3] QA as ionization enhancers for analysis of peptides at the attomole level [4]. Herein we design two new classes of quaternary ammonium isobaric tags for relative and absolute quantitation (QA-iTRAQ 2-plex). The first procedure is based on the combination of 18O exchange catalysed by trypsin followed by chemical modification of primary amines groups with isotopically labeled pyrylium salt[5] which allows isobaric labeling of tryptic digests. Each isobaric labeled peptide contains zero or four 13C atoms in the mass reporter group, which results in a unique reporter mass during tandem MS/MS for sample identification and relative quantitation. The second procedure is based on application of N-(4-iodobenzylpiperidinylcarbonyl)-β-alanine tag, with two deuterium atoms incorporated into the benzyl (stabile reporter group) or β-alanine (balance group) residues.[6] We tested the QA-iTRAQ 2-plex reagents on various peptides as well as protein tryptic digests and podocyte cells. Obtained results suggest usefulness of the isobaric ionization tags for relative and absolute quantification of trace amounts of peptides. This work was supported by a grant No. UMO-2016/23/B/ST4/01036 from the NSC, Poland.

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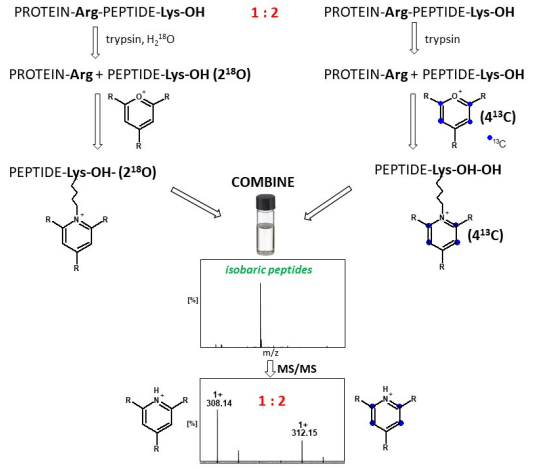

OP42

Development of serine protease inhibitors using the 1,2,3-triazole motif as a disulfide mimetic in the cyclic peptide sunflower trypsin inhibitor-1

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Disulfide-rich peptides are a diverse class of highly constrained molecules that have potential in drug design due to their ability to be adapted towards a broad range of therapeutic targets. Despite having high proteolytic stability, one potential therapeutic limitation of these peptides is the redox instability of the disulfide bond, with reduction or shuffling often rendering the peptide unstable or inactive. Applications of redox stable disulfide bond mimetics have demonstrated that 1,5- and 1,4-disubstituted 1,2,3-triazole motifs can be used to successfully replace disulfide bonds as a conformational restraint in certain applications [1-2]. In this project, our aim was to further explore the utility of the triazole bridge as a disulfide mimetic in highly constained backbone cyclized (N-C) Sunflower Trypsin Inibitor-1 (SFTI-1) and engineered variants. Using solution phase triazole bridge synthesis, we successfully synthesized a series of triazole bridged variants, with the best SFTI-1 based analogue exhibiting highly potent trypsin inhibition (Ki: 76 pM). Capitalizing on this success, we further implemented the use of the 1,5-disubstituted 1,2,3-triazole to target other serine proteases using engineered SFTI-1 based inhibitors which will be discussed in this presentation.

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OP43

Peptide-Bile acid Cyclisation as a Tool for the Development of a Universal Vaccine Against Influenza A

Dorien Van Lysebetten[1], Bert Schepens[2], Xavier Saelens[2], Annemieke Madder[1]

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[2]Ghent University, VIB, Belgium

Peptide cyclisation is considered to enhance the therapeutic properties of peptide drugs because it allows to modulate for example the conformational flexibility, susceptibility to proteases, potency and selectivity of such drugs. In this work, cyclisation is achieved by grafting the peptide sequence onto a bile acid scaffold, more specifically deoxycholic acid. Apart from being rigid, the scaffold is also very cheap and has amphipathic properties which equips the molecules with a hydrophobic side [1]. In this way, the scaffold can constrain the peptide as well as help to increase cellular uptake[2], bioavailability[2], albumin binding, protease stability[3], etc. This bile acid cyclisation can be used for a variety of targets. Here, we present a conjugate peptide antigen designed to generate broadly protective antibodies against Influenza A. We chose to graft a peptide corresponding to the extracellular part of matrix protein 2 (M2e) onto the scaffold because M2e is conserved across different influenza A virus subtypes and can thus act as a universal antigen for influenza A[4]. The M2e peptide adopts a U-shape when bound to a M2e-specific and protective monoclonal antibody (mAb 65)[5,6]. Therefore, we hypothesized that constraining this peptide can promote the induction of mAb 65-like antibodies. The grafting reaction involves an easy one-step synthesis delivering two regioisomers, which can be separated and purified with optimised HPLC conditions. Interestingly, the cyclic peptide-bile acid conjugates are recognized by M2e-specific monoclonal antibodies and polyclonal serum in an ELISA assay. In addition, the immunogenicity of the constrained M2e peptide that best competed with the linear M2e peptide for mAb 65 binding was evaluated in vivo. Immunisation of laboratory mice with the cyclic M2e peptide induced serum antibodies that were specific for M2e, and for M2 expressed by mammalian cells. We conclude that constraining a peptide antigen on a bile-acid scaffold is an efficient method to design a universal influenza A vaccine candidate.

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OP44

Stereochemically modified glucagon with improved biophysical parameters

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Glucagon serve seminal role in maintaining physiological homeostasis of the sugar metabolism buffering against insulin induced hypoglycemia. Despite its importance as the lifesaving therapeutics and missing element in artificial pancreas, development of the glucagon has been hindered by the peptide's poor solubility, high aggregation propensities and chemical instability. Over the years number of approaches has been deployed to overcome biophysical issues -predominately modification in amino acid sequence to adjust isoelectric point. On the other hand, introduction of the D-amino acids into the native sequence of the pharmaceutically relevant peptide has been popular mean to enhance peptides proteolytic resistance and increase circulation. In our minimalistic approaches to peptide optimization we demonstrated that single point steroinversions lead to dramatically increased solubility in physiological buffer with preservation of full in vitro and in vivo bioactivity. Furthermore, D-amino acid insertions are associated with significant decrease in glucagon's fibrillation.

OP45

Design, synthesis and study of multimeric peptidic conjugates for a new approach of anti-tumoral immunotherapy

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Despite significant progress in cancer therapy, current treatments are still controversial due to intolerable side effects. Targeted immunotherapy has emerged as an ideal alternative to improve treatment modalities but very limited approaches are available today and major issues remain to be addressed. Our objective is to design bi-modular systems called "Antibody-Recruiting Molecules" (ARM) with the objective of redirect endogen antibodies already present in the bloodstream against cancer cells, without preimmunization [1]. The first module (ABM for "Antibody Binding Module"), is composed of multivalent carbohydrate antigens for endogenous antibodies [2]. The second module (TBM for "Tumoral Binding Module") is composed of selective ligands for markers expressed by diverse cancer cell lines [3]. Several ARMs have been synthesized with variable size, geometry and valency by using peptide scaffolds and chemoselective ligations [4]. These molecular architectures have been tested in vitro with several cancer cell lines by Fluorescence Activated Cell Sorting (FACS). Here we report the synthesis and biological evaluation of the best compounds obtained so far.

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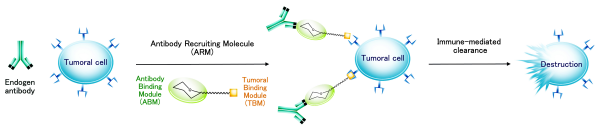

OP46

Engineered protein-protein interaction regulators for therapeutic applications

Nir Qvit

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Protein-protein interactions (PPIs) are intimately involved in almost all biological processes, including inter- and intracellular signal transduction, gene expression, cell proliferation and apoptosis. Therefore, they are important phenomena in basic research and promising targets for treating human disease. Nevertheless, targeting PPIs is challenging, as PPI interfaces are large, flat, and are usually endowed with a significant degree of conformational flexibility. Peptides are ideal candidates for targeting PPIs as they have demonstrated high conformational flexibility, increased selectivity, and are generally inexpensive. Moreover, peptide limitations, such as poor stability and inefficient crossing of cell membranes, can be overcome using peptidomimetics (modified peptides). Here we combine two techniques for the development of peptidomemetics. The first is an approach for the identification of the two critical PPI interfaces. We developed novel algorithms to detect specific PPI interfaces between a vital protein and only one of its many partners, using rational design, based on unique interaction sites. A second technique is an already established approach used for the development of peptidomimetics that retain the functional groups of the substrate side chain residues essential for their bioactivity. We thus convert active sequences into peptidomimetics while retaining their conformational flexibility. Together, these techniques allow the development of selective inhibitors of specific binding sites that are metabolically stable and highly active. One example of inhibition of specific PPIs is the selective inhibition of the phosphorylation of pyruvate dehydrogenase kinase (PDK) by protein kinase C (δPKC), which demonstrated its correlation with cellular processes following cardiac ischemia. Since δPKC phosphorylates many substrates, the extent to which this specific phosphorylation is critical for cardiac damage could not be previously explored. We developed an inhibitor of the δPKC/PDK PPI, ψPDK, which selectively inhibits PDK phosphorylation. In animal models of heart attack, ψPDK reduced infarct size by 70% and tripled ATP levels. ψPDK was found to be highly active (EC50 5 nM) and specific, and did not affect the phosphorylation of other δPKC substrates even at 1 μM. A second example of specific PPI inhibition involves proteins that regulate mitochondrial fission, dynamin-related protein 1 (Drp1) and mitochondrial outer membrane protein, Fis1. Excessive mitochondrial fission results in oxidative stress and contributes to the pathology of neurodegenerative diseases. We developed a selective inhibitor of the Drp1/Fis1 PPI, p110. The selective inhibition reduced ROS production, improved mitochondrial membrane potential and reduced apoptosis. Our overall findings demonstrated that P110 prevents mitochondrial fragmentation and cell death under pathological conditions. Inhibition of δPKC/PDK and Drp1/Fis1 PPIs are both examples of specific and selective inhibition by rationally designed peptidomimetics. These novel, highly selective PPI inhibitors may be used as lead compounds for therapeutic applications. Furthermore, similar approaches can be applied for

the development of various other specific PPI regulators.

OP47

Utilizing Combinations of New Approaches to Peptide and Peptidomimetic Design for G-Protein Coupled Receptors

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The renewed interest in peptides and peptidomimetics in drug development presents a welcome challenge for enhanced design of desired properties including selectivity, bioavailability, stability, etc. This requires enhanced integration of computational, design, synthetic and topographical considerations. G-Protein Coupled Receptors (GPCRs) are particularly challenging because they are the current targets of over one third of drugs. However, their inherent flexibility and instability and the fact that of their native ligands also are often flexible has made it very difficult to obtain x-ray crystal structures of the native proteins binding to their native ligands. To overcome these difficulties we have made increasing use of a combination of conformational and topographical constraint of the native hormones and neurotransmitters coupled with enhanced computational approaches for homology modeling of the receptors, and for docking of novel ligands. This is used in conjunction with state of the art receptor ligand design for selectivity to specific GPCR subtypes (both agonists and antagonists), for ligand bioavailability, and for enhanced penetration of the blood brain barrier. We will illustrate this approach by examples of the design of melanocortin 1 receptors (MC1R) selective ligands that converted the non-selective native hormone gamma-melanocyte stimulating hormone to a highly selective peptide ligand, using only eukaryotic L-amino acids. We also have designed MC3R and MC4R selective ligands with enhanced bioavailability and blood-brain barrier penetration that are conformational and topographically constrained, both agonists and antagonists. Supported in part by the U.S. Public Health Service, National Institutes of Health and by Tech Launch Arizona

OP48

Immobilising integral membrane proteins on silica

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The 19 amino acid R5 peptide is a repetition sequence from the diatom Cylindrotheca fusiformis sil1p protein. Even in the absence of post-translational modifications, it can be used as a silica precipitation tag for small molecules and proteins.[1] The silica structures can be obtained at ambient pressure and room temperature within minutes. Silica nanoparticles are non-toxic and have tunable bio-degradability.[2] Silica materials can be used for drug delivery into cells and improving enzymatic activity of immobilised proteins.[3] The aim of this project is to develop a system to incorporate functional membrane proteins into silica nanoparticles. To this end, nanodiscs, which use a truncated version of the human apo A1 protein to create nanometer-sized lipid bilayers in solution,[4] were combined with the R5 peptide. The new construct was shown by TEM to enable nanodisc formation and silica precipitation and will be used to embed functional membrane proteins and immobilise them in lipid-silica particles.

Acknowledgements We gratefully acknowledge financial support through the ProteinConjugates innovative science & training program which is funded under the Horizon 2020 Marie Skłodowska-Curie actions (Project ID 675007).

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OP49

Cathepsin B-Activatable Cell-Penetrating Peptides for Imaging Cancer-related Cathepsin B

Konstantin Kuhne[1], Lydia Behring[1], Birgit Belter[1], Robert Wodtke[1], Lara von Krüchten[2], Max Keller[2], Jörg Steinbach[1], Jens Pietzsch[1], Reik Löser[1]

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In our understanding of the many drivers of malignant progression and cancer metastasis, proteases are increasingly drawn into the spotlight as crucial modulators in cancer angiogenesis, invasion, and metastasis [1]. Elevated activities of multiple members of the cathepsin family have been shown to correlate with increased metastasis and high therapy resistance [2, 3]. Especially high expression levels of extracellular cathepsin B (CTB) indicate poor prognosis in neoplastic diseases, making CTB an interesting target for activity-based molecular imaging in cancer diagnostics as well as in cancer therapy monitoring for personalised therapies. It is our aim to develop such an imaging probe for CTB by combination of a polyarginine-based, activatable cell-penetrating peptide (ACPP), as first described by R. Tsien, and an optimised endopeptidase substrate for CTB [4]. Substrate optimisation proofed to be challenging as two entirely opposite factors needed to be balanced – high stability against serum proteases to prevent premature cleavage of the activation sequence, while retaining efficient and specific endoproteolytic cleavability by CTB. We have generated a CTB-endoprotease substrate by C-terminally elongating the CTB-carboxydipeptidase substrate Abz-GIVR*AK(Dnp)-OH (Abz - aminobenzoyl, Dnp – dinitrophenyl, * – cleavage site), described by Cotrin et al. in 2004, to the octapeptide Abz-GIVR*AK(Dnp)GX-CONH2, which could be used as activation site in the final ACPP [5]. Introduction of any amino acid other than glycine at the P4' position resulted in hysteretic kinetics for the CTB-catalysed hydrolysis of the octapeptides, which might indicate the displacement of the occluding loop from the active site upon interaction with the substrates. Using LC-ESI-MS-based analysis of serum-incubated substrates, the positions P1 and P3' where determined to be primary determinants of serum stability. After suppression of the P3' instability by Nα-methylation and optimisation within the positions P1-P3, we were able to increase serum half-life from < 5 min to > 1440 min under concomitant improvement of kinetic substrate efficiency towards CTB. Currently, the substrate is optimised towards CTB-specificity within the cathepsin protease family. Additionally, cell uptake studies of a fluorescently labelled ACPP using the optimised CTB-endoprotease substrate sequence are ongoing. Using this fluorescent probe, we plan to study cell uptake, in vivo stability and initial biodistribution. Furthermore, ACPPs conjugated to radiometal-chelating entities are currently prepared, which will allow for PET imaging in vivo.

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OP50

Designing antibiotic peptide polymer adjuvants for multidrug resistant bacteria

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The continuing rise in antibiotic and multi-drug resistant bacterial infections is a major global medical health issue, which is not being met by traditional antibiotic research. Here, we show that a new class of antimicrobial agents, termed 'Structurally Nanoengineered Antimicrobial Polypeptide Particles' (SNAPPs), synthesized using ring-opening polymerization (ROP) of α-amino acid N-carboxyanhydrides (NCAs) to produce star-shaped polypeptide nanoparticles exhibit antibiotic-adjuvant properties resulting in colistin-multi-drug resistant (CMDR) bacteria becoming sensitive to antibiotics once more. Synergistic interactions were demonstrated between a model SNAPP and ampicillin, imipenem, doxycycline, gentamicin, or silver ions. We show that different peptide architectures alter the activity of the SNAPPs. We also show that the effective antibiotic dose could be decreased by 3.7 to 16 fold from the original lethal dose while retaining synergistic interactions with SNAPPs against CMDR bacteria. Further, we demonstrate that the combination treatment approach using SNAPPs is able to attenuate toxicity. Interestingly, our studies show that SNAPPs sensitise bacteria to antibiotics that were previously ineffective. Overall, this study demonstrates the potential of synergistic combinations of SNAPPs with conventional antibiotics where the SNAPP not only has antimicrobial activity in its own right but also acts as an adjuvant for the conventional antibiotic to treat CMDR bacterial infections

OP51

Tuning the Catalytic Activity and Substrate Specificity of Peptide-Nanoparticle Conjugates

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The rational design and synthesis of complex systems that mimic the outstanding substrate specificity and catalytic properties of enzymes, is still a challenging task. The self-assembly of cysteine-containing peptides onto the surface of gold nanoparticle (Pep-Au-NP) emerged as a promising strategy towards the development of artificial enzymes. The high local peptide concentration leads to dense functional monolayers that create novel, cooperative features and catalytic properties, different from the monomeric peptide in solution.1,2 In this context we studied the effect the location of the catalytic unit within the peptide-monolayers relative to the nanoparticle surface has on the esterolytic activity and substrate specificity of three Pep-Au-NP, that only differ in the position of the catalytic unit (surface proximal, intermediate, surface distal). Rates of ester hydrolysis were found to correlate with the hydrophobicity of the substrate and the position of the catalytic unit. Highly hydrophobic ester substrates are cleaved more efficiently surface proximal, whereas less hydrophobic substrates showed higher rates of hydrolysis in the intermediate region of the monolayer. Additionally, positive cooperativity in substrate binding was observed and presents another striking property of natural enzymes achievable in the here described artificial systems. The results point to the importance the position of the catalytic center has on the catalytic activity and substrate specificity of peptide-nanoparticle conjugates.

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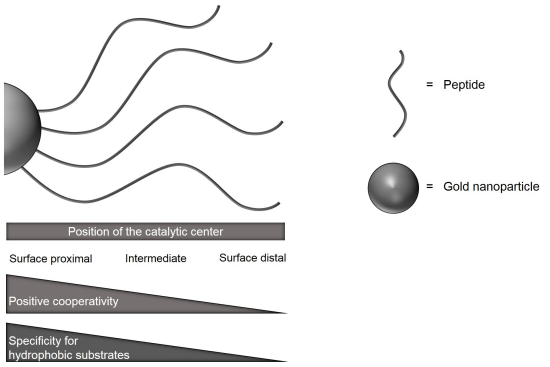

OP52

Lighting up programmed cell death in real-time: apoTRACKER as an in vivo compatible apoptosis diagnostic tool

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Programmed cell death (apoptosis) is an essential biological process that stands out as a potential key biomarker of development and progression of several diseases. Therefore, there is the need for sensitive and broadly applicable methods for detection of apoptotic cells as diagnostic tools for unraveling the mechanisms accounting for disease pathogenesis. The current gold standard in optical imaging of apoptosis are fluorescent versions of Annexin V, a protein that binds with high affinity to phosphatidylserine (PS), a membrane phospholipid which is translocated to the external side of the plasma membrane in the early stages of the apoptotic process, including apoptotic bodies [1]. However, Annexin requires high concentrations of Ca2+, which can translate into functional damages. Herein we introduce a novel peptide-based fluorogenic probe (apoTRACKER), which represents a Ca2+-free imaging platform for selective staining and detection of cells undergoing programmed death.

Our vision was that amphipatic peptides could display affinity for negatively-charged phospholipids exposed in apoptotic cells, while retaining selectivity over viable ones. The presence of the environmentally sensitive Trp-BODIPY amino acid allows for fluorogenic imaging of the interaction between peptide and their cellular target in the green GFP channel [2]. We designed a panel of peptides to study the hydrophylic and aromatic sequence requirements that define the optimal binding to PS. From a chemical point of view, the sensitivity of the BODIPY chromophore towards acid needs to be taken into account when planning the synthetic strategy [3]. Therefore, mild acid-labile CTC resin and hydrogen sensitive side-chain protecting groups were employed. Peptides were cyclized using COMU to ensure desirable proteolytic stability in vivo. Fully cyclic, unprotected peptides were obtained after hydrogenolysis using Pd-catalysis in mild conditions.

apoTRACKER emerged as the most promising analogue of the evaluation against apoptotic neutrophils in vitro. We have successfully used apoTRACKER to image apoptosis in real-time in a wide variety of cell lines and demonstrate selective binding to apoptotic. Using a combination of optical and biophysical methods we have verified that apoTRACKER binds with strong affinity to PS. Finally, we have effectively used apoTRACKER to image apoptosis in vivo in a murine model of inflammation. apoTRACKER stands out as the next gold standard in the field of apoptosis imaging, avoiding the need for high Ca2+ media, and allowing in vivo labelling of cells undergoing apoptosis.

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OP53

Synthetic integrin-binding immune stimulators target cancer cells and prevent tumor formation

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High-affinity monoclonal antibodies are seen as promising cancer therapeutics, leading to the development of antibody derivatives as well as other protein-based scaffolds that couple selective targeting of cancer cells with the stimulation of an adaptive immune response.(1) Peptides can also bind with high affinity to cancer cells and are intermediate in size between antibodies and small molecules.(2) Furthermore, chemical peptide synthesis and chemoselective ligations can be used to generate a variety of cancer-targeting molecules with different numbers and combinations of binding moieties in a modular and homogeneous fashion. In several recent studies, we have designed and synthesized a suite of synthetic 'immune system engagers' (ISErs) that mimic the functions of antibodies by binding specifically to cancer cells and stimulating an immune response.(3) To explore avidity and valency effects, we constructed ISErs bearing different numbers and combinations of two 'binder' peptides that target integrin $\alpha 3$ and ephrin A2 receptors linked via monodisperse PEG-based polymers to an 'effector' peptide that binds to formyl peptide receptors and stimulates an immune response.(4) ISErs are able to activate human neutrophils after targeted binding to cancer cells and injection of the integrin $\alpha 3$-targeting ISEr led to immune cell infiltration and prevented tumor formation in guinea pig and mouse models. The anti-tumor activity and synthetic accessibility of ISErs demonstrate that these novel compounds could be applied to a wide variety of cancer cell targets as well as towards other diseases with such specific markers.

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OP54

Activity of the viral-derived peptide pepR against Staphylococcus aureus biofilms and insights into the mechanism of action

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Bacterial infections are a major human health problem given not only the increasing incidence of drug-resistant bacteria, but also the ability of bacteria to form biofilms. Biofilm-related infections are particularly difficult to treat due to their reduced susceptibility to conventional antibiotics. Thus, it is urgent to develop new and effective antimicrobial agents against bacterial biofilms. Antimicrobial peptides (AMPs) have been considered potential alternatives for this purpose. However, the development of new peptide sequences and the study of their mechanism of action against biofilms is important to expand the potential use of peptides as antibiofilm agents. We investigated the activity of pepR, a multifunctional peptide derived from the Dengue virus capsid protein, against Staphylococcus aureus biofilms, with a focus on the peptide's mechanism of action at the molecular level. We show that pepR is able to both prevent the formation and act on preformed S. aureus biofilms. Mechanistic studies using flow cytometry and confocal fluorescence microscopy show that pepR targets the bacterial membrane. Prevention of biofilm formation results from the fast bactericidal membrane permeabilization of bacteria in the planktonic form. More important, the peptide is able to diffuse through a preformed biofilm and rapidly kill biofilm-embedded bacteria through the same mechanism. Overall, our work demonstrates the potential of pepR as a lead towards the development of novel membrane-active antibiofilm peptides.

OP55

Development of Mirror-Image Monobodies for Targeted Cancer Therapies

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Monoclonal antibody therapies for targeted cancer treatment are a tremendous clinical success. CD20 alone is the target of six different monoclonal antibodies (mAbs) approved for clinical use, across a variety of therapeutic indications including chronic lymphocytic leukemia, Non-Hodgkin's

lymphoma, rheumatoid arthritis, and multiple sclerosis. These and other mAbs targeting CD20 are under investigation for yet more indications. However, these mAbs possess significant downsides in common with other mAb therapies, including serious potential side effects and the possibility of severe immune responses. One strategy to ameliorate these disadvantages is to use mirror-image protein therapies. Since mirror-image proteins use unnatural D-amino acids, these proteins are resistant to proteases and are non-immunogenic. However, since proteins consisting entirely of D-amino acids (and achiral glycine) cannot be produced in biological systems, mirror image proteins cannot be directly used in protein libraries in order to select synthetic binding proteins. Instead, development of mirror-image protein therapies requires chemical synthesis of the mirror-image protein target, a selection step to identify or produce a protein therapeutic effective against the D target, then chemical synthesis of the D-protein therapeutic effective against the natural target. Our protein scaffold of choice is the monobody, an attractive class of molecules that retain the essential features of antibodies while ameliorating their disadvantages. Specifically, monobodies are based on the fibronectin type 3 domain with a highly stable beta-sandwich core structure with three randomizable surface loops and one randomizable beta-strand presents a large surface for potential protein-protein interactions. Crucially, at 100 amino acids in length they are obtainable by chemical synthesis. We report here the development, characterization, and testing of a mirror-image monobody targeting the membrane protein CD20, including the synthesis of the mirror image of the target, the selection of a monobody that binds the target using both phage and yeast display technologies, the total chemical synthesis of both enantiomers of the selected monobody, and in vitro characterization of the binding of the synthetic mirror-image monobody.

OP56

Subtle modifications to oxytocin produce ligands that retain potency and improved selectivity across species

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Poor oral availability and susceptibility to reduction and protease degradation is a major hurdle in peptide drug development for peripheral or central nervous system candidates. In contrast, druggable receptors in the gut present an attractive niche for peptide therapeutics. Here we demonstrate, in a mouse model of chronic abdominal pain, that oxytocin receptors are significantly upregulated in nociceptors innervating the colon. Correspondingly, we have developed novel chemoselective strategies to engineer non-reducible stable oxytocin analogues that are equipotent to native oxytocin. Moreover, we identified a selective and more stable oxytocin receptor (OTR) agonist by subtly modifying the pharmacophore framework of human oxytocin andvasopressin. [Se-Se]-oxytocin-OH displayed similar potency to oxytocin but improved selectivity for OTR, an effect that was retained in mice.. Nuclear magnetic resonance structural analysis of native oxytocin and the seleno-oxytocin derivatives reveals that oxytocin has a pre-organized turn structure in solution, in marked contrast to earlier X-ray crystallography studies. These seleno-oxytocin analogues potently inhibit colonic nociceptors both in vitro and in vivo in mice with chronic visceral hypersensitivity. Our findings have important implications for the clinical use of oxytocin analogues and disulfide-rich peptides in general.

OP57

Cyclotides as novel inhibitors of the human prolyl oligopeptidase

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Cyclotides are plant-derived mini-proteins. Their characteristic circular cystine-knot motif confers them structural stability. Cyclotides have been discovered in the plant families of Cucurbitaceae, Fabaceae, Solanaceae, Poaceae, Rubiaceae and Violaceae and recently their distribution in the Psychotria tribe (Rubiaceae) was further refined [1]. They are expressed as natural peptide libraries essentially with a high molecular diversity and hence these peptides are an interesting starting point for screening toward molecular proteins. In general, native cystine-knot peptides are potent and selective inhibitors of some serine-type proteases. Here we present the discovery of the first cyclotide as a specific inhibitor of the human prolyl oligopeptidase (POP) using a bioassay-guided fractionation approach combined with target-based pharmacology. Plant extracts of four species of the Psychotria and one Viola species were characterized for inhibition of human POP in-vitro at concentrations of 100 to 400 μg/ml. The most promising Psychotria solitudinum extract submitted to a pharmacology guided isolation resulted in the novel cyclotide psysol 2 (IC50 25 μM) as the most abundant compound in this plant peptide library. The molecular structure and amino acid sequence of psysol 2 was characterized by manual de-novo sequencing using tandem mass spectrometry. The specificity for POP inhibition was determined by comparison of the inhibitory activity towards other serine-proteases, namely trypsin and chymotrypsin, which both appeared unaffected by psysol 2 up to 100 μM. Preliminary structure activity studies suggested that proline residues might by important for the observed POP inhibition since kalata B1, a cyclotide with high sequence homology to psysol 2, also inhibited POP activity with an IC50 of 5.6 μM [2]. The enzyme POP is well known for its role in memory and learning processes, and it is currently being considered as a promising therapeutic target for cognitive deficits and neurodegenerative diseases, such as schizophrenia and Parkinson's disease. In the context of discovery and development of future POP inhibitors for therapeutic applications, cyclotides may be suitable starting points considering that small molecule POP inhibitors fail to provide enough selectivity for the enzyme class of post prolyl cleaving endopeptidases.

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OP58

Near-infrared photoactivatable oxygenation catalyst of amyloid peptide

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Aggregation of peptide/protein is intimately related to a number of human diseases [1]. More than twenty have been identified to aggregate into fibrils containing extensive beta-sheet structures, and species generated in

the aggregation processes (i.e., oligomers, protofibrils, and fibrils) contribute to disease development. Amyloid-beta (designated Abeta), related to Alzheimer disease (AD), is the representative example. Artificial chemical transformation of toxic, aberrant Abeta to less toxic forms at the disease site might be a new candidate for AD treatment. Thus, covalent installation of hydrophilic oxygen atoms to Abeta using aerobic oxygen and visible light as oxygen atom and energy sources, respectively, in the presence of a catalyst (i.e., catalytic photooxygenation) was used. Selective, cell-compatible photooxygenation of Abeta by a flavin catalyst attached to an Abeta-binding peptide markedly decreased aggregation potency and neurotoxicity of Abeta [2]. Moreover, we designed photooxygenation catalysts that can be turned on only when binding with the higher-order structures of amyloid aggregates [3]. This on/off switchable activity of the catalyst that senses amyloid structure enabled highly Abeta-selective oxygenation in the presence of other bioactive peptides and living cells. In this context, we recently succeeded to develop new switchable oxygenation catalysts that can be activated by a near-infrared light [4], and demonstrate the applicability of the catalyst to the disease mouse. Thus, the photooxygenation in living AD model mice significantly decreased the Abeta level. Therefore, chemical transformation of Abeta by use of photooxygenation catalyst would be a novel strategy for AD prevention and treatment.

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OP59

Development of the MC1R Selective Ligands for the Melanoma Prevention

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Seeking effective treatments of melanoma is very hot in current era of science and technology. However, prevention of melanoma has been neglected. Here, we propose developing selective hMC1R selective ligands for the melanoma prevention agents. We have been very successful in developing selective hMC1R ligands. Lately we first successfully developed only natural amino acid made peptide, [Leu3, Leu7, Phe8]-γ-MSH-NH2, which is a potent selective hMC1R agonist; and many other hMC1R selective agonists which are more druggable and bioavailable. In vivo studies demonstrated these peptides can cause immediately pigmentation. The natural skin color can be resumed less than 20 hours. The high selectivity of the [Leu3, Leu7, Phe8]-γ-MSH-NH2 for the hMC1R and shorter half-life provides a safer and reduced side-effect agent for the prevention of melanoma skin cancer. This research will be more applicable and benefit for most of people for skin cancer prevention.

OP60

Is the mirror image a true reflection? Lipid chirality in the activity of the prototypic cyclotide kalata B1

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Cyclotides, a family of cyclic disulfide-rich peptides from plants, possess an outstanding structure, intrinsic biological properties (e.g. insecticidal, anticancer, anti-HIV), cell penetrating properties and potential as a scaffold for drug design and intracellular delivery. In previous studies, we have shown that some cyclotides have an unusual ability to specifically recognise phosphatidylethanolamine (PE)-phospholipids in cell membranes. This lipid selectively is intimately related with the highly conserved three-dimensional structure, biological activities and cell-penetrating properties of cyclotides. It is normally assumed that membrane-active peptides and their mirror images have identical activity, but in previous studies, we have shown that the mirror form of the prototypic cyclotide kalata B1, D-kalata B1, although active in all the activities tested, is less potent than the native kalata B1. The lower activity of the D-kalata B1 correlated with a lower affinity for PE-containing lipid bilayers. In the current study, we were specifically interested in investigating whether the chiral environment created by biological membranes can discriminate between kalata B1 enantiomers and would explain the lower potency of D-kalata B1. We have synthesised unnatural enantiomers of two phospholipids (referred here as D-phospholipids) and compared the L- and D-kalata B1 enantiomers in their ability to bind model membranes composed with either L- or D-phospholipids. Using kalata B1 analogues, cell-based assays, NMR spectroscopy, fluorescence spectroscopy and surface plasmon resonance we showed that kalata B1 recognises PE-headgroups in a chiral-independent manner, but inserts into lipid bilayers via a mechanism dependent on membrane chirality. This study shows that, contrary to the generally assumed, chiral environment of lipid bilayers can modulate the activity of peptides and proteins.

OP61

Substitution of Aromatic Residues in the Macrocyclic Opioid Peptide [D-Trp]CJ-15,208 Alters the Opioid Activity Profile in vivo

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The macrocyclic peptide [D-Trp]CJ-15,208 (cyclo[Phe-D-Pro-Phe-D-Trp]) exhibits kappa opioid receptor (KOR) antagonist activity in vivo in the mouse 55 oC warm-water tail withdrawal assay, but the alanine scan analogs of this peptide also unexpectedly exhibited robust antinociceptive (agonist) activity in this assay (Aldrich et al., Br. J. Pharmacol. 2014, 171, 3212–3222). As part of our exploration of the structure-activity relationships (SAR) of this peptide, additional substitutions were made for the phenylalanine residues, including both aliphatic residues and substituted aromatic residues. Most of the analogs examined to date retained KOR antagonist activity in the antinociceptive assay after intracerebroventricular (i.c.v.) administration, although the potency varied substantially depending upon the substitution. One analog evaluated exhibited robust KOR antagonist activity after oral administration (10 mg/kg). The agonist (antinociceptive) activity, however, varied substantially depending on the substitution, with some analogs exhibiting full agonist activity (EC50 = 20-35 nmol i.c.v.). Selected peptides were also shown to prevent stress-induced reinstatement of morphine seeking behavior in a conditioned place preference assay. These results advance our understanding of the SAR of these novel opioid peptides and support further exploration of these peptides for the treatment of drug abuse. This research supported by grants PR141230 and PR141230P1 from the U.S. Army and grant R01 DA018332 from the National Institute on Drug Abuse.

OP62

Developing novel bi-specific peptide as an immunosuppressant using directed-evolution optimization

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The serine/threonine phosphatase calcineurin targets the nuclear factors of activated T cells (NFATs) and leads to the activation of cytokine genes. Calcium influx activates calcineurin to dephosphorylate multiple serine residues within the 400 residue NFAT homology region; this triggers NFAT nuclear translocation. The binding of the two proteins relies on the interaction between two sites in calcineurin, harboring the conserved motifs PxIxIT and LxVP, located at the N- and C-terminal to the phosphorylation sites in NFAT's homology region. NFAT's PxIxIT motif binds calcineurin's catalytic domain at a location that is a distance from the catalytic site of calcineurin. The LxVP motif binds at the interface of calcineurin's regulatory and catalytic domains; however, it can also bind to the same site on the catalytic domain as the PxIxIT, but with a lower affinity. Owing to the role of calcineurin-NFAT in T-cell activation, calcineurin is the target for immune suppression drugs such as cyclosporine and tacrolimus, thus obscuring the latter catalytic site. However, the vast range of cellular processes regulated by the calcineurin-NFAT interaction has aroused great interest in the discovery of protein-protein interaction inhibitors that will interfere with the calcineurin-NFAT complex formation while keeping calcineurin's catalytic site free. Recently, we developed a new Enzyme Linked Immuno Sorbent Assay (ELISA) that is based on the interaction of the heterodimer calcineurin, i.e., catalytic and regulatory domain, with a NFAT homology region that includes the phosphorylation sites flanked by PxIxIT and LxVP binding motifs. This experimental scheme provides valuable data regarding the interaction of the two binding motifs of NFAT with calcineurin. Based on this setup, we are currently developing an optimized di-peptide constructs by linking peptides derived from the PxIxIT and LxVP motifs. Furthermore, directed-evolution is being used for the optimization of this chimeric peptide and for the creation of a novel protein-protein interaction inhibitor that can act as a new immunosuppressant.

OP63

The Use of Peptide-Membrane Interactions in the Design of Selective and Potent Sodium Channel Inhibitors

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Peptide toxins isolated from spiders are potent inhibitors of human voltage-gated sodium channels (NaV). Some of these peptides are selective against subtypes NaV1.7, reported to be involved in nociception, and may thus have potential as pain therapeutic leads. Peptide toxins can inhibit NaV activity by blocking the pore domain (i.e, pore blockers) or by binding to the membrane-embedded voltage sensor domain of the sodium channel (i.e. gating-modifier toxins). It is now well established that some gating-modifier toxins also interact with lipid membranes surrounding the voltage-sensor domains. However, it is less well-known to what extent these peptide-lipid interactions are relevant for the inhibitory activity and/or sodium channel selectivity. Using a range of biophysical techniques, we have examined the importance of membrane binding on the inhibitory activity of a subset of peptides, showing a direct correlation between membrane binding affinity and NaV selectivity, highlighting the importance of considering potential membrane-binding events when designing sodium channel voltage-gating modifier inhibitors. Using this information, we have designed modified gating-modifier toxins with improved potency and off-target selectivity for the therapeutically relevant sodium channel subtype NaV1.7.

OP64

Dipeptide-derived Alkynes as Novel Irreversible Inhibitors of Cathepsin B

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Until recently, alkynes were considered to be bioinert. Thus, they are popular reaction partners in bioorthogonal click reactions in vitro and in vivo. Despite the virtual chemical inertness of the alkyne moiety, two research groups observed the irreversible inhibition of a cysteine protease by an alkyne-functionalised substrate derivative: both EKKEBUS et al. and SOMMER et al. independently described the unexpected inactivation of de-ubiquitinating enzymes by ubiquitin or ubiquitin-like modifiers bearing propargylamine in place of C-terminal glycine [1, 2]. We intended to harness that finding for the design of inhibitor-based probes for the imaging of tumour-associated cysteine proteases. Cysteine cathepsins play an important role in tumour progression. In particular cathepsin B is involved in a variety of tumour progression-related processes and elevated extracellular levels are linked to increased malignancy and poor prognosis [3]. Therefore, this enzyme represents a promising target for the therapy and imaging of tumours. GREENSPAN et al. reported a potent and highly selective dipeptidyl nitrile-based cathepsin B inhibitor (N-[2-[(3-Carboxyphenyl)methoxy]-1(S)-cyanoethyl]-3-methyl-Nα-(2,4-difluorobenzoyl)-L-phenylalaninamide) [4]. Based on that lead compound, cathepsin B-targeting dipeptide alkynes were designed by isoelectronic replacement of the nitrile nitrogen atom by a methine group and consecutive variation of the 2,4-difluorobenzoyl and (3-carboxybenzyl)oxymethyl residue. Formation of the C-C triple bond by reaction of the corresponding open-chain serine-derived aldehyde with the Bestmann-Ohira reagent was accompanied by partial enantiomerisation. Therefore, the synthesis was performed via Garner's aldehyde, which accounted for a high stereochemical purity of the final compounds. The inhibitory potential was investigated against cathepsin B, S, L and K. The most potent compound exhibited irreversible inhibition of cathepsin B with an inactivation constant (kinact/KI) of 771 1/(M*s). Values for cathepsins L and S were significantly lower; no irreversible inhibition was observed for cathepsin K. In addition, inhibition of cathepsin B activity in human glioblastoma cell lysates and living cells has been demonstrated. Based on these promising results, dipeptidyl alkynes have the potential to become a valuable tool for imaging due to the expected low activity towards other cysteine proteases. In further studies, selected inhibitors for cathepsin B will be labelled with suitable radionuclides to obtain an inhibitor-based probe directed towards cathepsin B.

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OP65

Potent Antimicrobial Peptide Dendrimers against Multi-Drug Resistant Pseudomonas aeruginosa and Acinobacter baumanii

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Multi-drug resistant bacteria represent a public health threat that needs urgent handling. In particular, resistant Gram-negative bacteria are difficult to treat and the arsenal of antibiotics still effective is dwelling. Peptide based antimicrobials offer an attractive opportunity to control these pathogens [1].

While natural peptide antimicrobials are normally linear or cyclic sequences we have explored branched and bridged bicyclic topologies for antimicrobial activity. Branched peptide dendrimers with high activity against multi-resistant clinical isolates of P. aeruginosa and Acinetobacter baumanii were designed and further optimized by screening a third generation peptide dendrimer library. The third generation dendrimer G3KL incorporating natural lysine and leucine residues in the branches and as branching point (37 amino acid residues) gave a MIC (minimal inhibitory concentration) value of 2 $\neq$ g/mL against P. aeruginosa and good activity against a large panel of multi-resistant strains [2,3]. We have now discovered second generation peptide dendrimers (17 amino acid residues) incorporating a C10 lipid chain with high potency against the multidrug resistant strains. The lipophilic alkyl chain is essential for interaction with the lipopolysaccharide in the bacterial cell wall. Mechanistic studies indicate that the lipid chain may fold onto the peptide dendrimer branches in water and unfold in contact with the bacterial membrane to expose the hydrophobic lipid chain leading to membrane disruption and rapid cell death. [4] The antimicrobial peptide dendrimers showed low toxicity to red blood cells and, in contrast to linear peptides, stability and high activity in human serum. The combination of topology, peptide sequence and a lipid chain represents a new design to discover antimicrobials to fight multi-resistant bacterial pathogens.

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OP66

Modifying short phenylalanine-phenylalanine peptide sequences to create multifunctional nanomaterials with biomaterial and drug delivery applications

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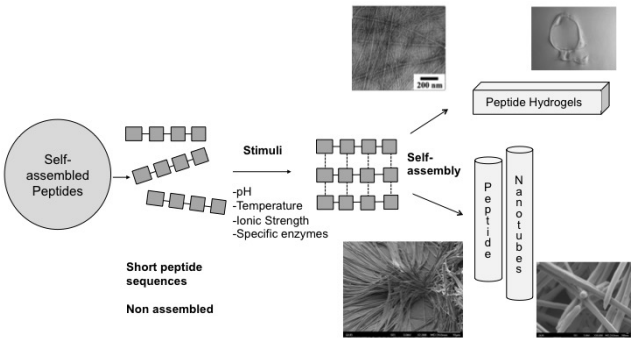
Background: The threat of antimicrobial resistance to society is compounded by a relative lack of new clinically effective licensed therapies reaching patients over the past three decades. Phenylalanine-phenylalanine (FF) peptides have attracted increased interest due to their ability to self-assemble into nanotubes or nanofibrous hydrogel structures that can be utilised as drug delivery and biomaterial platforms. The chemical versatility of the peptide motif enables antimicrobial and anti-inflammatory activity to be built into a peptide capable of self-assembling into a nanomaterial structure.

Methods: The antimicrobial activity of FF peptide nanotubes and FF (including naphthalene, Fmoc and naproxen) hydrogelators were investigated against planktonic and biofilm forms of microorganisms implicated in hospital-acquired infections (Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa and Candida albicans) using viable counting methods. Scanning electron microscopy was used to visualise the nanomaterial's effect on biofilms. Cell cytotoxicity was evaluated using LIVE/DEAD, MTS and LDH assays against a variety of relevant cell lines (e.g. NCTC 929).

Results: Both peptide nanotube and hydrogel platforms demonstrated potent activity against planktonic forms of hospital superbugs. NH2-FF-COOH nanotubes demonstrated potent activity against planktonic and biofilm forms of Gram-positive bacteria, possessing minimal cell toxicity. 5mg/mL peptide nanotubes demonstrated 6.25 log10 reduction in colony forming units per ml (CFU/ml) against planktonic S. aureus and a 5.4 log10 reduction in CFU/ml against S. aureus biofilm. SEM images showed bacterial membrane damage had occurred and this was supported by a bacterial membrane permeability (NPN) assay.An ultrashort cationic hydrogelator (naphthalene-2-ly)-acetyl-diphenylalanine-dilysine-OH (NapFFKK-OH) possessed broad-spectrum antimicrobial efficacy including ability to eradicate surface attached biofilms form associated with increased tolerance to standardly employed antimicrobials. Naproxen attached FFKK-OH (NpxFFKK-OH) possessed additional anti-inflammatory activity (COX inhibition) which may be of benefit to chronic wounds.

Conclusions: Peptide nanomaterials demonstrate significant promise as future drug delivery and biomaterial platforms. This is the first study to prove the activity of FF peptide nanotubes against microbial biofilms, a major healthcare and industrial problem. The architecture of these peptide nanostructures mean antibiotics could be potentially encapsulated within the hollow hydrophilic pores of FF nanotubes or within their hydrophobic phenylalanine walls to provide synergistic antibiofilm action leading to improved therapy and at lower antibiotic concentrations. Multifunctional ultrashort peptide hydrogels also demonstrate potential as wound healing products or biomaterial coatings. Peptide hydrogel dressings may resolve issues in the case of chronic wounds which fail to heal (e.g. diabetic ulcers).



OP67

Reviving Old Protecting Group Chemistry for Site-Selective Peptide-Protein Conjugation

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Given the increasing importance of biopharmaceuticals in the modern drug scene, methodologies to conjugate proteins to property-enhancing entities including peptides are highly sought after. Use of a thiol functionality in a protein via a cysteine residue provides an orthogonal attachment point for conjugation of these entities.[1] Cysteine's relatively low abundance in proteins coupled with the fact that it can be uniquely nucleophilic due to its low pKa (8.2), makes it an ideal handle for site-selectively conjugating proteins to groups of interest via alkylation or crossed-disulfide formation. An advantage of the disulfide bond is that intracellular release of the moiety of interest is possible.[2] For effective reaction to occur, the protein's reaction partner needs to harbour a thiol group for oxidative reaction with the cysteine. In general, in order to reduce the formation of homodimeric disulfide species, either the protein cysteine or thiol partner is activated. We report herein a remarkably simple strategy for conjugating proteins bearing accessible cysteines to unprotected peptides containing a Cys(Scm) (Carbomethoxysulfenyl) protecting group. The Scm group was introduced on-resin via a Cys(Acm) (Acetamidomethyl) building block.[3] The peptides employed for this proof of principle study were highly varied and structurally diverse and underwent multiple on-resin decoration steps prior to protein conjugation. The methodology was applied to three different proteins and proved to be site-selective. Moreover, the overall synthesis is facile, efficient and versatile, and takes advantage of solid phase peptide chemistry strategies for the decoration of proteins with free Cys residues. On the peptide partner, the Acm protecting group is stable to numerous conditions, allowing different functionalisations to be carried out on-resin, prior to quantitative conversion to Scm. The use of Fmoc-(Cys) (as opposed to Boc-MTS or Boc-DTNP) allows the use of milder peptide synthesis conditions. Conjugations of the resulting unprotected peptides to proteins are carried out in ambient conditions, and protein-peptide conjugates are easily isolated from excess peptide for characterisation. In addition, we found that for some peptides synthesised, prior purification was not required pre-disulfide formation, and pure protein conjugate was obtained. Obviously, where strictly homogeneous conjugates are required, employed peptides can be purified. The methodology should be generally applicable to many systems, as evidenced by our use of multiple proteins and peptides. Crucially, Cys can be introduced into proteins by simple site-directed mutagenesis. Further biological applications of the conjugates formed are currently being explored in our group, as well as application of the method to other biomolecules.

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OP68

Use of selenoester for the efficient synthesis of protein

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We have been developing efficient methods for protein synthesis based on the ligation methods, such as the native chemical ligation [1] and the thioester method [2]. One of the remaining issues in these methods is that it is necessary to isolate the intermediate segments after each ligation reactions, which leads to the low recovery yield from the HPLC column as well as the low efficiency of the entire synthetic process. Many researchers are engaged in developing the one-pot ligation method, which can eliminate this problem. We have developed one-pot four-segment ligation without requiring any purification and any protecting group-removal, combining the use of peptide seleno and thioester. The efficiency of the method was demonstrated by the synthesis of human superoxide dismutase composed of 153 amino acid residues [3]. Another topic of this presentation is the synthesis of selenocysteine-containing protein, such as selenoinsulin [4] and selenoferredoxin. The synthesis of the segments, ligation and folding reaction will be described.

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OP69

Eukaryotic macrocyclases enhance biocatalytic production of cyclic peptides

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Peptide macrocycles display enhanced structural rigidity, protease resistance cell membrane permeability as well as ability to modulate protein-protein interaction. Thus, rendering a major interest in drug development. Chemically diverse cyclic peptides can be challenging to synthesis chemically. In vitro approaches using biosynthetic enzymes have been proven successful in combining cyclic peptide biosynthesis with solid phase peptide synthesis accessing cyclic peptides containing non-proteinogenic amino acids. However, macrocyclization of these compounds has been the bottle-neck within this process. PCY1 from Saponaria vaccaria and GmPOPB from Galerina marginata are recently discovered macrocyclases involved in the biosynthesis of segetalins and amanitin toxin, respectively, belonging to the class of prolyl-oligo peptidases (POPs). Extensive structural characterization of substrate complexes of both enzymes coupled with kinetic and thermodynamic studies shed light on mechanistic insights which informed the design of substrates with a minimal C-terminal recognition sequence, allowing a more effective usage of these two enzymes as biocatlytic tools in the (bio)synthesis of cyclic peptides.

OP70

Cost Effective peptide Purification via ZEOsphere DRP Mixed-Mode Chromatography

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Cost Effective Peptide Purification via ZEOsphere DRP Mixed-Mode Chromatography Alessandro Butté [1], Jürgen Machielse [2], Andrea Wild [2], Timo Nuijens [3], Marcel Schmidt [3] 1. ETH-Zürich / DataHow, Zürich, Switzerland 2. Zeochem AG, Rüti, Switzerland 3. EnzyPep BV, Geleen, The Netherlands

Peptides are important API's for modern pharmaceuticals and have to be produced on preparative scale with increasing demand on separation costs. RPC and Ion Exchange are well-established chromatographic modes within the available tool-box of methods and procedures for the purification of this class of compounds in preparative scale production.

The presentation will show the beneficial use of ZEOsphere DRP (Doped Reversed Phase) Mixed-Mode stationary phases in the repulsive-attractive mode compared to non-doped RP or IEX stationary phases on crude peptides. The orthogonal Doped Reversed Phase materials combines the dual action of strong IEX groups (acidic or basic) and Reversed Phase ligands like octyl chains on the packing surface.

Usually, Mixed-Mode stationary phases are applied under conditions which add the retention power of IEX groups by electrostatic attractive forces to the retention received from hydrophobic surface groups to the solutes. Both species, e.g. the peptide and the IEX group carry opposite charges.

It can be shown that in the majority of real life cases tested so far with ZEOsphere DRP in repulsion-attractive mode, improved selectivities and increased resolution at decreased retention time and solvent consumption can be obtained.

Young Investigators' Mini Symposium

MS1

Generation and characterisation of novel antibodies from selectively Advanced Glycation Endproduct (AGE) modified Collagen Model Peptides (CMPs)

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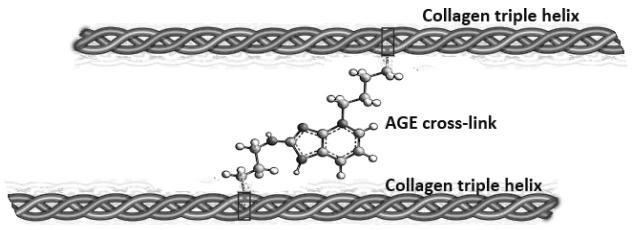
The University of Auckland, New Zealand

Advanced Glycation Endproducts (AGEs) are naturally occurring protein modifications found in humans that are derived from the non-enzymatic reaction of sugars with proteins which alters the structure and biological properties of modified peptides. AGEs accumulate over time in long-lived peptides, particularly in diabetes patients due to persistent high blood-sugar levels. A positive relationship between increased AGE formation and pathological disease state has been established for diabetes, cancer, cardiovascular and Alzheimer's diseases, making AGEs a group of highly interesting biomarkers. Collagen is one of the most abundant proteins in humans, and is the main component of skin, cartilage, bones and blood vessels. Cross-linking via uncontrolled glycation in collagen alters the structural and biological properties of the native peptide contributing to diabetes-related failure of tissue, arteriosclerosis and oxidative stress. Selective AGE modification of Collagen model peptide (CMPs) can be achieved by utilizing Fmoc based solid phase peptide synthesis (SPPS). The target AGE need to be synthesised first and similar to common SPPS protected. AGEs such as glyoxal lysine-dimer (GOLD) and methylglyoxal lysine-dimer (MOLD) have already been successfully incorporated into collagen peptides in a site-selective matter by our group, whereas more complex AGEs (Pentosidine, Vesperlysine) have only been described as nonspecific protein modifications in the literature to date. Our group is interested in investigating the effect of different cross-linking AGEs on the structural and biological properties of CMPs. Furthermore, we are focusing on new methods to detect these modifications using second generation antibodies derived from these biomimetic haptens. Pentosidine and Vesperlysine cross-linked CMPs are the most attractive target as both AGEs have been frequently used as a biomarker for pathological conditions associated with hyperglycaemia. Therefore, a reliable and accessible method to measure and investigate AGEs in complex biological samples is needed. Several techniques are available to determine the quantity and identity of AGEs in biological samples, with the most prominent being UV-Vis, fluorescence spectroscopy and HPLC analysis. These methods require highly specialised equipment and personnel and are thus expensive in terms of both time and cost. Selective antibodies, generated to recognize AGE modifications in peptides, offer a more versatile alternative. However, the antibodies currently available are primarily generated from a direct but unselective AGE modification of carrier proteins such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH), raising the question of their epitope specificity. Designing a biomimetic immunogen is crucial for the generation of highly specific antibodies that are capable of recognising an AGE on a particular human protein. Particularly since the epitope of an antibody is likely to encompass not only the AGE modification but also the modified protein, making the backbone of the peptide an important factor for antibody generation.



MS2

Light-controlled inhibition of MLL1 methyltransferase by azo-containing peptides: towards optoepigenetic leukemia regulation

Lea Albert[1], Jing Xu[2], Vasundara Srinivasan[1], Yali Dou[2], Olalla Vázquez[1]

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Epigenetics is the study of changes in gene expression unrelated to changes in the DNA sequence. Since genetics alone cannot explain human variation and disease, epigenetics is one of the most promising fields in biomedicine. Combinatorial post-translational modifications (PTMs) on histones, often referred to as the histone language,[1] regulate the structure of chromatin and affect transcriptional activity by recruiting a large variety of proteins through protein-protein interactions (PPIs). One widely studied PTM is the specific methylation at lysine 4 of histone 3 (H3K4) by the mixed-lineage leukemia (MLL) enzymes. MLL1 is an H3K4 methyltransferase enzyme and a promising cancer therapeutic target since its deregulation has been implicated in various cancers and solid tumors.[2] MLL1 exists as a part of a protein-core complex that enhances its activity. Particularly, the protein WD40-repeat protein 5 (WDR5) controls MLL1 activity by forming a bridge between units and via binding to the argenine 3765 of MLL1, which is essential for methylation activity. Therefore, blocking WDR5-MLL1 interaction is an effective strategy for its inhibition. There are some precedents of small molecules[3,4] and peptides[5] as potent MLL1 antagonists. However, all these precedents do not offer the possibility to exert spatiotemporal control over the highly dynamic epigenetic signalling processes. To solve this, our group has recently synthetized and studied cell-permeable photoswitchable peptidomimetics that are able to target the key WDR5-MLL1 interaction[6] with high affinity (Ki = 1.25 nM). Importantly, upon irradiation, the change of conformation of our peptidomimetics triggers a significant difference in MLL1 activity, leukemia cell proliferation and even transcription of the MLL1-target gene Deptor.

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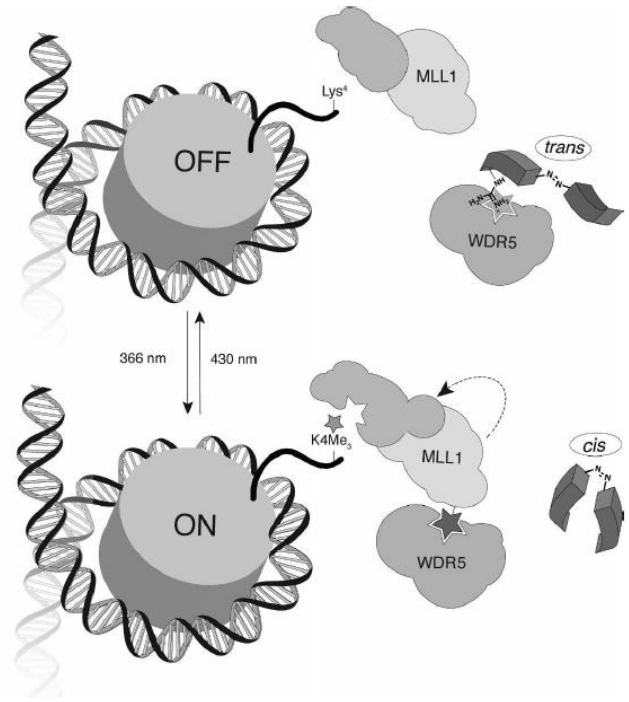

MS3

Development of NaV-Selective Agonists with Potential for Treatment of Dravet Syndrome Epilepsy

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Dravet syndrome (DS) is a catastrophic epileptic encephalopathy characterised by childhood-onset polymorphic seizures, multiple neuropsychiatric comorbidities, and increased risk of sudden death. Most DS cases result from de novo loss-of-function mutations in one allele of the SCN1A gene that encodes the ion-conducting α-subunit of the voltage-gated sodium channel 1.1 (NaV1.1). In the brain, NaV1.1 is primarily found in the axon initial segment of fast-spiking GABAergic inhibitory interneurons; selective deletion of NaV1.1 in these interneurons in mice recapitulates the symptoms of DS. Thus, the principle mechanism proposed to underlie seizure genesis in DS is loss of inhibitory input due to dysfunctional firing of GABAergic interneurons. In support of this hypothesis, antiepileptic drugs such as phenytoin, lamotrigine and carbamezipine that non-selectively inhibit NaV channels are contraindicated in DS, whereas compounds that enhance GABAergic neurotransmission such as benzodiazepines are therapeutically beneficial.

We hypothesised that DS symptoms could be ameliorated by a drug that activated the reduced population of functional NaV1.1 channels in DS interneurons. Toward this end, we identified and characterised two homologous disulfide-rich spider-venom peptides (Hm1a and Hm1b) that selectively potentiate NaV1.1. Hm1a rescues DS interneurons from action potential collapse, while i.c.v. infusion of Hm1a abolishes seizures in DS mice and rescues them from premature death. However, Hm1a is unstable in vivo and difficult to produce via both recombinant and chemical methods. We therefore produced recombinant Hm1b using periplasmic E. coli expression system and examined its selectivity against a panel of human NaV subtypes using whole-cell patch-clamp recordings. Hm1b is a potent and highly selective agonist of NaV1.1 (EC50 12 nM). Hm1b is a gating modifier that shifts the voltage dependence of channel activation and inactivation to hyperpolarised and depolarised potentials respectively, presumably by interacting with the channel's voltage-sensor domains. It has minimal effect on peak current amplitude but robustly inhibits fast inactivation to cause a large increase in sustained currents. Hm1b has no effect on other Nav subtypes except NaV1.3. Like Hm1a, the structure of Hm1b determined using NMR revealed a classical inhibitor cystine knot motif in which the peptide is cross-braced by three disulfide bonds. However, we show that Hm1b is an order of magnitude more stable than Hm1a in human cerebrospinal fluid. Taken together, our data suggest that Hm1b is an exciting lead for a precision therapeutic targeted against DS.

MS4

Exploiting thioamide reactivity in peptide synthesis

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Over recent decades, the preparation of proteins and long peptides has been achieved using either recombinant methods or total chemical synthesis employing native chemical ligation (NCL). These methods each have limitations; recombinant expression techniques cannot be used for unnatural amino acids and modified amino acids, while native chemical ligation requires cysteine at the ligation site. To overcome limitations of NCL, several methods have been developed including desulfurization of cysteine to alanine. Nevertheless, all NCL-type methods rely on thioester (or selenoester) exchange with an N-terminal residue possessing a thiol or selenol functional group as the critical step. To overcome the limitation of NCL methods we are developing new residue-independent ligation strategies. Previous work in the Hutton group developed novel chemoselective reactions of thioamides with peptide C-terminal carboxylic acids, which has been exploited in the synthesis of peptide imides and enables coupling of amino acids for N-C direction peptide synthesis. The N-C direction peptide synthesis involves the generation of isoimide intermediates, which can rearrange to imides via an O-N acyl transfer.1 We have now developed a novel chemoselective peptide ligation strategy that further exploits the reactivity of peptides containing backbone thioamides. We have successfully synthesized madanin-1 (60 amino acid peptide) using this thioamide-based ligation method. Further, we are exploiting the thioamide-based ligation in an intramolecular reaction to synthesize naturally occurring cyclic peptides, including gramicidin S and mahafacyclin B.

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MS5

Zn-catalyzed tert-butyl nicotinate-directed amide cleavage for applications in peptide synthesis and peptidomimetic design

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An amide is the key functional group that makes up the backbone of pep-

tides and proteins. Consequently, the selective formation of this group has been extensively studied by peptide scientist with the development of a well-established catalogue of so-called coupling reagents as a result. Nevertheless, the general method remains the direct acylation of an amine by means of an in situ activated carboxylic acid.[1] In endeavors to overcome the inherent limitations associated with these coupling reagents, chemical ligation involving the assistance of other functional groups or amine rather than carboxylic acid activation has been explored.[2] To date, amides have rarely been considered as a carboxylic acid surrogate because of their poor electrophilicity and intrinsic stability. While the activation of secondary amides via Bocylation delivers substrates which are cleavable with alcohols and amines under Ni-catalysis,[3] our research focused on the cleavage of primary amides via directing group activation. This directing group is easily introduced onto primary amides via Pd-catalyzed amidation with tert-butyl 2-chloronicotinate. Zinc(II)acetate was identified as a suitable catalyst for nicotinate directed amide cleavage with alcohols.[4] The activation mechanism can be regarded as 'biomimetic': the C3-ester substituent of the pyridine in the directing group populates the trans-conformer suitable for Zn-chelation, C=O(amide)-Zn-N(directing group), and Zn-coordinated alcohol is additionally activated as a nucleophile by hydrogen bonding with the acetate ligand of the catalyst. Additionally, the acetate ligand assists in intramolecular O-to-N proton transfer. The method could be extended to amines. Nicotinate activated amino acid amides proved to be suitable substrates. Transamidations with amino acid esters/amides allow diverse applications in peptide research, exemplified by segment condensations and macrocyclisations.

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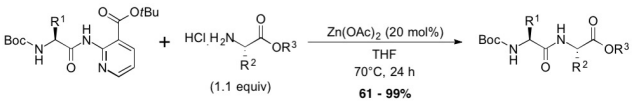

MS6

Synthesis of peptides glycated at Lys residues

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Institute of Biomedical Chemistry (IBMC), Moscow, Russia

Glycated proteins are considered putative triggers of diabetes complications. Glycated peptides are needed as standards for proteomic studies of pathological protein glycation and as antigens for producing specific antibodies. We studied several approaches for the preparation of peptides containing glycated (1-deoxyfructosyl-, or Amadori product (AM), carboxymethylated (CM) and carboxyethylated (CE)) Lys residues: 1) modification by glucose in water solution; 2) Schiff base formation with further reduction; 3) alkylation (for CM-and CE-modifications). Peptide modification versus Fmoc-Lys modification with further application of the latter in SPPS was evaluated. Yields and purity of glycated peptides obtained with the use of different approaches were compared and yields and purity achieved by the tested approaches were compared. The suitability of each studied approach (except the 1st one) was shown to depend upon the type of the glycation and the position of the modified Lys residue in peptide chains. Modification of peptides by glucose in solution resulted in low yields and a mixture of various glycation products difficult to separate selectively by HPLC. The study was performed in the framework of the Program for Basic Research of State Academies of Sciences for 2013-2020. Peptide synthesis was done with the use of the equipment of the "Human Proteome" Core Facility of the Institute of Biomedical Chemstry (Moscow, Russia) supported by Ministry of Education and Science of the Russian Federation (unique project ID RMEFI62117X0017).

MS7

Targeting Ghrelin Receptor Homodimer: Bivalent Ligands with Exceptional Binding Affinity and Potency

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The ghrelin receptor (GHS-R1a) is a 7-transmembrane G protein-coupled receptor (GPCR) that is activated by a 28-amino acid peptide, ghrelin. GPCRs are known to form dimers and the literature investigating ghrelin receptor dimerization has largely focused on heterodimerization with other GPCRs. The formation of GHS-R1a homodimers is less studied and chemical tools to explore this phenomena would allow for a better understanding of mechanism and functional relevance of homodimerization. In the present study we report the design, synthesis, and binding affinity evaluation of bivalent ligands targeting GHS-R1a. Bivalent ligands, based on the peptidomimetic G7039, were designed through a rational structure-based design approach. The optimal linker length was determined through the synthesis of eight bivalent ligand variants and these were evaluated using an in vitro competitive binding assay. Our studies showed that the bivalent ligand B5-58 possesses exceptionally high binding affinity (IC(50high) = 0.43 nM; IC(50low) = 0.42 pM) and can bind to both receptors within the GHS-R1a homodimer in HEK293 cells, which is indicated by a bivalent binding curve. In addition, we demonstrated that B5-58 is a strong agonist by its activation of beta-arrestin-1 and beta-arrestin-2 recruitment in the low nanomolar range (EC(50) = 1.81 nM for beta-Arr-1 and EC(50) = 2.09 nM for beta-Arr-2). The monomeric version S5-58 (the single peptidomimetic with the linker attached), the unmodified G7039 peptidomimetic, and the natural ligand for the receptor, ghrelin, are 2-15x less potent then B5-58 based on EC(50) values for beta-arrestin-1 and beta-arrestin-2 recruitment. GHS-R1a was then inserted into both a yellow fluorescent protein (YFP) vector and Renilla luciferase (rluc) vector for use in a BRET assay to assess the induction of the homodimer with the addition of B5-58. We determined that there was 3-fold increase in homodimerization BRET response with B5-58 compared to ghrelin, G7039, and the monomer. Currently, B5-58 is being evaluated in cell signaling assays and confocal experiments to visualize the induction of the homodimer. Ghrelin receptor activation triggers several signaling pathways that can be harnessed for therapeutic response in disease states (i.e. inflammation, diabetes, and hypotensive effects), leading to the potential for B5-58 to be used as a therapeutic agent. Therefore, we conclude that B5-58 is able to induce GHS-R1a homodimer formation and is a bivalent ligand that has high binding affinity and receptor activation. The bivalent ligand described herein is a powerful tool to explore the functions of the GHS-R1a homodimer and constitutes a novel therapeutic entity for GHS-R1a related pathological conditions and diseases that can be controlled through the activation increase of the ghrelin receptor.

MS8

De novo designed proteins catalyzing amide bond forming reactions

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Chemical synthesis of peptides and proteins is of great interest as a tool for studying protein structure and function and as a method for preparation of biomedically important proteins.[1] Our goal is to design de novo proteins which can accelerate the amide bond forming reactions and can be used as peptide ligases or for peptide/protein labelling. In this study, we explored N-terminus of α-helix with unpaired hydrogen-bond donors as a structural motif to stabilize negatively charged tetrahedral intermediates of nucleophilic addition-elimination reactions at carbonyl group. The catalytic cysteine residue acting as a principal nucleophile was engineered at either of the two key positions, so-called Ncap (residue preceding the first amino acid that adopts α-helical conformation) and N2 (second residue at N-terminus of α-helix) in a designed Domain-Swapped Dimer (DSD) three-α-helix protein scaffold.[2] We demonstrated efficient transthioesterification of peptide-αthioesters and, subsequently, aminolysis of the thioester intermediate in the presence of a large excess of tris(hydroxymethyl)aminomethane (Tris), an amine with pKa similar to N-terminal amino group of peptides. To improve catalytic parameters, the iterations of computational design, X-ray crystallographic structural studies, as well as combinatorial synthesis of libraries of variants are currently undergoing.

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MS9

Synthesis and biochemical evaluation of GnRH-III-drug conjugates

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Gonadotropin releasing hormone-III (GnRH-III, <EHWSHDWKPG-NH2; <E – pyroglutamic acid, isolated from sea lamprey), a native isoform of the human peptide hormone GnRH-I, represents a promising starting point for the development of efficient peptide-based drug delivery systems for targeted cancer therapy. Beneficially hereby is that GnRH-III specifically binds to GnRH receptors on cancer cells without revealing a significant endocrine effect [1]. Based on these findings, a variety of GnRH-III-drug conjugates have been developed and characterized in our laboratories. We could demonstrate that the side chain of 8Lys provides a suitable ligation site for cytotoxic agents without substantial decrease of receptor binding affinity or antiproliferative activity [1]. Moreover, we developed an efficient strategy to link the anthracycline daunorubicin (Dau) to GnRH-III via oxime bond by insertion of an aminooxyacetyl moiety [1]. To achieve an improved antitumor activity, we designed and synthesized a series of oxime linked GnRH-III-Dau conjugates in which the targeting peptide sequence was modified by different unnatural amino acids [2]. Initially, the cytostatic effect of the 20 novel bioconjugates was studied on MCF-7 breast and HT-29 colon cancer cells revealing an enhanced antiproliferative activity for one of the compounds. Due to this, the best candidates were chosen for further biochemical evaluation like cellular uptake studies, plasma stability or lysosomal degradation. Furthermore, the two most promising peptide carriers of these studies were selected as targeting moiety for the development of novel drug conjugates. Thus, the classical anticancer drugs Dau and paclitaxel (PTX) have been successfully linked to GnRH-III derivatives by using a self-immolative p-aminobenzyloxycarbonlyl (PABC) spacer between a cathepsin B cleavable dipeptide (Val-Ala, Val-Cit) and the drug. For comparative purpose, the non-cleavable GnRH-III-drug conjugates were also synthesized. The resulting bioconjugates have been analyzed for their in vitro cytostatic effect on A-2780 ovarian and Panc-1 pancreatic cancer cells and the release of the drug was followed by lysosomal degradation studies in presence of rat liver lysosomal homogenate. Next to this, the highly efficient drug candidate cryptophycin was conjugated to a GnRH-III homing device by using different cleavable and non-cleavable linker systems [5]. The corresponding in vitro evaluation including the antitumor activity of the compounds as well as stability and drug-release studies are ongoing. This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No 642004, and from the National Research, Development and Innovation Office (NKFIH K119552), Hungary.

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MS10

Directing PYY3-36 internalization through half-life extenders

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Peptide YY3-36 (PYY3-36) is an endogenous ligand of the neuropeptide Y2 receptor (Y2R), on which it act to reduce food intake. Accordingly, PYY3-36 analogues are interesting as potential anti-obesity pharmaceuticals. However, native PYY3-36 is rapidly cleared from circulation, and half-life extension is thus a prerequisite for PYY3-36 based pharmaceuticals. This is typically achieved by covalent attachment of PYY3-36 to macromolecules (e.g. PEG) or through lipidation which promotes non-covalent interactions to albumin. Many peptide drugs, like PYY3-36, require binding to a specific G protein coupled receptor (GPCR) to exert their effect. GPCRs are desensitized and internalized following intracellular binding of arrestins, which bind to the ligand-activated conformation of the receptor.[1] GPCR-bound arrestins can in some cases confer signalling with distinct and important biological consequences, but will also inhibit G-protein signalling, which is often the primary signalling pathway.[1]

Beck-Sickinger and co-workers recently reported how PEGylation and lipidation differentially direct arrestin-3 (Arr3) recruitment and receptor internalization of pancreatic polypeptide (PP), another peptide of the neuropeptide Y receptor system.[2] Hence, the half-life extender selected for PYY3-36 therapeutics may alter its efficacy. Accordingly, we aimed to investigate how PEGylation (PEG20), hexadenoic acid (C16), and octadecanedioic acid (C18 di-acid) lipidation, direct Y2R-mediated internalization of PYY3-36. We report how PEGylation, confer a G-protein bias and a reduced Y2R internalization. We further report how lipidation with both C16 and C18 di-acid confer a slight Arr3 bias, whereas only C16 increase Y2R internalization. Next, the mechanism underlying the half-life extender's control of receptor-internalization were investigated through Y2R binding studies. We report how affinity alone cannot explain differences in internalization. Moreover, we demonstrate how binding kinetics may be the root-cause of internalization differences, with increased koff leading to reduced internalization.

In summary, peptides including PYY3-36 may achieve enhanced pharmacodynamic- and pharmacokinetic properties, if receptor-mediated internalization is minimized. Half-life extending techniques can differentially direct signalling bias and internalization of the Y2R-PYY3-36 complex. Here, we demonstrate for the first time, how binding kinetics under-

lay these differences.

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MS11

Mechanisms of bacterial membrane permeabilization of snake venom-derived peptides crotalicidin (Ctn) and Ctn[15-34]

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Against the alarming rise of multi-resistant bacteria, antimicrobial peptides (AMPs) have emerged as a promising therapeutic choice, due to their potency, broad spectrum and mechanisms of action that minimize the appearance of resistance. In this context, we recently identified a new cathelicidin-like AMP from the venom gland of the South American Crotalus durissus terrificus rattlesnake, named crotalicidin (Ctn), which demonstrated potent antimicrobial and antitumoral properties. Subsequent studies allowed us to identify a minimal motive, Ctn[15-34], with enhanced selectivity and serum stability while preserving activity. In the present study we investigate the antibacterial mechanism of both Ctn and Ctn[15-34], focusing on their membrane-disruptive properties. Although both peptides are shown to permeabilize E. coli membranes in a three-step process, i.e., (i) recruitment, (ii) accumulation, (iii) membrane disruption, slight differences between them were detected by time-resolved flow cytometry, suggesting different modes of action. Additionally, the effect of the peptides on bacterial cells was directly visualized by atomic force microscopy, and their bacterial surface localization by confocal microscopy. Ctn[15-34] demonstrated preference for vesicles that mimic bacterial or tumoral cell membranes, confirming its potential as an anti-infective lead.

Poster Presentations

P1

Convenient method of peptide hydrazide synthesis using a new hydrazone resin

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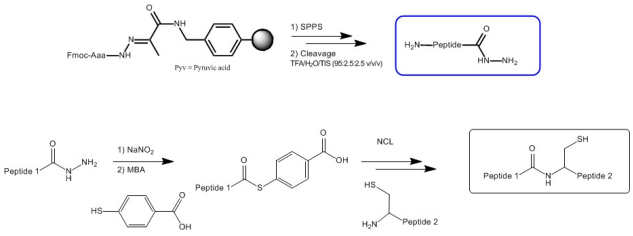
Peptide hydrazides can be easily synthesized using a new hydrazone resin, obtained via acylation of aminomethyl polystyrene by Fmoc-hydrazone of pyruvic acid. It was shown that the hydrazone linker is completely stable in the course of standard Fmoc SPPS. Moreover, it can tolerate a treatment with 5% TFA/DCM thus permitting selective removal of Mtt or related acid-labile protecting groups. Subsequent application of cleavage cocktails containing net TFA permits to obtain the desired peptides in a reasonable yields and purity. Synthesized peptide hydrazides can be applied as building blocks for the conjugation with different carrier molecules using hydrazone ligation technique.



P2

On the challenge of cysteine-rich peptide synthesis: the heat-stable toxin from Enterotoxigenic Escherichia coli (STh) and its toxoids

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Enterotoxigenic Escherichia coli (ETEC), and in particular those that express the heat-stable toxin (ST), with or without the heat-labile toxin (LTB), are an important cause of diarrheal disease and death in children under five years of age.1 Porcine and human ST (STp and STh) are antigens of interest in the search for a broad coverage ETEC vaccine. Owing to their similarity with guanylin and uroguanylin (Figure 1), toxoid analogues are the only option when considering STh as antigen. In this context, selected mono- and double mutants of STh have emerged as lead candidate STh toxoids.2 STh and selected analogues are 19 amino acid peptides encompassing three disulfide bridges, the synthesis of which is a challenging issue. Correct cysteine pairing is of upmost importance for antigenicity, and therefore for immunogenicity. A panel of toxoids and reference STh were produced either by recombinant synthesis or solid phase peptide synthesis combined to direct air-oxydation folding or to site-selective cysteine pairing. Whilst native STh adopted the right cysteine pairing independently of the protocol, important divergences have been observed for toxoids, with a strong impact on their antigenic properties. Using selected toxoids, the various synthesis strategies and their outcome are discussed in line with the antigenicity profiles of the obtained peptides. Our data suggest that the fully orthogonal chemical strategy is the only way to ensure that the isolated peptides are the properly folded STh toxoid isomers.

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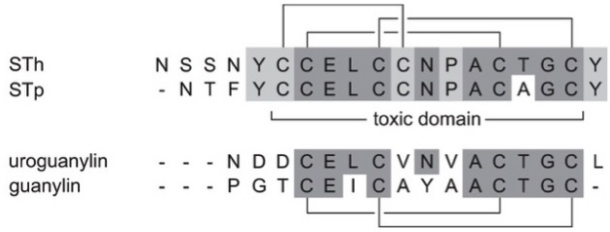
Figure 1. The STp and STh toxins and their sequence similarities with uroguanylin and guanylin.



P3

Photo induced electron transfer across transmembrane peptides

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Electron transfer through proteins is a controlled process playing an important role in biological processes such as photosynthesis and respiration. But it still remains to be investigated in detail which of the 20 natural amino acids are present or influence the electron pathways and what role transmembrane peptides play. Previous investigations of ribonucleotide reductase , DNA photolyase , and photosystem II have shown the importance of aromatic amino acids like tyrosine (TyrOH) and tryptophan (TrpH) for electron transfer in these proteins. To gain better insight about electron transfers across peptides as key components of biological energy conversion systems our idea is to create a transmembrane peptide in which by pulsed laser excitation the progress of electron transfer through the various amino acids can be monitored by time resolved spectrophotometry. At first, a polyproline peptide model was chosen to confirm the stepwise electron transfer across the peptide using TrpH and TyrOH as electron stepping stones and a Flavin modified amino acid as electron acceptor. This model showed a fluorescence quenching dependent on the distance between the Flavin amino acid at the N terminus and the first Trp stepping stone. Furthermore, a polyproline II as secondary structure was found in dependency on the amount of neighbouring prolines for the peptide models. These results already indicate an electron transfer which has to be confirmed by transient absorption spectroscopy. Hereafter, the system can be transferred to the transmembrane peptide EGFR and electron transfer across membranes can be achieved.

P4

Multivalent Ligand Design – Chemistry and Applications

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Multivalent interactions are widely observed in nature yet relatively underexplored in ligand design and drug development. Dendritic structures of neurons, vasculature, viral surface proteins, roots and river deltas are just a few examples where biological function is transferred in a branched fashion. Chemically, multivalent interactions and dendritic structures have been explored in biomaterials, catalysis, gene delivery and vaccine development, however, the therapeutic prospect of multivalent ligands has yet to be established. Our lab is interested in chemical strategies to efficiently assemble a diverse range of monodisperse, well-defined, multifunctional peptide dendrimers, so we can explore their potential as a novel class of molecular probes, drug leads and theranostics.

Here we present our synthetic strategies to produce different sets of multivalent (2x, 4x, 8x, 16x) peptide ligands. Design rationale, the different chemistries employed, pharmacological and biophysical characterisation, and the synthetic challenges that were faced will be discussed. Biological applications of these multivalent ligands include G protein-coupled receptor interaction which resulted in substantial potency improvements, and the use of poly-arginine peptide dendrimers to improve intracellular delivery of nanoparticles decorated with drug cargo. The chemical strategies employed in this work are broadly applicable for the synthesis of other complex peptide constructs, and our biological results strongly highlight the versatility of multivalent ligands as molecular probes and potential drug leads.

P5

Peptide Couplings by Reactive Extrusion: Efficient, Continuous and Green

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Peptides play a central role in the therapeutic solutions of the future. Unfortunately, conventional peptide production techniques (i.e. solution-phase and supported-phase) suffer from both very long reaction times and huge amounts of toxic solvents and reactants that are required during synthesis. These limitations have a highly negative influence on environmental impact and cost of production, thereby inhibiting economical attractiveness of peptide production. Therefore, the development of efficient and environmentally sound processes for the production of peptides is of utmost priority. To reach this goal, we have developed innovative peptide synthesis technique based on the use of a twin-screw extruder. Thus, by using this equipment that enables mixing of extremely concentrated solid-containing reaction mixtures, peptide couplings could be performed solvent-free in a continuous way, thereby offering a direct and scalable approach to efficient and sustainable production of peptides at the industrial scale.[1]

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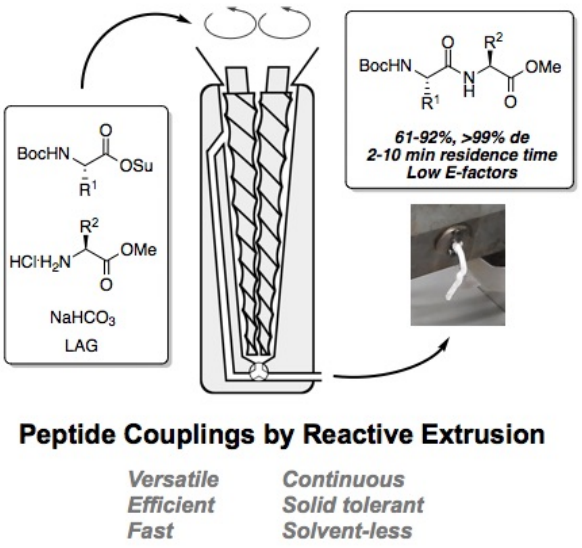

P6

Ribosomal Synthesis of a Thioamide Bond

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In the past few years, numerous reports of ribosomal synthesis of polypeptides containing non-natural peptide backbones including N-alkyl-amide or ester backbones have appeared in the literature, where the amine nucleophile was modified to either N-substituted amine or hydroxyl group without changing the carbonyl carbon electrophile. In the present study, we demonstrated the catalysis of a thioamide bond by the ribosome, where a thiocarbonyl carbon was utilized as an electrophile. First, we developed a strategy for the preparation of amino(thio)acyl-tRNAs using the flexizyme technology developed in the Suga lab, which allowed for reassignment of the genetic code to a thio-amino acid. Later, by using the amino(thio)acyl-tRNAs in flexible in vitro translation (FIT) system, linear and cyclized peptides having one thioamide backbone were synthesized by the ribosome. Moreover, ribosomal synthesis of an N-methyl-thioamide bond was shown for the first time. Many examples have demonstrated the application of thioamide backbone in improving the pharmacological properties of a peptide as well as in studying protein folding and dynamics. So far, polypeptides bearing thioamide backbones are accessible by synthetic or semi-synthetic methods. Using the method described in this study, direct ribosomal translation of peptides and proteins with thioamide backbones is now possible. This method will enable the generation of an mRNA displayed peptide library containing one or more thioamide backbone, that will possibly lead to the discovery of biologically active macrocyclic peptides with improved pharmacological properties

P7

New Synthesis of Stapled Peptide Alcohols and Their Activity as Mdm2 Inhibitor

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The use and testing of peptide alcohols (PAs) in SAR are limited because of the lack of commercially available resins that enable their reliable and fast synthesis. At A*STAR, we have developed a set of resins that enable the synthesis of PAs using the conventional 9-fluorenylmethoxycarbonyl (Fmoc)/tBu SPPS strategy.

It is well known that peptide C-terminus can have a strong influence on the activity. These new resins provide a great opportunity to evaluate the effect of changing the amide or carboxylic group to their reduced form (alcohol). Here we are presenting the first solid phase synthesis of stapled PAs and a comparison with their peptide amide counterparts. The change of the C-terminal amide to alcohol increases the hydrophobicity and doubles the p53 activation over their C-terminus amide counterparts. Even though the amide and PAs have similar Kd, an increase in hydrophobicity can enhance the activation of p53 by making the PAs more cell permeable.

P8

One-Pot Assembly of Oligopeptides in Solution-Phase

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Synthetic peptide chemistry in solution-phase makes use of two main strategies for peptide coupling: the use of pre-activated peptide fragments and in situ activation procedures. These strategies are being employed throughout the peptide chemistry disjointedly. This work describes a methodological advancement in solution-phase peptide synthesis via the development of a convenient and operational protocol to synthesize oligopeptides in a one-pot three-step cascade method, in which two peptide bonds are introduced chemoselectively. This protocol comprises the coupling between a fully deprotected peptide fragment and a pre-activated fragment in its succinimide ester form, followed by in situ activation of carboxylic acid moiety of the intermediate which undergoes aminolysis with last amino-peptide fragment. Following this protocol, tri- to hexapeptides were obtained in high global yields (80–95%) with no need of purification of intermediates and with virtually no epimerization as determined via HPLC and variable-temperature NMR experiences. This methodology represents a faster, easier and milder approach to the synthesis of peptides, and it operates at equimolar amounts. In this study, we checked the compatibility with common N-protecting in peptide chemistry (Z, Boc and Fmoc) the formation of secondary and tertiary amides as well as the use of D/L and non-proteinogenic amino acids. This protocol may represent an attractive alternative to the preparation of larger peptides by facilitating convergent synthesis which otherwise would entail in exhaustive cycles of protection/deprotection and coupling steps.

P9

The Thioamide Ligation: A Residue-Independent Peptide Ligation

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The most widely used peptide ligation strategy is native chemical ligation (NCL). The requirement for ligation at cysteine – the least common of the proteinogenic amino acids – has led to the development of modified ligation methods. Thiol- or selenol-functionalized amino acid derivatives have been employed in NCL, which after dechalcogenation facilitate ligations at a variety of amino acid residues. However, all NCL-type approaches require a side-chain functionalized N-terminal amino acid residue. We have developed a residue-independent ligation process in which a backbone thioamide group is incorporated at the N-terminal amide position. This thioamide directs a chemoselective reaction with a peptide carboxylic acid to generate, after O–N acyl transfer of an isoimide intermediate, the ligated peptide.

P10

Inhibitory Effect of 4 Novel Synthetic Peptides on Food Spoilage Yeasts

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The spoilage of foods caused by the growth of undesirable yeast species is a problem in the food industry. Yeast species such as Zygosaccharomyces bailii, Zygosaccharomyces rouxii, Debaryomyces hansenii, Kluyveromyces lactis and Saccharomyces cerevisiae have been encountered in foods such as high sugar products, fruit juices, wine, mayonnaise, chocolate and soft drinks. The demand for new methods of preservations have increased because of the negative stigma attached to chemical preservatives. A novel peptide sequence (KKFFRAWWAPRFLK-NH2) was modified to generate three versions of this original peptide. These peptides were tested for the inhibition of the yeasts mentioned above, allowing us to better understand their residue modifications. The range of the Minimum Inhibitory Concentration was between 25 and 200 μg/mL. Zygosaccharomyces bailii was the most sensitive strain to the peptides, while Zygosaccharomyces rouxii was the most resistant. Membrane permeabilisation was found to be responsible for yeast inhibition to some extent at a two-fold concentration (400 μg/mL) of the MIC. The possibility of the overproduction of Reactive Oxygen Species was also assessed but was not recognised as a factor involved for the peptides' mode of action. Cytotoxicity and haemolytic activity of the peptides were assessed to address the safety concern of using synthetic peptides in food products. This concern was dismissed as the rate of haemolysis (less than 10% at the highest concentration) and cytotoxicity were found to be relatively low. These efficient novel peptides represent a new source of food preservation that can be used as an alternative for current controversial preservatives used in the food industry.

P11

Improving Throughput in Therapeutic Peptide Manufacturing with Parallel Automated SPPS

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Process development for the manufacture of therapeutic peptides remains a vital part of the commercial peptide production process. An optimized solid-phase synthesis protocol can be challenging to develop, however the benefits range from increased crude product purity and yield to reduced synthesis time and reduced production costs. Many of the recent advances in peptide therapeutics focus on peptides with greater structural complexity compared to naturally occurring peptides, allowing for the design of more physiologically stable products with increased target specificity and membrane permeability [1]. Examples of more complex peptides include cyclic, hydrophobic and chemically modified peptides, both of which can be difficult to synthesize and subsequently purify, which calls for more efficient synthesis protocols to overcome these manufacturing challenges. The advent of the neoantigen and personalized medicine sectors adds a further requirement for faster peptide synthesis protocols and reduced processing times, including adherence to regulatory guidelines and GxP requirements during manufacture.

Solid-phase peptide synthesis (SPPS) of linear peptides using heat (>50 °C) and highly reactive coupling reagents have resulted in products with increased crude purities and yields when using significantly shorter coupling cycles compared to room-temperature methods [2]. Some structural modifications such as cyclizations have traditionally been performed post-SPPS using solution-phase methods, however factors such as ring size and sequence can compromise reaction efficiency. Development of automated solid-phase (on-resin) protocols for cyclization, potentially using heat, may provide numerous advantages for the development of complex peptide products [3]. Parallel automated synthesis is a useful tool for quickly and efficiently synthesizing multiple peptide analogs simultaneously for structure-activity relationship (SAR) studies of synthetically challenging sequences.

Here we describe the process development and parallel optimization of SPSS relating to different biologically relevant peptides. Solid-support screening, reagent screening and temperature screening are demonstrated using an automated peptide synthesizer as part of the optimization process for difficult peptides such as Aib-Enkephalin (90% optimized crude purity vs 21% crude purity) and JR 10-mer (67% optimized crude purity vs 21% crude purity). A number of automated solid-phase protocols are also shown to be more efficient replacements for traditionally manual or solution-phase manipulations, resulting in reduced processing times and high crude product purities and yields. We report the use of heat to increase the crude purity of Melanotan II peptide from 43% to 60% using solely on-resin synthetic methods.

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P12

An Alternative Disconnection for Native Chemical Ligation

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Chemical modification of proteins is a powerful tool for the investigation of natural systems and the development of therapeutic conjugates. The refinement of methods for protein total synthesis remains a key objective of synthetic organic chemistry. To date, Native Chemical Ligation (NCL) represents the gold standard for the thioester-mediated, chemoselective peptide bond-forming condensation of unprotected peptide residues.[1a] Despite its widespread use, NCL still has certain limitations, such as the essential requirement for an N-terminal cysteine (Cys) at the ligation site.[1a] Although several synthetic advancements have been reported over the years,[1b] the development of efficient alternatives to NCL is an expanding area of research.[2] The recent emergence of the dehydroalanine aminoacid (DAla) and its successful application in protein modification,[3] prompted us to develop a novel ligation methodology based on the DAla chemical reactivity. DAla is an excellent electrophilic moiety, which can rapidly react with sulphur nucleophiles to generate alkyl cysteine analogues; the ligation with a specific amino thio-acid gives a stable thioester intermediate, which spontaneously undergoes to an S-to-N acyl shift forging a native peptide bond (Figure 1). The reported methodology enriches the state-of-the-art protein ligations set and undoubtedly represents a valid alternative disconnection approach within the NCL strategy.

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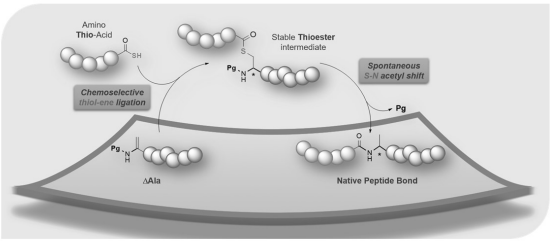

P13

Chemical synthesis of venom peptides using directed-disulfide bond formation

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Venoms come from thousands of years of evolution that allowed venomous species to develop complex mixtures of peptides and proteins known as toxins. Such molecules have received a growing interest due to their potential power as drugs in the medical field or even as eco-friendly insecticids . These toxins are particularly well folded due to the natural high abundance of cysteine which form several disulfide bonds and give to these molecules their specificity and robustness in organisms. Usually, toxins are first iso-

lated from venoms, but the low quantity of material that can be produced remains a major hurdle to overcome. To address this issue, chemical peptide synthesis is typically used. Nowadays, numerous synthesis techniques and automated synthesizers permit the preparation of the linear sequence of a toxin without major difficulties. The key step is then to correctly fold the biomolecule. Oxidative buffers are the easiest way to fold a cysteine-containing peptide, but generally lead to a complex mixture of the desired product with peptide misfolding, and sometimes to exclusively undesired disulfide bond configurations. In order to selectively synthesize the desired pattern, we herein describe chemical scheme to direct disulfide bond formations. This strategy uses a combination of orthogonally protected cysteines which are successively deprotected and oxidized in cystine and can be applied to venom peptides that contain up to three disulfide bonds. This approach has been applied with success to several toxins. Moreover, the directed-disulfide bond formation has been demonstrated in one-pot, then avoiding fastidious and time-consuming purifications of intermediates. This also enables the access to numerous analogues and modified toxins that cannot be extracted from venoms.

P14

Incorporation of Cystine and its Mimetics in Insulin and other Disulfide-rich Peptides

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Disulfide bonds are important structural elements in peptides and proteins and play a critical role in maintaining their biologically active conformation. Chemical synthesis of peptides can enable sequential formation of multiple cystine bonds through exploitation of judiciously chosen regioselective thiol protecting groups. The ortho-Nitroveratryl moiety can be utilised as an orthogonal cysteine protecting group and is efficiently cleaved via photolysis under ambient conditions. In combination with complementary S-pyridinesulfenyl activation, disulfide bonds are formed rapidly in situ. We also report the efficient incorporation of lactam and cystathionine bridges in insulin and related peptides as stable cystine mimetics for the development of peptide-based therapeutics with enhanced thermal stability.

P15

Revisiting ligation at selenomethionine: insights into native chemical ligation at selenocysteine and homoselenocysteine

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Selenomethionine (Sem), though not present naturally in human proteins, can serve as a structural probe in NMR and X-ray crystallography. Sem has been incorporated recombinantly into proteins many times to elucidate their structure and function. We revisited incorporation via chemical protein synthesis to shed light on the mechanism of native chemical ligation. The effect of chalcogen position on ligation was investigated, and selenium-containing peptide ligation was optimized. Additionally, selective methylation was performed on selenolates in a peptide in the presence of unprotected thiols.

P16

Synthesis of RGD Peptides Inducing Multicellular Spheroids Formation

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The 2D monolayer cell culture model widely applied for drug screening is not able to adequately mimic the in vivo response. Sutherland et al.[1] were the first to propose multicellular tumor spheroids as 3D model of small solid tumors. Later, multicellular spheroids (MS) composed of normal cells were applied for the screening of neuroprotective agents, 3D bioprinting and tissue regeneration. Recently we demonstrated the practical utility of a cyclic RGD peptide containing a triphenylphosphonium (TPP) moiety, namely cyclo-RGDfK(TPP), in a novel and highly reproducible one-step approach for MS formation [2]. Here we describe an optimized procedure for the synthesis of cyclo-RGDfK(TPP) and related peptides using our novel hydrazone resin [3]. In order to compare the efficiency of various reaction protocols, the synthesis was performed in parallel on different polymer supports, namely Wang resin, MBH-Br resin and Fmoc-hydrazono-pyruvoyl-aminomethylpolystyrene resin. In preliminary experiments it was shown that attachment of the TPP moiety to the cyclic peptide in solution complicated the purification of the final product. Therefore, it was achieved by selective deprotection of Lys(Mtt)-containing peptidyl resin, followed by acylation with 4-carboxybutyltriphenyl phosphonium bromide. Unexpectedly, using MBH resin the Mtt-deprotection with 1% TFA in DCM resulted in peptide cleavage from the polymer support, while application of a mixture of AcOH/TFE/DCM (1:2:7) was inefficient. Synthesis on Wang resin provided linear peptide in reasonable purity. Its cyclization with HCTU proceeded smoothly without significant formation of dimeric side product. However, subsequent removal of the OBzl group from the aspartic acid residue by catalytic hydrogenolysis showed very low efficiency. The final peptide deprotection requires two successive treatments with HBr/TFA for 30 min and 1 h. The best results were obtained using hydrazone resin. Selective removal of the Mtt group had no influence on the stability of the hydrazone bond. Peptide cleavage from the solid support and removal of all protecting groups proceeded in one step. Cyclization of the resulting peptide hydrazide was achieved in good yield and purity using the azide method. These data evidence the practical utility of hydrazone resin for the synthesis of cyclic RGD peptides or their analogs containing cargo molecules. The biological experiments have shown that cyclo-RGDfK(TPP) is able to generate MS formation from tumor, normal or stem cell lines of various origin. This methodology can be applied in the design of efficient in vitro models for the screening of novel drugs and drug delivery systems.

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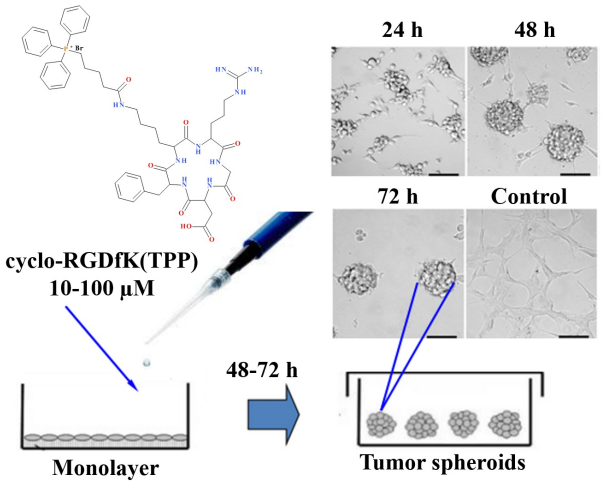

Fig. 4. Experimental procedure for MTS formation using cyclo-RGDfK(TPP) method

P17

Solid-phase peptide synthesis: the Greener, the Better

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The concept of solid-phase peptide synthesis (SPPS) pioneered by Merrifield in the 60s and the later development of the Fmoc/tBu strategy are the cornerstones that have made peptides the subject of study in many research areas. Thus, the demand for synthetic peptides is increasing, and considerable efforts have been channelled into optimizing the synthetic process itself, i.e. the coupling reagents, protecting groups, automatization of the process, etc. However, little attention has been paid to making the process greener. The concept of "solid-phase" itself is green in that, for instance, the synthesis of a peptide comprising 20 amino acids involves 41 chemical steps in only one reactor, and there is a clear minimization of mechanical losses and mass transfer. However, SPPS involves the use of large excesses of reagents, which are removed by simple filtration and exhaustive washing steps between each reaction, and therefore the consumption of solvents the main impediment to it being considered an eco process. DMF, the main solvent used in SPPS, is a highly reprotoxic solvent and is classified as a Substance of Very High Concern (SVHC). However, it is considered to be difficult to replace because of its properties. Our research demonstrates that the complete substitution of DMF by the biomass-derived organic solvent GVL in all the steps of SPPS, coupling, deprotection and washes can be achieved. Moreover, GVL is compatible with the application of microwave-assisted automated SPPS because it is stable at high temperatures. This solvent has been tested in the synthesis of peptides with a wide range of lengths and using polystyrene- and polyethylene glycol-based resins. The peptides were obtained in high purity, comparable to that achieved using standard methodologies. Although, the incorporation of the first protected amino acid into most resins is carried out by carboxylic acid activation, this reaction on CTC resin takes place by a SN reaction, which is normally done in DCM, a solvent called to be removed from practical use. We propose to replace DCM with the green solvent 2-MeTHF. We have also studied the peptide precipitation step after peptide cleavage from the resin. Diethyl ether (DEE) is the solvent used for this purpose and it is classified as a highly hazardous chemical. It is highly flammable and is sensitive to light and air, and it tends to form explosive peroxides. We propose to replace DEE with cyclopenthylmethyl ether (CPME), which is not hampered by the aforementioned previous hazards and is considered a green solvent. CPME gave similar performance to DEE. In general, the work we will present is a first and important step toward greener SPPS in terms of the use of solvent, waste generated, and energy efficiency.

P18

The Attempt of Solid-Phase Peptide Synthesis of Lassopeptides - Part 1

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Lassopeptides are specific class of peptides characterized by „knot" structure motif. These compounds are synthesized rybosomally and post-translationally modified. These peptides are built from macrolactam ring formed from isopeptide bond between N-terminal amino acid residue (e.g. glycine, alanine) and the side chain of aspartic or glutamic acid. The remaining C-terminal chain is threaded and divided into a loop and tail.[1] The broad interest of lassopeptides results from their biological activity. Recently, the biological study of lassomycin showed the activity against Mycobacterium tuberculosis.[2] The previous attempts of chemical synthesis of natural lassopeptides have been unsuccessful due to the lack of specific "knot" structure. The access to these compounds obtained by chemical synthesis would allow in our opinion to design the new analogs and study on their biological activity. In our research group we have made attempt to synthesize a natural lassopeptide Sungsanpin using solid-phase peptide synthesis. This is 15-amino acids peptide, which was discovered from Streptomyces in Korea.[3] For this purpose we synthesized a linear precursor of Sungsanpin, which was then subjected to on-resin cyclization using several solvent and elevated pressure during the coupling. The obtained products were controlled by LC-MS using selected ion monitoring mode. According to literature data, the threaded and unthreaded form of lassopeptide exhibit different chromatographic behavior, which in general leads to clear separation of these two form. Our recent study revealed, that the application of high pressure and non-polar solvent during the synthesis of Sungsanpin leads to formation of two form of peptide. The LC-MS analysis of the product showed the presence of two separated signals in the chromatogram, characterized by the same molecular mass. Additionally, these two forms of peptides exhibit different fragmentation properties during the MS/MS analysis. These facts are consistent with the literature data and may indicate the formation of threaded peptide. Moreover, the large scale purification of these two forms using high-pressure liquid chromatography was feasible.

This work was supported by Grant No.UMO-2015/19/B/ST5/00659 from the National Science Centre, Poland

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P19

The Attempt of Solid-Phase Peptide Synthesis of Lassopeptides - Part 2

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Lasso peptides are recently discovered class of natural peptides of ribosomal origin [1]. These compounds exhibit a wide range of biological functions. The most striking feature of lasso peptides is topology of these compounds. They are composed of a loop - macrocyclic ring stabilized by amide bond and peptide tail which is threaded through the loop. The unique structure of lasso peptides is formed in the enzyme active centre during posttranslational modification of precursor peptide. The effect of high pressure on the solid phase cyclization of linear lassomycin precursor was investigated. The synthesis of linear analogue of natural peptide lassomycin was performed on ChemMatrix® resin using the combination of protecting groups (2-phenylisopropyl ester group for carboxylic group in sidechain and FMOC for N-terminal amino group) allowing the solid phase cyclization [2]. The cyclization at various pressures was monitored by LC-MS and MS/MS techniques. However the chromatographic properties and fragmentation pattern of synthetic lassomycin obtained under atmospheric pressure and the sample cyclized under high pressure were identical. This behavior is distinct from the Sungsanpin which during cyclization under high pressure forms of two isomers with different retention times.

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P20

Green Chemistry Approach for Peptide Synthesis

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'Green chemistry is the utilisation of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products.' (Anastas and Warner, Oxford University Press, 1998).

Peptide synthesis involves amide bond formation between protected amino acids. The synthesis of peptides is a vital process in biological, medical and pharmaceutical research, however it usually employs the use of hazardous solvents and reagents which are used in large excess. REACH, a European Union regulation concerning the Registration, Evaluation, Authorisation & restriction of Chemicals, has identified aprotic solvents such as N,N-Dimethylformamide (DMF), dimethylacetaamide (DMAc), N-methyl pyrrolidone (NMP) and halogenated solvent dichloromethane (DCM), which are the most widely used solvents for peptide synthesis, as hazardous and problematic for pharmaceutical process scale-up. DMF, DMAc and NMP have been identified as reprotoxic and are classified under REACH regulations as a Substance of Very High Concern (SVCH) and is included in the Candidate list for Authorisation.[1,2,3] The classification of these solvents has highlighted the necessity to find alternative greener solvents and processes for peptide synthesis.

Currently, we are investigating alternative solvents for peptide synthesis and introducing continuous flow chemistry into our processes with the aim of reducing solvent/reagent consumption and improving the 'greenness' of our processes. We are also concerned with educating and training the scientific community with regards to green chemistry. All of our efforts to date are presented in our poster.

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P21

Peptides going sweet: Synthesis of glycopeptide hybrid structures using Passerini and Ugi reactions

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α-Acyloxy and α-acylamino carboxamides are the building blocks for the synthesis of various natural products such as peptide and peptoide derivatives. Multicomponent reactions (MCRs) offer an attractive one-pot strategy for generating a library of these highly functionalized and complex organic compounds. The Passerini and Ugi reactions are isonitrile-based MCR that yields α-acyloxy and α-acylamino carboxamides, respectilvely. Passerini reaction involves an aldehyde, an isonitrile, and a carboxylic acid while Ugi reaction involves an aldehyde, an isonitrile, a carboxylic acid and an amine component. Utilization of carbohydrates and their derivatives in MCRs is based on their polyfunctional character and stereochemical diversity.[1] Carbohydrates represent the most abundant class of natural products with distinctive role in different biological processes and they are highly valuable pool of chiral molecular scaffolds.[2] Our efforts were directed toward exploitation of carbohydrate derivatives as components in the Passerini and Ugi reactions. We used for the first time highly strained carbohydrate-derived aldehydes, isocyanides and amines in the Passerini and Ugi reaction with different commercially available carboxylic acids. As acidic non-sugar components acetic acid, benzoic acid and Boc-protected phenylalanine were used, and as a sugar component isopropylidene protected gulonic acid (GulA) was used. Three different sugar moieties were used as isopropylidene protected carbohydrate-derived components (aldehydes, isocyanide, and amine) in the Passerini and Ugi reaction: fructose (Fru), galactose (Gal) and sorbose (Sor). The prepared Passerini products bear up to three sugar components, while Ugi products comprise up to four sugar components. The Passerini reactions conducted with prepared sugar aldehydes were highly diastereoselective, with d.r. 90:10, and the crystal structure analysis revealed that the stereochemistry of newly formed chiral center is S. The diastereoselectivity of Ugi reactions depends on component's structure and varies from d.r. 1:1 to d.r. 90:10 with S isomer being predominant one.

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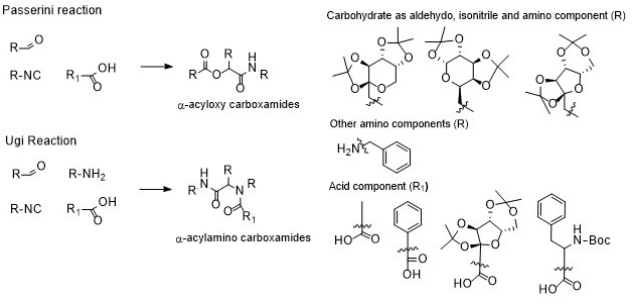

P22

Introducing the Petasis Reaction for Multicomponent Peptide Labeling and Stapling

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Multicomponent reactions have lately evolved from versatile methods for the synthesis of small molecules to powerful tools for the post-synthetic derivatization of peptides and proteins. Among the relevant multicomponent transformations recently conducted with peptides are cyclization, lipidation and labeling. The possibility of implementing these transformations in a multicomponent manner opens new venues of applications, as e.g., multiple reactive handles, affinity or fluorescent tags, and even additional biomolecular fragments can be simultaneously incorporated while conducting the desired transformation. Here, multicomponent labeling and stapling of peptides by means of the Petasis reaction is described. The Petasis reaction, also known as the borono-Mannich reaction, is a three-component process comprising the condensation of an aldehyde or ketone, an amine and an aryl/vinyl boronic acid or ester. The report includes the solid-phase derivatization of peptides at Lys and Nε-MeLys side chains and its simultaneously functionalization with boronic acids containing fluorescent tags, lipidic or biotin moieties in conjunction with relevant oxo-components such as dihydroxyacetone, glyceraldehyde, glyoxylic acid and aldoses, thus encompassing a powerful complexity-generating derivatization procedure. The multicomponent stapling is conducted in solution by linking Nε-MeLys or Orn side chains – positioned at i, i+7 and i, i+4 – with (bi)aryl tethers forcing the peptide to adopt helical conformations, while the hydroxyl-carbonyl moieties were introduced as exocyclic fragments. This multicomponent method enables the simultaneous incorporation of aryl/vinyl moieties and aldoses and ketoses at the peptide side chain. In addition, the solution-phase multicomponent stapling proves highly efficient, enabling the installation of rigid aryl tethers and hydroxylated exocyclic element. This successful application of the Petasis reaction in peptide chemistry shows promise for the future development of peptide pharmaceuticals.

P23

Bioactive fluorescent cyclopeptides through on-resin Suzuki cross-coupling reaction

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RGD containing peptides can be used as potential drugs for the treatment of osteoporosis, restenosis, ocular disease, and cancer [1]. Cyclic RGD peptides based on the tripeptide sequence Arg-Gly-Asp (RGD) are well-known to bind preferentially to the $\alpha V\beta 3$ integrin and in particular have been shown to exhibit improved affinity, receptor selectivity, and enzymatic stability relative to their parent linear peptides. Recently labeling RGD-peptides with a variety of fluorescent probes have been reported for imaging [2, 3]. A series of phenothiazine derivatives with carboxylic acid and boronic acid substituents was produced and introduced in the RGD peptide coupled to the enzymatically synthesized 7-Br tryptophan (W(7-Br)) [4], using solid phase peptide synthesis (SPPS). Successively, a new on resin Suzuki cross-coupling procedure was developed and allowed to obtain new fluorescent RGD cyclopeptides, presenting an absorption maxima at 303 nm and an emission maxima at 460 nm. The investigation of the cyclopeptide structure by NMR spectroscopy demonstrated the axial diastereoselection of the cyclization. The affinity of the cyclopeptides to bind with $\alpha v\beta 3$ integrin was evaluated by Enzyme-Linked Immunosorbent Assay (ELISA) [5]. This assay showed a relatively good activity only for the cyclopeptide containing oxidized form of phenothiazine (IC50=52 nM). In conclusion, we developed a new method that allows to modulate the inhibitory activity and the fluorescent property of RGD cyclopeptides, by using various synthesized phenothiazin and 7-Bromo tryptophan.

Acknowledgments: This work was supported by Alexander von Humboldt foundation Reference [1] Dayam, R.; Aiello, F.; Deng, J.; Wu, Y.; Garofalo, A.; Chen, X.; Neamati, N. J. Med. Chem. 2006, 49, 4526–4534. [2] Götzinger, A.C.; Michaelis, C. S.; Müller, T. J. J. Dyes and pigments, 2017, 143, 308-316. [3] Belei, D.; Dumea, C.; Samson, A.; Farce, A.; Dubois, J.; Bîcu, E.; Ghinet, A. Bioorg. Med. Chem. Lett. 2012, 22, 4517-4522. [4] Frese, M.; Sewald, N. Angew. Chem. Int. Ed. 2015, 54, 298-301. [5] Kapp, T, G.; Rechenmacher, F.; Neubauer, S.; Maltsev, O. V.; Cavalcanti-Adam, E. A.; Zarka, R. Sci. Rep. 2017, 7, 39805.

P24

A Photolabile Backbone Amide Linker for the Synthesis of Cyclic and C-terminal Modified Peptides

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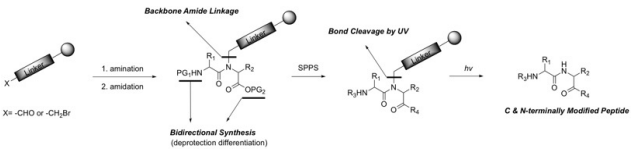
Photolabile linkers provide an alternative to the classic acidic or basic cleavage protocols used in solid phase organic synthesis. The backbone amide linker (BAL) approach involves a solid-phase linker for providing free C- and N-termini of a resin-bound peptide by anchoring the resin to the amide backbone of a peptide. Traditional BAL, an acid labile linker, is not orthogonal with any acid sensitive functional groups, which makes application to multi-dimensional solid phase synthesis difficult. Here our efforts focus on the developmnent of a photolabile BAL (pBAL), whereby the synthesized peptide is released only by UV irradiation, therefore permitting the general compatibility to the attachment and detachment of amino acids as well as tolerance of diverse functional groups. Initially, p-oxyphenacyl (Op), alpha-Methyl-p-oxyphenacyl (Mop), alpha,o-Dimethyl phenacyl (Dmp), alpha-Phenyl-p-oxyphenacyl (Pop), and 6-nitroveratryl (Nve) functional groups were screened, and o-nitrobenzyl such as Nve was found to be the most efficient functional group at the photolytic release of the backbone amide. To improve the efficiency of loading amino acids to the linker, a hydroxy group was added to the other ortho position of the o-nitrobenzyl group. A carboxylate was added to the para position of the 2-hydroxy-6-ntrobenzyl group to provide a resin attachment point as well as an additional electron withdrawing group to increase photoreactivity and tramsamidation. Finally, the 2-hydroxy-4-carbonyl-6-nitrobenzyl (Hcnb) linker was chosen as the optimal pBAL motif. Its reactivity was tested with more than eight amino acids in solution with respect to amination, amidation, and photolysis. The synthesis of several cyclic and C-terminally modified peptides will be discussed.



Posters

P25

Total chemical synthesis of hyper-phosphorylated p53 Transactivation Domain

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Chemical synthesis is a systematic tool for the creation of protein molecules with desirable characteristics as it allows enormous variations in the covalent structure resulting in protein molecules with precisely introduced site-specific modifications (1). Our goal is to develop novel and efficient chemical methods for the synthesis of post-translationally modified protein molecules. In this study, we chose the 61 residue N-terminal transactivation domain (TAD) of the human tumour suppressor p53, which contains a total of 9 phosphorylation sites (2). Phosphorylation along with acetylation, acts as a switch between the degradation and activation of the p53 (3). We devised one-pot, three fragment sequential ligation strategy towards the synthesis of the full length hyper-phosphorylated TAD. The fragments were synthesized using Fmoc-SPPS and the phospho residues were incorporated using building block approach. Through our strategy, we demonstrated the synthesis of the hyper-phosphorylated TAD containing 9 phosphorylations. Future directions include the optimization and development of efficient ligation strategies towards the synthesis of the combinatorial library of p53 TAD variants.

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P26

Enhancing Affinity of RGD Peptides by Incorporation of Bromotryptophan obtained by Enzymatic Halogenation

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Halogenases are a powerful tool to incorporate halogens (Br, Cl) into indol derivatives as tryptophan under mild reaction conditions requiring only oxygen and halide salts. Enzymatic halogenation has recently emerged as a method to introduce halogen substituents that are known to increase biological activity in natural products.[1-2] For preparative synthesis of halotryptophans immobilization by cross-linked enzyme aggregates (CLEAs) gave higher enzyme stability and allowed gram scale synthesis of 7-bromotryptophan.[3] This lead us to apply this method for introducing bromotryptophan residues into RGD peptides to increase their biological activity. The lead structure c(RGDwV) is derived from Cilengitide, c(RGDf(NMe)V), and displays similar low nanomolar affinities to different integrins ($\alpha v\beta 3$: 1.6 nM, $\alpha 5\beta 1$: 9.2 nM).[4] The affinity to $\alpha v\beta 3$ (0.4 nM) is increased fourfold by incorporating D-Trp(7Br) and a superior selectivity against $\alpha 5\beta 1$ (6112 nM) is observed. We elaborated our approach to synthesize a library of RGD derivatives containing different halogens (F ,Cl,Br,I) in different positions using halogenases and tryptophan synthase.

P27

Combining Synthetic Biology and Synthetic Chemistry to Dial in to New to Nature Peptidic Natural Products in vitro and in vivo

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Peptidic natural products and peptide-polyketide hybrid natural products play a central role in medicine, key examples include daptomycin, a drug used to treat skin and skin structure infections1 as well as vancomycin2, a last resort antibiotic. The GenoChemetic approach that we are developing affords access to diverse series of natural product analogues. One approach that we have been employing, within the GenoChemetics framework, is the utilization of halogenation and cross-coupling, in concert, to enable C-H activation. This poster focuses on applying this strategy to the peptidic natural product pacidamycin4. GenoChemetics involves the introduction of a gene into the natural product generating host. This gene acts in concert with the existing biosynthetic pathway, installing a chemically orthogonal handle. By developing mild chemistries and compatible fermentation media we can selectively carry out chemical diversification in the presence of the living cultures. For example we have constructed a bacterial host that can produce bromopacidamycin and have developed air and water tolerant Suzuki-Miyaura chemistry for its diversification in vivo. This exciting new approach draws flux through the system, enables labeling and tagging for tracking and purification and could potentially enable the production and testing of antibiotics in 1 step.

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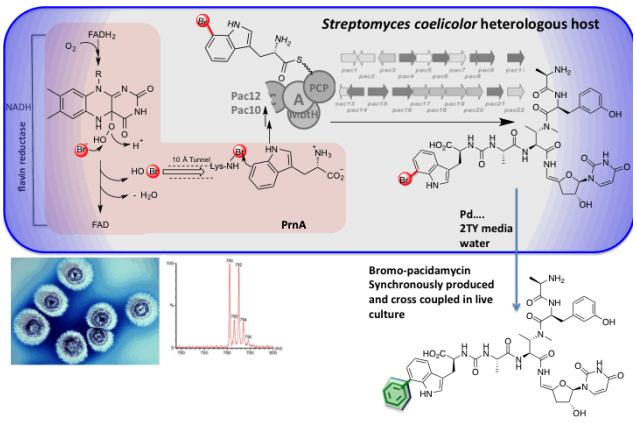

P28a

Quality Peptide Versus Speed: Conventional Synthesis Versus Microwave

Hossain Saneii, Farshad Karimi, William Bennett, Mandar Maduskar, Mostafa Hatam

AAPPTec, United States

Purification of crude peptides is time consuming. Utilizing methods that produce higher quality crude peptides can reduce the over-all production time of pure peptides and reduce costs. We have compared conventional synthesis with microwave synthesis for a number of peptides. In general, conventional methodology takes 32% longer, but the higher quality crude peptide reduces purification time by 200% to 500%.

P28b

Most Efficient Method For Synthesis and Purification of Peptides

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Many peptides are produced and marketed by pharmaceutical companies and the number is growing. In addition, peptides are utilized in increasing quantities in cosmetics, the food industry and other applications. Current methods for synthesis and purification of peptides are slow and costly. The increasing demand for large quantities of high purity peptides makes the development of fast, low cost, highly efficient methods of synthesizing and purifying peptides essential. We disclose a most efficient method of purifying peptides. By this method, the majority of impurities are extracted from the crude peptide before final purification. As a result, purification is accelerated and costs are reduced to approximately one seventh of standard HPLC purification costs. A further advantage of our method is that highly hydrophobic peptides with poor solubility can be purified readily. Additionally, because the extraction and HPLC processes rely on different methods of purification, our method can resolve impurities that overlap or co-elute with the desired peptide product in HPLC.

P29

Studies on the Regeneration of Aged DMF for Use in Solid-Phase Peptide Synthesis

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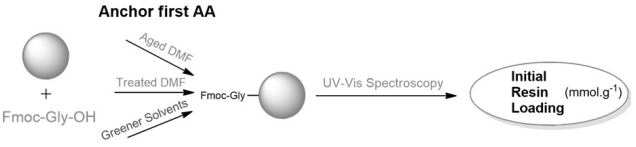
Peptide therapeutics have immense commercial value that is estimated to reach $46.6 billion USD by 2024 (Al Musaimi et al. 2018). Fluorenylmethyloxycarbonyl-based solid-phase peptide synthesis (Fmoc-SPPS) is one of the main techniques used for the synthesis of peptides, with dimethylformamide (DMF) as one of the most widely used solvents. However, it has long been known that DMF is not stable over time and degrades to dimethylamine (DMA) which can remove the Fmoc protecting group (van Regenmortel et al. 1988). The initial loading (mmol/g) has been identified as a critical parameter in SPPS since its magnitude ultimately dictates the maximum possible yield of a synthesised peptide (Forns & Fields 2000). Photometry-based resin loadings typically involve the quantification of dibenzofulvene-piperidine adduct which is formed during Fmoc removal with piperidine in DMF (Eissler et al. 2017). Significant amounts of DMA in aged DMF can lead to unwanted Fmoc removal and ultimately, inaccurate loading measurements.

During our studies, we treated aged DMF to reduce DMA content and compared the initial resin loading between treated aged DMF and newer DMF samples. Within the last decade, several research groups have proposed possible greener alternative solvents, which do not have the issue of DMA contamination, for SPPS (Kumar et al. 2018). For comparison, the loading values when using greener solvents for the initial loading step of SPPS, were also determined. Our studies show that the use of aged DMF can lead to a significant reduction in initial resin loading and the use of treated aged DMF can improve the measured loading. Our results thus far on the comparative initial loading measurements, when using aged DMF, treated aged DMF and greener solvent systems, will be presented.



P30

Solid-phase Synthesis of Tyrosine Cyclic DepsiPeptides from Fmoc-MeDbz-resin

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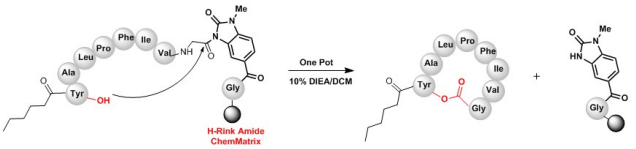
Desipeptides are peptides that, within their structure, at least one of the amide links has been replaced with ester, thioester or amide links with molecules that are not amino acids. In general, they are cyclic compounds that are found as natural products in different living organisms of marine origin amongst others. They present themselves with a wide spectrum of biological activity, they have reported that depsipeptides act as enzyme inhibitors that are used as anti-tumour agents such as Romidepsin1 (FK228); antiviral properties such as Neamphamide2 and antibacterial or antibiotic properties such as Etamycin3 amongst others. In this work we studied the conditions to obtain cyclic depsipeptides from Tyrosine in solid phase using the MeDbz-resin that permitted after activation, in a single step, the formation of the ester and the cleavage from the resin.

1.-J. Med. Chem. 2007, 50, 5720-5726; 2.- J. Org. Chem. 2005, 70, 6842-6847; 3.- J. Antibiotics, 2010, 63, 219–224



P31

Fluorogen-based amino acids with Aggregation-Induced Emission features for bioprobes design

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Fluorescently labelled peptides are widely used as tool for molecular imaging to provide biological information on living systems. However, most of the conventional organic fluorophores used for such purpose aggregate when dispersed in aqueous media.1 This aggregation generally implies a self-quenching and thus, a drastic decrease of the fluorescence ("turn-off") which is a thorny problem for developing efficient bioprobes. At the opposite, luminophores such as tetraphenylethylene (TPE) and silole derivatives with unique aggregation-induced emission (AIE) features have been reported.2 These dyes are found to be non-emissive when molecularly dissolved but highly emissive when aggregated, which is due to a restriction of the intramolecular rotations (RIR) in these dyes, a well-known source of nonradiative deactivation. Unlike conventional organic fluorophores, they also exhibit higher photobleaching resistance.3 Among the AIEgens,4 siloles exhibit great light emission and are used for preparation of organic light-emitting diodes and optoelectronic devices. The silole core is a dienic five membered ring containing a silicon atom. The low-lying of the LUMO favoured interaction between the σ^* and the π^*, conferring fast electron mobility and remarkable fluorescent properties to silole moiety.5 Hence, the unusual fluorescence and higher photostability of siloles make them excellent candidates to develop a new class of fluorescently labelled peptides.

Based on our previous work on silylated amino acids,6,7 we describe here the synthesis of the first silole-based amino acids and their corresponding fluorescent properties. After selective deprotection of carboxylic or amine functions, C- or N- peptide coupling with alanine proved their possible incorporation into peptides. The synthesis of a model tripeptide was also achieved by SPPS with the N-Fmoc protected silole amino acid derivative. As expected, such silole amino acids and peptides exhibit AIE properties with λem around 500 nm and large Stokes shift ($\Delta\lambda$ 100 nm) offering new opportunities to design AIE fluorogens-peptides conjugates.

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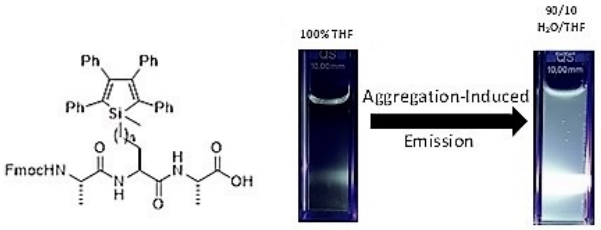

P32

Stabilization of Secondary Structures by Cross-Coupling Reactions

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Protein-protein interactions (PPi) related to signal transduction are involved in many biological processes. Hence, dysregulated PPi's are responsible for the origin of many diseases such as cancer, infections or neurological disorders. In particular, peptides as potential therapeutics are very promising due to their high selectivity. However, small peptides often suffer from low conformational stability and stability against proteolytic digestion as well as poor membrane permeability. In contrast, cyclic peptides have a more constrained conformation resulting in better binding affinities to their target and moreover, cyclization improves the stability against proteases.[1] The cyclization can be performed by backbone or side chain to side chain connection. For side chain to side chain crosslinks, reactions such as ring closure metathesis,[2] cycloadditions,[3] lactam-,[4] disulfide-[5] and thioether-formation[6] are described and can be used for stabilization of secondary structures such as α-helices or β-sheets. The availability of C5-, C6- and C7 regioisomers of bromotryptophan on a preparative scale using FAD-dependent tryptophan halogenases gave rise to utilize Pd-catalyzed cross-couplings for stabilization of secondary structures by side chain to side chain connection.[7] A main advantage of Pd-catalyzed bioorthogonal reactions is the selective derivatization in presence of a broad range of functional groups in biomolecules. The intramolecular SUZUKI-MIYAURA cross-coupling between bromotryptophan and a suitable aryl boronic acid provides a promising tool for those crosslinks, because it was shown to facilitate the derivatization of bromotryptophan and bromotryptophan containing peptides and natural products.[8] Moreover, other cross coupling reactions such as MIZOROKI-HECK reaction are investigated as suitable approaches.

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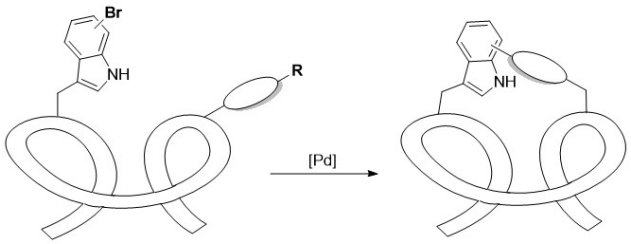

P33

Synthesis of A-ring Analogues of the Bioactive Peptide Nisin

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Institute of Technology Tallaght, Ireland

Significant numbers of bacteriocins have recently been discovered with high potential for use as human therapeutic agents [1]. Nisin, the most common lantibiotic, which has a polycyclic structure consists of 34 amino acids, is currently used worldwide as a food preservative in dairy, meat and tinned food products. It is a potent antimicrobial agent against Gram-positive bacteria and contains unusual amino acids like dehydroalanine (Dha) and dehydrobutyrine (Dhb) within its structure [2]. An interesting feature of many lantibiotics, from very diverse sources, is the similarity of their A and B ring fragments (Figure 1). They contain the same number of amino acid residues, with the Dha and (methyl)lanthionine (thioether links) residues present in all the structures. The major difference between the various A-rings is the amino acids residues flanking the Dha moiety. The strategy towards the synthesis of different analogues of the A-ring of nisin, and other type A lantibiotics, will be presented.

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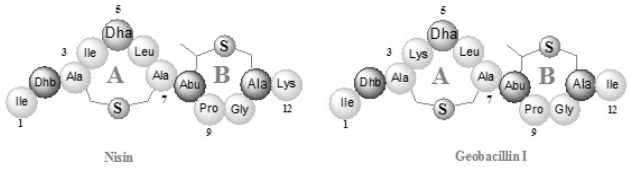

P34

Synthesis of different lengths of highly monodisperse cholesteryl-PEGn-peptide conjugates

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Cholesterol (Chol) is an essential component of eukaryotic cell plasma membrane and has multiple functions regulating fluidity and permeability of the membrane structure. In lipid-based nanovesicles (L-NVs) such as liposomes, Chol is used as a key component stabilizing these nanostructures, reducing the permeability of encapsulated drugs, and enhancing liposome drug loading.1 In this sense, cholesteryl-polyethylenglycol (Chol-PEG) conjugates are usually employed as stabilizing anchors for liposomes favouring the increase of their circulation time.2 Chol-PEG conjugates also behave as solubilizing surfactants which serve to prevent aggregation of vesicles. In this sense, the use of monodisperse PEGs (M-PEGs) has a burgeoning interest in therapeutic delivery systems due to the well stablished correlation between erratic biological activities and polydispersity. This issue can originate a range of problems in their applications in regulatory phases. Herein, we describe the synthesis of highly monodisperse Chol-PEGn derivatives (n=200-1000) by iterative introduction of macrocyclic tetraethylenglycolsulphate.3 As a proof of concept, we have conjugated a cyclic (RGDfK) peptide, a well known integrin ligand, to all these Chol-PEGn moieties with good yields and excellent purities (up to 99%). The versatility of this platform has been demonstrated by chemoselective ligation of other peptides to Chol-PEGn maleimide derivatives. All these Chol-PEGn-peptide conjugates can be achieved with good yields, high purity and showing highly monodispersity.

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Acknowledgements This study was funded by the Spanish government (RTC-2014-2207-1), CIBER-BBN (Lipocell, CB06-01-0074) and European Community (Smart4Fabry).

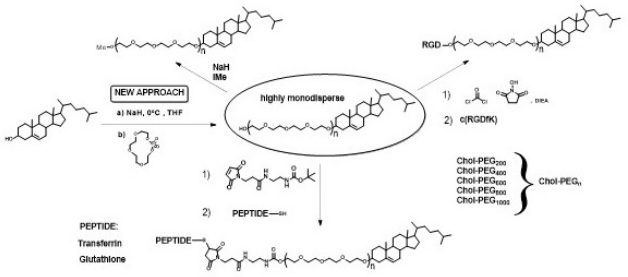

Figure 1. Synthesis of Cholesteryl-PEG$_n$-conjugates.

P35

Design and Synthesis of a μ-KIIIA Conotoxin Peptidomimetic that Blocks Human Nav1.6

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The conotoxins are a family of naturally occurring peptides which are used as defensive and offensive neurotoxic agents by predators, such as the cone snail. These disulfide containing mini-proteins act by blocking ion channels and disrupting normal cell membrane potentials. Selective inhibition of ion channels has the potential to treat conditions such as epilepsy and chronic pain. Previous studies into these compounds have elucidated the active region of the peptide which is important for activity and have replaced the disulfide networks with other covalent constraints which are more stable and synthetically accessible. 1

Physical constraints for peptide backbones have been well studied with a variety of strategies employed. Hydrocarbon staples have undergone significant investigation due to the relative ease of incorporating alkene bearing amino acids and performing a ring closing metathesis reaction. A unique and emerging chemical constraint is a diyne bridge formed by a Glaser coupling of two unnatural amino acids bearing terminal alkyne groups. This strategy produces no cis/trans isomers, gives a more rigid cross bridge and has the potential for label free imaging using Raman spectroscopy. 2,3

This work focuses on the asymmetric synthesis of alkyne bearing amino acids and investigation of Glaser coupling conditions for intramolecular diyne formation. Electrophysiological data using a patch clamp assay showed it to be a good inhibitor of human Nav1.6.

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P36

High-throughput parallel synthesis optimization of Glucagon-like Peptide 1 receptor agonists

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[2]Gyros Protein Technologies, United Kingdom

Type 2 diabetes represents a major health burden, with over 400 million patients worldwide [1]. Glucagon-like Peptide 1 (GLP-1) agonists are an important treatment for the management of Type 2 Diabetes. GLP-1 agonists improve glycemic control through multiple mechanisms with a low risk of hypoglycemia [2]. Type 2 diabetes is usually coupled with obesity and thus molecules targeting both GLP-1 receptor, mediating food intake suppression and the glucagon receptor, which modulates energy expenditure, are of great interest and some have progressed into the clinic1. Many SAR studies have been completed on GLP-1 related peptides in the search for stability and increased target affinity, with improvement via modifications such as peptide cyclizations and the attachment of PEG-based monomers. These peptides have been prepared by fragment synthesis and solution phase condensation in order to reduce impurities and maximize yields for pharmaceutical manufacturing. Recently, dual GLP-1/glucagon receptor peptide agonists have been described by Evers et al.[1] Here we show the synthesis optimization in parallel of GLP-1 related peptides and dual GLP-1/glucagon receptor agonists, testing multiple resins and coupling reagents searching for optimal crude purity that may translate into ease of purification during the manufacturing process.

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P37

Synthesis of an HIV-targeted stapled peptide: Fully automated on-resin peptide cyclization via olefin metathesis

James Cain[1], Daniel Martinez[1], Andrew Kennedy[2], Cyf Ramos-Colon[1]

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Cyclic peptides are an important tool for development of peptide therapeutics. Their increased rigidity allows for increased membrane permeability and increased stability to proteases, two major drawbacks of peptide drug development. Peptide cyclization can be achieved several ways, including head-to-tail cyclizations or side chain-to-side chain cyclizations, which can be done by lactam bridge formation, disulfide bonds or hydrocarbon stapling via olefin metathesis, to name a few. Hydrocarbon stapling stabilizes a-helical structures and thus increases cell permeability and stability. Other advantages of stapled peptides include the targeting of protein-protein interactions, important for targeting many disease states such as cancer. Zhang et al. have described the synthesis and properties of an i, i+4 stapled peptide, NYAD-1, which targets the capsid and inhibits HIV-1 in cell culture [1]. Here we show the fully automated synthesis, from linear, on-resin cyclization, and resin cleavage, of NYAD-1 and other examples.

Reference:

[1] Zhang, H. et al. Dual-acting stapled peptides target both HIV-1 entry and assembly. Retrovirology 10, 1–20 (2013).

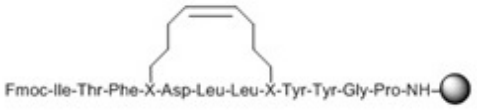

Figure 1. NYAD-1 structure.

P38

Non-natural Amino Acid and Peptoid Building Blocks for Optimization of the Cell-Penetrating (R-X-R)n Motif

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Facilitating efficient cell membrane permeability is a crucial step in unlocking the therapeutic potential of drug candidates that are otherwise limited by poor inherent cellular uptake. Cell-penetrating peptides (CPPs) are short peptides that most often are cationic in nature, which confers a propensity to enable efficient intracellular delivery of a variety of cargo molecules (e.g. other peptides) across the cell membrane. CPPs containing the (R-X-R)n motif have proved to be among the most effective in facilitating membrane penetration.

Herein, we report the optimized synthesis of a number of non-natural amino acid and peptoid building blocks that are ideally suited for the synthesis of analogues of the (R-X-R)n motif. The cationic character of typical CPPs is resulting from the presence of both Lys and Arg residues. However, especially the guanidino functionality of the arginine residues has been found to be crucial for the cell-penetrating properties of highly cationic CPPs. The ideal presentation of guanidinium groups as well as an optimal ratio of hydrophobic-hydrophilic character was shown to be critical for the modulation of their activity. Incorporation of the described non-natural amino acid building blocks allows for the synthesis of a variety of analogues aiming at optimization of the hydrophobic-hydrophilic components and the spacing between guanidinium groups, which both are expected to influence the cellular uptake properties. Furthermore, incorporation of related peptoid building blocks is expected to increase proteolytic stability of the resulting analogues.

All building blocks can be synthesized and purified in large scale, and they are compatible with use in microwave-assisted automated solid-phase-peptide synthesis (SPPS). They can be used efficiently with standard coupling protocols and the resulting peptides are obtained in very high purity.

P39

Disulphide cyclization of somatostatin and its analogues with selected oxidizing agents

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Somatostatin is a peptide hormone produced by hypothalamus, thyroid, placenta, and mucous membranes of the stomach and small intestine. It is a cyclic peptide with a disulphide bridge that occurs in two forms, the 14- and 28-amino acid residue long. Somatostatin regulates the endocrine system and affects neurotransmission and cell proliferation. Moreover, it inhibits the secretion of growth hormone (GH), serotonin, insulin, and glucagon. Radiolabelled analogues of somatostatin can be used to localize scintigraphically neuroendocrine tumours on account of the increase in expression of somatostatin receptors on tumour cells. Hence, it seemed expedient to obtain a peptide analogue, octreotide with extended biological half-life, conjugated with a chelator (hydrazinonicotinic acid - HYNIC). A co-ligand and a radionuclide are added to the peptide before use. The aim of this study was to examine the potential of selected oxidizing agents in intramolecular disulphide bridge formation.

Somatostatin and octreotide analogues – HYNIC-TOC and HYNIC-TATE, were obtained by manual synthesis on solid support using Fmoc/tBu strategy. Coupling reactions were carried out for 1.5 h in DMF and DCM mixture using a fourfold excess of substrates (Fmoc-AA : DIC : Oxyma Pure, 1:1:1, mol/mol) based on the resin. Deprotection step was accomplished with a 20% piperidine in DMF for 15 min. The peptides were cleaved form the resin in TFA and a scavengers mixture (TFA : EDT : TIS : water : phenol, 90:2.5:2.5:2.5:2.5, v/v) for 1 h. Iodine, ozone, and CLEAR-OX™ (a polymer-supported oxidant) were examined as oxidizing agents in peptide cyclization. Oxidation was performed in peptide solution (1 mg/mL) in 20% acetic acid in water. To monitor the progress of oxidation reaction the Ellman test and LC-MS analyses were performed.

In this study we determined the efficiency of different oxidizing agents. At early stages of oxidation, the cyclized peptide was identified in all samples. However, ozone rapidly degraded the desired product. On the other hand, iodine provided an efficient and fast oxidation. It was found to be an appropriate agent for cyclization of somatostatin, but HYNIC-TOC and HYNIC-TATE were degraded. Only CLEAR-OX™, as a mild oxidant, did not degrade the peptides. Nonetheless, one of the weaknesses of the procedure is that CLEAR-OX™ requires a relatively long time (2 h) to cyclize the peptides. Air appears to be the most universal oxidizing agent. However, after two days of oxidation there was still a few percent of free sulphydryl groups left in the samples.

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P40

Evaluation of the "ResPep continuous flow synthesizer" with real-time UV-monitoring, automated feedback & heating in solid phase peptide synthesis

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Automation in assembly of peptides via solid phase synthesis following the Fmoc-strategy [1] is a well-established method and commonly used for peptide synthesis today. Mostly due to length of the peptide, the presence of hydrophobic stretches and sterically hindered amino acids, difficulties during peptide synthesis are sequence inherent. Therefore, the choice of the proper conditions like coupling reagent and temperature have tremendous influence on the stepwise amide bond formation yielding the crude product in the highest purity possible. Here, we present examples of automated peptide syntheses of so called "difficult sequences" [2], [3] using different coupling reagents and varying temperature on the new ResPep continuous flow synthesizer with real-time UV monitoring and feedback. Four classes of coupling reagents were applied: triazine-, uronium-, phosphonium- and carbodiimide-based reagents were used in solid phase peptide synthesis. The results were discussed and compared in terms of quality of the crude product, coupling efficiency, costs and cycle times.

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P41

A simple method for raising the temperature in manual peptide synthesis

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Methods for obtaining synthetic peptides have undergone only a few changes over many years. Chemists do not like to modify synthesis protocols. This involves the need to change reagents and re-training employees. With the emergence of the Fmoc methodology, peptides have been obtained in the same way for over 30 years. After the success of microwave devices, many synthesizer manufacturers return to heating techniques in peptide synthesizers. The present research shows how to easily implement heating to the manual synthesis without the need to use water-heated vessels. Synthesis of three model peptides were performed using two reference methods (either the classic synthesis at room temperature or automatic synthesis on a microwave synthesizer) and two classic methods with dry heating blocks. The classic experiments were performed using the device Skiller 1 (heating block with a magnetic stirrer) and in the laboratory heating clamp. The syntheses with the heating block were carried out in a glass vessel enabling thermostatting with air (the Kamysz vessel). Synthesis with Skiller 1 and heating clamp was performed at 60°C. The reference syntheses were carried out either at room temperature (classic synthesis) or following the synthesizer protocol. All peptides were synthesized using the Wang resin and a threefold excess of reagents (Fmoc-AA : DIC : OxymaPure, 1:1:1, mol/mol) dissolved in DMF based on the resin. Coupling was carried out for 2 h in the classic method and for 15 min when heated. Deprotection was performed with a 20% piperidine in DMF for 2 and 10 min in the classic method and for 2×2 min when heated. The peptides were cleaved form the resin in TFA and a scavengers mixture (TFA : TIS : water : phenol, 94:2:2:2, v/v) for 1 h. The quantity of the crude peptides was determined and their purity was tested by HPLC. The resins used in the experiment were examined independently for their mechanical degradation. The results clearly indicate that the heating in dry heating blocks is an effective method to accelerate peptide synthesis. This approach does not require specialized equipment and the temperature used (60°C) is not dangerous for the user. At the same time, it was found that the synthesis of long peptides using a magnetic stirrer is either not recommendable (although possible) or demand the use of mechanically resistant resin (TentaGel type).

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P42

Peptide Production from mg to Kg with Automation and Microwave Assisted Heating

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Peptide therapeutics are an attractive alternative to their small molecule drug counterparts [1]. With several high revenue peptide drugs on the market and a pipeline full of potential candidates [2], the demand for highly robust and efficient synthetic methods is of great importance. Microwave assisted SPPS has established itself as the primary chemical method to produce high quality peptides while drastically reducing synthesis time and waste [3]. This poster will highlight new R&D and technology which encompasses GMP peptide production from mg to Kg scale. On the R&D scale, our research team has developed a standardized methodology for synthesis of varied peptides, providing a powerful tool that simplifies peptide therapeutic research. To validate this comprehensive strategy that utilizes microwave (MW) heating and rapid automation, a variety peptides from current literature were linearly synthesized. Key features of this methodology include:

- Improved carbodiimide coupling which increases purity and suppresses side reactions such as epimerization - One-pot coupling and deprotection process for increased efficiency - Reduction of up to 95% generated waste compared to conventional methods

Rapid scale-up for clinical trials and peptide production has been accomplished using similar technology with a focus on elevated temperatures. Crude purity from R&D to production scale is preserved if not improved and unwanted side reactions such as epimerization and aspartimide formation are easily controlled. The result, easier purification and reduced labor cost. Cycle times at the production scale range from 10 – 60 min with the capability to produce up to 1 KG crude peptide in a single batch. Several examples, including process development, will be presented.

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P43

Microwave-Assisted Synthesis of Symmetrically and Unsymmetrically Branched Peptides

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Branched peptides represent a class of peptides with highly desirable physio-chemical and biological properties. For example, peptides with symmetrically branched cores such as multiple antigenic peptides (MAPs) or peptide dendrimers have been shown to have increased biological activity compared to their linear counterparts due to multivalent binding to a protein target or due to an improved resistance to proteases.1 On the other hand, unsymmetrical branching allows for the synthesis of chimeric peptides that contain different peptide sequences in a single structure. As a result of their useful properties, branched peptides have found use in a variety of applications including the development of antimicrobial and antiviral drugs2–4, tumor-targeting agents5,6, drug delivery vehicles7, and other novel applications8,9.

Synthesis of branched peptides via SPPS is often challenging because of the inherent close proximity of the elongating peptide chains on a branched scaffold, which leads to steric clashes and poor peptide coupling. Here we report on the application of microwave-assisted SPPS to the synthesis of branched peptides. Microwave energy helps overcome steric challenges, allowing for more efficient coupling and the rapid synthesis of difficult branched peptides with fewer deletion products.

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Automated Synthesis of Cyclic Disulfide-Bridged Peptides

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Cyclic peptides containing disulfides represent a class of compounds with a profound array of biological functions ranging from venoms to integral hormones(1). The disulfide bonds help stabilize the secondary structure and conformation of peptides, which can contribute favorably to proteolytic stability and target affinity(2). Because of their promising therapeutic potential, interest in the synthesis of cyclic disulfide-bridged peptides has grown steadily. Peptides with disulfide bridges can be prepared by SPPS by using orthogonally-protected cysteine amino acids such as Fmoc-(S)-Cys(Mmt)-OH and Fmoc-(S)-Cys(STmp)-OH. The Cys(Mmt) group can be deprotected using a dilute solution of trifluoroacetic acid (TFA), whereas the Cys(STmp) group is orthogonally deprotected using dithiothreitol (DTT) as a reducing agent. After deprotection, disulfide bond formation can be achieved using N-chlorosuccinimide (NCS) as a mild oxidant(3).

Here, we report that cyclic disulfide-bridged peptides can be prepared rapidly with good purity using automated microwave-enhanced SPPS. Synthesis of the peptide hormone oxytocin4 was achieved in under 3 h with 69% purity. Preparation of a peptide agonist of BMP receptor activin-like kinase 3 (Alk3), THR-1235, was completed in 3 h with 77% purity. Finally, a peptide venom from cone snails containing two disulfide bridges (conotoxin-SI)6 was synthesized in under 4 h with 67% purity.

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P45

3-Substituted isocyanopyridines as mildly convertible isocyanides

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A powerful tool to generate peptidomimetics are multicomponent reactions, such as the Ugi reaction, often combined with post-condensation modifications. An Ugi reaction typically gives an α-acylaminocarboxamide that has a dipeptide like structure. The downside of the Ugi reaction is the formation of a stable amide bond at the C-terminus of the product, which cannot be cleaved under mild reaction conditions. This limitation can be circumvented by the use of convertible isocyanides that allow the chemoselective transformation of this amide bond.[1] Throughout the years, different convertible isocyanides have been developed all with their advantages and restrictions such as low stability, cleavage requires acidic or basic conditions or a multistep procedure. There is, however, still a need for efficient convertible isocyanides that can easily be cleaved under neutral conditions. With this in mind, we set out to develop a convertible isocyanide that can be cleaved using a Zn-catalyzed nicotinate-directed transamidation. Hence, the synthesis of different 3-substituted-2-isocyanopyridines was pursued. To compare their efficiency towards the amide cleavage, all isocyanides were subjected to the Ugi-4C conditions. These Ugi products were in turn used in the zinc catalyzed transamidation reaction. It was demonstrated that the 3-methyl ester generates the most labile amide bond, followed by the bromo and chloro directing groups, which resulted in comparable conversions, while the least labile amide is obtained for the methoxy substituted directing group. Nevertheless, the amide bond at the C-terminus was easily and selectively cleaved in all cases.

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Strategies toward optimizing automated on-resin disulfide bond formation in disulfide rich peptides

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Disulfide rich peptides exhibit exquisite stability due to the covalent stabilization of their secondary structure mediated by disulfide bonds. While many of these compounds already display bioactivity, they have also demonstrated a unique plasticity when alternative sequences are grafted into various loop regions. After a particular sequence has been identified, these peptides are often folded in solution under redox conditions, assuming that the thermodynamically stable conformation will be the predominant species. However, there are many scenarios that multiple disulfide bonding patterns are observed upon folding completion, demanding additional purification and characterization before any biological assays can be performed. A simplified on-resin synthesis strategy is attractive as the purification and characterization steps can be minimized, if not completely eliminated, increasing the overall yield of the peptide with the proper disulfide bond pattern. Herein we describe a fully automated, optimized solid phase synthesis of apamin, an 18 amino acid peptide conformationally constrained by two disulfide bonds. Using Branches™, the synthesis and on-resin disulfide bond formation was readily visualized and programmed, simplifying the total synthesis and ensuring that the proper disulfide bond pattern was achieved.

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Synthesis and characterization of new Hemorphin-5 analogues containing azobenzene units with potential optical switching properties

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The development of agents to control bio macromolecular function with light offers the potential to change the properties of defined molecules in biological systems with minimal disturbance to the rest of the system. Photodynamic control of peptides, proteins or drugs provides a non-invasive way to disorder these networks to investigate their effect or cause a defined outcome. Azobenzene containing peptides could be acting as photoswitches that interconvert between E and Z isomers around –N=N– group offer highly predictable light state structural properties. These molecules are relatively small and adopt predictable conformations they are well suited as tools to interrogate cellular function in a spatially and temporally controlled fashion and for applications in photopharmacology and nanotechnology. In order to elucidate the influence of azobenzene-peptides we introduced the different substitution azobenzenes to the N-side of Hemorphin-5 peptide analogues. This report refers to the solid-phase synthesis and characterization of novel azobenzene containing Hemorphin-5 analogues. Hemorphin-5, also known as Valorphin, is a naturally occurring, endogenous opioid peptide of the Hemorphin family with affinity for opioid receptors and morphinomimetic properties. These neuropeptides are increasingly being used in the treatment of various diseases such as hypertension, epilepsy, chronic pain, cancer, and etc. The synthesis and characterization of a photochromic compound, in which Valorphin analogues are linked through their N-terminal amino group by an azobenzene units, are reported. The synthesis was achieved by a modified solid-phase peptide synthesis by Fmoc-strategy, based on the reaction between different substitution azobenzenes with N-terminal amino group of heptapeptide Hemorphin-5 (Val-Val-Tyr-Pro-Trp-Thr-Gln) directly to the resin. The crude neuropeptides were purified on an RP-HPLC and the molecular weights were determined, using ES-MS, and also determining of the specific angles of optical rotation and photoisomerization.

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P48

A preparation of isopeptide mimetics by the peptide ligation using a thiirane linker

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The site specific modification of proteins such as post-translational modification (PTM) and fluorescence labeling is important for studies of protein functions. Histone proteins form an octamer as a core of the nucleosome, which is a basic unit of chromatin and which consists of two copies

of each four different core histones, H2A, H2B, H3, and H4 with DNA. The PTMs of histones, such as acetylation, methylation, phosphorylation, and so on, play an important role in the epigenetic regulation of gene expression. We previously reported on the preparation of histones H3 and H4 containing a trimethylated Lys residue [1,2]. Ubiquitination is also one of important PTMs, in which the C-terminus of ubiquitin (Ub), a 76 amino acid residue peptide, is attached to the side-chain amino group of Lys residues by an isopeptide bond. Histone H3 was reported to be ubiquitinated at Lys18 or 23 to regulate maintenance DNA methylation by DNA methyltransferases [3]. Here we report on a convenient method for preparing an isopeptide-mimetic structure, in which thiiran-2-ylmethanamine (2-aminomethylthiirane) is used as a linker for peptide ligation [4]. Using this procedure the ubiquitinated histone H3 was prepared [5].

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P49

Synthesis of Homogeneously Glycosylated Soluble Fas Ligand Variants

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Glycosylation is one of the most abundant and complex posttranslational modifications of proteins. Since its alteration is frequently observed in many pathogenic conditions [1-3], it is crucial to be able to investigate the impact of individual glycan structures at specific positions in proteins and access to homogeneously glycosylated protein variants is urgently needed [4]. To obtain such homogeneously N-glycosylated variants of soluble Fas ligand (sFasL), an important player in cancer proliferation, a chemoenzymatic approach was chosen. The protein will be assembled from three segments, each of them synthesized by Solid Phase Peptide Synthesis (SPPS), using Native Chemical Ligation (NCL). The C-terminal peptide, containing two native N-glycosylation sites, will be equipped with an N-terminal selencocysteine for NCL and subsequent selective deselenisation and a polyethyleneglycol (PEG) chain attached to a tobacco etch virus (TEV) protease cleavage site to facilitate peptide purification between cycles of enzymatic carbohydrate chain elongation [5]. After the incorporation of asparagine-N-acetyl-glucosamine building blocks by SPPS at the sites of N-glycosylation, glycan extension is achieved by using glycosidases/glycosynthases and a homogeneous, activated N-glycan core structure isolated from egg yolk [6]. Subsequent elongation with suitable glycosyltransferases increases the number of accessible glycopeptide variants [7]. These glycopeptide variants will be linked to the larger N-terminal segment, which in turn is synthesized from two smaller SPPS fragments by NCL. In this way, several N-glycan variants of soluble Fas ligand will be obtained and subjected to further chemical and biological investigation. Here, the latest results on the synthesis of all three peptide segments and different ligation reactions will be presented.

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P50

Pd-catalyzed cysteine modification of peptides and proteins

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Posttranslational modifications such as phosphorylation, acylation and lipidation play an important role for the control of protein function and localization. Among these, prenylation is especially important for the association of certain proteins to specific membranes.[1] The current knowledge about the physiological function of many of these proteins is mostly based on the use of prenylated peptides that are linked to a target protein by chemoselective modification reactions in order to obtain prenylated protein variants. For these semisynthetic approaches especially proteins where almost all lipidations occur within the last C-terminal amino acids are well suited. As the attachment of lipid chains to C terminal peptides occurs mostly via thioethers and thioesters, chemistry not easily compatible with standard Fmoc-based solid phase peptide synthesis and cleavage conditions, complex synthesis strategies were developed.[1,2] Another approach to prepare protein variants with either natural or synthetically modified residues is the use of chemical protein modification. This allows to decide which modification should be attached to which residue in order to create proteins with the desired properties such as natural posttranslational modifications, fluorophores or reactive tags.[3] But the synthesis of chemically modified proteins still represents an important challenge due to problems with selectivity, reaction conditions and yield.[3,4] Here we show initial experiments that support our hypothesis that Pd-catalyzed Tsuji-Trost-allylation can be used for the prenylation of Cys-containing peptides and proteins, thereby allowing direct access to prenylated peptides and proteins with high n/iso ratio and excellent chemoselectivity. Furthermore, peptides with other modifications such as a fluorophore and an affinity tag can be synthesized.

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P51

Accessing semisynthetic Hsp27 carrying site-specific non-enzymatic post-translational modification implicated in disease

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Non-enzymatic posttranslational modifications (nPTMs) are believed to affect at least 30% of human proteins, many of which are implicated in var-

ious pathological conditions e.g. cataract, diabetes, neurodegenerative diseases and cancer. A general lack of structural information of many of such nPTM target proteins coupled with the difficulty in pin-pointing the site of such modifications impede further studies and development of therapeutics against such modifications. Since most of these nPTMs are not included in the available chemical libraries or protein expression systems, semisynthesis remains to be the only feasible way to access such proteins carrying site-specific and homogeneous modifications. Methylglyoxal (MG), a reactive electrophilic species formed during glycolysis is known to modify proteins at amino acids carrying nucleophilic side chains (e.g Arg, Lys etc). One of such modifications, argpyrimidine (Apy) has been detected in human small heat shock protein Hsp27 (HSPB1) in various tissues of patients suffering from pathological conditions, such as hyperglycemia, cataract and cancer. Although, there are as many as 16 arginines in this 205-amino acid long protein susceptible to Apy modification, previous studies based on mutating arginines in Hsp27 to glycine residues followed by expression in HEK-293 cells and subsequent immunoblot analyses, revealed that only mutation at Arg188 leads to loss of recognition by an anti-Apy antibody. This particular point mutation also results in partial dissociation of the usual higher order oligomeric structure of Hsp27 to lower order ones, accompanied with a loss of chaperone activity, as we have previously observed with a semisynthetic Hsp27 analogue carrying an R188Apy mutation. We were interested to understand the impact of such individual point mutations of arginine residues to Apy, precisely, how it influences the quaternary structure and thus associated functions of Hsp27. Herein, we present a study combining semisyntheses and biophysical as well as biochemical assays on Hsp27 analogues with single point Apy mutations at five different arginine sites (R4, R5, R12, R20, R27) within the N-terminal domain of Hsp27.

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Deciphering the interplay role of phosphorylation and glycosylation on the aggregation of tau proteins by using semi-synthesis

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The tau protein is a major target in Alzheimer disease (AD) research, since it was found to be the main component of insoluble, aggregated neurofibrillary tangles (NFTs) in the brains of AD patients [1]. It was observed that phosphorylation and glycosylation played an important role during tau aggregation process, where the pattern of modification suggests that some motifs are unique to the disease [2]. Among these are the post-translational modifications (PTMs) on the AT-8 epitope within the proline-rich domain [3] and on the PHF-1 epitope in the microtubulin-binding domain [4], both of which are recognised by several disease-relevant antibodies [5] and are consequently of high interest for tau aggregation studies. While biochemical methods, like pseudo-phosphorylation and enzymatic modifications, are valuable tools to evaluate the effect of these motifs on the structure and function of tau, none of them can produce homogeneously modified, natural protein.

By utilising native chemical ligation (NCL) [6] and expressed protein ligation (EPL) [7], Haj-Yahya and Lashuel have recently demonstrated the preparation of full-length native tau[8]. Currently, we aim to produce homogeneously PTM tau proteins bearing phosphorylation and glycosylation, both of which were already successfully applied in the semi-synthesis of tau fragments and proteins [9,10]. In this study, we will present our recent efforts to obtain new tau constructs and show the potential interplays between phosphorylation and glycosylation on the aggregation of tau protein.

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P53

Synthesis of a new generation auxiliary for cysteine-independent native chemical ligation and chemoenzymatic modification

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From expanding the spectrum of biological and chemical tools for the synthesis of complex modified proteins, various fields of research would greatly benefit. Semisynthesis in combination with auxiliary-mediated native chemical ligation methods present an incredibly valuable tool for this purpose. [1] A recently developed PEGylated auxiliary by Bello et. al. exploits the advantages of ligation auxiliaries and adds functionality by introducing a polyethylene glycol (PEG) polymer to its scaffold. Thereby, the fast and quantitative recovery following repeating rounds of enzymatic glycosylation of a glycopeptide was possible. [2] Here we present a synthesis route for a new generation of this ligation auxiliary. Its modified structure enables investigation of alternative coupling strategies, with the prospect of expansion of the auxiliary's versatility beyond glycine residues. Furthermore, a straightforward and short synthesis scheme improves its applicability and allows us to extend its usage in semisynthesis and chemoenzymatic modification reactions of peptides. We were able to couple this newly generated auxiliary to a peptide N-terminus by Mitsunobu-Fukuyama alkylation, via the newly introduced hydroxyl moiety. The conditions employed are mild, in agreement with standard Fmoc peptide chemistry and tolerate the presence of O-linked N-acetylgalactosamine. In combination with the general strategy of a PEGylated photocleavable auxiliary, this revisited approach should enable further expansion of its application to previously not accessible ligation sites.

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P54

Chemical synthesis of transactivation domain (TAD) of tumor suppressor protein p53

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Abstract: Intrinsically disordered proteins (IDPs) have evolved as a key structural component of functional proteins in the last decade in the midst of traditional fixed 3D protein structure.1 The intrinsic disorder enables flexibility in the protein which helps it to attain different conformations for interacting with distinct biomolecules subject to different biological conditions and functions. The tumor suppressor protein p53, whose incorrect behavior is responsible for many cancers,2 possesses an intrinsically disordered transactivation domain (TAD) in its N-terminus.3 Chemical synthesis of this full-length TAD, rarely reported in the literature, is extremely important to address the questions of the role of the combinations of post-translational modifications (PTMs) in p53 function as most PTM sites of the full-length p53 are located in this domain.4,5 To achieve this, a detailed synthetic procedure for preparation of TAD p53 is presented in this work based on the combination of Fmoc/tBu-solid phase peptide synthesis (SPPS) and native chemical ligation (NCL). Both the C-to-N and N-to-C one-pot three-segment strategies were applied and three different thioesters of the middle segment (PEP2) were used for the N-to-C native chemical ligation during the synthesis and their comparative yields are presented.6,7 The chemically synthesized negatively charged TAD interacts with a positively charged nuclear receptor coactivator binding domain (NCBD) of CREB-binding protein as clearly visible from the NMR and CD experiments which are in concurrence with the previous reports.4,5

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P55

Calibration of analytical HPLC to generate preparative LC gradients for peptide purification

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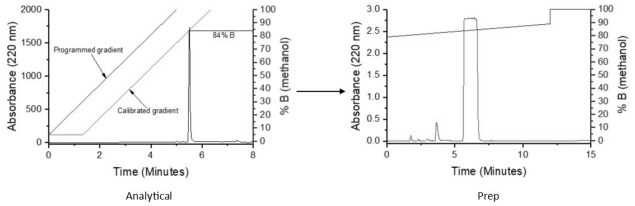
Preparative LC (liquid chromatography) is widely used to purify synthesized peptides. One bottleneck in the purification process is method development. Significant time can be required to produce an efficient preparative purification method that resolves the desired peptide from impurities and minimizes both time and solvent usage. This work describes a simple method of calibrating analytical HPLC systems to match the preparative LC system using the existing scouting gradients typically employed by a research group. After the calibration is complete, the determined delay volume is applied to the scouting gradient. This delay volume encompasses any dwell volumes, column volumes, mixing volumes, solvent misproportioning, and other corrections that are needed to match the analytical system to the preparative system. After the calibration is complete, the user only needs to enter the retention time of the desired compound from the analytical HPLC scouting run to calculate a preparative method. Although the calculated gradient is designed to run over 12 minutes with targeted peptides eluting at 6 minutes, other gradient lengths may be run.



P56

Development of a strategic and quantitative route for the chemical synthesis of membrane-associated proteins

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Membrane proteins are vitally important for cells providing communication between outer and inner compartment, the transfer of ions, small molecules and the transduction of stimuli.[1] These numerous competences make them a suspenseful drug target leading to an increased demand on models for fundamental as well as individual research. Membrane proteins are embedded and integrated into a lipid bilayer, thus displaying a highly hydrophobic behavior. This feature consequently leads to difficulties in handling not only the protein itself during experiments and recrystallization but also during synthesis, that being either expression or chemical synthesis. The synthetic production of membrane proteins features many benefits. These involve obtaining amounts of product in the milligram range which can further be used for structural analysis as well as the ability to rather easily insert mutations, isotopic labels and other desired post-translational modifications. To obtain the demanded membrane protein, solid phase peptide synthesis (SPPS) reaches its limit with the elongation of the peptide sequence. To date the synthetic production of sequences of membrane-associated proteins containing more than 50 – 60 amino acids are still a great challenge. Native chemical ligation (NCL) is the method of choice whereupon at least two fragments are needed, one displaying a N-terminal cysteine and the other a C-terminal thioester.[2] Modern-day research focuses on the C-terminal part with the integration of rearrangement groups which generate thioesters during NCL through O- to S or N- to S-acyl shifts. This approach represents the basis for our research on the Hmp (2-hydroxy-3-mercaptopropionic acid) rearrangement group.[3] Using this building block to evade difficulties that come along with thioester-groups and integrating solubilizing tags that facilitate handling as well as solubility, NCL of several membrane proteins was successful leading to the development of unique strategies given from the requirements of different membrane protein sequences. Herein we present the synthesis of the structurally unsolved part of the M2 proton channel from influenza B virus as well as results towards the smallest known viral potassium channel.

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P57

Second generation N-2-hydroxybenzyl-cysteine peptide crypto-thioesters for native chemical ligation

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Recent advances in Fmoc-based solid phase synthesis of peptide α-thioesters for the convergent synthesis of proteins via native chemical ligation (NCL) significantly contributed to push up the boundaries of the field. In particular, promising beta-mercapto amide-based thioesterification devices have emerged. If most systems require an acid catalysis, a

few of them can undergo an N → S acyl shift under NCL conditions (neutral pH): peptide bearing such devices are called crypto-thioesters.1 We recently reported an N-(2-hydroxy-4-nitrobenzyl)cysteine-based device (N-Hnb-Cys),2-5 which allows routine automated synthesis of crypto-thioesters from inexpensive building blocks. Conveniently, no post-SPPS steps are required, and these thioester surrogates are perfectly stable to handling, storage and purification. This strategy is thus easily and readily usable by non-specialists. We illustrated the potential of this methodology for the synthesis of long,2,3,5 cyclic4 and N-terminal Cys-containing3 disulfide-rich peptides. To assess the scope and limitations of the method, we undertook systematic kinetic studies that showed that NCL reactions using N-Hnb-Cys-based crypto-thioesters are only 4-to-5 fold slower than those using a benchmark preformed alkyl thioester. These relatively fast kinetics are thought to arise from a bio-inspired design, aimed at mimicking intein-like intramolecular catalysis with a well-positioned phenol group.2 In order to rationally design further optimized second-generation devices, we aimed at finely understand the molecular basis underlying the fast rearrangement. These efforts gratifyingly led to a promising second generation device with a 2 fold increase of the NCL kinetics.

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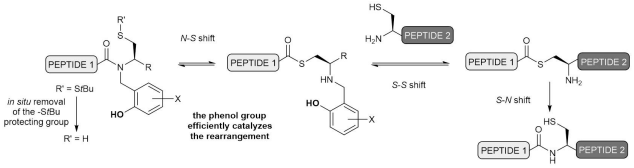

P58

Gold-Titania Nanocatalyst Generated by Mineralization Using Two Artificial Peptides with DNA

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Organic-inorganic hybrid nanostructures[1] could be used in diverse applications in nanotechnology, nanoelectronics and biotechnology. However, their production method by general top-down and bottom-up strategies is unsatisfactory. Biomineralization (precipitation of inorganic compounds) in a biological system is one of the most powerful approaches for overcoming this problem. However, inorganic compounds formed by mineralization precipitate randomly in solution. This system's function is to create inorganic compounds on organic compounds such as proteins and peptides[2]. Therefore, the mineralization control at nanometer level is necessary for the manufacture of functional organic-inorganic hybrid nanostructures. Our previous study, we had developed a site-specific control of an inorganic compound (silica) on DNA using an artificial peptide[3]. Furthermore, we succeeded in a limited site-specific precipitation of multiple inorganic compounds (silica and calcium carbonate) using a DNA and an artificial peptide[4]. However, there were some problems of direct multiple inorganic precipitation using one DNA and one peptide. 1) DNA can precipitate only a specific inorganic compounds with positive charge. 2) Direct inorganic precipitation on DNA constructs only rod or fiber like nanostructures. Therefore, by using template DNA and two precipitating peptides corresponding to two inorganic compounds, we attempted to produce more complicated organic-inorganic hybrid nanostructures as nanobuildingblocks were built. In this study, focusing on construction of gold-titania nanocatalyst with VIS light excitation, we demonstrated more complicated site-specific precipitation control of gold and titania on DNA using two artificial peptides. At first, we performed micro-scale techniques such as atomic force microscopy (AFM) and transmission electron microscopy-energy dispersive X-ray microscopy (TEM-EDX) and macro-scale techniques such as dynamic light scattering (DLS) and UV-VIS spectroscopy for the nanocomposite. These results suggested that the site-specific precipitation of titania and gold on DNA was successfully achieved. Additionally, we evaluated their visible light excitation characteristic by UV-VIS diffuse reflectance spectroscopy (UV-VIS DRS) and photocatalytic activity under visible light irradiation using photodegradation reaction of methylene blue (MB). As a result, the generated gold-titania nanocatalyst had visible light excitation characteristic. Our method for site-specific precipitation thus represents a powerful and fundamental tool for use in nanotechnology.

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P59

Nanostructures generated by protease digestion of amyloid fibrils towords an application to cell culture substrate

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Amyloid fibril formed by aggregation of amyloid bata peptide (Aβ) have been involved Alzheimer's disease [1]. The Aβ-nanomaterials studies have been demonstrated because amyloid possesses high regularity of molecular structure, thermodynamic stability and stiffness [2-4]. In addition, some studies showed that the morphologies in mixture of Aβ and another peptide were changed from fibrils of Aβ. [5] Focusing on application to cell culture substrate, we attempted to generate nanostructure (digested Aβs:dAβs) composed of some Aβ fragments by protease digestion of fibrils formed by aggregation of Aβ. Furthermore, we identified Aβ fragments in dAβs and synthesized these Aβ fragments. Finally, we performed cell adhesion assay using these Aβ fragments to compare with dAβs. We first generated amyloid fibrils by aggregation of Aβ(1-40) and performed digestion of the amyloid fibrils using three proteases (trypsin, chymotrypsin and subtilisin). The results of TEM (transmission electron microscopy) showed that the morphologies of dAβs samples remarkably were different from fibril structure of Aβ without digestion. We performed cell adhesion assay using three dAβs. The results showed that the number of adhered cells in Tryp-dAβs (Aβ digested by trypsin)-coating well is larger than that in other dAβs-coating wells. Then, we identified some Aβ fragments in Tryp-dAβs using HPLC and MALDI-TOF MS. The results showed that Tryp-dAβs included Aβ(17-28) and Aβ(6-40). Futhermore, we synthesized identified Aβ fragments and performed monitoring by ThT (Thioflavin-T) of aggregation using these Aβ fragments. The ThT moni-

toring showed that Aβ(17-28) does not form amyloid-like nanostructure as amyloid fibril. Finally, we demonstrated the cell adhesion assay using these Aβ fragment samples. Wells with Tryp-dAβs showed better cell adhesion than wells with Aβ(17-28) or with Aβ(6-40). Throughout this study, we successfully produced nanostructures (dAβs) consisting of some Aβ fragments and Tryp-dAβs (mixture of Aβ fragments) was more useful than Aβ fragment in cell culture substrate. Our method thus was indicated as a powerful and fundamental tool for use in nanomaterials.

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P60

Use of peptides with defined secondary structure in the catalysis of asymmetric aldol reactions

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The development of organocatalysts, which are metal-free organic catalysts, has shown a great potential in the last decade for various asymmetric transformations such as aldol, Mannich and Michael reactions. [1] Asymmetric aldol reaction provides chiral β-hydroxy carbonyl units and constitutes one of the most important and useful carbon-carbon bond-forming reaction. Among the various organocatalysts, bifunctional catalysts bearing both free secondary amine and carboxylic acid functions that are involved in the enamine activation and in the control of the geometrical position of the reagents, are particularly relevant. In particular, short peptides lead to efficient catalysts when both the amino and carboxylic acid functions are correctly positioned, which makes the secondary structure of such peptides an important characteristic. [2]

In the work presented here, a series of N-pyrrolidine-based α,β-peptide catalysts incorporating a constrained aminobicyclo[2.2.2]octane-2-carboxylic acid (ABOC) residue were synthesized and evaluated in the asymmetric aldol reaction from acetone and some p-substituted benzaldehydes. Their catalytic properties were shown to be highly dependent on the amino acid sequences and on the absolute configuration of the ABOC residue that played a determinant role. DFT calculations helped to rationalize this role. Among the peptides tested, the heterochiral tripeptide H-Pro-(R)-ABOC-Asp-OCH3, that adopts a turn conformation in the solid state, proved to be the most efficient catalyst affording β-hydroxy ketones in high yields and good enantioselectivities (up to 87%). [3]

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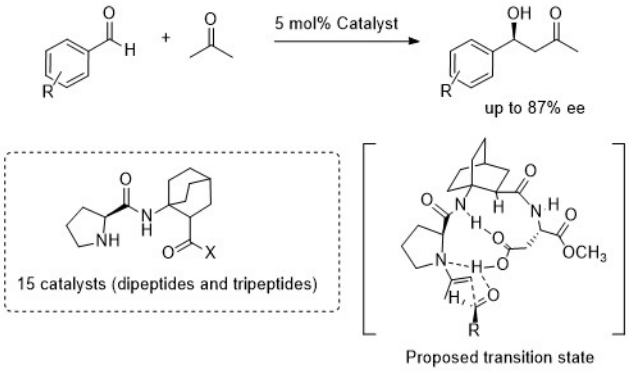

P61

De novo designed protein folding motifs as reaction scaffolds

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De novo designed biomolecules have a tradition in being applied as models to mimic natural systems or to create new functional assemblies, which work under physiological conditions. Among the explored protein folding motifs the coiled coil is probably best understood, and designs for a variety of coiled-coil assemblies are available.

We aim to use well-understood small self-assembling protein folding motifs as scaffolds to mediate chemical reactions. In this respect, the parallel heterodimeric coiled coil is highly interesting. Due to its heterogeneity it is not simply a dimerization tool but also provides control over the association process. By now, the specificity of the interaction between the two coil strands can be fine-tuned via the sequence pattern or chain lengths of the individual coil strands.[1-3] Furthermore, we found that based on the differences in strength of association, coil strands can be readily exchanged to form the more stable coiled-coil assembly.[4] These properties make the heterodimeric coiled coil a perfect building block to develop strategies for proximity-induced reactions and even reaction sequences.

Another protein structure of interest is the WW domain, a small β-sheet structural motif, which mediates protein-protein interaction. As the WW domain is not as well-understood as the coiled-coil motif, we envisioned a combinatorial approach to modulate function. We use fragmented WW domains and link the fragments on the strands of an antiparallel heterodimeric coiled coil, respectively. The three-dimensional structure of the split-WW domain is reconstituted upon coiled-coil association. With this tool in hand, we plan to establish the WW-domain-scaffold as platform for the design of small peptide-based catalytic entities.

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P62

Functionalizable oligoprolines as a platform for the development of extended self-assemblies with tunable morphologies

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Self-assembly of π-conjugated building blocks has become increasingly important for the fabrication of functional nanostructures. Towards this goal, peptides, which readily adopt well-defined secondary structures and are highly modular, have been used to direct chromophore self-assembly into well-organized, chiral nanostructures. The assembly of these conjugates has primarily been controlled by exploiting hydrogen-bonding networks within the peptides. Here we will present the formation of highly ordered supramolecular structures built on non-self-assembling peptidic scaffolds. Using oligoprolines as scaffolds to direct the self-assembly of conjugated systems, we achieved the hierarchical self-assembly of perylene monoimides into fibrils, sheets, and advanced, novel topologies, and the self-assembly of quaterthiophenes into extended sheets and ribbons wrapped in a double helix.[1, 2] This work culminated in the discovery of the first extended triaxial supramolecular weave, which consists of wholly organic threads. The weave proved to be more robust than non-woven self-assemblies of the same building block and is capable of hosting iridium nanoparticles.[3] Variation in length of the oligoproline and choice of chromophore and linker has allowed for facile access to a variety of supramolecular structures that do not rely on H-bonding between peptides. Thus, oligoproline π-system conjugates constitute novel and efficient tools for self-assembly towards functional nanostructures.

+This work was performed in collaboration with Prof. K. Müllen (Max Planck Institute, Mainz) and Prof. Peter Bäuerle (University of Ulm)

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P63

Single step recombinant human follicle stimulating hormone purification by peptide affinity chromatography

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Human Follicle Stimulating Hormone (hFSH) is used clinically for women ovulation and men spermatogenesis induction, in assisted reproduction technologies. As FSH-based biopharmaceuticals are parenterally administered, their purity must be high. Current methods for hFSH purification include several chromatographic steps to reach the required purity. However, these involve a decrease of the hFSH total yield, thus rising the cost of the process. Short peptides have been described as useful ligands for AC because of their low cost, simple chemical synthesis and high stability in comparison to protein-based ligands. In previous works, Ryu et al (1998) and Sohn et al. (2002) studied the hFSH receptor and examined the interaction of its exoloop 3 with the hormone, testing each amino acid of that exoloop by Ala substitutions. From those works, the mutant with higher affinity: (580)KVPLITVSKAK(590) was selected to design a synthetic ligand for affinity chromatography (AC): Ac-KVPLTVSKAKVAC-NH2. The peptide was synthesized as amide and was acetylated to avoid its polymerization during the coupling to the chromatographic support and to improve its stability to degradation by exopeptidases. A Cys was incorporated at the C-termini to facilitate its subsequent immobilization to the chromatographic activated SulfoLink agarose resin. A sample of crude recombinant FSH (rhFSH) was loaded to the peptide affinity column using 20 mM sodium phosphate, 0.5 mM Met, pH 5.6 and 7.2 as adsorption and elution buffers respectively. The column was overloaded, and the dynamic capacity obtained was 54.6 mg rhFSH/mL chromatographic resin. The purity obtained after AC purification was 95 %. Purified rhFSH quality was analyzed: the percentage of oxidized rhFSH was 3.4 % and the percentage of free subunits was 1 %, both of them in the range established by the European Pharmacopeia, as also were the sialic acid content and the isoforms profile. The method here designed allows obtaining a high quality rhFSH using a low-cost affinity matrix based on a short peptide ligand.

P64

Bevacizumab Purification by Peptide Affinity Chromatography

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Therapeutic Monoclonal Antibodies (mAbs) are widely used in many diseases' treatments such as cancer, rheumatoid arthritis, multiple sclerosis, among others. Bevacizumab (trade name: Avastin) inhibits the vascular endothelial growth factor and hence the angiogenesis process and it is applied to treat brain, breast, colorectal, lung and renal cancers. Its biotechnological production process involves host cell optimization, cultivation and mAb biosynthesis (upstream processing) and finally, the mAb recovery and purification from the culture broth (downstream processing). Due to its parenteral administration, its degree of purity must be extremely high. Nowadays, that is achieved with affinity chromatography (AC) with immobilized protein A, a highly expensive ligand that increases the cost of the process. In addition, due to the high affinity between antibodies and protein A, harsh elution conditions are required, impairing the protein ligand and reducing the chromatographic matrix half life. On the other hand, short peptides are ideal ligands for AC due to their higher stability, easier synthesis and lower cost in comparison to protein A. In this work, short peptide ligands with affinity for Bevacizumab were selected from a short one-bead-one-peptide combinatorial library obtained by the divide-couple-recombine method using the HMBA-ChemMatrix resin and using the Fmoc strategy. Bevacizumab (AGC Biologist (USA) was labeled with Texas-Red, and after mixing it with the library, fluorescent beads were selected and the peptide immobilized in each bead was identified by MALDI-TOF MS/MS. Those peptides that appeared most frequently were synthesized in larger quantities on Rink Amide resin by Fmoc strategy, separated from the solid support with TFA cocktail, and afterwards immobilized on NHS-activated agarose. The affinity chromatographic matrices were evalu-

ated by measuring the adsorption isotherms and affinities between 105-106 M were obtained. Those peptides that selectively adsorbed Bevacizumab without interacting with the CHO cell supernatants proteins were selected for future process scale-up. In conclusion, an efficient one-step-method based on AC with an immobilized short peptide allows the purification of the mAb Bevacizumab not only in a single step, but also at low cost.

P65

Switch of DNA G-wire Formation Using a PNA Peptide by Protease Activity

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Switchable molecular devices whose structure and function can be dynamically regulated are necessary for nanomedicine, nanomaterials and nanomachines. DNA molecules and their derivative such as PNA (peptide nucleic acid) [1] are attractive for the design of such as nanodevices because they have nanostructures, and they can be easily designed logically and systematically based on complementary binding. Guanine nanowire (G-wire) [2], one of the DNA nanostructure, is based on a four-stranded DNA helix called a G-quadruplex and is growing interest as functional elements in molecular electronics and nanotechnology. We previously constructed a regulating system for the G-rich DNA sequences, using a PNA peptide (Lmyc) that could switch the DNA secondary structures through protease (calpain I) activity [3]. In this study, we tried to develop a DNA nanostructural switch system (G-wire <-> particles) using Lmyc with calpain I toward nanotechnological applications [4]. First of all, Lmyc was synthesized. Lmyc consists of a G-rich PNA sequence for inducing G-rich DNA to form DNA-PNA hybrid structures and a calpain I substrate sequence for switching moiety that is depending on the activity of calpain I. Thus, Lmyc would induce the DNA to form hybrid secondary structures resulting in forming nano particles, and once calpain I existing the peptide would be digested and simultaneously loose the induction ability resulting in forming G-wire in the presence of calcium ion. We used the micro-scale analyses such as AFM and TEM, and the macro-scale analyses including zeta potential and DLS, to demonstrate the nanostructural switching of a DNA. AFM and TEM showed that the DNA alone formed G-wires in the presence of calcium ion, and that the peptide disrupted this formation and formed particles with the DNA. In the addition of calpain I, the peptide was digested and G-wire was regenerated. The results of macro-scale analyses agree with those of the micro-scale analyses. Our findings imply that a secondary structural change in the G-wires (higher-order structure) using the peptide provides the conformational change of DNA into a different nanostructure (higher-order structure). Throughout this study, we have constructed a switching system of nanostructural morphology through enzyme activity using a PNA peptide. This system would be promising tool for controlling the formation of DNA nanostructures for various applications, including electronic circuits for use in nanotechnologies.

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P66

Ultrashort Self-Assembling Peptides: De Novo Design, Nano Structure Analyses and Antimicrobial Activity

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Supramolecular chemistry was defined by the Nobel Laureate Jean-Marie Lehn as 'Chemistry beyond the molecule' and deals with complex molecular architectures that result when two or more species organize into higher order structures which are held together by intermolecular forces [1]. A common example of biopolymer self-assembly is the formation of amyloid fibrils, which are implicated in several diseases [2]. However, the importance of self-assembled peptides as nanomaterials for technological applications has also been realized [3-4]. Ultrashort peptides as well as designed beta peptides have been investigated for their self-assembly properties and as biomaterials with intrinsic antimicrobial activity [5-7]. Peptides can assemble into beta-sheets and form entangled fibrillar networks, resulting in water-trapping hydrogels [8]. Hydrogels have several potential applications such as drug delivery vectors, wound healing treatments and tissue engineering matrices [9]. Additionally, self-assembly in water provides a green chemistry approach towards generating soft biomaterials. We have designed and characterized a new class of ultrashort peptides (3 to 5 residues) that rapidly form hydrogels and possess promising broad spectrum antimicrobial activity. Modifying key amino acid residues enhances the self-assembly potential of these peptides resulting in improved kinetics and biophysical properties. Results from extensive biophysical and imaging techniques indicate that these peptides self-assemble into extended β-sheet nanofibrils. Investigations into the potential biomedical applications of these peptides are currently underway.

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P67

Peptide-based recovery of high-tech metals

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High-tech metals are almost ubiquitous in our everyday lives. Due to their great importance for the electronics industry, the demand is continuously growing. The supply of these important raw materials is currently mainly covered by primary raw material sources. However, with increasing technological progress, the supply situation on the global market becomes tense. The recovery of high-tech metals from secondary raw mate-

rial sources could help to ease the situation. This task is a major challenge due to a strongly mixed matrix and sometimes low concentrations of valuable metals from such sources. However, highly innovative strategies are required to meet this challenge. Modern biotechnology offers promising concepts for the efficient, economical and sustainable recycling of high-tech metals [1]. Recently we've established a phage display technology platform for the highly specific recognition of mineral particles as well as of metal ions in polluted water streams. This is escorted by a newly developed system for the heterologous expression of identified peptides [2-4]. Here we report in detail about the development of high-affinity peptide ligands for the recovery of gallium from industrial wastewater. Various gallium binding peptide sequences were identified by applying a commercial dodecamer peptide library (Ph.D.-12, NEB, US). Biopanning conditions were optimized for the enrichment of metal ion binding phage clones, which allows a more precise selection process. By single clone binding studies and competitive binding experiments, 3 sequences were characterized to show high binding affinity and selectivity for gallium above other metals, especially arsenic. Gallium binding peptides are now produced for further spectroscopic characterization and evaluation of binding properties. In addition immobilization strategies to create peptide-based materials for the recovery of gallium binding peptides will be discussed.

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P68

Designing a Minimal Synthetase

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Minimal enzymes are enzymatic units reduced to the bare essential with a preservation of their initial catalytic activity. The most famous example is SerHis dipeptide - a minimal hydrolase. Such minimal enzymes are thought to be a missing link between chemistry and the first organisms in the abiotic Earth environment and hold potential to use instead of enzymes in the future. As far as SerHis dipeptide can be used to condensate substrates, this reaction is unfavorable by the environment. In aqueous conditions direction of the reaction will be shifted from polymerization to hydrolysis. There is, however, another type of enzymes that deal with this problem by coupling unfavorable reactions with promoted ones. Synthetases are driven by hydrolysis of the pyrophosphate bond which they couple with the reaction of synthesis.

By studying the active sites of various enzymes belonging to the class of synthetases we have extracted crucial residues in their active sites and subsequently transferred them onto smaller, peptidic units.

In my presentation, I will present the inside to the structure of minimal enzymes we have designed and discuss features behind their activity. I will also compare peptides with synthetase-like activity with peptides without any desired properties yet similar sequence.

P69

Functionalized coiled-coil peptide hydrogels as potential ECM mimics

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Biomaterials have numerous applications in the fields of tissue engineering and regenerative medicine, which rely on the use of stem cells or progenitor cells. Various approaches using peptides as Extracellular Matrix (ECM) mimics for directing stem cell differentiation have been reported. Coiled-coil peptides have been increasingly investigated as highly suitable substrates for stem cell culture applications due to their self-assembly properties, which allow multivalent ligand presentation.[1] To date, however, there are very few examples of 2D and 3D coiled-coil based substrates presenting functional ligands of ECM proteins and subgroups of glycosaminoglycans (GAGs) to mimic proteoglycans and glycoproteins and further influence stem cell behavior.[2] GAGs for example play crucial roles in a variety of biological processes and are found covalently attached to proteoglycans, a critical biomacromolecule of ECM in mammalian tissues.[3,4]

Herein we present a self-assembling coiled-coil peptide hydrogel which is modified with a carbohydrate moiety to evaluate whether this peptide is a suitable substrate for stem cell culture applications. The impact of the nature and density of GAG or other ligands on stem cell proliferation and differentiation is currently evaluated and will allow tailoring of the ECM for a variety of applications.

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P70

Bioactivity of silicate foams selectively and covalently functionalized with protease-resistant adhesive peptides

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Bioceramic foams, based on as wollastonite and diopside, can be prepared from the thermal treatment of preceramic polymers (silicone resins) containing micro- and nano-sized filler powders [1]. This novel process presents limited processing temperature, microstructural homogeneity and leads to foams that can simulate the natural porous internal structure of human bones [2]. Currently the discovery of many physiological molecules with an active role on cell behaviour, opens the perspective to create biomimetic materials. In our studies, we demonstrated that the nonapeptide (HVP) from the h-Vitronectin is able to enhance human (h)-osteoblast adhesion through an osteoblast-specific mechanism [3-4]. In this work, polymer-derived silicate foams were covalently and selectively functionalized with adhesive peptides. In addition to HVP sequence, a dimeric analogue (2HVP) has been prepared in order to increase ionic interactions with cellular GAGs, together with two retro-inverted sequences (DHVP and D-2HVP) [5] in an effort to avoid the enzymatic degradation of HVP peptide in serum-containing medium. Both mechanical and biological as-

says were carried out to compare the differently decorated bioactive foams. The mechanical strength at crushing remained unchanged after functionalization. All functionalized samples showed increased cell proliferation at 2, 4 and 6 days from the seeding with respect to the control and silanized foams. In particular, retro-inverted sequences doubled the proliferation with respect to HVP and 2HVP at 2 days; D-2HVP gave a dramatic proliferation increase (more than 15 times) at 6 days. All anchored peptides enhanced mRNA specific transcript levels coding IBSP, VTN, RUNX2 and OPN. LDH test confirmed the absence of cytotoxicity. In vivo assays were performed inserting samples/controls under-skin in a murine model for 45 days. D-2HVP functionalized foams enhanced calcium deposition in vivo and histological analysis revealed the presence of new calcium deposits inside the pores only on functionalized bioceramics. In vivo assays demonstrated the osteoconductive and osteoinductive properties of D-2HVP peptide grafted to bioceramic foams.

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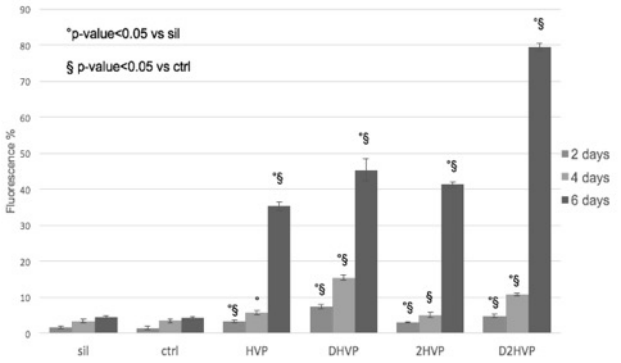

HOB proliferation on functionalized foams determined using CFSE probe assay."Sil" sample refers to the silanized scaffolds; "ctrl" refers to samples functionalized with a non-adhesive peptide. °,§ P-value<0.05 Student's t-test compared to "Sil" and "ctrl" respectively.

P71

Catalytically Active Peptides for Conjugate Addition Reactions

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Peptides of the type H-Pro-Pro-Xaa (Xaa = any amino acid) are highly reactive and stereoselective catalysts for organocatalytic C-C bond formations, such as aldol reactions (H-Pro-Pro-Asp-NH2)[1] as well as conjugate addition reactions of aldehydes to nitroolefins (H-D-Pro-Pro-Glu-NH2)[2] and unprotected maleimide (H-D-Pro-Pro-Asn-NH2), respectively.[3] The peptide catalysts are so reactive that loadings of less than 1 mol% suffice to obtain the products in high yields, enantio- and diastereoselectivities and the peptides can be immobilized and used in flow chemistry.[4] ESI MS, React-IR and NMR spectroscopic studies allowed for detailed insight into the mechanism.[5] Recently, our interests focused on the structural features of H-Pro-Pro-Xaa type peptides and in particular the trans/cis conformer ratio and hence its consequence for reactivity and stereoselectivity of the catalyst.[6] The poster will focus on the expansion of the scope of peptide catalyzed conjugate addition reactions to even more challenging substrates.

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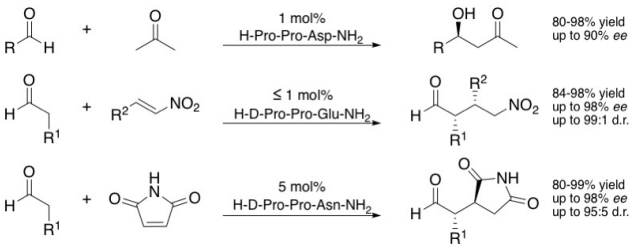

P72

Peptidic Stabilization of Gold Nanoparticles for Biological Applications

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Peptide-capped gold nanoparticles (GNPs) offer a multitude of possible bio-based applications including imaging, labelling, electron contrast enhancement, bio-sensing, photothermal therapy or as a drug delivery platform. GNPs act as a defined solid core, providing a tunable size and shape template for peptide-functionalized particle formation, and can serve as an optical read-out1. Peptides provide biocompatibility, solubility, stability under physiological conditions, and allow for the possibility of functionalization with drugs or other small molecules2-3. The peptide sequence CALNN was proposed by Lévy4, and was designed for GNPs to be used for photothermal therapy. The use of this peptide produced the highest grafting density ever reported, which ensured GNP stability in vivo. In this study, we modified the CALNN sequence to obtain a small peptide library and employed this to determine optimal peptide sequences for stabilizing different in respect to the GNPs sizes. All the peptide sequences are a short beta-structured unit, forming a shell of 1 nm thickness around the GNPs, and contain a free thiol at the N-terminus to covalently bind to the GNP surface. By adding or substituting a beta-sheet forming residue, we managed to stabilize a range of differently-sized GNPs. The effect of surface curvature defined the composition of the peptide required, with the inclusion of bulkier residues necessary for the stabilization of large GNPs. Our peptide-capped GNPs can be modified with extra moieties to demonstrate their potential as imaging and therapeutic agents. For example, a click-handle can be attached to enable orthogonal cell-labeling, and to act as an imaging tool. Alternatively, the stabilizing peptide can be extended to incorporate a therapeutic sequence, thus yielding bioactive peptide-capped GNPs capable of generating immunogenic, bio-sensing, or specific targeting properties.

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P73

Tyrocidines and the creation of antimicrobial cellulose

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Agriculture and processing sectors are plagued with continual bacterial and fungal infections that lead to great losses in terms of product spoilage and costs of recall. Since treatment and factory reset can be costly, it is favourable to invest in the prevention of surface infection. This can be done by creating antimicrobial surfaces by covalently or non-covalently attaching an antimicrobial agent to a surface. Antimicrobial peptides have been identified as possible candidates for this purpose. Tyrocidines (Trcs), antimicrobial cyclodecapeptides, have a broad spectrum of activity, as well as a limited resistance potential. Moreover, its inherent bio-stability and tendency to adhere to various surfaces make Trcs ideal peptides for creating antimicrobial materials. We observed that these peptides have a preference to bind to celluloses while still maintaining its activity against Gram-positive bacteria. Furthermore, it was shown that Trc-treated cellulose would maintain activity over broad pH and temperature ranges, as well as after multiple water washes. Which begs the question: How does the Trcs associate so tightly with cellulose? The inherent fluorescence of Trp residues in the Trcs was used to study the peptide association/dissociation to cellulose. Quenching of the fluorescence at low acetonitrile (ACN) was observed, indicated the stacking of the tryptophan aromatic rings eluding to the peptides forming high order structures. Trcs also only bound to the cellulose at 5-10% ACN (v/v). After binding, only a third of the bound Trcs could be removed over 20 minutes of washing with 50-60% ACN (v/v). Trc binding to the cellulose is driven by the concentration of peptide available usually binding half of the peptide in the first hour, regardless of the starting concentration, indicating layering by self-association or oligomerisation. The oligomerisation of the Trcs was studied with circular dichroism (CD) and ion-mobility mass spectroscopy (IMMS), specifically comparing 5% and 50% ACN to mimic a polar and amphipathic environment. As IMMS is performed in vacuo, only polar interactions remain. Similar oligomeric structures, from dimers to octamers, were observed for both conditions. The Trcs displayed CD spectra with a pronounced deepening of the minima at 206±2 nm and 215±2 nm from 5% to 50% ACN. Based on the results obtained and known amphipathic nature of the dimeric Trcs, the current hypothesis is that if higher order oligomers occur, it will selectively bind to the hydrophilic cellulose. We further propose that the first layer of peptide could bind covalently to the cellulose via the Mailard reaction between the Lys/Orn residue and glucose moieties. The Trcs are then layered via self-assembly in higher order oligomers and the amount of deposited Trcs is depended on concentration. The antimicrobial Trcs-containing cellulose can be utilised in agriculture, paper industry and food packaging, ultimately limiting surface contamination and product spoilage.

P74

Modification of metal surfaces using TCM-derived cyclotides: an antibacterial and antibiofilm study

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Modification of metal surfaces with antimicrobial peptides is a promising approach to reduce bacterial adhesion. Here, cyclic peptides or cyclotides, possessing remarkable stability and antimicrobial activities were extracted and purified from a traditional Chinese Medicine (TCM)- Viola philippica Cav., and identified using mass spectrometry. Cyclotides were subsequently utilized to modify stainless steel surfaces via polydopamine-mediated coupling. The resulting cyclotide-modified surfaces were characterized by Fourier transform infrared (FTIR) spectroscopy and contact angle analysis. The antibacterial capacity of these cyclotides against Staphylococcus aureus was assessed by Alamar blue assay. The antibiofilm capacity of the modified surfaces was assessed by crystal violet assay, and scanning electron microscopy (SEM). A composite of Varv A, Kalata b1, Viba 15 and Viba 17 (P1), Varv E (P2), and Viphi G (P3) was isolated and identified. FTIR analysis of the modified surfaces demonstrated that cyclotides bound to the surfaces and induced reduction of contact angles. Antimicrobial effects showed an order P3 > P1 and P2, with P3-treated surfaces demonstrating the strongest antibiofilm capacity. SEM confirmed reduced biofilm formation for P3-treated surfaces. This study provides novel evidence for cyclotides as a new class for development of antibacterial and antibiofilm agents.

P75

Selective gold recovery by peptides from homogenous aqueous solutions containing noble metal ion contaminants

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In these days, noble metals are used as decorative items, electrodes, etc. However, because of limited production of noble metals, recovering noble metals from electronic materials and liquid wastes are quite important. For instance, liquid-liquid extraction method enables to stepwise recovery of noble metal species from liquid waste, but it uses large amount of organic solvents and extracting agents, and takes time for completion due to biphasic reaction. In the previous studies, it was found that RU006, a 9-residue-peptide formed self-assembled nanostructures and reduced gold ions to afford metallic gold [1, 2]. The mechanism would be applicable to selective gold recovery from homogeneous aqueous solution containing metal ion contaminants. In this study, aromatic ring-containing RU006 and its derivatives were employed to develop selective and stepwise separation/recovery of gold ions in the presence of platinum ions. These peptides were allowed to self-assembly in the mixture of HAuCl4 and H2PtCl6 at 40°C for 24 hours. Formations of metallic particles were detected by UV-vis spectroscopy. Elemental analysis of the precipitates after centrifugation of the reaction mixtures was conducted by EDS-FE-SEM. Efficiency of noble metal recovery by peptides was conducted by ICP-OES. We found that all peptides successfully recovered gold ions in 90% efficiency from the mixtures and one of them enabled recovery of gold in 7.5 times more selectively than platinum from homogeneous aqueous solutions. It would contribute to the development of sustainable society.

This study was financially supported in part by The Joint Research Center for Science and Technology, Ryukoku University (Japan).

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P76

Detection and characterization of new blood peptide analogues using different voltamperometric techniques

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The oxidation and adsorption reactions of the peptides at an electrode surface in different electrolyte media are relevant in order to be investigating the interfacial behavior of these compounds and to be calculating their acid-base dissociation constants. In this regard the voltamperometric properties of some analogues of valorphin (Val-Val-Tyr-Pro-Trp-Thr-Gln) have been studied using hanging mercury drop electrode (HMDE) and a platinum electrode as working electrodes, Ag/AgCl, (3 mol l-1) KCl electrode as a reference electrode and a carbon electrode as an auxiliary electrode. The redox potentials and dissociated constants of the compounds were determinated under various experimental conditions, e.g. pH of the electrolyte solution, scan rate and mode of sweep (differential pulse DP and cyclic voltamperometry CV). Electrochemical oxidation of peptides at two type working electrodes have shown that there is strong adsorption of all species at anodic potentials in neutral solution (phosphate buffer solution: pH = 7.0 $\pm$ 0.1). Analysis of the wave shape, pH dependence, and concentration dependence of the oxidation process leads to the conclusion that the overall reaction of the peptides at mercury electrode could be described by the equation 2Vlf(Trp) + Hg $\longleftrightarrow$ Hg(Trp)2 + 2H+ + 2e- where Vlf(Trp) is valorphine containing tryptophan: Val-Val-Tyr-Pro-Trp-Thr-Gln. The fact that the mercury complex of Vlf(Trp) derivatives are strongly adsorbed at mercury electrodes leads to the possibility of employing differential pulse polarography with deposition of the mercury drop for the determination of low concentrations of this class of peptides. Limits of detection in the 1x10-6 to 1x10-5 mol L-1 concentration range are available.

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P77

Peptidyl Microbeads for Modification of a Direct Peptide Reactivity Assay (DPRA) in Skin Sensitization Assessment

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A direct peptide reactivity assay (DPRA) is one of the most promising method as a robust alternative to animal testing for skin sensitization analysis. In DPRA, a chemical substance is mixed with peptides. After 24 h reaction, HPLC is used to measure unreacted peptides, and skin sensitization is predicted based on the percent decrease in unreacted peptides [1]. Since the test substance and peptides are both in aqueous solutions during DPRA, DPRA cannot appropriately assess poorly water-soluble substances. In addition, HPLC measurement has relatively cumbersome procedures. This precludes the ready assessment of numerous types of samples in a short period of time. In this study, we immobilized the DPRA peptides to amphiphilic microbeads. Then using these peptidyl microbeads, two modified skin sensitization assay systems were developed in order to address these limitations in DPRA. We utilized a photo-labile linker [2, 3] and an unreacted peptide sequence as an internal standard in the first system, and thiol or amino group indicators [4, 5] for detecting amount of unreacted peptides were used in the second system. We examined test chemicals including poorly water-soluble substances by our two modified methods. The results indicated that the both two methods were able to appropriately assess all test chemicals including poorly water-soluble substances that conventional DPRA was unable to assess. Additionally, our method offered easy handling and general versatility, and more reproducibility and accuracy than the conventional DPRA. Thus, these studies successfully improved the conventional DPRA. Our method provides a high-throughput testing for skin sensitization with easy handling.

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P78

Label-free analysis of proteins in human cells in relation to cytotoxicity, viability and differentiation processes

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In the last years, vibrational spectroscopies have been increasingly used to investigate a large variety of biological samples, from complex tissues to single living cells. The label free nature of these techniques, together with their applicability in physiological environments, allows to study biochemical variations occurring in biological processes without damaging or altering the cell status. Following characteristic bands, we studied alterations of the protein content of different cell types. We used attenuated total reflection infrared spectroscopy (ATR-FTIR) to study the formation of prefibrillar aggregates in cord blood samples and HuDe (Human Dermal) adherent fibroblasts related to the cytotoxic effect of dimethyl sulfoxide (DMSO) (1). Due to superior spatial resolution and reduced background signal from the culture medium and intracellular water, Raman microspectroscopy allows the analysis of single living cells in their physiological environment. We were, thus, able to follow variations of the protein content without cell fixation and staining in single neuroblastoma and glioblastoma cells in adhesion on substrates of interest in neurosciences (2). Moreover, using a 532 nm exciting wavelength, it was possible to observe the strong resonant Raman scattering of cytochrome c. Since it acts as a trigger of caspase cascade activation resulting in the disassembly of proteins, the observation of the dynamics of cytochrome c reveals the initiation of the apoptotic process before morphological changes are visible. Therefore, the analysis of the cytochrome c band provided important information regarding cell viability in stress conditions and during the differentiation process. This work confirms the potential of Infrared and Raman spectroscopies to detect protein alterations that are indicative of the cell status and that can be possibly used for diagnostics or as a tool for evaluating new therapies. This research project was supported by PAT (Autonomous Province of Trento) (GP/PAT/2012) "Grandi Progetti 2012" Project "MaDEleNA", "Fondo d'Ateneo per la ricerca di base 2014" - D.D.N. 40/2015 DCBB-UniPG and by Fondazione Cassa di Risparmio – Perugia with the project 2015.0332.021 "Analisi ed ottimizzazione del processo dicrioconservazione di linee cellulari mediante tecniche spettro-

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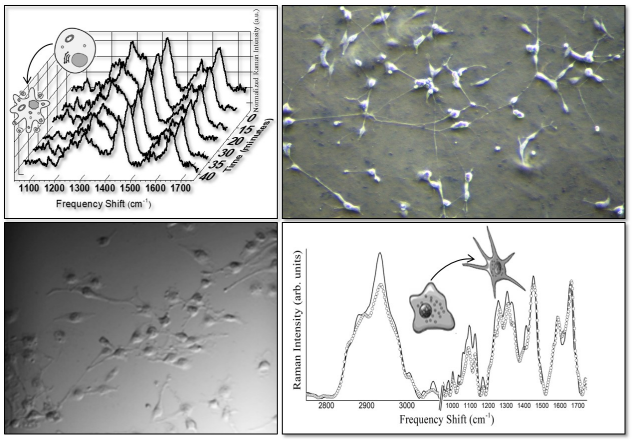

P79

Extracellular vesicles oriented by shear force can be studied using polarized light spectroscopy

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Extracellular vesicles (EVs) are currently in the scientific focus showing a great potential to revolutionize the diagnosis and therapy of various diseases. Despite intense investigation, however, many properties and mechanisms remain indefinable due to the lack of standardization of isolation and characterization methods which hinders the translation of EV-based diagnostics into clinical use. Our knowledge may rapidly increase on EVs if these systems could be the subject of additional structural methods used commonly for lipid systems. Research of EVs, having lipid composition very similar to those of the secreting cells have opened the way to potentially use complex lipid vesicles in biophysical characterization of biomolecules, such as antimicrobial peptides or membrane associated proteins. Monitoring the orientation of biomolecules associated to EVs can provide valuable information on their structure and interaction with the lipid membrane.

Extracellular vesicles were isolated from human erythrocytes and characterized by flow-linear and circular dichroism spectroscopy (flow-LD, CD), freeze-fracture electron microscopy (FF-TEM), dynamic light scattering (DLS) and attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR). The Soret-band of the LD spectra demonstrates that hemoglobin molecules are attached to the lipid bilayer in freshly released EVs. During storage this interaction ceases coupled to major protein conformational changes relative to the initial state. In overall, we propose that both LD and circular dichroism (CD) spectra provide simple, rapid, yet efficient ways to track changes in membrane-protein interactions of EV components at the molecular level which may also give insight to processes occurring during vesiculation.

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Acknowledgment:

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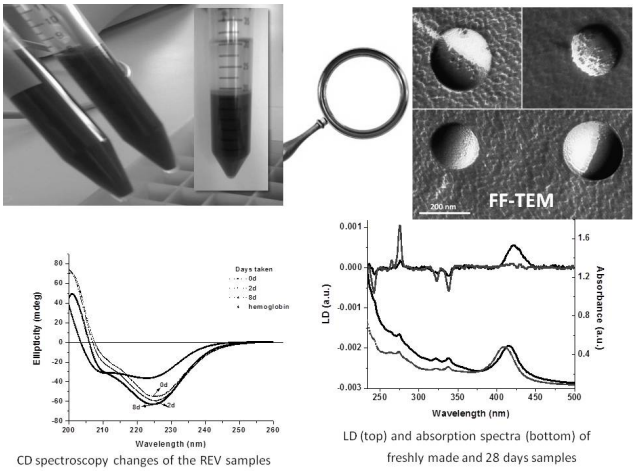

CD spectroscopy changes of the REV samples

LD (top) and absorption spectra (bottom) of freshly made and 28 days samples

P80

Modulating peptide hydrogel strength through halogenation

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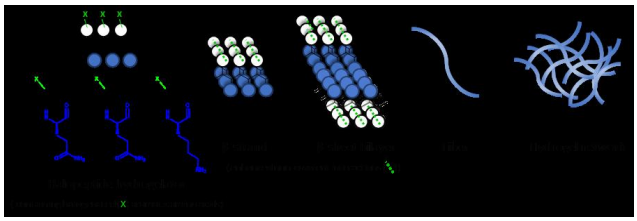
Due to their biocompatibility, low toxicity and physical properties, peptide hydrogels represent a promising class of biomaterials for medical applications. Previously, we investigated amphipathic peptide sequences able to form hydrogels [1]. The developed hexamer sequences possess alternating hydrophilic and hydrophobic residues. Because earlier findings have shown that the hydrophobicity of the composing amino acids plays a key role in the gelation process, further elaboration of the hydrophobic amino acid was scoped to improve the strength of the peptide fibers, while keeping the peptide length to a minimum. Preliminary in-house calculations and quantifications of the non-covalent interactions index (NCI) [2] between halogenated aromatic amino acids, have determined a significant increase of the NCI once the aromatic amino acids phenylalanine (Phe) and tryptophan (Trp) are halogenated. To experimentally validate the calculations, commercially available halogenated Phe were directly incorporated into SPPS and selected halogenated Trp residues were synthesized, via chemical synthesis followed by an enzymatic deconvolution and Fmoc-protection, yielding building blocks compatible with SPPS. Consequently, a library of halogenated Phe and Trp-based peptides was synthesized. Subsequently, quantitative results were needed to confirm the hypothesis and the rigidity of the peptide hydrogels was evaluated through dynamic rheometry. These measurements indicated that the halopeptides have a higher rigidity, in comparison with the non-halogenated lead sequences, with up to a hundred-fold increase in the storage modulus G'.



P81

A Self-assembling Peptide Hydrogel for Ultrarapid 3D Immunoassays

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Diagnostic array platforms rely on the intimate structure-function relation of immobilized probes. In this context, hydrogels are appealing semi-wet systems to locally confine biomolecules while preserving their structural integrity and function. Yet, limitations on biomolecule diffusion rates or fabrication difficulties have hampered their broad application. Here, using micromolar concentrations of a self-assembling peptide, a printable and self-adhesive hydrogel was obtained and applied to the 3D immobilization of biomolecules on microarray slides. This soft matrix represents a robust and versatile material allowing selective tuning of analytes diffusion, that is here exploited to run in-gel immunoassays under solution-like conditions in an unprecedented (<10 min) time frame. The developed material overcomes major limitations associated to hydrogel for immunoassays, widening the perspectives of easy fabrication of multifunctional biointerfaces for high-throughput, molecular recognition assays.

P82

Development of new supramolecular nanostructured materials based on peptide hydrogelator Ac-L-Phe-L-Phe-L-Ala-NH2 with embedded liposomes for potential biomedical application

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In recent years design and synthesis of self-assembled nanomaterials with diversified structures and functionalities via fine tuning of supramolecular building blocks increased rapidly (1). Self-assembling peptides have been widely recognized as nanomaterials with high potential for a extensive range of biomedical applications from drug delivery to tissue enginering, owing to their hydrophilic character and biocompatibility (2,3). The aim of the present study was preparation and characterization of supramolecular systems based on peptide hydrogelator(4) Ac-L-Phe-L-Phe-L-Ala-NH2 and liposomes with incorporated model proteins. The gelling properties of the hydrogelator Ac-L-Phe-L-Phe-L-Ala-NH2 with addition of liposomes and proteins were studied. Liposome formulations of BSA and FITC-BSA incorporated in the hydrogel were characterized by electron and confocal microscopy. Transmission electron microscopy (TEM) confirmed incorporation of liposomes into the hydrogel. In gel samples with built-in BSA, the gel network preserved integrity, but it was noticed that the gel fibers were thinner and thicker. Confocal microscopy proved the incorporation of liposome formulations of FITC-BSA in the hydrogel. It was also shown that liposomes do not impair the gel network of hydrogelator at lipid concentrations lower than the gelator concentration. We confirmed that use of higher amounts of protein albumin (BSA)/FITC-BSA resulted in slower gel formation or inability to transition into gel. In order to investigate the potential of using the prepared nanostructured supramolecular systems in immunodiagnostic and immunotherapy, complex protein mixture isolated from allergen pollen of Ambrosia elatior plant was successfully incorporated into the tested system. References: 1. J. Boekhoven , W. E. Hendriksen , G. J. M. Koper , R. Eelkema , J. H. van Esch , Science 2015 , 349 , 1075 2. X. Du, J. Zhou, J. Shi, B. Xu, Chem. Rev. 2015, 115, 13165−13307,DOI: 10.1021/acs.chemrev.5b00299 3. R. Dong, Y. Pang, Y. Su, X. Zhu, Biomater. Sci., 2015, 3, 937. 4. T. Pospišil, L. Ferhatović Hamzić, L. Brkić Ahmed, M. Lovrić, S. Gajović, L. Frkanec, Biomater Sci. 2016; 4: 1412–1416.

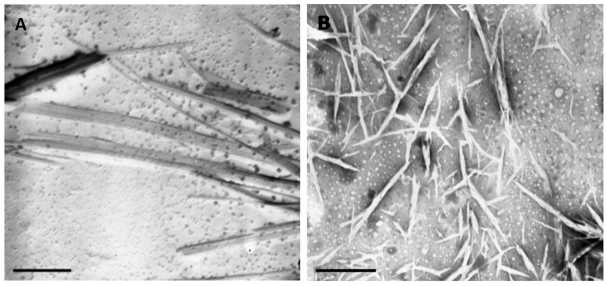

Figure 1 TEM images of liposomes in hydrogel. (A) Pd-shadowed. Lipids:gelator = 1:2.5, (B) PWK-stained. Lipids:gelator = 1:5. Magnification 10 000x, scale bar= 1 µm.

P83

Improving Peptide Studies with Miniaturised Mass Spectrometry

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Peptides are important building blocks of many biologically active compounds so they must be carefully characterised, and their quality checked throughout processing. Mass Spectrometry (MS) has always been a well-used tool in the analysis of peptides, however not always accessible at the point-of-need. Miniaturised MS allows the capability of mass detection to be available for real-time, on-line analysis. This study shows the integration of a miniaturised Mass Spectrometer (MS) with a Nano-LC system. The data obtained of the commonly studied cytochrome C digest, shows an order of magnitude increase in sensitivity compared with traditional UV detection. This study showcases the possibility to improve analytical capabilities in peptide studies with little increase in footprint or resources required.

P84

Correlation between the structure and oligomerisation of the three main cyclodecapeptides from the tyrocidine group

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[2]Dr Harisingh Gour Central University Sagar, India

The tyrocidines (Trcs) are a group of cyclodecapeptides [cyclo(D-Phe1-L-Pro2-L-X3-D-X4-L-Asn5-L-Gln6-L-Tyr7-L-Val8-L-X9-L-Leu10] produced by the soil bacterium Brevibacillus parabrevis. The three major analogues, TrcA, TrcB and TrcC, contain an ornithine residue in position X9 and vary at the aromatic dipeptide moiety (L-X3-D-X4) with TrcA containing L-Phe3-D-Phe4, TrcB L-Trp3-D-Phe4 and TrcC L-Trp3-D-Trp4. These Trcs have potent but differential antimicrobial activity toward a broad range of pathogens including Gram-positive bacteria, fungi and protozoa (Plasmodium falciparum). Differences in antimicrobial activity seem to be dependent on the identity of the aromatic amino acid residues in L-X3-D-X4. Trc dimers have been proposed to be the membrane active moieties. Electrospray mass spectrometry (ESMS) analysis and ion mobility mass spectrometry (IMMS) supported the formation of highly stable dimers by all three Trcs. However, the Trcs oligomerise into high order structures and we detected up to octamers with ESMS and IMMS. Analysis of 24-hour incubated Trc samples by circular dichroism (CD) indicated differences between the three Trcs regarding their hydrogen bonded structures. All three Trcs displayed CD spectra with a pronounced deep minimum at 206±2 nm and shallower minimum at 215±2 nm in 50% trifluoreoethanol (TFE). However, the ellipticity minima of TrcC was significantly less than that of TrcA and TrcB, both in water and in 50% TFE. Visualisation of nanostructures formed by the Trcs through scanning electron microscopy showed freshly prepared TrcA formed well defined vesicles, in contrast to aggregated vesicles observed for TrcB, while vesicles were only detected for TrcC after 24 hours of incubation. Comparison of the nanostructures formed by the three peptides after 24 hours by atomic force microscopy showed both TrcA and TrcB formed vesicles with a diameter of ±0.3 μm while the TrcC vesicles were smaller than 0.1 μm. The nanostructures could be related to the activity of the peptides with the more hydrophobic TrcA that rapidly forms higher order and nanostructures being more active against slower growing fungi and protozoa. TrcB forming abundant dimers, but less defined macrostructures have good activity against protozoa, but much weaker activity against fungi, while being much more active against bacteria. TrcC forming less dimers and smaller nanostructures has appreciable activity against fungi and bacteria, but low activity against protozoa. These data show the importance of D-Phe4 in activity and the association of the Trcs into higher order structures by forming π-π stacking interactions complementing the anti-parallel β-sheet structures formed by the Trcs.

P85

Label-free detection of high-density peptide arrays

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Protein-protein or protein-peptide interactions are crucial for most cellular and biochemical processes. Especially peptides represent interesting emerging drug targets. Therefore, the demand for high-throughput screenings of large peptide libraries is rising. In this study, we used high-throughput label-free screening technology to analyse the binding of a target to a large number of peptides in a fast and simultaneous assay. The high-density peptide array consisting of two different FLAG epitope variants and five different streptavidin-binding peptides was generated through photolithographic immobilisation. Each of those different peptides was generated in different spot sizes (7.4 – 111 μm) and multiple replicates among the array to evaluate data reproducibility. We then observed the binding of an anti-FLAG antibody to the two FLAG epitopes and the binding of streptavidin to the five streptavidin-binding peptides by using label-free single colour reflectometry (SCORE). Kinetic analysis on all binding events was performed to further characterise the protein-peptide interactions. We could show that this technology enables a precise, fast, and reliable characterisation of protein-protein interactions and represents a novel and unique combination of high-density peptide microarrays with label-free read-out.

P86

Chemical labeling associated to Mass spectrometry as a powerful tool for peptide detection and quantification in biology

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IBMM, France

Mass spectrometry represents a method of choice to identify and characterize peptides and proteins present in complex biological mixtures at low concentrations. In the attempt to develop potent MS methodologies, we work to the design and synthesis of chemical tags able to increase detection sensitivity and specificity through direct MS detection and/or directed fragmentation. On one hand, we aim at developing a novel approach that relies on the joint use of MALDI mass spectrometry and original labeling chemistries designed to specifically enhance the ionization of the tagged molecules. MALDI is well-known to provide very sensitive detection but not commonly used for quantitative measurements. Using MALDI for such purposes is challenging and has been successfully applied to track peptides at low concentrations in various media. We developed a methodology associating HCCA-targeted peptide to be analyzed by MALDI-MS in a matrix such as HCCE. This original approach allowed to selectively enhance and discriminate the MALDI-MS signals of targeted peptides. This concept was successfully applied to protein structure issues illustrated by the cross-linking of a model protein and peptide quantification for pharmacological studies of receptor/ligand systems. On the other hand, we focused on N-terminal positively charged peptide derivatization as efficient agents for directed ESI MS fragmentation. We explored labeling by pyridinium-based molecules, well known in chemistry literature, generally for enantio-separation, pharmaceutical or biochemical analysis, showing a great tendency to meet some of the desired requirements to investigate the field of ESI-MS qualitative and quantitative analysis of biomolecules like proteins or peptides. This technology should have a great impact in biosciences, in particular in research laboratories dealing with pharmacology. We will give you an account on the development of these methodologies.

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P87

Dynamic stereoselection of kinetically-inert DNA-binding metallopeptide cylinders

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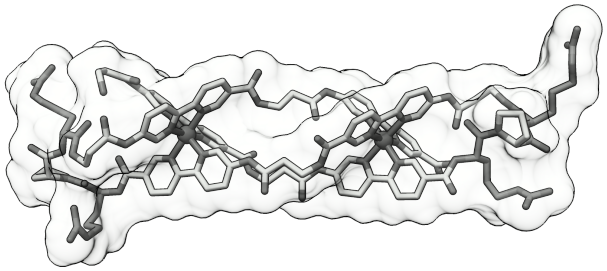
Helicates, discrete metal complexes in which one or more organic ligands are wound around and coordinate two or more metal ions. Helicates are inherently chiral species that can appear as right-handed, or left-handed helicity, according to the orientation in which the ligands coil around the helical axis defined by the metal centers. Indeed, the supramolecular chirality of helicates represents an additional challenge for the synthesis of these complexes and, despite some noteworthy examples, no general approach for the efficient and versatile stereoselective synthesis of helicates is yet available. We propose that peptides represent ideal platforms for the programmed assembly of helicates—and, in general, of supramolecular metallostructures. In this context, we present the synthesis and study of peptide helicate precursors featuring 2,2'-bipyridine ligands that predictably fold into complexes with defined chirality in the presence of metal ions. The folding of the peptide ligands into bimetallic helicates is directed by a particular heterochiral combination residues in the loops connecting the coordinating bipyridines that induce a tight turn in the sequence and allow the thermodynamic control over the supramolecular chirality of the final three-stranded metallocylinders after the coordination of Co (II) or Fe (II) ions. Furthermore, the Co(II) peptide helicates can be locked into defined configurations by oxidative treatment with cerium(IV) ammonium nitrate, thus providing a straightforward method for stereoselective selection of kinetically inert metallopeptides in aqueous media. The DNA-binding properties of these labile Co(II), Fe(II), and inert Co(III) peptide helicates was studied by fluorescence titrations and electroforetic mobility shift assays with three-way junctions and double-stranded DNAs. Both the labile and the inert complexes display good DNA-binding properties and selectivity towards three-way junction DNA.



P88

Peptide Segments of Type I Collagen Preferably Preserved in Archaeological Bones and Animal Glues

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Collagen is arguably one of the oldest proteins found to survive in archaeological specimens. In the tryptic digest of α1 and α2 chains of type I collagen, each of which has 1,000-amino acid-long triple helical region, our mass spectrometric study has identified a few peptides almost invariably appeared irrespective of the animal species and the ages as old as 10,000 years before present (BP). These include a peptide with the sequence GLTGPIGPPGPAGAPGDKGEAGPSGPAG PTGAR corresponding to residue 763-795 of bovine α1 chain (C763A1). We also identified the sequence GAPGAIGAPGPAGANGDRGEAGPAGPAGPAGPR of bovine α2 chain (C763A2), which is precisely the same length (33-amino acid-long) as that of C763A1 and homologous, characteristically conserving the pair of acidic and basic amino acids DK and DR in the middle of the peptide chain. The similar pair of α1 and α2 sequences has been found for those of GETGPAGPPGAPGAPGAPGPVGPAGK (bovine α1, 1036-1061; C1036A1) and GYPGNAGPVGAAGAPGPQGPVGPVGK (bovine α2; C1036A2). The peptides in each pair C763A1/A2 and C1036A1/A2 are homologous but definitely contain different amino acids. Furthermore, mutations at a few amino acid residues in the sequences, or the difference in animal species, did not affect the detection of the respective peptides. These features allowed us to identify the animal species by amino acid sequencing. In fact, the peak of peptide C763A1 in the mass spectra of collagen digest is particularly useful as a marker in zoological mass spectrometry of archaeological specimens in which collagen is often the sole protein surviving while DNA has been diminished. It is most likely that these peptides are involved in considerably stable triple helical regions in which two α1 chains and one α2 chain are contacting very tightly to form the triple helix. Considering that these collagen peptides have been identified not only in archaeological animal bones up to 10,000 years BP but also animal glues dated to more than 4000 years BP, we could assume that the stability of the triple helix is responsible for the resistance of the respective peptide against degradation due to ageing. Note that animal glues in mural paintings used as a binder are exposed to natural environment without being protected from chemical and biological degradation. In particular, a tightly folded structure can serve to avoid digestion by proper proteases by shielding the peptide bonds in an interior of the triple helix. We will discuss the relationship between the amino acid sequences of these peptides and the stability of collagen.

P89

Use of Peptide Nucleic Acids for the Chemoselective Ligation of Biomolecules

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The DNA-like, base-pair-specific hetero-dimerization of peptide nucleic acids (PNA) has been utilized for a range of applications, including the alignment and pre-organization of biomolecules, generating spatial proximity, and enabling otherwise unlikely covalent reactions. Here, we explore the possibility of using complimentary PNAs as adapter domains for the chemoselective, site-directed covalent ligation of intrinsically multireactive molecules, such as peptides. In a first study using match and mismatch 5-mer to 10-mer PNAs, we could show that hybridization of matching sequences, with subsequent covalent stabilization of the resulting heterodimer via thioether bond, is a promising strategy for the highly selective and site-directed ligation of biomolecules.

P90

Chemoselective Modifications of Elastin-like Polypeptides Towards Synthetic Glycoconjugates

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There are relevant opportunities to develop innovative biomaterials at the frontier between biotechnology, peptide chemistry and traditional organic chemistry. The main challenge relies in the design of a robust and reproducible synthetic approach that will allow the establishment of structure-activity relationships. In this project, protein-engineering techniques are used to access functional precision polypeptide scaffolds with exquisite control over monomer sequence and length, namely with an exact "primary structure" [1]. Orthogonal bioconjugation strategies are subsequently applied to chemoselectively modify specific residues of the genetically-engineered polypeptides so as to introduce biologically relevant motifs, as a means to access thermoresponsive, well-defined and high molecular weight multivalent bioconjugates.

This project is specifically applied to the synthesis of chemical tools for glycobiology, where there is a critical need for the rational design of glycopolypeptides mimics for drug-targeting and vaccination strategies. To achieve this goal, a set of elastin-like polypeptide (ELPs) scaffolds was designed and produced recombinantly in Escherichia coli bacteria. Precision functional ELPs and diblock ELPs are subsequently modified chemoselectively onto specific residues lateral chain (i.e., methionine and lysine) to introduce pendant carbohydrate motifs at defined positions within the ELP backbones [2]. Thanks to their perfectly defined primary structures, these will be subjected to structure-activity relationship studies. Their propensity to recognize and bind specific lectins and receptors will be assayed as soluble chains and self-assembled nanoparticles in order to determine optimal ligand design for receptor binding and answer relevant biological questions.

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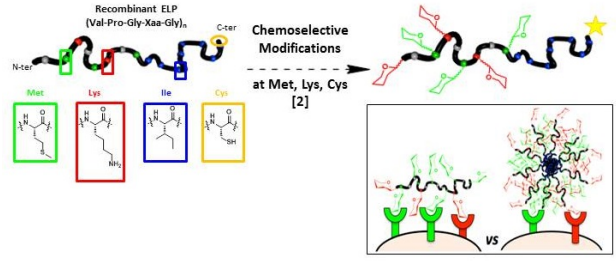

P91

Interactions of fructosyllysine and phenylboronic moieties in amphipathic peptides

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Glycation, one of the post-translational modifications of proteins, is a nonenzymatic reaction of amino groups of proteins and peptides with glucose yielding Amadori products (fructosyllysine derivatives).[1] These derivatives are the key intermediates in the formation of advanced glycation end products (AGEs).[2] Fructosyllysine moieties interact selectively with phenylboronic acids[3] as well as borate ions[4]. The affinity of phenylboronic acids towards Amadori products found the application in enrichment and isolation of glycated peptides as well as their selective detection.[5] Herein we designed and synthesized series of penetrating peptide (MAP) analogues. MAP (Ac-KLALKLALKALKAALKLA-NH2) is an α-helical amphipathic peptide that shows high cellular uptake.[6] The designed analogues contain in the sequence both the glycated lysine moiety and phenylboronic acid. We investigated the intramolecular interactions between the fructosyl moiety and phenylboronic acid incorporated in various positions of peptide chain using high-resolution mass spectrometry (HR-MS). Additionally, we studied the influence of these interactions on secondary structure of peptides using circular dichroism (CD) and nuclear magnetic resonance (NMR).

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P92

Double-head lipopeptide surfactants as potential antimicrobial agents

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Short cationic lipopeptides are an interesting group of antimicrobial compounds. They satisfy the amphipathicity and total positive charge conditions, essential features of antibiotic peptides. Additionally, due to the surfactant-like structure they exhibit surface-active properties. Thus, they can act as both preservatives and stabilizers in multiphase systems. A group of compounds of our particular interest are double-head lipopeptide surfactants. This class of compounds can be obtained by lipidation of the side chain of one of the middle lysine or its shorter homologs (Fig. 1). To our knowledge, such peptide-like compounds have not been tested as antimicrobials so far.

The lipopeptides studied are expected to undergo self-assembly in solution above a certain concentration referred to as the critical micelle concentration. Both the larger headgroup charge and size enhance electrostatic repulsions among surfactant molecules. Consequently, a larger micellar surface is required, and progressively fewer surfactant molecules can be accommodated in a single micellar aggregate. Therefore, double-head lipopeptides are expected to form smaller and more dynamic aggregates in comparison with the single-head ones. It is believed that smaller aggregates of antimicrobial amphiphiles can penetrate more deeply into the bacterial cell surface that the large ones, this resulting in enhanced antimicrobial activity. Smaller aggregates are also expected to bind to a greater number of bacterial cells, and consequently operate more efficiently. All the lipopeptides were tested for antimicrobial activity. The isothermal titration calorimetry (ITC) was employed to determine thermodynamic parameters of the binding of the lipopeptides to anionic liposomes being a model of bacterial membrane. Molecular dynamic simulations were used to visualize the self-assembly properties. The results of our studies were discussed with regard to the parameters involved in the potency and cell specificity of cationic lipopeptides and their potential to be developed as antimicrobial agents.

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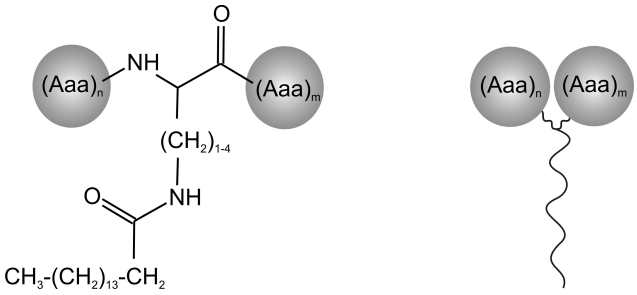
Figure 1. Double-head lipopeptide and schematic representation of the double-head surfactant.



P93

Studies of Bio-orthogonal self-assembled peptide-carbohydrates

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Oligogalacturonic acid (OGA) is known to dimerize into "egg-box" like structure in the presence of suitable amount of Ca(II).1 The dimerization between OGAs can be used as an intermolecular Ca(II) bridge to connect peptide units in order to form Ca(II) sensitive nanoassemblies, where the bridge can be disrupted again by adding monovalent cations or chelating agents. Here, we conjugated a 10-mer OGA chain at the N-terminus of a 23-mer and a 30-mer α-helical coiled coil peptide through an oxime linkage.2,3 The peptide-carbohydrate conjugate can assemble via two bio-orthogonal self-assembly principles; hydrophobic collapse between peptides, and egg-box dimerization between OGAs triggered by Ca(II). The dynamic light scattering studies showed that the size of self-assembly is affected by two important factors; the ratio between divalent and monovalent cations, and the number of heptad repeats in the peptide sequence. The size change of the nanoassemblies inspired us to redesign our peptide-carbohydrate conjugates by chromophore labeling. The chromophore will be labelled on the outer face of the coiled coil unit which allows us to study the shape of the nanoassemblies by using stochastic optical reconstruction microscopy.

P94

Synthesis of Aryl-Thioglycopeptides Through Chemoselective Pd-Catalyzed Conjugation

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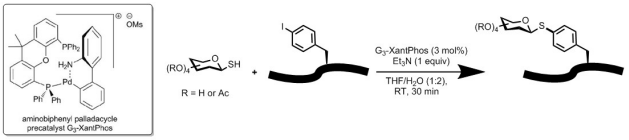
We describe herein a methodology for the thioglycoconjugation of p-iodophenylanaline (p-IPh) containing-peptides and aminoacids through palladium-catalyzed coupling with glycosyl thiols. Palladacycle pre-catalyst G3-XantPhos[1] was found to be highly efficient for the C¬S bond formation under semi-aqueous conditions. The S-glycoconjugation is operationally simple and displays broad substrate scope. A wide range of thioglycosylated amino acids building blocks were synthesized with complete control of anomeric stereochemistry. We studied the suitability of the synthesized building blocks in standard Fmoc-based SPPS protocols. Thioglycoconjugation was carried out at Tyr1 of a lipo-triazolo-peptide analogue of the neuropeptide kisspeptin-10 (KP10)[2] with the aim to improve its physicochemical properties (low water solubility, unwanted gel formation[3]). The expected thioglyco-lipo-triazolopeptide was obtained in similar yields as for the parent compound, demonstrating the compatibility of our N-Fmoc thioglucoaminoacid building block with standard Fmoc-SPPS protocols, including couplings, piperidine-mediated Fmoc deprotections and TFA-based cleavage. The thioglycopeptide was tested for its agonist activity towards the GPCR KiSSR, and showed similar subnanomolar EC50 as the parent compound. Its physicochemical properties were also briefly evaluated, however, no marked differences were observed as compared to the non-glycosylated peptide.[4] The strategy was also successfully applied to the thioglycoconjugation of unprotected peptides: to demonstrate the broad potential of this technique for late stage functionalization, we incorporated challenging β-S-GlcNAc- and α-S-GalNAc-derivatives into very long unprotected peptides derived from the mucin MUC1. The generated thioglycoconjugates were used as biochemical tools to study their recognition by O-GalNAc-specific lectins, suggesting future in applications of this method in chemical (glyco)biology. References: 1. See for example: (a) A. Bruneau, M. Roche, A. Hamze, J.-D. Brion, M. Alami and S. Messaoudi, Chem. Eur. J. 2015, 21, 8375–8379; (b) N. Probst, R. Lartia, O. Thery, M. Alami, E. Defrancq, S. Messaoudi, Chem. Eur. J. 2016, 22, 15006–15010. 2. (a) M. Beltramo, V. Robert, M. Galibert, J.-B. Madinier, P. Marceau, H. Dardente, N. De Roux, D. Lomet, A. F. Delmas, A. Caraty and V. Aucagne, J. Med. Chem., 2015, 58, 3459−3470; (b) C. Decourt, V. Robert, K. Anger, M. Galibert, J. B. Madinier, X. Liu, H. Dardente, D. Lomet, A. F. Delmas, A. Caraty, A. E. Herbison, G. M. Anderson, V. Aucagne and M. Beltramo M. Sci. Rep. 2016, 6, 26908–26918. 3. N. Nishizawa, Y. Takatsu, S. Kumano, A. Kiba, J. Ban, S. Tsutsumi, H. Matsui, Shin-ichi Matsumoto, M. Yamaguchi, Y. Ikeda, M. Kusaka, T. Ohtaki, F. Itoh, and T.Asami J. Med. Chem.2016, 59, 8804–8811. 4. The article of this work has been submitted



P95

Verification of mannan (polymannose)-peptide conjugation by SDS-PAGE

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Myelination of the nerve axons ensures the unhindered transmission of neuron impulses. Multiple sclerosis (MS) is an autoimmune disease whereby the myelin of the central nervous system (CNS) is destroyed, leading to paralysis and serious health problems. Myelin consists of proteins and lipids while in CNS, myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG) and myelin-associated glycoprotein (MAG) are the main proteins of the membrane sheath of myelin. MOG is an autoantigen associated with the pathogenesis of MS and experimental autoimmune encephalomyelitis (EAE, animal model of MS). The 35-55 epitope of MOG has been implicated in the induction of EAE in mice. Conjugation of this epitope with mannan polysaccharides via Schiff base has been extensively studied in our laboratory with the conjugates showing potential against EAE. In the presented study, the conjugation reaction and the quantification of the unconjugated peptide

were followed and verified by SDS-PAGE and high-performance liquid chromatography (HPLC). The optimum time needed for complete conjugation was six hours (yield of more than 95 %). The developed analytical methodology could prove useful for the study of conjugation between peptides and polysaccharides.

P96

Nucleus penetrating peptidomimetic synthesis and application

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Arginine rich peptides, belong to class of cell penetrating peptides that are able to transport the wide variety of attached chemical entities (cargos) through the cell membrane. Diversity of transported molecules is impressive and includes: DNAs, siRNAs, peptides, proteins and small drugs. Polyarginines comprise over five residues of L-arginine efficiently penetrate the cell membrane, and in some instances are part of more complex molecule which is able to deliver cargos to nucleus through nuclear localisation signal (NLS). The general aim of this work is to reveal the mechanism and properties of novel cell penetrating peptidomimetic. Such molecule contains six instances of L-2,3-diaminopropionic acid (DAP) connected through the peptide bond and modified on its side chain amino groups by substitution of 8-amidino-3,6-dioxaoctanoic acid (PEG) further abbreviated as (GO2). For group of compound we proposed the abbreviation DAPEG that is merged DAP and PEG. Such compound was synthesized on solid phase using Fmoc/Mtt chemistry by applying semi-monomeric approach. Obtained peptidomimetic is labelled by fluorophore moieties efficiently penetrates cell membrane and is transported directly to nucleus of primary and transformed cell without significant toxicity. Such compound is also resistant to protease action inside the cells. Such compound forming strong non covalent complex with DNA, which is resistant to nuclease action. In this work the panel of techniques aiming to deeply characterized novel nuclear penetrating peptidomimetics with different types of cargo moieties will be presented including DNA binding constant (thermophoresis, nanocalorimetry and surphase plasmon resonanse) and interactions with cell membrane.

P97

Design and Synthesis of Novel Silicon-containing Small Molecule Peptidomimetics with Nanomolar Anticancer Activities

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The problems associated with existing anticancer drugs are that many patients are unable to tolerate the high toxicities associated with chemotherapy (serious side-effects and toxicity-associated death). Thus, there is an urgent need to develop new first-line agents with reduced toxicity and side-effects. There is also a need for new drugs for cancer prevention in high-risk patients. More than a decade ago we developed a highly potent bradykinin (BK) antagonist peptide dimer, B9870 (also known as B201, CU201 and Breceptin) with excellent anticancer activity against small cell lung cancer (SCLC) [1]. The transformation of our B9870 BK anticancer peptide dimer into peptidomimetics led us to the discovery of BKM570, the first generation of our small molecule peptide mimetic with a simple 3 component "A-B-C" structure [1]. Structural optimization of BKM570 resulted our new generation of peptidomimetics with aminobisphosphonate and NSAID components [2,3]. In this presentation, we will discuss the synthesis and the structural modification of our anticancer "A-B-C" small molecules introducing silicon-containing components [4]. Using silicon over carbon has many advantages: a) compounds are more lipophilic than their carbon equivalent, b) silicon can improve compound permeability, c) silicon can lower compound toxicity, d) silicon can change receptor selectivity, e) silicon can enhance anticancer activity of compounds, f) silicon compounds can treat drug- resistant tumors. In our new compounds, GH1501, GH1503 and GH1504, the "A" component was modified to contain a silicon atom. These compounds were tested by the National Cancer Institute (NCI) in vitro against 60 human tumor cell lines. The NCI results showed that these small molecules are potential anticancer drug candidates with specificity against prostate and colon cancer cell lines. These compounds were less active against other cancer types including breast, central nervous system (CNS), leukemia, melanoma, non-small cell (NSC) lung, ovarian, and renal cell lines. GH1503 is the best silicon containing compound to date for prostate cancer cell lines (average IC50 less than 500 nM). GH1501, GH1503 and GH1504 are all active against colon cancer with similar activity (IC50 less than 500 nM). The Eli Lilly Open Innovation Drug Discovery Program suggested the target for GH1503 is EZH2, a Histone H3 Lys 27 (H3K27) Methyltransferase. These new silicon containing peptidomimetics were synthesized in solution phase using Boc-chemistry. They were purified by Reversed-phase high-performance liquid chromatography (RP-HPLC) and characterized by LC-MS. Our anticancer results will be discussed.

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P98

Pyrrole-based Macrocyclic Small-Molecule Inhibitors Targetting Oocyte Maturation

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Polo-like kinase 1 (PLK1) plays crucial roles in various stages of oocyte maturation. Recently, we reported that the peptidomimetic compound AB103-8, which targets the polo box domain (PBD) of PLK1, affects oocyte meiotic maturation and the resumption of meiosis[1]. However, to overcome the drawbacks of peptidic compounds, we designed and synthesized a series of pyrrole-based small-molecule inhibitors and tested them for their effects on the rates of porcine oocyte maturation. Among them, the macrocyclic compound (E/Z)-3-(2,16-dioxo-19-(4-phenylbutyl)-3,19-diazabicyclo[15.2.1]icosa-1(20),6,17-trien-3-yl)-propyl dihydrogen phosphate (4) showed the highest inhibitory activity with enhanced inhibition against embryonic blastocyst formation. Furthermore, the addition of this compound to culture media efficiently blocked the maturation of porcine and mouse oocytes, indicating its capacity to penetrate the zona pellucida and cell membrane. We investigated mouse oocytes treated with compound 4, and the resulting impairment of spindle formation confirmed PLK1 inhibition. The molecular modeling studies with PLK1 PBD also confirmed the presence of significant interactions between compound 4 and PLK1 PBD binding pocket residues, including those in the phosphate, tyrosine-rich, and pyrrolidine binding pockets. Collectively, these results suggest that the macrocyclic compound 4 may serve as a promising template for the development of novel contraceptive agents. [2]

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P99

Amphipathic Triazine based small molecules with Potent Anti-bacterial and Anti-atopic Dermatitis Properties

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The steady rise of drug resistant bacteria remains the major threat to the public health.1 Even though cationic peptide antibiotics (CPAs) found to be a potential source of treating the drug resistant bacteria, their transformation into a therapeutic is hindered by series of disadvantageous including, high protection cost, proteolytic stability, poor cell permeability and salt resisitance.2 To overcome this,we report the development of triazine based amphiphilic small molecular antibacterial agents as a mimics of lysine- and arginine-based cationic peptide antibiotics (CPAs). From the antimicrobial screening of these compounds against a panel of both Gram-positive and Gram-negative bacterial strains, we identified some potent compounds. Further, evaluation of these compounds against anti-inflammatory studies led to an array of four efficient compounds, DG-5, DG-6, DL-5, and DL-6. Moreover, these molecules displayed good potency against drug-resistant bacteria, including MRSA, MDRPA, and VREF. In addition, they inhibit the biofilm formation, and also maintains their antimicrobial activity in the presence of physiological salts and serum. Mechanistic studies, including cytoplasmic membrane potential, confocal imaging and flow cytometry suggest that DG-5, DG-6, and DL-5 kill bacteria by targeting bacterial membrane, while DL-6 follows intracellular targeting mechanism. We also demonstrate that these molecules are having therapeutic potential by showing the efficiency of DG-5 in preventing the lung inflammation of LPS-induced ALI mouse model. More intriguingly, DL-6 exhibited impressive potency on atopic dermatitis (AD)-like skin lesions in BALB/c mice model by displaying the proficiency in suppressing the pro-inflammatory cytokines. Collectively, these results suggest that they can serve a new class of antimicrobial, anti-inflammatory and anti-atopic agents with promising therapeutic potential.

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P100

Synthesis & Analysis of β-Peptides For The Specific & Non-specific Aggregation On Model Membranes

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In biological systems protein-membrane interactions form complex structures, which fulfill numerous functions such as signaling, regulation and domain formation. Furthermore, these interactions are of great importance to the functional and structural integrity of bio-membranes.1 Yet many aspects of the mechanism and dynamics of the protein-membrane interactions remain unknown, therefore artificial model systems might give an insight into the complex nature of protein-membrane interactions.2,3 The goal of this study is to establish model systems of aggregational peptide-membrane interactions by exploiting β3-peptide scaffolds. These scaffolds are known to form stable, well-defined 14-Helices as secondary structures and can be obtained via manual Solid Phase Peptide Synthesis (SPPS).6,7 These peptides are modified with molecular recognition units to enable specific and non-specific aggregation among each peptide, as well with hydrophobic moieties to achieve membrane interactions. These lipid-like modifications can be alkyl chains as well as cholesterol, which are able to insert into membranes spontaneously.8,9 The Peptide aggregation is achieved by pursuing two approaches. One approach is the incorporation of a chelating agent like a bipyridine derivative. These non-specific aggregation can be achieved by the addition of metal cations.10,11 The other approach is the use of nucleobase modifications, which offers a means of specific aggregation by Watson-Crick base pairing.12

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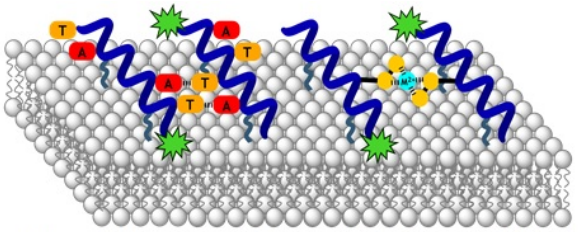

*Figure 1 Schematically illustration of the two different approaches for the aggregation of the β^3-peptides. **Right site**: Specific aggregation among the peptides through Watson-Crick base-pairing. **Left site**: Non-specific aggregation among the peptides through Metal-ligand-interaction.*

P101

Enopeptin A analogs: Synthesis & Activity

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The therapy of life-threatening infections significantly weakened by the global spread of antibiotic resistance has prompted a need for the development of novel, effective, and safe antibiotics. Novel antibacterial agents with unprecedented mechanisms of action, which are devoid of pre-existing cross-resistances, are therefore very necessary [1]. Natural products from soil bacteria (Actinomycetes), have served as a promising source of new structural leads in this area [2], in particular the cyclodidepsipeptides (CDP). CDP's show promising pharmacological activities and meet all criteria for good solubility and permeability. CDP's are capable like natural peptides, to interact with numerous proteins and show a large panel of activities (antitumor, anthelmintic, insecticidal, antibiotic, antifungal, immunosuppressant, antiinflammatory and antimalarial). We will present most recent achievments with our novel solid-phase assembled Enopeptin A analogs varying the R and R' positions, and discuss their structure-activity relationship following cell bio-activity screening assays. (1) Hinzen B. et al., Med. Chem. 2006, 1, 689 – 693 (2) Jordan D. Goodreid, J. Nat. Prod. 2014, 77, 2170−2181

P102

Immobilization by Surface Conjugation of Cyclic Peptides for Effective Mimicry of the HCV-Envelope E2 Protein as a Strategy toward Synthetic Vaccines

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Mimicry of the binding interface of antibody−antigen interactions using peptide-based modulators (i.e., epitope mimics) has promising applications for vaccine design. These epitope mimics can be synthesized in a streamlined and straightforward fashion, thereby allowing for high-throughput analysis. The design of epitope mimics is highly influenced by their spatial configuration and structural conformation. It is widely assumed that for proper mimicry sufficient conformational constraints have to be implemented. This paper describes the synthesis of bromide derivatives functionalized with a flexible TEG linker equipped with a thiol-moiety that could be used to support cyclic or linear peptides. The cyclic and linear epitope mimics were covalently conjugated via the free thiol-moiety on maleimide-activated plate surfaces. The resulting covalent, uniform, and oriented coated surface of cyclic or linear epitope mimics were subjected to an ELISA to investigate the effect of peptide cyclization with respect to mimicry of an antigen−antibody interaction of the HCV E2 glycoprotein. To the best of our knowledge, the benefit of cyclized peptides over linear peptides has been clearly demonstrated here for the first time. Cyclic epitope mimics, and not the linear epitope mimics, demonstrated specificity toward their monoclonal antibodies HC84.1 and V3.2, respectively. The described strategy for the construction of epitope mimics shows potential for high-throughput screening of key binding residues by simply changing the amino acid sequences within synthetic peptides. In this way, leucine-438 has been identified as a key binding residue for binding monoclonal antibody V3.2.

P103

Trifluoromethylated proline surrogates as part of 'Pro-Pro' turn-inducing templates for the design of β-hairpin mimetics.

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Proline is often found as a turn inducer in protein domains. Exploitation of its restricted conformational freedom led to the development of a D-Pro-Pro (pP) segment as a 'templating' unit, frequently used in the design of β-hairpin peptidomimetics. Recent studies revealed the stabilizing effect of CF3-proline surrogates on β-turn conformation, especially by controlling the cis-trans isomerization of the amide bond. In view of finding alternative templates with a stronger capacity to fix the β-hairpin conformation, we investigated different fluorinated analogs of the well-established D-Pro-Pro segment as β-turn promoter. A conformational study was performed on a set of 12 non-natural variants of pP hairpin sequence, incorporating TfmPro and TfmOxa. Out of the investigated combinations only TfmPro-Pro, TfmPro-TfmPro, TfmOxa-Pro, D-Pro-TfmPro, D-Pro-TfmOxa exhibited a strongly stabilized β-turn conformation, relative to the 'parent' pP. Considering synthetic feasibility, D-Pro-TfmOxa was selected for the incorporation into peptides. The increased preference of the selected peptide for the β-hairpin conformation was confirmed by molecular dynamics. To support those calculations, synthetic methodology was elaborated to prepare the peptide Fmoc-DPro-TfmOxa-Val-OH as a suitable building block for incorporation by SPPS in a cyclic β-hairpin mimetic sequence. Subsequent NMR conformational study is carried out to evaluate the β-turn-inducer propensity of these trifluoromethylated diproline surrogates.

P104

Functionalized Collagen Model Peptides

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Collagen is the most prevalent protein in mammals.[1] The protein has a fibrous structure that arises from right-handed triple helices that are formed from three, parallel, left-handed all-trans polyproline (II)-like single strands. Studies with short-chain collagen model peptides (CMPs) showed that the cis/trans isomerization of the amide bonds is the rate-limiting step for triple helix formation.[2] Preorganization of the amide bonds within collagen single strands into trans conformation is therefore expected to accelerate triple helix folding and result in increased triple helix stability. In 2015, our group showed that an apolar environment favors the trans isomer of acetylated dimethylamide of proline over the cis isomer.[3] Based on this result, we reason that a hydrophobic environment pre-organizes the single strands in a trans conformation and should result in stable, fast-folding triple helices. To create such a hydrophobic microenvironment, we covalently linked hydrophobic residues to CMPs, while still maintaining the solubility in an aqueous environment. Thermal denaturation experiments and temperature jump experiments showed that the CMPs with the hydrophobic attachment are remarkably more stable and faster-folding than CMPs without a hydrophobic moiety. NMR studies and native MS confirmed the observations. The results of these experiments as well as those obtained with other functionalized CMPs[4,5] will be presented.

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P105

Design of new cyclic plasmin inhibitors

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Plasmin (Plm) is involved in the fibrinolysis pathway and pathologic events

including angiogenesis, tumor invasion, metastasis, and alters the expression of cytokines. A growing body of data suggests that a Plm inhibitor is a potential candidate as an anti-inflammatory and anti-cancer agent. The X-ray crystal structure of YO-2 [trans-4-aminomethylcyclohexanecarbonyl-Tyr(Opicolyl)-NH-Octyl: IC50 = 0.53 μM for Plm] [1] in complex with microPlm (serine protease domain of Plm) reveals its binding mode [2], in which the cyclohexylmethylamine moiety inserts into the S1 subsite. At the S1' subsite, the Tyr moiety forms a hydrogen bond with K607, and the pyridine moiety forms an imperfect π-π stacking. Contrarily, the hydrophobic octyl moiety does not have extensive interaction with the S2' subsite; it points away from the protease surface. In this work, we describe cyclic Plm inhibitors, in which YO-2 was cyclized between the side chains of P2'and P3'site residues with the aim of creating extensive interactions at the S2'site. The ring segment of the inhibitor could fit well into the S2'site to enhance the binding affinity. The cyclization was performed via ring closing metathesis (RCM) or lactam formation: 16-, 18-, 20-, and 22-membered ring compounds were obtained. The cyclic compounds inhibited Plm more strongly than the corresponding liner compounds. Furthermore, the compounds having an olefin exhibited stronger Plm inhibition than that bearing a lactam. Among compounds, the cyclic compound possessing an olefin with 20-membered ring showed the strongest Plm inhibition (IC50 = 3.68 μM), which was ten-times weaker than YO-2. It implies that the ring structure is useful to occupy the S2' site; however the other elements such as hydrophobic interaction are required to increase the contact points.

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P106

ON/OFF repetitive control of protease activity using peptidomimetic ligands through biotin-streptavidin affinity

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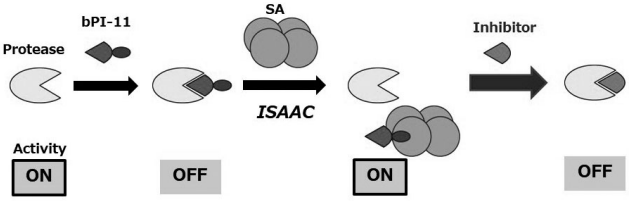
Peptidomimetic protease inhibitors targeting the enzyme active site are useful as research tools and drugs. Such small compounds are difficult to remove from the proteases once they bind, requiring dilution or protein denaturation procedures. Our research group developed "removable inhibitors" that was designed with a direct conjugation of biotin and a strippable property, through extremely strong affinity to streptavidin (SA) even after the binding to the target protease, in which the methodology was named ISAAC. To extend the utility of removable inhibitors, we studied the ON/OFF repetitive control of enzymatic activity derived from affinity-purified proteases.

Protease inhibitors containing an amino group were coupled with biotin using mixed anhydride method. Peptidomimetics with a carboxylic acid were biotinylated through an additional hydrazide bond. The target proteases were purified from human serum or cellular lysate using magnetic beads possessing the conjugated inhibitors with a long spacer. The bound protease was eluted by a solution containing removable inhibitors and the eluate was examined for activity detection using the corresponding FRET substrates along with SA addition.

Enzymatic activity of HIV-1 protease was inhibited by a removable inhibitor, bPI-11, which was sufficiently recovered by SA addition, and then inhibited again by extra addition of the inhibitor. The result showed ON/OFF/ON/OFF repetitive control of the enzymatic activity of HIV protease. Based on this result, we performed the activity detection of the wild-type protease purified from human serum. The substrate cleavage was confirmed by addition of SA and suppressed by following addition of clinical protease inhibitor drugs such as darunavir, lopinavir and atazanavir. Similar experiments using the mutated protease, A17m5, which is lopinavir-resistant, were performed, revealing its lower enzymatic activity and different inhibition by the clinical drugs from that of the wild-type protease. Some biotinylated derivatives of pepstatin A, were also utilized to detect cathepsin D activity purified from HCT116 cells and the activity was inhibited by addition of pepstatin.

These results showed the ON/OFF repetitive control of proteases using removable inhibitors, suggesting useful applications for susceptibility or drug resistance testing before treatment with protease inhibitor drugs.



P107

Polymer-based peptidomimetics of Cell Penetrating Peptides and Antimicrobial Peptides

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Peptides are an emerging class of new therapeutic candidates. The total peptide drug market was estimated at $50 billion in 2015 and, with a projected annual growth rate of approximately 10%, is expected to exceed $70 billion by 2019. They are approved/in development in a large number of therapeutic areas, including metabolic diseases, endocrinology, oncology, gastroenterology, cardiovascular, neurology... Antimicrobial Peptides (AMPs) and Cell Penetrating Peptides (CPPs) are also promising peptide candidates for therapeutic applications, as active entities and delivery agents, respectively. Peptides, however, have a number of limitations impending their direct clinical application. They are essentially a lack of oral bioavailability and metabolic stability; a reduced serum half-life; an inability, generally, to reach intracellular targets; a potential toxicity; a high cost of production. Accordingly, the chemical and drug developments of therapeutic peptides generally require a technology addressing these clinical shortcomings.

Peptidomimetic conversion and conjugation to biocompatible polymers are archetypal techniques of pharmacokinetic and pharmacodynamic properties improvement for peptide therapeutics. The combination of these two techniques in a method to generate polymeric mimics of peptides has successfully been applied for the peptidomimetic conversion of CPPs and AMPs (deRonde BM and Tew GN, Biopolymers, 2015, 104(4), 265-280; Lienkamp K and Tew GN, Chemistry, 2009, 15(44), 11784-11800). These peptidomimetics include guanylated poly((oxa)norbornenes), polymethacrylamides and oligocarbonates, as well as a guanylated polymethacrylates, for CPPs, and poly(acrylics), nylon-3 and poly(norbornenes), for AMPs. Novel polymeric mimics of CPPs and AMPs, based on a biocompatible polymer backbone not previously exploited in peptidomimetics, have also been prepared and will be presented. Their activities as transfection reagents (CPP mimetics) or antimicrobial/antibiofilm agents (AMP mimetics) surpass, or match those of the parent peptides, respectively, while being metabolically stable. Also, as demonstrated with the CPP mimetics, these polymer-based peptidomimetics are devoid of toxicity and cheaper (30 fold) to produce than the parent peptide.

P108

Comparison of the Cell Permeability of Linear and Cyclic Peptoids

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Cyclic peptoids are emerging as powerful class of peptidomimetics. Peptoids are known to have enhanced of cell permeability compared to peptides, which means that they could serve as enhanced cell permeable molecules. Also, cyclic peptoids, which have increased rigidity compared to linear peptoids, could exhibit increased binding affinity against target protein compared to linear counterparts due to reduced entropy penalty when forming complex with the target. [1] However, no studies have shown the experimental results of enhanced cell permeability of cyclic peptoids compared to linear peptoids that contain same residues. Here, we, for the first time showed that the cell permeability of cyclic peptoids are more cell permeable irrespective of side chains and size. Peptoids that were used in the experiment were designed to be compatible with combinatorial library and high-throughput screening technologies. Thus, cyclic peptoids will serve as a promising protein binding molecules with high affinity that could target intracellular proteins without the matter of cell permeability and proteolytic ability.

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P109

Incorporation of trifluoromethylated pseudoproline in an entomotoxic knottin: effects on oxidative folding, 3D structure and biological activity

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Chemical tools to explore the trans/cis isomerization of the peptide bond have been studied for decades. Trans/cis Pro isomerization is known to be a late and relatively slow event on the oxidative folding time scale, and is considered a rate-limiting step in the folding of cis bond-containing proteins. Cis bond-inducing (4S)-fluoro-proline and 5,5-dimethyl proline were shown to accelerate the folding of cis bond-containing proteins [1]. We recently reported on the incorporation of a trifluoromethylated proline surrogate (CF3-ΨPro) into short peptide sequences and its positive impact on the stabilization of a cis peptide bond [2]. To enlarge the scope of CF3-ΨPro to finely tune physicochemical and biological properties of polypeptides, we incorporated CF3-ΨPro in PA1b (37 residues), an entomotoxin effective per os, isolated from pea seeds. PA1b is cross-linked by three interlocked disulfide bridges, and contains a cis amide bond suspected to slow down its oxidative folding [3]. CF3-ΨPro-containing PA1b was synthesized by Fmoc solid phase peptide synthesis and CF3-ΨPro was incorporated as a Fmoc-Ala-CF3-ΨPro dipeptide. The oxidative folding was conducted under thermodynamic control in the presence of a redox couple as optimized [3a] and compared to native PA1b. The biological activity of CF3-ΨPro-containing PA1b is of the same order of magnitude as that of native PA1b. To evaluate subtle modifications introduced by a non natural amino acid, we solved by quasiracemate crystallography [4] the 3D structure of a mixture of L-CF3-ΨPro-containing PA1b and a D-form incorporating D-Pro instead of D-CF3-ΨPro. This 1.1 Å resolution structure allowed us to unambiguously attribute the cysteine pairing of interlocked disulfide bridges and to gain atomic-level insight of CF3-ΨPro modified knottin derivatives.

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P110

Evaluation of Cell Permeability of Bicyclic Peptoids

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Macrocyclic peptoids, in particular bicyclic peptoids are of considerable interest as a promising class of potential protein capture agents. Compared to their linear and monocyclic counterparts, bicyclic peptoids have significantly increased conformational rigidity and preorganized structures, thereby enabling them to bind more tightly to the target proteins without a major entropy loss. Owing to their relatively large size and conformational constraint, bicyclic peptoids might be well-suited molecules to target many challenging protein targets such as protein−protein interactions, which cannot be easily modulated by traditional drug-like small molecules. Because macrocyclic peptoids lack the amide protons in their backbones like linear peptoids, it was perceived and recently investigated that cyclic peptoids are more cell permeable than linear counterparts. However, despite the cell permeability of monocyclic peptoids were studied, that of bicyclic peptoids have not been investigated yet. Here, we compared the cell permeability of bicyclic peptoids and their linear counterparts from 6mer to 10mer. Halotag-labeled linear and bicyclic peptoids were synthesized and their penetration into the cell was analyzed by chloroalkane penetration assay (CAPA). We could conclude that the bicyclic peptoids are quite more permeable than linear peptoids. This result suggests that compared to the linear peptoids, bicyclic peptoids will be a useful source of protein binding molecules, especially for targeting intracellular proteins, given their excellent cell permeability in addition to their conformational rigidity and proteolytic stability.

P111

Structure-Activity Study of α-Peptoid/α-Peptide Peptidomimetics: Influence of Hydrophobicity on Antimicrobial Activity and Cytotoxicity

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Bacterial resistance to conventional antibiotics is a rising global challenge and discovery and development of novel classes of antimicrobial agents are urgently needed. In particular, multidrug-resistant Gram-negative strains resistant to all currently used clinical antibiotics constitute a severe health problem, since the new classes of antibiotics, launched in the period 2000-2012 primarily target Gram-positive infections.

Antimicrobial peptides (AMPs) have in the last decades produced promising leads, as they often exhibit broad-spectrum activity and high selectivity towards bacteria over mammalian cells. However, their therapeutic potential is generally limited by several disadvantages e.g., low plasma stability due to proteolytic susceptibility and decreased activity in vivo due to binding to serum proteins. To circumvent these drawbacks, several types of peptidomimetics with a high content of unnatural residues have been investigated as potent AMPs. Peptoid-containing peptidomimetics constitute an interesting class of such compounds as they have been found to possess high antimicrobial activity, while being stable and display increased activity in plasma.

Previously, lysine-based alpha-peptide/beta-peptoid hybrid oligomers were found to display activity against multidrug-resistant bacteria. Generally, a 1:1 ratio of lysine to hydrophobic peptoid residues were found to result in the highest antibacterial activity and lowest toxicity, but only a small number of hydrophobic peptoids have been investigated. Previous studies have shown that phenylalanine analogues and substituted variant are favorable. Thus a more comprehensive investigation of the role of hydrophobic residues for activity of peptoid-peptide hybrids with an alternating cationic-hydrophobic design appeared to be lacking in literature.

In the present study, it was decided to explore a reversed design with the cationic residues being the alpha-peptoid anologue lysine (i.e., NLys) since we found that the corresponding Fmoc-protected building block readily could be obtained in multigram scale without chromatography. Thus, a wide range of polarity could be investigated by complementing the natural hydrophobic amino acids with unnatural alpha-amino acid displaying appropriate side chains. Thus, a library of >20 α-peptoid/α-peptide hybrid oligomers were obtained by automated microwave-assisted solid-phase synthesis using the Fmoc-protected peptoid building block Fmoc-NLys(Boc)-OH and various Fmoc-protected amino acids. Subsequently, the antimicrobial activity (MIC) of the compounds was tested against a panel of pathogenic Gram-negative bacteria (Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa). Somewhat surprisingly, most compounds displaying hydrophobic aliphatic side chains proved to be inactive, with the exception of cyclohexyl, while most compounds with hydrophobic aromatic side chains exhibited moderate to high activity.

P112

Synthesis of novel arylazepinone dipeptide mimetics and 1,5-benzothiazepinones as local constraints in peptidomimetic design

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Incorporation of conformationally constrained amino acids is a well-known technique to design peptides that are more potent and/or selective towards their targeted receptors. Moreover, these modified peptides or peptidomimetics display an increased metabolic stability, as compared to their natural counterparts.[1] For this reason, many research groups direct their attention to the application of conformationally constrained amino acids in the development of novel peptide therapeutics. Presented herein is the use of novel conformationally constrained arylazepinone dipeptidomimetics to screen the χ-space of the aromatic Phe3 side chain within dermorphin's sequence. 4-Amino-8-bromo-2-benzazepin-3-one (1) (8-Br-Aba), 3-amino-3-4-dihydroquinolin-2-one (Dhq, not shown), and regioisomeric 4-amino-naphthoazepinone (2-3) (1/2-Ana) scaffolds were synthesized in solution via N-acyl iminium ion cyclization. These conformationally constrained scaffolds were incorporated into dermorphin-like tetrapeptides, evaluated in vitro and resulted in compact and high affinity MOP/DOP opioid receptor ligands.[2] Secondly, 3-amino-1,5-benzothiazepin-4(5H)-One dipeptidemimetics (5) were constructed through an Ugi-4CR-Ullmann Cross-Coupling sequence. The seven dipeptides were conformationally investigated in silico and by NMR analysis, all lowest energy conformers stabilize γ-and β-turn structures.[3]

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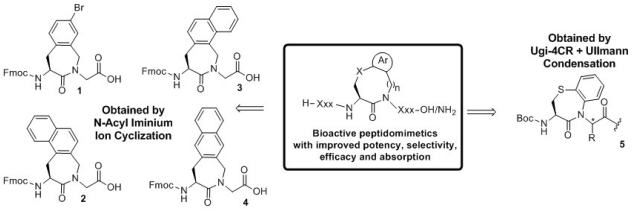

P113

Impact of Bulky Dehydroamino Acids on the Structure and Proteolytic Stability of Peptides

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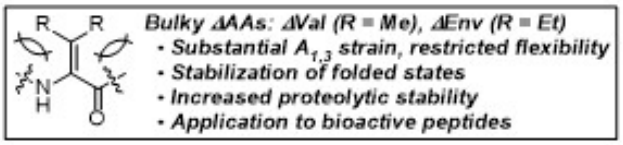
New strategies for increasing the stability of peptides to proteolysis are needed in order to facilitate the translation of bioactive peptides into pharmaceutical agents. The inherently high levels of A1,3 strain present in bulky alpha,beta-dehydroamino acids such as dehydrovaline and dehydroethylnorvaline make them promising candidates for rigidifying the backbones of peptides and increasing their proteolytic stability. However, synthetic challenges have hindered investigations of the impact of bulky dehydroamino acids on peptide structure and stability. Recent advances

from our laboratory have enabled the construction of peptides containing dehydroVal or dehydroEnv. We have shown that these bulky dehydroamino acids can substantially increase the stability of model beta-hairpin peptides to degradation by Pronase, an aggressive mixture of nonspecific proteases. We have correlated this enhanced proteolytic stability to an increased preference for the folded state versus random coil states. A summary of these findings will be presented along with new data describing the effect of bulky dehydroamino acids on other secondary structures. We envision a host of exciting future applications for dehydroVal, dehydroEnv, and other bulky dehydroamino acids as stabilizing and constraining elements of peptides.



P114

Study of β-sheet Helix Interactions Based on Conformational Properties of Synthetic D,L-alternating Decapeptides

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De novo peptide design is based on the ability to construct peptide sequences with predictable folding patterns. Helices and β-sheet have been the focus of considerable synthetic attention in attempts to assemble mimics for helical bundles and all β-motifs. In contrast, strategies for construction of β-sheet helix have been less widely explored. The β-sheet helical architecture is constructed from polypeptides that are coiled into a large helix, formed by stacks of β-sheets separated by loops. β-Sheets helices are present in the fibrous form of transyretin, that play an important role in bovine spongiform encephalopathy (BSE) and form the crucial structural elements in insect antifreeze proteins. We report on synthetic analogues able to form β-sheet helices and on the structural comparison with β-helices formed by D,L-alternating peptides. Alternating D,L peptides are able to assume very specific conformations, including, among others, various kinds of single and double stranded β-helical structures. β-Helix conformations share an important number of structural features with β-sheet ones, as a set of hydrogen bonds between amino and carbonyl backbone groups and ΦL, ΨL,ΦD, and ΨD values in poly-L- and poly-D-peptides. Double stranded β-helices, both parallel and antiparallel, are stabilized by systematic interchain NH-CO hydrogen bonds between adjacent peptide chains and by intrachain hydrogen bonds equivalent in pairs. They are symmetrical structures in which the backbone CO and NH groups are oriented quasi-parallel to the helical axis but in alternating opposite directions. The side chains are oriented perpendicular to the helical axis on the outside of the helix. Insertion of proline residues within a homochiral segment has been used to nucleate β-sheet helical conformation. The juxtaposition of proline within repetitive motifs supports the formation of β-turns which are labile and impart dynamic and flexibility upon sliding between successive Pro-containing repeats. Furthermore, the periodicity with which Pro and Xaa amino acid are arranged with the peptide directs the formation of specific secondary structures. We have observed –XPXPXPXP- periodicity with the synthetic homologous of β-casomorphin-8 and a -PXXP- periodicity using a synthetic, homochiral sequence having X=F with 13 amino acid. Here we report about synthesis and conformational behaviour of both peptides.

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P115

Modulation of the properties of elastin-like polypeptides by structure variations

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Elastin-like polypeptides (ELPs) are artificial biomacromolecules derived from hydrophobic VPGVG sequence of extracellular matrix protein tropoelastin. Their ability to form coacervates at specific temperature has huge potential in drug delivery, ELPs are also used in areas of protein purification or tissue engineering. Non-natural, synthetic ELPs consist of repeating pentapeptide motives VPGXG, where X is represented by any of encoded aminoacids except proline to preserve β-turn conformation [1]. Phase transition behavior is significant feature of this type of peptides. Under transition temperature, these molecules are soluble in water. On the other hand they form unsoluble clusters above the transition temperature. Subsequent cooling under their transition temperature results in the resolution of ELPs. The transition temperature can be influenced by both extrinsic and intrinsic factors. The intrinsic factors include changing primary sequence and chain length, whereas extrinsic variables comprise pH, ionic strength or solvents [2]. We present the set of new pentacontapeptides H-(VPGLG)2(VPGXG)2(VPGLG)6-H prepared using SPPS protocol by Fmoc/tBu strategy, where as the variable element X the hydrophobic (Gly, Ala, Leu), hydrophilic (Gln, Asn, Tyr), acidic (Glu) as well as basic aminoacids (His, Lys) were used. Transition temperatures of our peptides set mainly obtained using CD spectroscopy were defined and discussed with respect to their primary structure.

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P116

Heme binding beyond Cys-Pro-motifs? – A systematic Analysis of His/Tyr-Based Motifs in Heme Binding peptides and proteins

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The porphyrin derivative heme (Fe(III)-PPIX) is vital to the survival of all eukaryotic organ-isms, which mainly exploit its gas and electron transporting properties in hemoproteins such as hemoglobin, myoglobin, and cytochromes. In addition, a pool of biologically available heme has been recognized to regulate biological processes such as transcription, ion transport, circa-dian rhythm and inflammation.[1] Considering the amino acid sequence responsible for such a regulatory interaction it is meanwhile established that solvent-exposed sequence stretches pos-sessing distinct features including coordination site(s) for the iron ion (termed heme-regulatory motifs (HRMs)) are an indispensable prerequisite for heme binding. Apart from the known Cys-Pro (CP)-dipeptide motifs less was known with respect to consensus sequences for spe-cific HRMs beyond CP. Thus, we established an approach which facilitates the detection of heme-binding sequences and, in turn, novel heme-regulated proteins based on combinatorial peptide library screening.[2-6] In these earlier studies, we first focused on Cys-, His- and Tyr-containing sequences with a pri-

mary interest in CP-motif containing peptides and proteins.[7-9] However, a deeper analysis of the hit sequences revealed the frequent occurrence of multiple tyrosines and/or histidines in peptides with strong heme-binding affinities and in several well-known proteins.[10] Therefore, we hypothesized that distinct combinations of His and Tyr may depict a novel HRM beyond the well-established CP-motif. Here, we report a detailed structural analysis of the heme-binding capacity of a subset of H/Y-containing peptides and their inverted sequences using spectroscopic methods. In addition, we will introduce a web-based tool, which enables the user to screen protein sequences for HRMs, based on our findings. The presented study will provide insight into this new class of HRMs and explore the underlying structure-binding relationship. It will provide an improved understanding of the molecular mechanism of regulatory heme binding and may allow for the discovery of novel heme-regulated proteins based on defined H/Y-motifs.

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P117

Exploring the membrane activity of a designed antimicrobial peptide with fusogenic properties

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The ever-growing spread of antimicrobial resistance among bacterial pathogens is one of the major threats to public health. Especially concerning is the case of Gram-negative multi-resistant bacteria, intrinsically resistant to many hydrophobic molecules, due to the presence of a highly-packed extra membrane system, the outer membrane (OM). Antimicrobial peptides (AMPs) are interesting candidates to fight bacterial infections, including those caused by Gram-negative bacteria, as the antimicrobial activity is exerted through membrane disruption, a mechanism that is thought to be unlike to elicit resistance. We have studied the membrane activity and selectivity of EcAMP1R2, a designed AMP highly specific towards Escherichia coli. As model membrane systems, we have used large unilamellar vesicles (LUVs) of different lipid compositions, including those mimicking the outer (OML) and inner (IML) membranes of E. coli, and actual E. coli cells. Using a combination of fluorescence spectroscopy, flow cytometry and light scattering spectroscopy, we have found that EcAMP1R2 discriminates between zwitterionic (mammalian-like) and anionic (bacterial-like) membranes. Interestingly, the peptide causes hyperpolarization of E. coli cells, indicating inability for pore formation. Moreover, we have found that EcAMP1R2 promotes a cardiolipin-dependent (hemi)fusion of IML vesicles, without a concomitant neutralization of the zeta-potential. This result suggests that the interaction of EcAMP1R2 with IML vesicles results in major membrane lipid rearrangements able to alter the membrane curvature, with interesting mechanic implications. We propose that the peptide can induce cardiolipin demixing at the plasmatic membrane level, hampering the activity of the respiratory machinery, thereby causing membrane hyperpolarization. Molecular dynamics (MD) simulations with the Martini force field were performed to shed some light on the validity of our hypothesis, gathering additional information that could explain the fusogenic ability of EcAMP1R2. Simulations indicated dimer formation on the membrane, which prompted experimental validation by NMR.

P118

Conformational dynamics of an intrinsically disordered protein controlled by alpha-methylation of amino acid residues

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Intrinsically disordered proteins (IDPs) or protein domains readily fold into well-defined secondary and/or tertiary structures upon binding to their targets whereas in isolation they remain largely unfolded. Determining the mechanistic pathways that lead to well-structured complexes involving IDPs is a challenge. Here we demonstrate that mechanism of binding-induced folding of an IDP can be elucidated by using site-specific incorporation of alpha-methylated amino acids that locally stabilize alpha-helical conformations. Combinatorial chemical protein synthesis was applied to prepare protein library that contained more than fifty alpha-methylated variants of the p160 transcriptional co-activator ACTR and possessed up to six alpha-methylated amino acids incorporated at various positions. Complexation between ACTR variants and nuclear co-activator binding domain NCBD was studied using various biophysical techniques in conjunction with molecular dynamics simulations. Thermodynamic data for complexation between ACTR variants and nuclear co-activator binding domain (NCBD) revealed presence of entropy-enthalpy compensation, such that local conformational constrains (loss of entropy) resulted in more stable intra- and intermolecular contacts (gain of enthalpy). Anomalous compensation corresponded to functionally most important residues at ACTR/NCBD interface. Furthermore, several alpha-methylated analogs of ACTR have higher affinities to NCBD than wild type highlighting their potential for development into efficient inhibitors for cancer-related ACTR/NCBD interaction.

P119

Selective Metal-Ion Induced Folding of a Coiled-Coil Peptide

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A variety of small, well-defined, peptide scaffolds based on α-helices and β-strands have been engineered to produce metal-binding peptides. These designed metallopeptides have allowed researchers to probe the relationship between peptide-folding and metal-binding. One such scaffold, used to generate several metallopeptides, is the α-helical coiled coil.1 Coiled coils are attractive design scaffolds as the majority have a defined sequence repeat and several studies have uncovered rules enabling coiled-coils with specific oligomeric states and orientations to be reliably designed.2-4 For this study an existing, well-folded, trimeric coiled coil was employed,3 and histidine residues were introduced into different positions within the

hydrophobic core. Analysis of the resulting peptides using circular dichroism (CD) spectroscopy showed that the inclusion of one histidine per helix was well tolerated but when two histidine modifications were introduced, folding of the coiled coil was obliterated. However, folding could be induced in a selective manner when divalent metal ions were added to the peptide; Cu(II) and Ni(II) promoted folding of the peptide, while Co(II) and Zn(II) did not. A combination of CD spectroscopy, NMR studies, and analytical ultracentrifugation (AUC) revealed more about how the metal ions and the peptides interact to form a folded construct. In addition, the X-ray crystal structure of the Cu(II)-peptide complex was obtained and this revealed an unusual coordination mode of the Cu(II), which provided insights as to the reason for the observed metal-binding selectivity. It is envisaged that further modification of this scaffold would allow for other metals to be coordinated and potentially for metal-binding specificity to be achieved.

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P120

The multiHis-cyclopeptide as ligands for metal ions

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Wide spectrum of biological processes involving metal ions fuels the intensive research aimed at their detailed characterization. This research are generally carried out with the use of biomimetic systems which mimicking the structure and/or the activity of natural systems. These studies are conducted primarily on protein and/or peptide systems, because they allow to the introduction of a large number of modifications and tracking of their impact on efficiency of metal ion binding and its coordination sphere. The use of the linear models is restricted by high liability of their chain. This restriction may be waived by the cyclization of the peptide chain. The antibiotics, hormone peptides, toxins or transporters of metal ions though the membrane can be found among of the naturally occurring cyclopeptides. Additionally the cyclic structure influences the sterical arrangements of the side chains of the amino acid residues, which is significant for the interactions with metal ions or for the receptor binding in the biological systems.

The donor atoms for metal ions in the cyclopeptide molecule are nitrogens form peptide bonds and donors from the side chains of the amino acid residues as imidazole nitrogen of His or oxygen carboxylate side chain of the Asp residue. The binding mode of the metal ion depends on the number structural modifications e.g.: optical isomerism and acid-base properties of side chain group [1], the ring size [2] or presence of Pro residue in the peptide sequence [3]

We focused on the specially created cyclopeptides able to form bi-metallic systems mimicking the active centers of metalloproteins. The new cyclopeptides with two prolines in the peptide cycle have been designed. The insertions of two prolines allowed to create of two well defined metal binding sites. The performed studies have shown that these compounds are the promising tool for the creation of the biomimetic systems as e.g. active site of the superoxide dismutase (SOD). Due to the complexity of the binding processes and action of the metal ions in the living systems, presented studies are still continued.

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P121

Elucidating the effect of membrane composition on the structure of antimicrobial peptide

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GL13K is a thirteen residue, cationic antimicrobial peptide that is highly selective for both Gram-positive and Gram-negative bacteria. Monolayer studies coupled with spectroscopic techniques provide insight into the behaviour of the peptide at the outer leaflet of the membrane biointerface. We probe the impact of membrane packing on the behaviour of GL13K by incorporating cholesterol (Ch) or branched diphytanoylphosphatidylglycerol (DPhPG) into negatively-charged dioleoylphosphatidylglycerol (DOPG) model membranes. At the interface the peptide organizes into crystalline β-sheets. The membrane composition significantly impacts the depth of peptide insertion as well as the size of the inter-peptide β-sheet structures.

P122

Methylene thioacetal bond substitution reveals a new hidden pathway in BPTI folding mechanism

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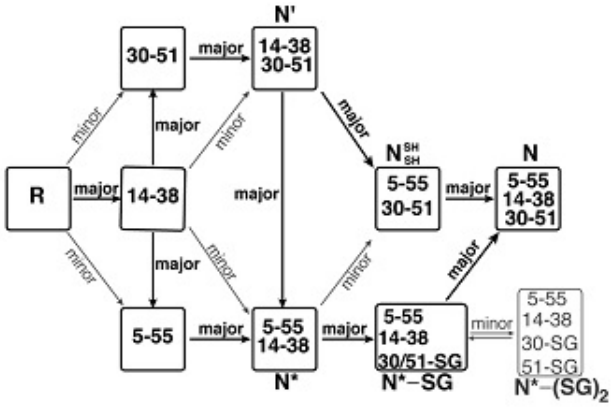
Bovine pancreatic trypsin inhibitor (BPTI) is a 58 residues protein that is stabilized by three disulfide bonds at positions 5-55, 14-38 and 30-51 (right). BPTI folding mechanism was extensively studied since it represents a folding model for many disulfide-rich proteins. The reduced protein folds via bifurcated pathway, roughly half of the reduced BPTI reach the native state via two intermediates: N' that lacks the 5-55 disulfide and N* that lacks the 30-51. Formation of the fully oxidized BPTI requires partial unfolding that reduces the solvent exposed disulfide bridge 14-38 and rearrangement to form another intermediate NSHSH . Therefore, we hypothesized that replacing the 14-38 disulfide bond with highly stable methylene thioacetal (MT), will cause the protein to be trapped at the two stable intermediates N' and N*, preventing the formation of the native state (N). We performed this experiment and found that this is not the case! The methylene thioacetal substitution revealed a hidden pathway involving direct reaction between N* and GSSG. This experiment revealed that the previously published mechanism does not present a complete picture of BPTI folding.



P123

Membrane Interactions of Cyclic Lipodepsipeptides

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The use of natural products isolated from microorganisms is becoming a promising alternative approach to control plant diseases caused by phytopathogens. In this respect, cyclic lipodepsipeptides (CLPs) are a diverse group of secondary metabolites produced by various bacteria with interesting biological functions. However, they have yet unresolved molecular mechanisms. CLPs are made up of an amino acid sequence consisting of both D- and L-amino acids. At the N-terminus, they are capped by a fatty acid moiety, in most cases a hydroxydecanoic acid. Depending on chemical properties, such as the amino acid sequence length, the CLPs can be classified into different groups. Our previous efforts have gone towards characterizing the three-dimensional conformations of several CLP groups using liquid-state NMR spectroscopy, X-ray crystallography or both. It is believed that CLPs exert their biological functions through interactions with the cellular membrane. However, a full understanding of their membrane interactions is essential to elucidate the exact working mechanism of CLPs. To obtain comprehensive structural information in a membrane environment, we have used various, complementary techniques including liquid-state NMR spectroscopy. Using this technique, the affinity, orientation and insertion depth of CLPs in a membrane environment can be investigated using diffusion NMR and paramagnetic relaxation enhancement measurements. The latter is achieved by introducing paramagnetic probes at various locations. By introducing a water-soluble paramagnetic complex, NMR signals from nuclei closer to the aqueous phase can be identified. Adding lipid molecules with covalently linked paramagnetic radicals at various positions deliver the orientation and the insertion depth of the peptides in the bilayer. The NMR results are complemented with other experimental techniques, including fluorescence spectroscopy, circular dichroism and infrared spectroscopy. We have also performed all-atom molecular dynamics (MD) simulations of CLPs within lipid membranes, which can be confronted with the experimental results.

P124

New insights into the structural and dynamics properties of collagen model peptides using CD spectroscopy, MD simulations and innovative NMR approaches

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Collagen, the most abundant protein in the animal kingdom, is also an important biomaterial owing to its great mechanical strength and its excellent thermal stability. This last decade, both the industry and the academia have been involved in the development of new collagen-related biomaterials.[1] One common strategy consists in using Collagen Model Peptides (CMP) as collagen surrogates. Numerous studies have demonstrated that short sequences, based on the repetition of the Pro-Hyp-Gly triplet, can be used to mimic the primary sequence of collagen.[2] Using such peptides, we have developed an approach combining CD and NMR spectroscopies in order to characterize the formation of the triple helix (TH) and its structural features in solution. MD simulations and NMR spectroscopy have allowed us to gain insights into the conformational ensembles explored by the monomeric CMP peptides and their conversion into larger oligomeric species. Innovative 13C, 15N strategies and NMR developments have been carried out to obtain structural information on these assemblies at the atomic scale. In addition, we have been able to introduce 19F nuclei in model CMP using previously reported strategies.[3-6] Kinetic and structural effects of this insertion onto the collagen triple helix will be discussed using the developed biophysical tools and MD simulations.

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P125

Structural and positional studies of the antimicrobial peptide brevinin-1BYa and its analogue

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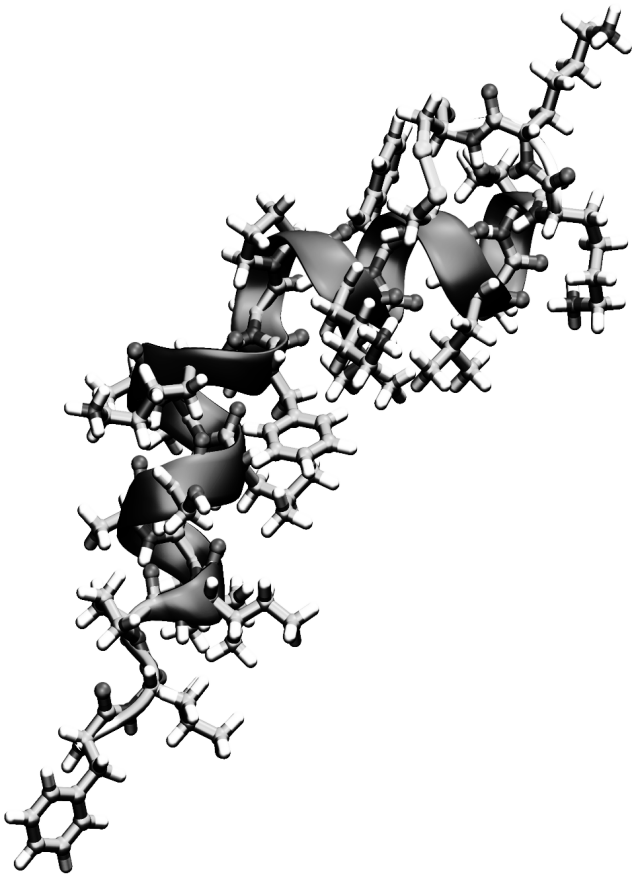
Brevinin-1BYa (FLPILASLAAKFGPKLFCLVTKKC), first isolated from skin secretions of the foothill yellow-legged frog *Rana boylii*, is particularly effective against *C. albicans*, and is also active against *E. coli*

and *S. aureus*. The structures of brevinin-1BYa and its less-potent analogue [C18S,C24S]brevinin-1BYa were investigated in various solution and membrane-mimicking environments by ^{1}H-NMR spectroscopy and molecular modelling. Brevinin-1BYa possesses two cysteine residues, one at the C-terminal end of the peptide and the other 6 residues prior to it, joined by a disulphide bridge. The peptide does not appear to possess a secondary structure in aqueous solution. In a 33% 2,2,2-trifluoroethanol (TFE-d_3)-H_2O solvent mixture, as well as in membrane-mimicking sodium dodecyl sulphate micelles, and dodecylphosphocholine micelles, however, the structure is characterised by a helix-hinge-helix motif, with a hinge located at the Gly^{13}/Pro^{14} residues, and the two α-helices extending from Ile^4 to Lys^{11} and from Lys^{15} to Thr^{21}. Positional studies of both the native peptide and its serine analogue in sodium dodecyl sulphate micelles using 5-doxyl labelled stearic acid and manganese chloride paramagnetic probes show that the N-terminal helical segment of the peptide lies parallel to the micellar surface, with the residues of the hydrophobic side of the amphipathic helix facing towards the micelle core and the hydrophilic residues pointing outwards, with similar results obtained for the C-terminal segment of the native peptide.



P126

Understanding how antimicrobial peptides interact with membranes

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Antimicrobial peptides (AMPs) are a first line of defence against many pathogens in a diverse range of organisms, including humans. The majority of AMPs act by disrupting the plasma membrane. Many models of membrane disruption have been proposed (e.g. barrel-stave pores, toroidal pores, disordered toroidal pores, carpet-like, micelle formation, etc.). This makes predicting whether a given peptide sequence will be antimicrobial highly challenging. Using the assumption that membrane disrupting peptides optimise the amphipathic arrangement of amino acids, I have examined the ability of evolutionary algorithms to predict the structures of 44 membrane-bound AMPs. I have compared the predicted structures to conformations determined by solution NMR in membrane mimetic environments and explored how these structures might be used to predict activity and mechanism of action.

Many of the structures predicted were curved helices, with alignment of hydrophobic residues on the concave surface of the peptide. For example, the curvature and the amphipathic arrangement predicted for crotalicidin matched well with the curvature and residue alignment observed by solution NMR. However, in the case of dermadistinctin K, the hydrophobic residues faced the convex surface; opposite to the alignment of residues seen by solution NMR. To investigate this disparity, the precise details of how dermadistinctin K and crotalicidin disrupt lipid bilayers were studied with molecular dynamics simulations. The antimicrobial activity of Dermadistinctin K is claimed to act by a carpet-like mechanism and crotalicidin is proposed to form pores. The implications of how these contrasting mechanisms would effect their interaction with the membrane, the membrane-bound structures and how they compare to the results of optimising amphipathicity, are investigated.

P127

Targeting Peptide Secondary Structures with Multicomponent Ligation and Cyclization Strategies

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This work discloses how peptide secondary structures (i.e., helices, beta-strands and beta-turns) can be either induced or stabilized in short peptide sequences with the use of isocyanide-based MCRs. Two different multicomponent strategies are described: i) the side chain-to-side chain macrocyclization of peptides to stabilize helices, bulges and turn structures, and ii) the ligation of a small peptide to a rigid, bulky template capable to fold back the peptide chain into a beta-turn. Applications of such strategies on the synthesis of biologically relevant peptide structures(e.g., beta-hairpins) are presented, together with the determination of the solution three-dimensional structures of the peptides by means of NMR and molecular dynamics simulation.

P128

Alpha-methylation scan of an intrinsically disordered protein to study and control its conformational dynamics and binding

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The activation domain from p160 transcriptional co-activator for thyroid hormone and retinoid receptors (ACTR, 47 amino acids) was found to be highly unstructured with only the N-terminal part partially adopting α--

helical conformation. It is a well-known intrinsically disordered protein which binds with high specificity and affinity to nuclear co-activator binding domain (NCBD, 47 amino acids) from the CREB protein forming a well-structured complex which exhibits six α-helices (PDB 1KBH).

In this work, we present a new way to study IDPs conformation dynamics and complex formation. The incorporation of non-canonical α-methylated amino acids, which are conformationally constrained with (φ/ψ) backbone dihedral angles corresponding to 310/α-helical conformation, was performed on ACTR at multiple sites using Fmoc/tBu-Solid Phase Peptide Synthesis (SPPS) and Native Chemical Ligation (NCL)(1). In total we synthesized more than 50 methylated variants of ACTR. By incorporation of α-methylated amino acids we were able to modify the conformation of ACTR variants in the free state and, furthermore, to alter the energetic landscape of binding-induced folding with NCBD. To observe changes in α--helical content and conformational changes upon α-methylation, nuclear magnetic resonance (NMR) and circular dichroism (CD) measurements were performed. To dissect the thermodynamics and kinetics of complex formation, isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR) were used, respectively.

We confirmed that incorporation of α-methyl amino acids leads to the enhancement in α-helical content of ACTR, and that the α-helical content plays a key role in complex formation with NCBD.

(1) Dissecting Mechanism of Coupled Folding and Binding of an Intrinsically Disordered Protein by Chemical Synthesis of Conformationally Constrained Analogues. B. Schmidtgall, O. Chaloin, V. Bauer, M. Sumyk, C. Birck,and V. Torbeev. Chemical Communications, (2017) DOI : 10.1039/C7CC02276J

P129

Activation of the Chemokine-like Receptor 1 by Cyclic Chemerin-9 Analogs

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The chemokine-like receptor 1 (CMKLR1) is a G protein-coupled receptor (GPCR), which is expressed mostly on the surface of cells of the innate immune system like macrophages and dendritic cells. Since activation of CMKLR1 by its endogenous ligand chemerin results in the chemotaxis of these cells, chemerin has been proposed to be an important regulator of inflammation. Detailed knowledge of receptor binding and activation at a molecular level is crucial for the development of agents that could be used in the treatment of diseases like psoriasis or inflammatory bowel disease. Wittamer et al. discovered in 2004 that a small nonapeptide derived from the C-terminus of chemerin, named chemerin-9, is able to activate the CMKLR1 with nanomolar potency similar to the full-length protein. Previous work in our group indicated that this peptide binds to its receptor in a hairpin-like conformation. Exploiting this information we could synthesize cyclic chemerin-9 analogs that have the same potency and increased stability compared to the native peptide. Running molecular dynamics (MD) simulations of these novel cyclic peptides in combination with signal transduction studies helped gaining a more detailed insight into the conformation necessary for receptor activation, which might be beneficial for the development of even more potent analogs.

P130

Synthesis and conformation of short peptides modeled after peptide LL-37

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Numerous tissues and cells of our body (e.g., epithelial or innate immune cells) are able to produce the 37-residue peptide cathelicidin LL-37 [1]: -LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES- LL-37 plays important roles in the innate immune system as it acts as a natural antibiotic against Gram-positive and Gram-negative bacteria, fungi, and even viruses [1,2,3]. Also, it can inhibit the viral replication [1], promote wound healing [1,4] and kill tumor cells [4,5]. Its possible mechanisms of action, based on the disruption of cell membranes, were recently reviewed [3,6]. LL-37 is an amphipathic, α-helical peptide with an overall positive charge (+6). The helical structure promotes cell killing by binding to the negatively-charged bacterial membrane. To make LL-37 industrially appealing as a drug, we started a program aimed at individuating shorter segments endowed with the same potency of LL-37. We synthesized short, helical peptides stabilized by one or two helix-promoting Aib (alpha-aminoisobutyric acid) residues: 1 Pal-RKSKEKIG-NH2 (Pal = palmitoyl) 2 Pal-KSKEKIG-NH2 3 Ac-RKUKEKIG-NH2 (Ac = acetyl; U = Aib)) 4 Pal-RKUKEKIG-NH2 5 Ac-KRUVQRUKDFLR-NH2 6 Pal-KRUVQRUKDFLR-NH2 Peptides 1 and 2 correspond to segments 7-14 and 8-14, respectively, of LL-37. To protect them from the enzymatic degradation we added at the N-terminus a palmitoyl moiety, able also to promote membrane insertion. In peptides 3 and 4 the helix-promoting and hydrophobic Aib residue replaces Ser9. We came to this choice after having observed that the hydrophilic Ser9 is located on the hydrophobic face of the alpha-helical LL-37. With peptides 5 and 6 we focused on the 18-29 segment of LL-37, known to be quite active. Also in this case we aimed at assessing the possible beneficial effects of palmitoylation and Aib insertion. All peptides were synthesized by the solid phase approach and purified by reverse-phase HPLC. Their 3D-structures were assessed by means of circular dichroism and 2D-NMR. For all peptides we observed alpha-/3-10-helix equilibria, strongly dependent on the solvent used. Preliminary experiments of interactions with model membranes reveal an interesting selectivity for peptide 4.

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P131

Synthesis and conformational investigation of hetero-chiral sequential oligopeptides based on the (alphaMe)Aze/Ala dyad

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Conformationally restricted alpha-amino acids are able to promote specific main-chain conformations in peptides. One of them, (S)-2-methylazetidine-2-carboxylic acid [(S)-(alphaMe)Aze], when combined in homo-chiral dipeptide sequences with (S)-Ala, has been shown to produce multiple gamma-turns.[1] Recently, some of us developed a practical synthesis of both (alphaMe)Aze enantiomers.[2] By use of (R)-(alphaMe)Aze we synthesized the hetero-chiral sequential oligopeptide series listed below:

Boc-[(S)-Ala-(R)-(alphaMe)Aze]n-OMe (n = 1-3) Boc-[(R)-(alphaMe)Aze-(S)-Ala]n-OMe (n = 2,3) Boc-[(S)-Ala-(R)-(alphaMe)Aze]n-(S)-Ala-OMe (n = 1,2)

An X-ray diffraction analysis unambiguously showed that in the crystal state the hetero-chiral tripeptide Boc-(S)-Ala-(R)-(alphaMe)Aze-(S)-Ala-OMe adopts a regular type-II beta-turn conformation. However, FT-IR absorption and 2D-NMR data provide evidence that in deuterochloroform solution the tripeptide is mainly folded in a gamma-turn conformation. Transition from the former conformation to the latter requires only a variation of the (alphaMe)Aze psi torsion angle from the crystallographically observed value of 3.8° to -45°. As for the higher homologs, the combined results of our conformational investigation in solution (by FT-IR absorption, NMR, and CD spectroscopies) strongly suggest that they are involved in conformational equilibria to which both beta- and gamma-turns may contribute. The relative weight of the beta-turn conformers seems to increase with increasing main-chain length. This outcome, quite unexpected if compared to the preference for multiple, non-consecutive gamma-turns displayed by the corresponding homo-chiral peptides, suggests that sequence chirality might be a dominant feature in directing type of backbone folding in these sequential oligopeptide series.

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P132

Synthesis of Fluorinated Ubiquitin Variants

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Ubiquitin is a structurally conserved protein found in many eukaryotic tissues. In the process called ubiquitination this protein is covalently attached to diverse target peptides and proteins, which is important in numerous biological processes, for example, proteolytic degradation.[1] Due to its highly conserved globular structure ubiquitin is an interesting model for an investigation of the effects that modifications in the primary structure have on protein stability, structure, and function. The incorporation of fluorinated amino acids into peptides and proteins has attracted broad interest due to the unique properties of fluorine (e.g., high electronegativity, low polarizability and small size). [2] In this context, our goal is to establish ubiquitin as a biologically relevant model for studying fluorine-specific interactions, in particular their impact on protein folding. To this end a protein library of site-specifically fluorinated ubiquitin monomers will be synthesized and ligated in different combinations with similarly fluorinated or not fluorinated variants. These complexes will be investigated regarding the role of fluorine-fluorine interaction in protein folding. [1] D. Komander, M. Rape, Annu. Rev. Biochem. 2012, 81, 203–29. [2] A. A. Berger, J- S. Völler, N. Budisa, B. Koksch, Acc. Chem. Res. 2017, 50, 2093-2103.

P133

Solution Studies of the Bioactive Peptide Nisin

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Nisin, a 34 amino acid lanthipeptide, is currently used as a food preservative worldwide and has been used for decades without significant bacterial resistance being reported.1 It is an extremely active molecule which kills a range of different Gram-positive bacterial species, in the nano-molar range. However, nisin, along with some other similar peptides, suffers from low stability at physiological pH, which severely restricts its possible use as a therapeutic in human and veterinary medicine.2

Above pH 6 nisin suffers from a dramatic decrease in both solubility and stability. The two histidine residues (27 and 31) may be involved in the dramatic decrease in solubility due to the side-chain pKa values being in this pH range. Nisin is supplied commercially with a very high NaCl content (>70% w/w), which may have an effect on both its conformational stability and bioactivity. NMR and Circular Dichroism (CD) studies of both naturally occurring forms of Nisin (A and Z) will be presented.

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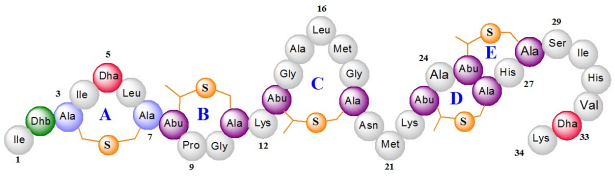

Fig. 1 Primary amino acid sequence of Nisin

P134

The 1,3-diyne linker as a tunable tool for peptide secondary structure stabilization

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In search of new, mild and functional group tolerant cyclization strategies towards novel constrained cyclopeptidomimetics, oxidative alkyne-alkyne coupling reactions were proposed as a straightforward method. The in-

troduction of 1,3-diynes in macrocyclic peptides can provide new reactive sites for post-cyclisation derivatization of the obtained macrocycles. This is of importance to reach libraries of conformationally distinct peptide organizations, starting from one parent macrocycle. A thorough optimization study of the Glaser-Hay coupling (> 50 conditions) using alkynylated amino acid derivatives revealed that O-propargylserines and analogues undergo much faster oxidative coupling as compared to propargylglycine derivatives. A series of tetrapeptides, synthesised via SPPS and a solution phase strategy, were cyclized under the optimised Glaser-Hay conditions using Cu(OAc)2.H2O and NiCl2 as catalysts in EtOH or DMF at 60°C in the presence of pyridine and triethylamine (14 examples; between 50 and >99% conversion).[1] Moreover, the cyclic peptides were used as parent molecules to obtain a variety of peptidic macrocycles containing a modified 1,3-diyne linker. Thiophenes and furans were introduced after reaction of the diyne moiety with NaHS and H2O respectively under mild conditions. New macrocycles were obtained after reaction with three different 1,2-bisnucleophiles (hydrazine, hydroxylamine and N-methylhydrazine). In all cases, a mixture of regioisomers was obtained. In contrast, the reaction of 1,2-bisnucleophiles with a macrocyclic non-symmetric 1-aryl-4-alkyl-1,3-diyne resulted in only one regioisomer.[2] Recently, we have shown that the 1,3-diyne linker can be used as a rigid "staple" for stabilizing an α-helical peptide containing 12 amino acids. The figure below shows one example where the linker stabilizes the helical fragment within the macrocycle (i,i+7). Because the seemingly straightforward alkyne alkyne coupling failed upon attempts to cyclize the O-propargylserine containing peptide sequence, the 1,3-diyne unit is introduced as a dipeptidic SPPS-compatible building block and the peptide is cyclised through head-to-tail lactamisation. Furthermore, the influence of the configuration of the O-propargylserines and the transformation of the 1,3-diyne towards heterocycles on the secondary structure is studied.

P135

Gaining and losing the activity of antimicrobial [KL]n peptides

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In the last decade, the development of peptides as drugs has experienced a renaissance due to finding more and more applications to treat various diseases. Further, they are generally expected to behave more predictably in vivo and be less toxic than synthetic small molecule drugs. However, their chemical and physical stability is susceptible to external factors, such as pH, temperature and surface interactions which can lead to degradation or aggregation/fibrillation and makes application and storage of peptidic drugs challenging.

To study the influence of surface interactions on the activity of antimicrobial peptides, amphiphilic β-strands can be designed in different fashions. In this study, the chosen design consists only of alternating lysine and leucine residues, resulting in perfect segregation of charged K residues and hydrophobic L residues and are thus excellent candidates to form β-pleated amyloid fibrils. These so called KLn peptides ([KL]n/2-NH2) show high antimicrobial activity against various bacterial strains which can be modified by variation of peptide length. Additionally the antimicrobial activity of KLn peptides can be turned on by diminishing contact time to free phosphate ions. From these properties guidelines towards the mode of action for these kind of peptides can be derived.

P136

Conformation and Domain Movement Analysis of Human Matrix Metalloprotease-2: the role of associated Zn2+ and Ca2+ ions

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Matrix Metaloprotease-2 (MMP-2) is a 550 amino acid residue extracellular Zn2+ protease that recognizes and degrades type I and IV collagens. Its Expression is associated with several inflammatory, degenerative, and malignant diseases. MMP-2 has three domains (fibronectin, catalytic, and hemopexin) and five crystallographically assigned divalent cations (2 Zn2+ and 3 Ca2+). In this study, we compared the conformational properties and domain movements of MMP-2 with attention to interactions between the protein and its associated metal ions using 1.2 μs NPT (at 310 K and 101.325 kPa) MD simulations using the CHARMM36m force field and metal ion parameters of Li et al.[1]. Dihedral principle component analysis revealed 10 families of conformations with the greatest degree of variability occurring in the loop connecting the catalytic and hemopexin domains. Cross correlation analysis indicated a domain movement corresponding to opening and closing of the hemopexin domain in relation to the fibronectin and catalytic domains. The root mean square fluctuations of the metal ions compared to the average conformation were as follows: Zn2+ ion 1: 0.59 nm, Zn2+ ion 2: 0.88 nm, Ca2 ion 1: 0.57 nm, Ca2+ ion 2: 2.91 nm, and Ca2+ ion 3: 1.06 nm. The catalytic Zn2+ ion 1 is closely associated (0.21$\pm$0.01 nm) with the Nε atoms of His403, His407 and His413. The non-catalytic Zn2+ ion 2 is closely associated (0.18$\pm$0.01 nm) with the Oδ atoms of Asp180. Interaction energies were calculated using the MMPBSA-interaction entropy analysis method [2,3] and are as follows:

Zn2+ ion 1: -1113.51 ± 27.12 kJ mol-1, Zn2+ ion 2: -598.09 ± 29.09 kJ mol-1, Ca2+ ion 1: -1153.69 ± 56.65 kJ mol-1, Ca2+ ion 2: -298.07 ± 27.33 kJ mol-1, and Ca2+ ion 33: -1147.73 ± 30.78 kJ mol-1. Present MD simulations demonstrated significant stability in the interaction between the protein and the catalytic Zn2+ ion. The second Zn2+ ion plays a minor role in conformational stability of the catalytic domain while the Ca2+ ions do not contribute significantly any of the structural properties.

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P137

Inhibitors of the Neuropilin-1 and Vascular Endothelial Growth Factor 165 Interaction

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Neuropilin-1 (NRP-1) has been found to be overexpressed in several kinds of malignant tumors, and it is postulated that in pathological angiogenesis, its interaction with the vascular endothelial growth factor 165 (VEGF165) leads to progression of tumor vascularization and growth. Moreover this signaling complex is also involved in suppression of immune response against tumor cells. It has been previously shown, that heptapeptide ATWLPPR (A7R) binds to NRP-1 and selectively inhibits VEGF-165 binding to NRP-1. Additionally in vivo treatment with A7R resulted in decreasing breast cancer (MDA-MB-231) angiogenesis and growth. In the frame of our work we synthetized peptidomimetics based on the C-terminal site of A7R and proposed two types of very potent analogues: linear and cyclic. In this poster we present structure-activity relationship studies which had led us to obtain strong inhibitors of VEGF-165/NRP-1 interaction, which might serve as prospective antitumor drugs.

P138

Use of Trifluoromethylated Pseudoprolines for the Design of Collagen Triple Helix containing Unusual C(5)-Substituted Proline Surrogates

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Collagen is the most abundant structural protein of mammals representing the major component of the extracellular matrix and is therefore widespread all over the body. Its tertiary structure consists in three individual left-handed polyproline II helices (PPII, $\Phi \approx -60°$, $\psi \approx 150°$) folded into a right-handed triple helix which is stabilized by inter-strand hydrogen bonds and that has a single-residue offset.[1] Each strand comprises the repeat of the (Xaa-Yaa-Gly) primary sequence where the (2S)-proline (Pro,P) and the (2S,4R)-4-hydroxyproline (Hyp,O) are the most prevalent at Xaa and Yaa positions, respectively.[2] Crystal structures of short-length Collagen Model Peptides (CMPs) show that Pro and Hyp residues exhibit preferentially C(4)-endo and C(4)-exo ring pucker, respectively.[3] As a paradigm, this alternation has been proposed to be a prerequisite for the formation of the triple helix since it preorganizes the individual strands in suitable conformation, thereby decreasing the entropic cost.[4] Since both Pro and Hyp residues exhibit the expected puckering, their prevalence imparts an excellent thermal stability to the collagen triple helix. Following these rational design principles, numerous CMPs have been engineered using (4S) and (4R)-substituted proline derivatives without a significant destabilization of the collagen triple helix, provided that they have conformational properties similar to those of Pro and Hyp residues and do not interfere with the interstrand H-bonds. Our group has developed the synthesis of the CF3-pseudoprolines (CF3-ψPro) and their incorporation into peptides.[5] We previously demonstrated that these proline analogues allow to locally tune the peptide bond geometry, the puckering of the 5-membered ring as well as the Φ and Ψ dihedral angles.[6] It was found that the diastereoisomers Tfmψ**P** and tfmψ**P** are acting as analogues of the Pro and Hyp residues, respectively, by adopting the prerequisite puckering necessary for the formation and stabilization of the triple helix. Here, we will present the synthesis of fluorinated host-guest CMPs incorporating in the middle of the sequence one or two CF3-ψPro. 1H and 19F NMR structural analysis of the CMPs along with the CD and NMR studies of the formation of the collagen triple helix and its stability will be also shown.

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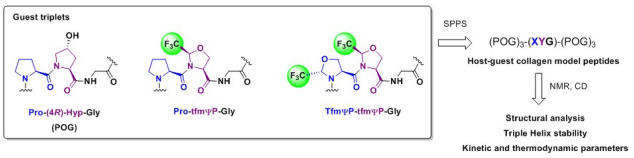

P139

Molecular dynamics simulations studies of Gonadotropin Releasing Hormone (GnRH) and GnRH Receptor

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Sexual maturation of human cells in ovaries and prostate is closely linked to the secretion of Gonadotropin Releasing Hormone (GnRH). The hormone binds to receptors (GnRH receptors) in the surface of the cells and instigates an extensive biochemical cascade that leads to production and release of luteinizing hormone (LH) and the follicle-stimulating hormone (FSH). Sequence analysis data classify GnRH receptors (GnRHR) as part of the G protein-coupled receptors (GPCRs). Structural data from GPCRs show the presence of a seven trans-membrane helical bundle. The lack of any structural data about GnRHR, impedes the rational design of agonist or antagonist GnRH peptides or non-peptide mimetics for use in the treatment of infertility and hormone dependent cancer. In this study, we employ these common structural and sequence characteristics for the construction of the homology model of GnRH receptor. Structural information from the human B2-adrenergic receptor as well as rhodopsin has been used in order to create a theoretical model of GnRHR. Furthermore, molecular dynamics (MD) simulations have been used to refine the proposed model and investigate the impact of the bilayer membrane in GnRHR conforma-

tion. Additionally, MD simulations were implemented for the analysis of the conformations of GnRH in aqueous solution. The results support the proposed model in the literature regarding the bent conformation of the hormone. The presence of a β-turn in the conformation of the peptide has been shown to be paramount in the binding of GnRH to the receptor and its subsequent activation.

P140

Structural Studies of Maximin-3

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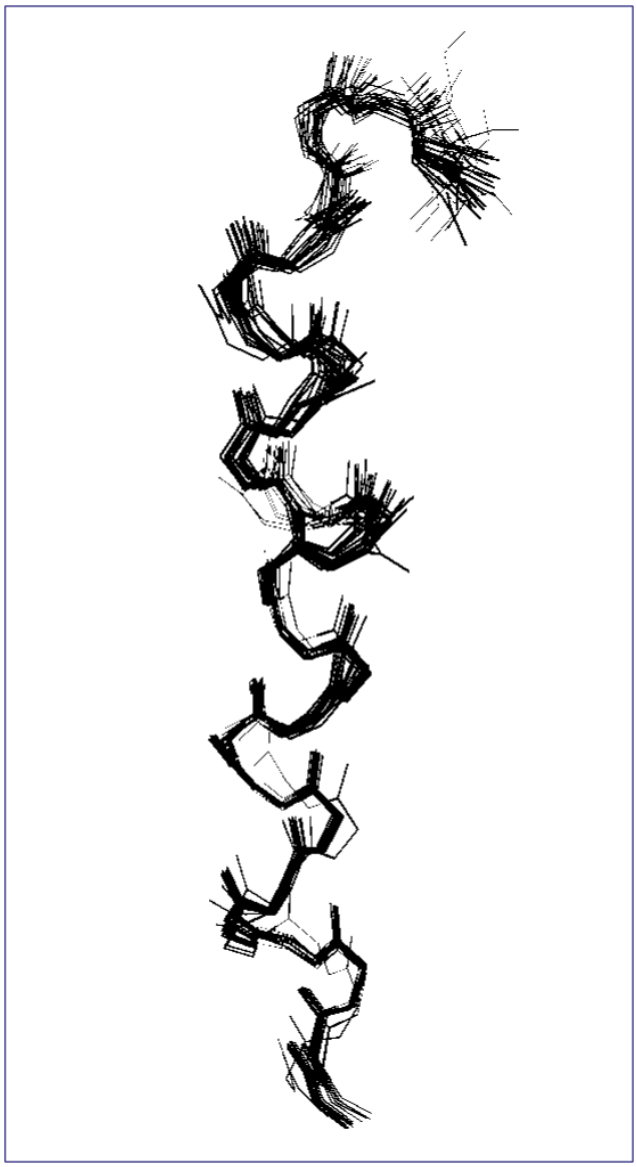
Maximin-3 (GIGGKILSGLKTALKGAAKELASTYLH) is a 27-residue long cationic antimicrobial peptide found in the skin secretion and brain of the Chinese red belly toad *Bombina maxima*. The peptide is interesting as it possesses anti-HIV activity, not found in the other maximin peptides, in addition to its antimicrobial, antitumor, and spermicidal activities. Its conformation was investigated in a 50/50% 2,2,2-trifluoroethanol (TFE-d_3)/water mixture using two-dimensional NMR spectroscopy. Maximin-3 was found to adopt an α-helical structure from residue Ile^2 to Ala^{22}, with a break around Lys^{15} and Gly^{16}, and a coiled structure extending from Ser^{23} to the C-terminus. The peptide has a well-defined separation between polar and hydrophobic residues, therefore it is amphipathic. The interactions with sodium dodecyl sulfate (SDS) micelles, a widely-used bacterial membrane-mimicking environment, are then modelled employing molecular dynamics simulations. The peptide maintains an α-helical conformation, occasionally displaying a flexibility around the Gly^9/Leu^{10} and Gly^{16}/Ala^{17} regions. It is found to preferentially adopt a position parallel to the micellar surface, with the C-terminal segment from Ser^{23}-His^{27} protruding into the aqueous solvent. The hydrophobic face of the amphipathic helix is in direct contact with the micelle core, while the polar residues face the aqueous solvent and form electrostatic interactions with the detergent's polar sulphate headgroups.



P141

Amino acid sequence requirements of laminin α2 chain peptide A2G80 (VQLRNGFPYFSY) for α-dystroglycan binding

Fumihiko Katagiri, Yuka Fukasawa, Jun Kumai, Kentaro Hozumi, Yamato Kikkawa, Motoyoshi Nomizu, Yasuhiko Yamada

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Dystroglycan (DG) is a component of the dystrophin-glycoprotein complex (DGC) in skeletal muscle. DGC is a molecular complex composed of dystrophin and membrane glycoprotein group and forms a structure linking the basement membrane and the actin skeleton. The association of basement membrane-cytoskeleton mediated by DGC is thought to be important for maintaining the strength of the muscle cell membrane which can withstand mechanical stress such as elongation and contraction of the muscle. DG binds with dystrophin directly under the cell membrane, it is bound extracellularly to basement membrane molecules such as laminin and play a role as a molecular axis linking the cytoskeleton and basement

membrane. DG consists of two subunits α and β, αDG, which is an extracellular subunit, binds ligands. βDG is a transmembrane subunit, which binds αDG to the cell surface extracellularly, and binds dystrophin within the cell. Laminin is known as a ligand molecule of αDG. Laminins are major components of basement membrane, consists of three different subunits, α, β, and γ chains, and so far, five α, three β, and three γ chains have been identified. We have constructed the synthetic peptide library derived from the laminin sequences, and identified functional peptides. We have reported that A2G78 (GLLFYMARINHA, mouse laminin α2 chain, 2796-2807) and A2G80 (VQLRNGFPYFSY, mouse laminin α2 chain, 2812-2823) peptides bind αDG [1]. In this study, we investigated the structure-activity relationship of A2G80 to identify the specific residues for binding to αDG. The αDG was rough purified from mouse muscle myoblast cell line C2C12. The A2G80 and its derivative peptides substituted each amino acid residue to alanine were synthesized by Fmoc-solid phase methods, furthermore, the biotinylated peptides of A2G80 and its Ala substituted peptides were prepared. The circular dichroism (CD) spectra of the peptides were measured for identification of secondary structure. The peptides substituted Asn5, Phe7, Pro8, Tyr9, Phe10, and Ser11 for Ala were different spectra to A2G80 peptide. The binding activity was evaluated by enzyme immunoassay using biotinylated peptides and crude αDG. The A2G80 peptide bound crude αDG with dose-dependent manner. The peptides substituted Val1, Gln2, Arg4, Gly6, Phe8, Tyr9, and Tyr12 to Ala lost the αDG-binding activity. From the result of X-ray crystallographic analysis, it is reported that the Gln2, Arg4, Asn5, Phe7, Tyr9, and Ser11 residue is exposed on the protein surface. We concluded that the Gln2 and Arg4 were critical residue for binding to αDG.

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P142

Expanding the chemical and genetic diversity of the cyanobactin family of natural products

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Natural products are low molecular weight metabolites with diverse chemical structures and potent biological activities. Cyanobactins are a class of natural products produced through the post-translational modification of short precursor peptides. Here we demonstrate that the capacity to produce cyanobactins is spread throughout the bacterial domain and many cyanobactin gene clusters encode potent antimicrobial peptides. We report novel cyanobactin post-translational modifications including prenylation, phosphorylation and disulfide bridge formation. Our results demonstrate that cyanobactin biosynthetic pathways encode rapidly evolving enzymes that catalyze the regiospecific and stereospecific modification of amino acids in macrocyclic and linear peptides. These findings broaden the structural diversity of the cyanobactin family to include highly modified linear and macrocyclic peptides with rare post-translational modifications.

P143

Isolation of phytochemicals from Camellia sinensis leaf with insulin releasing and glucose lowering effects for treatment of type-2 diabetes

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Background: Camellia sinensis, referred to as black tea, has been traditionally used as an antihyperglycemic agent. The present study of hot water extracts of C. sinensis leaf were investigated for insulin release and glucose homeostasis both in vitro and in vivo and possible mechanism/s of action. Materials and Methods: Effects of hot water extracts of C. sinensis on insulin release, membrane potential, and intracellular calcium were tested using rat clonal β-cells (BRIN-BD11 cells). Moreover, insulin secretory activity was observed using collagenase isolated mouse pancreatic islets. Effects on starch digestion, DPP-IV enzyme activity, and protein glycation were examined using in vitro model systems. High-fat fed rats treated for 9 consecutive days with extracts (250mg/5ml/kg) were used to evaluate the therapeutic impacts on glucose homeostasis. Result: The hot water extracts of C. sinensis, substantially (P<0.05–P<0.001) increased insulin release 1.3-4.3 fold (compared to 5.6mM/16.7mM glucose) in a linear manner between concentration 1.6 to 5000μg/ml from BRIN-BD11 cells. Comparable insulin secretory responses to 200-25 μg/ml C. sinensis extracts were observed using isolated mouse islets with stimulatory effects equivalent in magnitude to 1μM GLP-1. However, insulinotropic effects of C. sinensis at its non-toxic concentration (200μg/ml) on BRIN-BD11 cells were prominently reduced by verapamil (42%), diazoxide (40%) and calcium-free conditions (43%), while the secretion was further potentiated by the use of IBMX (200μM, 2.4-fold, P<0.001) tolbutamide (200μM, 2.2-fold, P<0.05) and KCl (30mM, 2.6-fold, P<0.001). C. sinensis (200μg/ml) depolarized membrane (6.2 fold) with an increase in intracellular Ca2+ by 4.5 fold suggesting the importance of ion channel and Ca2+ in the mechanism of action. Moreover, in vitro study resulted significant inhibition of starch digestion by 10-40% (125 -1000μg/ml, P< 0.05-P<0.001), DPP-IV enzyme activity by 5-60% (200-5000μg/ml, P<0.05-P<0.001), and reduced protein glycation by 17-53% (50-200μg/ml, P<0.01-P<0.001). Hot water extract (250mg/5ml/kg) exhibited a significant decrease in blood glucose and fluid intake by 13% and 18.5% in high-fat-fed rats over 9 days' treatment, correlating to this plasma insulin (P<0.05) increased. The significant decrease in energy intake was associated with decreased in body weight. Significant improvement has been observed in impaired oral glucose tolerance in fat-fed obese rats after 9 days' treatment with hot water extracts. High-fat diet significantly increased plasma DPP-IV activity in rats that were substantially inhibited by C. sinensis (P<0.05-P<0.01). Conclusion: These data suggest that hot water extracts of C. sinensis leaf possess insulin releasing and antidiabetic properties both in vitro and in vivo. This warrant further evaluation of the plant as a new therapy for the treatment of type 2 diabetes.

P144

On-bead Analysis of Substrate Specificity of Caspases using Peptide Modified by Qauternary Ammonium Group as Ionization Enhancers

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Caspases are proteolytic enzymes at the heart of networks that govern apoptosis and inflammation. Therefore the investigation of new substrates for monitoring of their activity is of great importance. The one-bead-one-compound (OBOC) peptide libraries are widely used in the investigation of new biologically active compounds and mass spectrometry (MS) is currently the method of choice for the identification of compounds on single beads. However, up to now, the analysis of on-bead enzyme activity and the determination of on-bead cleavage site have not been investigated by MS due to the necessity of analysis of trace amount of compound obtained from a single resin bead (femtomole level) which may be insufficient for reliable sequence analysis. Previously we invented the application of quaternary ammonium ionization tags for ultrasensitive sequencing of peptides by tandem mass spectrometry [1, 2]. Here we present a new, rapid and straightforward method of the on-bead caspase activity analysis by LC-ESI-MS/MS method. A series of model peptides derivatized by bicyclic quaternary ammonium groups at the N-terminus were synthesized and used in the investigation of substrate specificity and enzyme activity of caspases. The hydrolysis of peptide bond on the resin, occurring even with low efficiency, release the peptide fragment bearing fixed charge tag allowing sensitive detection and identification of fragments in supernatant obtained after enzymatic digestion performed even on single resin bead. The obtained results suggests the applicability of the proposed methodology in the analysis of on-bead enzyme activity. This work was supported by a grant No. UMO-2015/17/D/ST5/01329 from the National Science Centre, Poland.

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P145

Inhibition of angiotensin converting enzyme by hydrolysates and peptides from wheat germ

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Wheat germ flour has been used to generate protein hydrolysates (WGPH) and peptides with anti-hypertensive activity upon proteinase K digestion. The ACE-inhibitory activity of the hydrolysates increased in a concentration-dependent manner in the 100-1000μg/mL interval. The IC50 of 0.65 mg/mL determined suggests WGPH as an attractive source of ACE-inhibitory peptides. To further characterize the active components, WGPH was analyzed by reversed-phase semi-preparative HPLC into twelve fractions that were examined for anti-hypertensive activity. Fractions testing positive for ACE inhibition were then submitted to nano LC-ESI-QTOF-MS (liquid chromatography-electrospray ionization-quadrupole time of flight- mass spectrometry) sequencing. Five sequences from the most active peaks were selected to be synthetically replicated. The corresponding synthetic versions (IGGIGTVPVGR, SGGSYADELVSTAK, MDATALHYENQK, VDSLLTAAK and VALTGDNGHSDHVVHF) showed substantial IC50 values (125.7, 128.2, 303.6, 159.7 and 189.3 μg/mL, respectively) and may have potential as functional food additives, or nutraceuticals to improve human health.

P146

The novel lasso peptide pseudomycoidin can be matured in vivo by only one processing enzyme

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Ribosomally synthesized and posttranslationally modified peptides (RiPPs) are a major class of natural products with a high degree of structural diversity and a wide range of bioactivities. One intriguing family of RiPPs are the Lasso peptides, the small molecules having unusual topology resembling a lasso. The structure is insured by the single posttranslationally installed isopeptide bond between the N-terminal amino group and the side-chain carboxyl group of aspartate or glutamate at the position from 7 to 9, forming an N-terminal macrocycle ring. The biosynthesis of mature lasso-peptides occurs in two steps catalyzed by the processing enzymes – leader peptidase and macrolactam synthetase. All but few known lasso peptides exhibit the “lassoed” form in which the C-terminal tail is trapped through the ring. This knot-like fold provides high compactness, extraordinary stability and diverse bioactivities, such as antimicrobial, antiviral, receptor antagonistic or enzyme inhibitory activities. Via bioinformatic analysis of genomic DNA of Bacillus sp. in particular, we found the lasso-clusters in B. pseudomycoides DSM 12442, belonging to the recently discovered family featuring a novel cluster architecture, similar to the recently described lasso peptide paeninodin cluster found in genome Paenibacillus dendritiformis C454. The observed cluster consists of the biosynthetic genes coding the 43 residues precursor peptide, RiPP recognition element (RRE), leader peptidase and a lasso cyclase as well as the gene of ABC- transporter. The heterologous expression of five mentioned genes under individual T7 promoter each in E. coli resulted in the production of predicted lasso peptide, called pseudomycoidin, with mass-spectrometry detected average m/z = 2618. Quite unexpectedly, the cluster possessing only one processing enzyme gene coding macrolactam synthetase under heterologous expression in E. coli was still sufficient for the synthesizing unphosphorylated lasso. The identity of these compounds was confirmed by several biochemical methods and by MS-MS & NMR analysis. It is particularly interesting because all lasso peptides described to date require at least two essential specific enzymes for the lasso formation. Also, the biochemical and structural investigations showed the pseudomycoidin having a branched cycle rather than a threaded “lassoed” topology typical for characterized homologs. But either way, we consider the macrolactam synthetase from B. pseudomycoides as a promising candidate for the development of a universal tool for large-scale in vivo synthesis of various pseudomycoidin derivatives and, possibly, other lasso-compounds.

P147

Modulation of CYP3A4 by the RGD- and Neurotensin (8-13)-analogues

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RGD- and Neurotensin(8-13)-based drug design is a hopeful perspective for drug development. There is a great deal of data of the observed therapeutic effects of both sequences (e.g. anticancer, analgesic etc.), but little is known about the pharmacokinetic of these peptides and possible drug interactions in which they can be involved. Because of that, we decided to check whether these sequences can affect the activity of the most common cytochrome P450 enzyme CYP3A4, responsible for the most drug interactions. We used two RGD-analogues modified in the first position with nonproteinogenic amino acids - Agb (1) and Agp (2), Neurotensin (8-13) (3) and modified at position eight and nine with Lys-Cav (4). We used the fourth peptides at concentrations of 25 to 100 μM. Both RGD-analogues (1 and 2) showed minor modification in the activity of CYP3A4 and the most pronounced was in the largest used concentrations. Neurotensin (8-13) (3) and its analogue (4) did not show activity on CYP3A4. The results have shown that the use of the RGD-analogues (1 and 2) should be monitored for possible drug interactions, while using Nuerotensin(8-13)-sequences does not endanger drug interactions at the cytochrome P450 3A4 level.

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P148

Synthesis, characterization and nociceptive activity of new Hemorphin-5 analogues

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Hemorphin-5, also known as Valorphin (Val-Val-Tyr-Pro-Trp-Thr-Gln), is a naturally occurring, endogenous opioid peptide of the Hemorphin family with affinity for opioid receptors and morphinomimetic properties. Hemorphin-5 is short-chain peptide initially obtained from a particular region of the β-chain of hemoglobin. The Hemorphin peptides are increasingly being used in the treatment of various diseases such as hypertension, epilepsy, diabetes, chronic pain, cancer, and etc. In order to block the enzymes involving conversion of angiotensin peptides is required searching of non-toxic substances with more efficient anti-inflammatory and analgesic activity. This report refers to the investigation of nociceptive activity of newly synthesized hemorphin-5 analogues containing unnatural amino acids injected intracerebroventricularly in mice. The novel blood peptide derivatives have been prepared by replacement of Gln at position 7 with unnatural amino acids (Val-Val-Tyr-Pro-Trp-Thr-X) using SPPS, Fmoc-strategy. In order to inquiry of the influence of amino acid residues have been incorporated of Ile/Aib at position 1 (X-Val-Val-Tyr-Pro-Trp-Thr-Gln). The crude neuropeptides were purified on an RP-HPLC and the molecular weights were determined, using ES-MS, and also determining of the specific angles of optical rotation. The nociception was studied using acetic acid-induced writhing test. The mice were observed for typical movements (writhes) for 30 minutes after the injection of the irritant. Some of the newly-synthesized Valorphin-5 analogues showed significant time- and dose-dependent antinociceptive effect compared to both the saline-injected controls ($p<0.001$) and the reference compound at the same doses.

Acknowledgements: Financial support from BG05M20P001-2.009-0015 "Support for the development of capacity of doctoral students and young researcher in the field of engineering, natural and mathematical sciences" funded by the operational programme "Science and Education for Smart Growth" 2014–2020 the co-financed by the European Union through the European Social Fund is gratefully acknowledged.

P149

Synergy of short antimicrobial peptides with β-lactam antibiotics against MRSA resides in the degradation of peptidoglycan barrier

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The mode of action of cationic antimicrobial peptides (AMPs) involves interaction with the negatively charged phospholipid bilayer of bacterial cell membrane causing its disruption via pore formation or detergent-like disintegration, thereby leading to cell death. In Gram-positive bacteria AMPs have to pass through the cell wall that consists prevalently of a peptidoglycan layer, before reaching the cytoplasmic membrane. Here we focused on mechanism of the action of short cationic α-helical AMPs in the combination with common antibiotics against methicillin-resistant Staphylococcus aureus (MRSA). We found that the studied peptides showed synergy only with β-lactam antibiotics but not with vancomycin or gentamicin. To explain this phenomenon we prepared protoplasts of MRSA – the cells that lack a cell wall. For this purpose we used lysostaphin which digested peptidoglycan layer. Since β-lactams are known to inhibit cell wall synthesis, the protoplasts became resistant to them. On the other hand the protoplasts were more susceptible to all studied peptides. Assuming that the peptide would have to pass through peptidoglycan barrier before reaching the cytoplasmic membrane we studied its interaction with peptidoglycan isolated from Staphylococcus aureus. The study based on RP-HPLC methodology showed that up to 25% of 10 μM peptide was trapped into peptidoglycan. This occurred practically immediately after mixing of these two components. Under the same conditions, approximately the same amount of peptide was absorbed also into MRSA cells without killing them immediately, but the cells were killed during next two hours. Our results indicate that binding of AMP to peptidoglycan (and thus bacterial cell wall) causes considerable interference during AMP-mediated killing the bacteria due to a significant decrease of AMP concentration that is required for the disruption of cytoplasmic membrane. Based on presented results, we hypothesize that in the course of the combination treatment, β-lactams cause degradation of peptidoglycan layer, resulting in a weakening of the cell wall, thus allowing AMP easier approach to the cytoplasmic membrane and its subsequent disruption. Consequently, lower concentration of both antibiotic and AMP is needed to kill the bacteria that finally results to synergistic effect.

P150

Development of bioinspired antimicrobials: the new edge of pharmacological design

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Peptide rational design was here used to guide the creation of novel compounds that could help on resistant bacteria control. Firstly, two novel short beta-lactamase inhibitors with five amino acid residues length were generated. Molecular modeling associated to peptide synthesis improved bactericidal efficacy in addition to amoxicillin, ampicillin and cefotaxime. Docked structures were consistent with calorimetric analyses against bacterial beta-lactamases. These two compounds were further tested in mice. Whereas commercial antibiotics alone failed to cure mice infected with Staphylococcus aureus and Escherichia coli expressing β-lactamases, infection was cleared when treated with antibiotics in combination with peptides, clearly suggesting that peptides were able to neutralize bacterial resistance. Moreover, host-defense peptides derived from mastoparan and clavanin families were redesigned in order to improve antimicrobial activities and decrease mammalian cell toxicity. Both peptides were evaluated in sepsis and wound model infections showing the ability to control the infection caused by Gram-positive and -negative pathogenic bacteria. Moreover in all cases, immune response was also evaluated. In summary, the unusual peptides here described provide leads to overcome beta-lactamase-based resistance, a remarkable clinical challenge.

P151

Chemometric/cheminformatic - assisted approach in the analysis of bitter peptides originating from food proteins

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It is well-known that peptides derived from food protein sources show variety of bioactivities like e.g.: cardioprotective, antioxidant, antibacterial, immunomodulating etc. Additionally, biopeptides released during hydrolysis of proteins may affect the taste of hydrolysates. Usually it is an off-flavor bitter taste which is considered by as the one of limitations when producing biologically, chemically, and functionally active foods. Unwanted bitterness of food hydrolysates may be the obstacle in their practical use in food and nutrition industries. During the recent years, the popularity and continuous development of information technologies contributed to the increase of data repositories (databases) as well as programs helpful for prediction of some phenomena in the molecules like e.g. peptides and/or proteins. The aim of the study was the application of information provided in biological and chemical databases as well as multivariate analysis techniques to create bitter di- and tripeptide datasets and finally to find the relationships between the structure (sequence) and bitterness of sequences analyzed. The following in silico tools were used: BIOPEP-UWM database of sensory peptides and amino acids, AAindex database, ProtScale, Biological Magnetic Resonance Data Bank, Statistica13.1®. PCA and MLR carried out for bitter di- and tripeptide data matrices led to observe the impact of the following attributes on the peptides' taste: molecular weight, bulkiness, number of carbon and hydrogen atoms, polarity and hydrophobicity (N- and C-terminal amino acids in a dipeptide chain). In case of tripeptides there were: molecular weight, bulkiness (C-terminal and middle amino acid) and molecular weight, bulkiness and propensity to be exposed to solvent (N-terminal residue). The approach applied confirmed the results of other authors aiming the studies on structure-bitterness of peptides. However, our data matrices are universal and can also be applied to analyze structure-function of peptides with other activities. *** This work was financed by Warmia and Mazury University in Olsztyn (project number: 17.610.014-300)

P152

Self-Assembling Peptides as a Platform for Supramolecular Antitumor Vaccines

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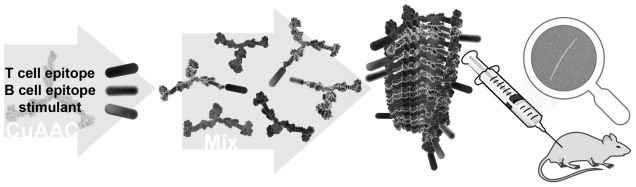
Peptides are ubiquitous in biological systems and present a class of biomolecules involved in controlling and modulating various processes in living organisms. Their chemical properties originate from the primary sequence and the related conformation. Knowledge of favorable structures such as α-helices or β-sheets can be harnessed to design monomers capable of self-assembling in a defined way into large supramolecular structures. Decorating the surface of this nano-sized objects with immunogenic molecular patterns results in a pathogen-mimicking particle thus potential vaccine candidate. To induce an immune reaction against tumor-associated thus endogenous structures, self-tolerance mechanisms must be overcome. Therefore, simultaneous presentation of different epitopes and immunostimulating agents is beneficial. Each supramolecular monomer can be specifically modified prior to self-assembly. Copolymerisation of these building blocks in water yields polymers equipped with epitopes in a multivalent manner. We describe the synthesis of a modular peptidic platform for antitumor vaccine development as well as their immunological evaluation. The three-armed star shape of the structural monomer (SM) originates from the 1,3,5-triazine core which bears three amphiphilic ethylene glycol (EG) modified tri-L-phenylalanine (F3) on the 2,4,6 positions. Immunogenic structures are conjugated via CuAAC to functional monomers (FM), which feature one alkyne terminated F3 sequence, instead of EG in SM. Three different cargos were loaded onto FM namely an immunostimulating agent (TLR7/8 agonist, imidazoquinoline), a T cell epitope (p30, tetanus toxin947-967) and a fully synthetic B cell epitope derived from the tandem-repeat sequence of the MUC1 glycopeptide. Furthermore, the latter incorporates the tumor-associated carbohydrate antigens Tn on threonine 11 and 2,3-sialyl T on threonine 18. C57B/L mice were immunized and the generated antisera exhibited high titers especially of the IgG2c isotype as determined from ELISA. FACS analysis revealed efficient binding of the generated antibodies to T47D tumor cells with high affinity. These results demonstrate the capabilities of supramolecular polymers to serve as a vaccine platform and highly encourage further investigations.



P153

Trans-Blood brain barrier peptides to enhace delivery of therapeutic molecules to treat Alzheimer's disease

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The delivery of therapeutic molecules to the central nervous system is hampered by poor delivery across the blood-brain barrier (BBB). Several strategies have been proposed to enhance transport into the brain, including invasive techniques and receptor-mediated transport (RMT) 1. Both approaches have several drawbacks, such as BBB disruption, receptor saturation and off-target effects, raising safety issues. In the quest to find new peptides that can be used as trans-BBB peptide vectors we studied natural viral proteins, because virus are molecular machines specialized in cell penetration. Some domains in these proteins have characteristics commonly found in cell penetrating peptides (CPP), such as being cationic and lipophilic. We tested the four helical domains of Dengue Virus type 2 capsid protein (DEN2C). Their mechanism of translocation is receptor-independent and consistent with adsorptive-mediated transport (AMT). One peptide in particular, PepH3, reaches equilibrium distribution concentrations across the BBB in less than 24 hours in a cellular in vitro assay. In addition, biophysical studies with pepH3 showed that this peptide interacts with anionic membranes, similarly to what is found in brain endothelial cells 2. Importantly, in vivo biodistribution data with radiolabeled peptide derivatives shows high brain penetration. To demonstrate the brain-shuttle capacity, the peptide was conjugated to a single domain antibody (VL) targeted to Alzheimer's disease (AD) 3. VL-PepH3 presents fast clearance from brain and high levels of excretion, showing PepH3 competence as drug delivery system to take a cargo in and out the brain. The activity of VL is retained in the several efficacy tests: antigen-ligand kinetics (VL-Aβ1-42 binding kinetics), inhibition of Aβ aggregation and recognition of senile plates in humans.

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P154

Probing N-terminal modification of ALM F50/5 with triazolo – dipeptide on its conformation and bioactivity

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As anti-bacterial chemotherapy is facing tremendous challenges, antimicrobial peptides are showing a ray of hope to be used as an effective alternative to the traditional drugs [1, 2]. Although the mechanism of interaction of this kind of peptide with membrane is not fully understood [3]. Here we particularly focus on a strategy of enhancing bioactivity through structural modification without altering its sequence. A bioactive peptaibol [4] like alamethicin was used in this study as a template for structural modification and investigating its influence on conservation of conformation and in turn, bio-activity. The study was conducted for two main reasons. First, the insertion of 'so called' peptidomimetic scaffold, 'triazole' at the N-terminal end of alamethicin replacing amide bond to observe whether or not the structure is perfectly poised and restore its conformation [5, 6,]. Secondly, how the insertion of fluorinated aromatic moieties impart hydrophobicity to the structure that lead to a better interaction. For the dipeptide synthesis, principle of 'Click chemistry' [7, 8] was exploited as it is a highly selective and reliable reaction to generate triazole moiety using copper as catalyst. These dipeptide fragments were subsequently used for generation of alamethicin analogues using Fmoc-based SPPS. Our main aim was to find a way to rationalize the interaction of alamethicin with lipid membrane through structural modification at the N-terminal end so as to augment the biological activity of the peptaibol.

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P155

Antibody Epitope of human α-Galactosidase A revealed by affinity-mass spectrometry

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Alpha-galactosidase (αGal) is a lysosomal enzyme that hydrolyses the alpha-galactosyl moiety from glycosphingo-lipids. Mutations in the αGal genes lead to defect enzymes resulting in substrate accumulation and pathophysiology. The deficiency of αGal, called Fabry's Disease (FD), belongs to the lysosomal storage diseases. Effective treatment of FD has been developed by enzyme replacement therapy (ERT) by infusion of recombinant enzyme to increase enzyme levels and reduce accumulated substrate. Immuno-reactivity and IgG antibody formation are major, therapy-limiting complications of ERT. Here we report the antibody epitope identification of human αGal, αGal(309-332), using a combination of proteolytic excision of the immobilized immune complex. The epitope peptide, αGal(309-332) was synthesized by solid-phase peptide synthesis; its affinity was determined by SPR (KD, 39 x 10-9 M) nearly identical to that of the full length enzyme (KD, 16 x 10-9 M). Proteolytic excision- mass spectrometry is shown to be an efficient tool for epitope identification of

immunogenic lysosomal enzymes. Since the full length enzyme and the antibody epitope showed comparable binding affinities, this provides a basis for reversing immunogenicity.

P156

The effects of glutathione analogues on Na,K-ATPase activity in the kidneys of the wildtype and the Wfs1 gene mutated mice in vitro and in vivo

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Protein S-glutathionylation, the reversible formation of mixed disulfides between glutathione and cysteinyl residues of proteins, is a potential mechanism for dynamic, post-translational regulation of a variety of regulatory, structural, and metabolic proteins in cells. Na,K-ATPase is a crucial plasma membrane enzyme in kidneys composed of α subunit, β subunits and a regulatory subunit belonging to the FXYD protein family, which all contain free cysteine residues. An α subunit have 23 free cysteine residues, the β have only one and FXYD protein two of them. The WFS1 gene encodes wolframin, an endoplasmic reticulum membrane glycoprotein, which regulates another protein folding and secretion, cellular transport and calcium homeostasis. Mutations in WFS1 gene lead to changes in Na,K-ATPase activity and in the expression of the α and β subunits. The aim of this study was to investigate the effect of glutathione (GSH) and its analogues UPF1 (o-methyl-L-tyrosinyl-g-L-glutamyl-L-cysteinyl-glycine) and UPF17 (o-methyl-L-tyrosinyl-L-glutamyl-L-cysteinyl-glycine) on Na,K-ATPase activity in two months old wildtype and WFS1 knockout mice kidney in vitro and in vivo. In our lab a small library of glutathione analogues (UPF peptides) was designed and synthesized. The activity of the Na,K-ATPase was determined as the ability to hydrolize ATP and expressed as μmol Pi/mg min. In vitro the samples were pre-incubated with GSH and UPF peptides for 10 and 45 minutes at room temperature. In vivo glutathione analoques were administrated i.p. 1 mg/kg for 5 days. The experimental results of the study showed that glutathione and UPF17 in concentrations 10-3 M inhibit the activity of Na,K-ATPase in wildtype and WFS1 knockout mice kidney in a time dependent manner. UPF1 in concentrations 10-3 M at pre-incubation time 10 min inhibits the activity of Na,K-ATPase in wildtype, but activates in WFS1 knockout mice kidney. Same concentration of UPF1 at pre-incubation time 45 min did not have any effect in both genotypes in vitro. Intraperitoneal administration of UPF1 and UPF17 inhibit the activity of Na,K-ATPase in wildtype mice kidney, but did not have any effect in WFS1 knockout mice in vivo. The various effects of glutathionylation on the activity of Na,K-ATPase may be caused both by structural differences between the GSH and UPF peptides and by conformational changes in the enzyme of WFS1 knockout mice. The present study was supported by Institutional Research Funding No IUT20-42.

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P157

New Tricks with Tridecaptins – Improving Peptidase Stability, Cost-Efficiency and Identifying Novel Mechanisms of Action

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The rapid emergence of multidrug resistant bacteria is a major global concern. A recent UK report predicts that by 2050, antibiotic resistance will cause 300 million premature deaths and kill more people than cancer.[1] Therefore, there is a pertinent need for new antimicrobial compounds and targets. In recent years antimicrobial peptides (AMPs) have become the vanguard of new antibiotic candidates. The tridecaptins are a family of non-ribosomally synthesized AMPs that show strong activity against Gram-negative bacteria, including multidrug resistant strains of Klebsiella pneumoniae and Acinetobacter baumannii.[2] These tridecapeptides operate by a currently unique mechanism of action – they bind specifically to lipid II on the inner membrane of Gram-negative bacteria and disrupt the proton-motive force.[3] The tridecaptins are inactive against Gram-positive bacteria and show low levels of cytotoxicity and hemolytic activity. They are short, linear peptides and therefore easily accessible by solid-phase peptide synthesis. Furthermore, the combination of L- and D-amino acids in their structure provides excellent stability to normally encountered peptidases. However, it was recently reported that the D-stereoselective peptidase TriF hydrolyzes the tridecaptins, abolishing antimicrobial activity.[4] Through analysis of the solution NMR structure of tridecaptin A1 bound to lipid II, we have rationally designed and synthesized new cyclic tridecaptin analogues that retain antimicrobial activity and are resistant to TriF. This was achieved by replacement of a key hydrophobic interaction with a covalent linkage. Another limitation of the tridecaptins is their cost of synthesis, owing to the presence of several non-proteinogenic amino acids. We have synthesized a library of new linear tridecaptin analogues that retain full antimicrobial activity and can be synthesized at a fraction of the price. We have also identified that the tridecaptins bind to a key enzyme involved in bacterial lipoprotein synthesis and are investigating the molecular basis of the inhibition mechanism.

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P158

Serpin A1 Derivatives as Collagen Turnover Modulators for Cosmeceut cal Uses

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One of the main enzymes responsible for the degradation of collagen is elastase, a protein secreted by neutrophils, which digests the components of the extracellular matrix, giving rise to inflammatory phenomena. The main physiological inhibitor of this enzyme is represented by serpin A1, a component of the serpins family, which are proteins endowed with anti-protease activity. Following to a preliminary report on the activity of the C-terminal portion of this protein [1], we showed that the decapeptide SA1-III is able to modulate collagen type I turnover in cultured human fibroblasts [2]. With the purpose of investigating the mechanism of

action of SA1-III, we synthesized four partially overlapping tetrapeptide fragments of this peptide, using the solid-phase strategy, followed by chromatographic purification and analytical characterization by HPLC-MS. All the peptides were used for in vitro studies on cell cultures derived from neonatal dermal human fibroblasts (NHDFs) in order to determine the concentrations of collagen type I produced in the presence of each tetrapeptide, in comparison with the basal concentration of collagen, measured in untreated fibroblasts. We performed Western blot test on culture medium derived from fibroblasts treated with the peptides, as well as in cell lysates. We chose to analyze specific protein bands, i.e. those related to the procollagen forms, which is the precursor of collagen type I, and collagen degraded by proteases released by fibroblasts in culture media. This analysis showed that the mechanism of action leading to increased concentration of collagen type I in cell treated with both SA1-III and its derivatives is an anti-protease action rather than a stimulation of de novo collagen synthesis. We excluded also a potential mitogenic effect of the tested peptides, which could explain the observed increase in collagen concentration, but which would determine toxicity in vivo. In particular, we assessed a significant ($p < 0.05$) activity of peptides SA1-III and AAT11, one of the tetrapeptides. Since the activity of collagen turnover modulators displayed by these short peptides appears very promising from a cosmeceutical viewpoint, we started a technology transfer activity, based on the new company Apotech srl, aiming to formulate, test, and commercialize anti-age cosmeceutical products based on the new peptides.

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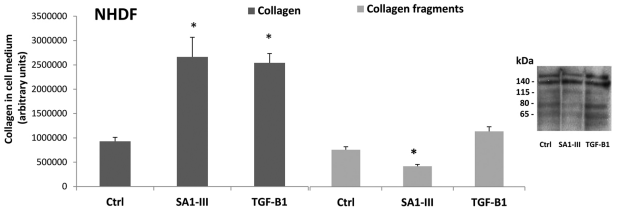

P159

Nature-derived peptides as molecular tools to study GPCR signaling

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Peptides are important bioactive molecules found in all organisms from bacteria to plants to mammals. They can function as hormones, neurotransmitters or defense molecules against host-pathogens. Bearing large diversity, naturally-occurring peptides represent novel ligands for a plethora of molecular targets and thus constitute attractive lead structures for drug development. An important group of such biological targets are G-protein coupled receptors (GPCRs), which are involved in a variety of physiological signaling processes and are therefore considered one of the most promising drug target classes; approximately 30% of all approved drugs are thought to act through modulation of these receptors. In this study we aim to investigate the modulation of GPCR signaling by nature-derived peptides. For this purpose, we will isolate and characterize peptides from plants and insects using reversed-phase liquid chromatography and mass spectrometry. These nature-derived peptides will be utilized as templates to design novel chemically-modified peptide analogs by applying solid-phase peptide synthesis. A focus of our research are oxytocin/vasopressin and opioid receptors as representative members of the GPCR family; pharmacological characterization of functionality and affinity of these receptors will be conducted using second messenger quantification as well as radioligand binding assays. To gain more insights into biased signaling properties of our nature-derived peptides we will be measuring selective activation of β-arrestin recruitment and receptor internalization by bioluminescence resonance energy transfer studies. The use of nature-derived peptide libraries will enable us to identify novel peptide ligands capable of modulating selective pathways of GPCR cellular signaling. These molecular tools may in the future be used for in vitro and in vivo efficacy studies to develop lead compounds for therapeutic indications such as multiple sclerosis, pain or autism.

P160

How to control Pseudomonas aeruginosa-induced pneumonia and keratitis? A lesson from the amphibian skin-derived peptide Esculentin(1-21) and its diastereomer

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The Gram-negative bacterium Pseudomonas aeruginosa is among microbial pathogens for which new anti-infective agents are urgently needed. It causes a large variety of infections including pneumonia especially in cystic fibrosis sufferers and keratitis in contact lens wearers. Naturally occurring antimicrobial peptides (AMPs) hold promise as novel therapeutics (1). Amphibian skin secretions are one of the richest sources for AMPs. We discovered that the frog skin-derived cationic AMP Esc(1-21) has (i) rapid killing kinetics against both free-living and biofilm forms of P. aeruginosa, with membrane-perturbing activity as a plausible mode of action which limits the emergence of resistance (2); (ii) the capability to preserve the barrier integrity of airway epithelial cells better than the human AMP LL-37; (iii) the ability to protect host from pulmonary Pseudomonas infection after a single intra-tracheal instillation at a very low dosage (0.1 mg/kg) without provoking an inflammatory response (3,4); (iii) the ability to stimulate migration of bronchial epithelial cells and, as a result, presumably to accelerate the recovery of an injured bronchial epithelium (5). Furthermore, a diastereomer of Esc(1-21), containing two D-amino acids was found to be less cytotoxic; more stable and with better in vivo efficacy. Importantly, one of the drawbacks in developing AMPs as new therapeutics is their inefficient delivery to the target site. We discovered that polymeric nanoparticles made of poly(lactic-co-glycolic) acid represent a promising tool for pulmonary delivery of peptides and their sustained release. We also demonstrated that the diastereomer is more efficient in treating Pseudomonas-associated keratitis in murine models, as well as in preventing bacterial attachment to soft contact lenses upon covalent conjugation to them. Overall, these peptides represent attractive alternatives to control Pseudomonas-associated pneumonia and keratitis.

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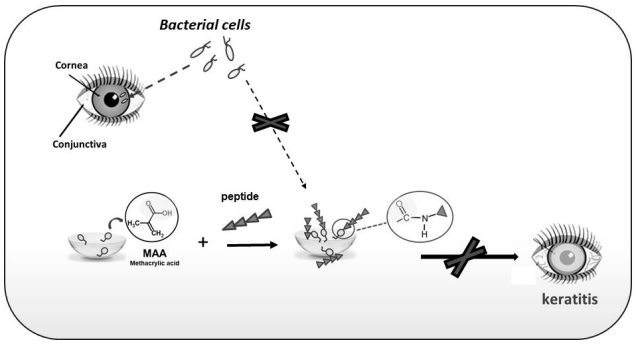

P161

Development of an IgG/IgY sandwich-ELISA for the bioactive polypeptide Prothymosin alpha

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Prothymosin alpha (ProTα) is a highly acidic polypeptide (109 amino acids in humans) expressed in all mammalian cells and acting both intracellularly and extracellularly as an anti-apoptotic and proliferation-enhancing factor or as an immunostimulating mediator, respectively [1]. Under certain cellular conditions ProTα may be enzymatically cleaved resulting in biologically active peptidyl fragments, such as the N-terminal fragment ProTα[1-28] (also known as thymosin alpha 1, Tα1) and the C-terminal decapeptide ProTα[100-109] [2,3]. Immunoassays distinguishing between intact parental ProTα and the active fragments thereof are considered valuable laboratory tools for elucidating the precise mechanism(s) of action of the polypeptide, thus facilitating its suggested clinical exploitation. In this work, we have employed rabbit IgG- and chicken IgY- antibodies -previously raised by our teams- to develop a sandwich-ELISA which is highly specific for intact ProTα and shows no cross-reactivity with ProTα[1-28] or ProTα[100-109]. More specifically, rabbit IgG-antibodies raised against a conjugate of synthetic ProTα[100-109] with keyhole limpet hemocyanin (KLH) have been indirectly immobilized on the ELISA microwells; standard solutions prepared by dissolving commercially available ProTα in various cell culture media, and chicken IgY-antibodies raised against a ProTα/KLH conjugate which show high specificity for the intact polypeptide [4,5] have been subsequently added to the microwells. Commercially available enzyme-labelled secondary antibodies have been used for signal development. The sandwich-ELISA shows very good reproducibility (intra- and inter- assay CVs < 8%) and a detection limit of < 2 ng/mL, while its high specificity for intact ProTα has been verified by cross-reactivity studies with synthetic ProTα[1-28] and ProTα[100-109] as well as synthetic peptides of the beta-thymosin family. The sandwich-ELISA is currently being applied to the supernatants of human cells cultured in the presence of various necrotic factors, which may release intact ProTα as a damage-associated molecular pattern (DAMP)/alarmin mediating responses of the innate immune system [1].

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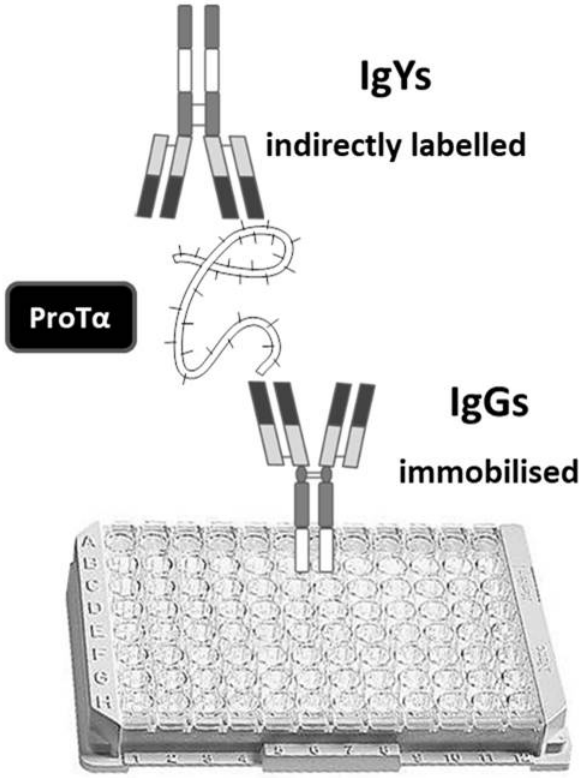

P162

Pore-forming antimicrobial peptides: design rules for amphipathic α-helices

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Antimicrobial peptides (AMPs), also known as host defense peptides, can kill microorganisms and constitutes a potential new class of antibiotics. It has been proposed that some AMPs form pores in bacterial membranes, thereby destroying the cell integrity. KIA peptides are cationic amphipathic α-helical AMPs based on the sequence KIAGKIA, with varying lengths from 14 to 28 amino acids. From antibacterial assays it was found that a minimum length of KIA peptides was needed to kill bacteria, which depends on the type of bacteria [1,2]. From vesicle leakage assays this length was identified as the membrane hydrophobic thickness; peptides must be long enough to span the membrane to induce leakage [1,2]. Using solid-state 15N-NMR, the orientation of the peptides in a membrane could be determined, and stable pores, formed with peptides in a transmembrane orientation, was observed in lyso-lipid containing membranes [3]. It was further found that the activity depends on the distribution of charges along the peptide sequence. For the KIA peptides, activity is highest when there are one or more positive charges at the N-terminus and the C-terminus is strongly hydrophobic. The distribution of charged and hydrophobic amino acids can be quantified by calculations of a 3D-hydrophobic moment (3D-HM) [4], and we show that a 3D-HM vector pointing along the helix axis of the peptides correlates with high activity. Based on this information we present a model of how the peptides insert into the membrane and form pores.

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P163

Synthetic peptides derived from Palmaria palmata show promising antidiabetic actions in cultured cells, as well as in acute in vivo studies in mice

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Background and Aims: Secretion of the incretin hormone, glucagon-like peptide-1(7-36)amide (GLP-1) from the intestinal enteroendocrine L-cells plays a key role in improving glycaemic control following a meal, but this peptide is rapidly inactivated by dipeptidylpeptidase-4 (DPP-4). Several component peptides identified from protein hydrolysates of the edible red seaweed Palmaria palmata, have been shown to possess DPP-4 inhibitory actions. Here we examined the efficacy of three of these short synthetic peptides to stabilise and improve the actions of GLP-1.

Materials and Methods: Peptides, Leu-Leu-Ala-Pro (LLAP), Met-Ala-Gly-Val-Asp-His-Ile (MAGVDHI) and Ile-Leu-Ala-Pro (ILAP) at preserving the stability of the incretin hormone GLP-1 was examined using a HPLC assay. GLP-1 stability was assessed over 24 h in the presence of porcine DPP-4 (5 mU) at 37ºC in triethanolamine buffer (50 mM, pH 7.8) with each peptide (10-6 M). In addition, peptides (10-12 to 10-6 M) were tested for their ability to promote acute insulin secretion from cultured pancreatic BRIN-BD11 cells at 5.6 mM glucose. The efficacy of peptides on stimulation of GLP-1 and GIP release from GLUTag and STC-1 cells respectively, was also assessed. Finally, peptides were administered by intraperitoneal (i.p.) injection individually (25 nmol/kg), or in combination with GLP-1 (25 nmol/kg) along with glucose (18 mmol, ipGGT) to healthy male NIH Swiss mice (aged 8-12 weeks) and tail blood samples collected at regular intervals between 0-120 min.

Results: All three peptides LLAP, MAGVDHI and ILAP demonstrated efficacy in inhibiting DPP-4 activity as assessed using an in vitro HPLC assay. GLP-1 control exposed to DPP-4 alone had a half-life of 1.5 h, but the above synthetic peptides extended the in vitro half-life of GLP-1 to 8, 10 and 13 h, respectively. LLAP and ILAP produced a dose-dependent (10-11 and 10-6 M) increase in insulin secretion (1.4- to 2.0-fold) from cultured BRIN-BD11 cells compared to 5.6 mM glucose controls ($P<0.01$ to $P<0.001$). During acute co-incubation using murine intestinal GLUTag cells cultured at 2 mM glucose, no secretion of GLP-1 above baseline was noted. However, co-incubation of ILAP and LLAP in STC-1 cells, caused significant secretion of GIP (by 1.8- and 1.5-fold; $P<0.05$, $P<0.01$) whereas in contrast MAGVDHI reduced GIP secretion by 40% ($P<0.05$). When tested in vivo in mice, LLAP and ILAP produced a 43-52% reduction ($P<0.05$) in the blood glucose area under the curve (AUC0-120 min). When co-injected with GLP-1, these peptides resulted in a further 13% (LLAP) and 16% (ILAP) reduction in blood glucose AUC0-120 min versus GLP-1 injection alone.

Conclusion: Synthetic peptides derived from P. palmata have displayed promising anti-diabetic actions helping to stabilise GLP-1 against DPP-4 degradation in vitro. Furthermore, LLAP and ILAP stimulated acute insulin secretion in cultured pancreatic cells, as well as providing additive therapeutic benefits when co-administered with GLP-1.

P164

Screening strategy to develop anticancer interfering peptides by SPOT synthesis

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Cyclin-dependent kinases (CDK) are heterodimeric kinases whose sequential activation regulates cell cycle progression. Overexpression or exacerbated CDK-cyclin activity has been documented in several cancers (e.g.: CDK4 and CDK6 are overexpressed in glioblastoma). Therefore CDK-cyclin complexes are attractive pharmacological targets for the development of anticancer drugs. To develop new interfering peptides targeting these complexes, we analyzed the interaction of CDK4 with Cyclin D1, D2, D3 and its inhibitor p16(INK4a). For this purpose, we used the following screening strategy using peptide libraries synthesized by SPOT synthesis: 1) Determination of the different Cyclin D and p16(INK4a) epitopes using a "PepScan" library; 2) Evaluation of the optimal epitope length using a "LengthAna" library and 3) Dissection of the key residues important for CDK4 interaction using a "SubAna" library. This strait forward screening strategy led to new interfering peptides targeting CDK4/cyclin complex. The best sequences have been synthesized as soluble peptides by SPPS (solid phase peptide synthesis) and tagged with a Tamra-dye for affinity measurements.

First results of these screenings will be presented. The next goal will be to covalently link the inhibitory peptides to a cell penetrating peptide in order to confirm their anti-proliferating activity in U87 cells.

Furthermore optimization of vectorization will be carried out with the aim for therapeutic application in terms of tissue targeting and plasma stability, to increase bioavailability of the therapeutic molecule at the site of the tumor and to reduce non-specific side effects.

P165

A novel frog derived peptide with promising anti-tuberculosis activity

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The rapid emergence and spread of multidrug-resistant tuberculosis (MDR-TB) to currently available drugs highlights the importance of screening for novel anti-TB agents. Cationic antibacterial peptides from amphibian skin could be a potential alternative therapeutic agent against Mycobacterium tuberculosis because of their unique mode of action. In our study, a peptide that belongs to brevinin family, isolated from the skin secretion of an Indian frog showed potent in vitro mycobactericidal activity and inhibited M. tuberculosis H37Rv strain. This peptide is non-toxic to macrophages but eliminated intracellular mycobacteria in a concentration dependent manner. It showed potent membranolytic activity and induced morphological changes on the bacterial cell wall. This amphibian peptide could be developed into a potential alternative therapeutic agent for the treatment of

tuberculosis. This is the first study demonstrating an amphibian derived peptide with anti tuberculosis activity.

P166

Synthesis and in vitro biological activity of new analogs of BIM-23052 containing unnatural amino acids

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Somatostatin is an endogeneous cyclic tetradecapeptide hormone that exerts multiple biological activities via a family of five receptors. The dual actions of natural somatostatins (inhibition of hormone release and cell growth) make them logical candidates as anticancer drugs, as well as for the treatment of neuroendocrine disorders. Some peptide analogues of this enzyme are widely used in clinical practice. However, their clinical efficacy is limited because of some disadvantages. In this respect, the search for new drugs of this type is an important task. BIM-23052 (DC-23-99) D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH2 is a linear SST analog with established in vitro GH-inhibitory activity in nM concentrations. It is characterized by high affinity to str5, sstr3 and sstr2, as sstr5 and sstr2 are present in high level in the cell membranes of a large number of tumor cells. This gave us the reason to synthesize modified analogues of BIM 23052, which are expected to possess anti-tumor activity. To study the relationship structure-biological activity a series of new analogs of BIM-23052 have been synthesized, where Thr, at position 6 was replaced with conformationally hindered Tle, Aib, Ac5c and Ac6c. The peptides were synthesized by standard solid phase peptide chemistry methods Fmoc-strategy.The cytotoxic effects of the compounds were tested in vitro against a panel of tumor cell lines: HT-29, MDA-MB-23, Hep-G2, HeLa and normal human diploid cell line Lep-3. All five somatostatin receptor types were modeled and docking was performed in order to determine the binding affinity of the analogues. The new peptides exhibited different concentration-dependent antiproliferative effect against the tumor cell lines after 24 h of treatment. The compound 3B (Aib6) demonstrated the most pronounced antiproliferative effects on HepG-2 cells with the IC50 = 0.01349 nM. This effects were proved with docking as the compound forms more stable complexes with somatostatin receptors within all new peptides tested.

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Structure-guided design and synthesis of peptides targeting protein synthesis in bacteria

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The rapid increase in drug-resistant infections poses a serious challenge for common antimicrobial therapies. There is thus an urgent need to combat this threat and to develop potent pharmaceutical agents that are effective against multi-drug resistant pathogens. Among antibacterial targets developed in our laboratory, we will illustrate how high resolution structural studies lead to an improved structure-based design strategy and optimized peptides that specifically bind and inactivate complex molecular machine. Our work focuses on proline-rich antimicrobial peptides (PrAMPs) a particular class of Antimicrobial Peptides (AMPs), whose mechanism of action involves specific inhibition of protein biosynthesis. AMPs are an abundant and diverse group of natural molecules that are produced by many tissues and cell types from prokaryotes to humans. Their unique antibiotic spectrum is generally determined by amino acid sequences and conformational properties. In contrast to many AMPs whose mechanism of action often involves disruption of the bacterial cell membrane, PrAMPs are actively transported inside the bacterial cell where they bind and inactivate specific targets. It was found that a number of these peptides inhibit protein synthesis by binding to the bacterial ribosome in the peptide exit tunnel. The recently solved crystal structure of the complex between the bacterial ribosome and Onc112, a representative member of the PrAMP family has been used as a starting point to further optimize these peptides and gain additional insight into the molecular determinants of this interaction.

P168

Fighting melanoma - human lactoferricin derived peptides kill cancer cells by inducing severe stress and apoptosis

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Treatment of melanoma still remains a challenge. Poor cancer toxicity

and side effects are often caused by lack of specificity for cancer cells. Furthermore drug resistance and relapse of disease, due to weak treatability of metastases, are major problems. We demonstrated that the negatively charged lipid phosphatidylserine (PS) is specifically exposed by cancer cells and metastases, thus representing a novel target for cancer therapy and the basis for the design of cationic antitumor peptides derived from human Lactoferricin (hLFcin) [1]. The hLFcin derivatives show highly increased activity and specificity against malignant melanoma and melanoma metastases compared to the parent peptide hLFcin. We improved the peptide specificity towards cancer cells by variations of peptide length, net positive charge and hydrophobicity, consequently affecting the secondary structure and the killing mechanism. Our studies reveal characteristic features of hLFcin derivatives facilitating specific killing of cancer cells. 2D and 3D in vitro studies show that highly active but nonselective peptides act through a direct membranolytic mechanism whereas selective peptides trigger apoptosis, which is also membrane-mediated by primary interactions with PS and subsequent interactions with intracellular targets like the Golgi apparatus. Besides PS plasma membrane cholesterol is crucial for the specificity of peptide-membrane interactions. Model systems mimicking cancer and non-cancer cells with different ratios of PS, phosphatidylcholine and cholesterol reveal different mode of actions for the peptides. The peptides alter the lipid distribution and fluidity of cancer cell membranes, which may lead to changes of the permeability of the plasma membrane and to secondary effects like the loss of function of membrane proteins or the release of stress signals like ROS (reactive oxygen species) and ATP. The specific cytotoxic effect of the designed peptides on cancer cells was demonstrated in in vivo studies in mouse melanoma xenografts showing tumor regression and suggesting them as potential candidates for further (pre)clinical development. Acknowledgment: Austrian Science Fund (FWF; grant no. P24608-B23) and Austrian Research Promotion Agency (FFG; grant no. 855671) [1] Riedl et al. BBA 1808(11): 2638-2645, 2011

P169

Lipopeptides derived from BP100 containing a D-amino acid or a His residue

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Plant diseases caused by bacteria and fungi are currently one of the major problems in agriculture, producing important economic losses. The solution to this problem relies on the use of copper derivatives, antibiotics or fungicides. These compounds are efficient; however, they are regarded as serious environmental contaminants. Moreover, antibiotics are not allowed in many countries and their use is hampered by the appearance of resistant strains [1].

Antimicrobial peptides have emerged as a good alternative to traditional pesticides. They display a broad spectrum of activity and do not easily facilitate the development of microbial resistance. In this context, the LIPPSO research group, in collaboration with the Laboratory of Plant Pathology, has identified the peptide H-Lys-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Tyr-Leu-NH2 (BP100), which displays high antibacterial activity in vitro and is low hemolytic [2].

The acylation of antimicrobial peptides is a strategy to increase their biological activity and their stability to protease degradation. In fact, the incorporation of an acyl chain into linear or cyclic peptides has rendered lipopeptides with interesting biological activity, being the length of the hydrophobic chain crucial for the antibacterial activity [3]. Based on these considerations, a collection of lipopeptides derived from BP100, incorporating an acyl chain at the N-terminus or at the side-chain of each residue of the sequence, was synthesized. Lipopeptides with an interesting biological activity profile were identified.

Herein, with the aim of improving the hemolysis and the stability in front of protease degradation of the above sequences, 21 new lipopeptides containing D-amino acids or a histidine residue were designed and synthesized. Their antimicrobial activity, hemolysis, phytotoxicity and proteolytic stability will be presented and discussed.

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P170

Discovery of Dermaseptin-PC from frog skin secretion and rational design of broad-spectrum truncated analogues against both planktonic and sessile bacteria

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Background: Antimicrobial peptides (AMPs) derived from the amphibian skin secretions are promising drug prototypes against ESKAPE strains. However, their clinical trials to date have shown efficacy only as topical agents, partly due to potential cytotoxicity. Material & Methods: Dermaseptin-PC (DM-PC) was discovered by the combination of 'shotgun' cloning and MS/MS fragmentation sequencing. Peptide design was carried out based on bioinformatics to enhance bio-efficacy and therapeutic index. The parent peptide and its analogues were synthesized using Tribute® synthesizer (Protein Technologies). The secondary structures of natural peptide and the analogues were validated by circular dichroism, and their antimicrobial efficacies were tested both on planktonic and sessile bacterial cells. Results: DM-PC possesses potent and broad-spectrum antimicrobial activity, but induce considerable haemolytic property that precludes it for further development. Here, truncated mimetics DMPC-19 and DMPC-10 were designed to improved selectivity against microorganisms. DMPC-19 maintained the activity and produced moderate haemolysis, while DMPC-10 shows significant decreases on both antimicrobial and haemolytic activities. Additionally, DMPC-10A, which contains Cha substituted at the C-terminus of DMPC-10, exhibits potent biological activity and higher therapeutic index. Moreover, DM-PC, DMPC-19 and DMPC-10A effectively inhibited the S.aureus and P. aeruginosa biofilms. Conclusion: With the optimisation of length of peptide chain and hydrophobicity, the therapeutic index can be improved. Additionally, the study of dermaseptin peptide also provides new insight on lead antibiotic drug discovery especially against Gram-negative bacteria and resistant strains.

P171

Synthesis and antimicrobial activity of the bacteriocin pediocin PA-1 and analogs thereof

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Bacteriocins are a family of ribosomally-synthesized antimicrobial pep-

tides produced by a wide variety of bacteria. With their attractive properties and activities, these peptides are promising alternatives to conventional antibiotics in the food and animal production industry as well as veterinary and human medicine. Among class IIa bacteriocins, we were particularly interested in pediocin PA-1, a peptide of 44 amino acids containing two disulfide bonds produced by Pediococcus acidilactici able to inhibit the growth of several clinically relevant pathogens such as Listeria spp. Despite its great potential as an antimicrobial agent, the problems associated with its production continue to limit its applicability and delay regulatory approval. Using a combination of solid- and solution-phase strategies, we were able to overcome difficulties due to instability and undesired reactions and produce synthetic pediocin PA-1 in good yields. Pediocin PA-1 thus synthesized was a potent inhibitor of Listeria monocytogenes (MIC = 6.8 nM), similar to the bacteriocin produced naturally by Pediococcus acidilactici. Pediocin-derived peptides were also prepared to perform structure-activity studies and structural analysis by NMR. Of particular interest is that linear analogs lacking both of the disulfide bonds characterizing pediocin PA-1 were as potent. One linear analog was also a strong inhibitor of Clostridium perfringens (MIC = 37.8 nM), another important food-borne pathogen. The total synthesis, characterization and antimicrobial activities of pediocin PA-1 and analogs thereof will be presented and discussed in light of conformational analyses and structure-activity relationship studies.

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Identification and characterization of cyclotides from Brazilian Psychotria species

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Psychotria genus below to tribe Psychotrieae (Rubiaceae) and comprises approximately 2,000 species; some of them are reported as cyclotide-containing plant species. Cyclotides are characterized by a head-to-tail cyclized backbone usually composed of 28-37 amino acids, and a unique knotted disulfide topology involving six conserved cysteine residues termed the cyclic cystine-knot (CCK) motif. The CCK motif is responsible for their exceptional resistance to chemical, enzymatic and thermal degradation. This topology confers them remarkable stability and wide range of biological and/or therapeutic applications, between them, anti-HIV, insecticidal, antimicrobial, uterotonic, cytotoxic, hemolytic, trypsin inhibitory, and immunosuppressive activities. This work deals with the identification and characterization of cyclotides accumulated in the Brazilian plant species: Psychotria vellosiana (stems and leaves) and Psychotria leiocarpa (aerial parts). The dried plants were ground prior to solvent extraction with a mixture of MeOH:CH2Cl2 (1:1; v/v) for 24 hours (4 times). The crudes extracts were dissolved in ACN:H2O (1:9; v/v) and immediately used for solid phase extraction (SPE). C18 SPE cartridges (Strata-Phenomenex C18 55um, 70Å, 500 mg) were activated with MeOH and subsequently equilibrated with of aqueous 1 % FA. After application of the extract, the cartridges were washed with ACN:H2O (2:8; v/v) and ACN:H2O (8:2; v/v). To determine whether these fractions contained peptides with disulfide bonds, they were treated with the reducing agent 10 mM dithiothreitol and alkylation agent 100 mM iodoacetomide, and the mass increase of 348 Da was observed, suggesting that three disulfide bonds had been reduced. The three extracts showed the presence of peptides with masses in the range of 2900-3700 Da as analyzed by MALDI-TOF mass spectrometry (MS). HPLC chromatography allowed to isolate the novel möbius cyclotides, with m/z 2889 and 2905 from P. vellosiana and to identify some cyclotides in P. leiocarpa. The cyclotides sequencing were performed by reduction, alkylation and enzymatic digest with endoproteinases Glu-C, trypsin, chymotrypsin and followed by MS/MS analysis. The spectra were carefully examined and the sequences were proposed, based on the presence of both b- and y-series of ions (N- and C-terminal fragments).

P173

Discovery of A Novel Membrane-Disruption Peptide against Multi-Drug Resistant Gram-Positive Strains Isolated from Cystic Fibrosis Patients

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The prevalence of the infection by multi-drug resistant gram-positive strains, especially methicillin-resistant Staphylococcus aureus (MRSA), is increasing in the airways of cystic fibrosis (CF) patients. The survival of CF patients with the presence of the associated infections was even worse. Although antibiotic therapies have been employed to treat the infections, the environment of CF respiratory tract increases the biofilm-forming ability, which makes bacteria intrinsically resistant to be eradicated. Antimicrobial peptide (AMP) is a promising candidate against drug-resistant bacteria as well as the biofilm. In this study, we reported a novel antimicrobial peptide identified from the skin secretion of Limnonectes fujianensis by the combination of cDNA cloning and MS/MS sequencing, namely Japonicin-2LF. The peptide possesses an α-helical segment and a typical heptapeptide loop, rana box, at the C-terminus. The solid peptide synthesis was performed using Tribute® synthesizer (Protein Technologies), and the synthetic replicates exhibit potent antimicrobial activity against both wide-typed and CF clinic-isolated gram-positive strains. The fluorescent staining reveals that Japonicin-2LF efficiently permeabilise the cell membrane of bacteria, leading to cell death. In addition, Japonicin-2LF can inhibit the formation of MRSA biofilm and eradicate it in vitro. To further investigate the antimicrobial effect of Japonicin-2LF in vivo, the acute infection module induced by CF isolated MRSA was employed, using larva of the wax moth, Galleria mellonella. The results demonstrated that 25 mg/kg of Japonicin-2LF increase the survival rate of MRSA infected larva significantly, without producing any toxicity. The study suggests that Japonicin-2LF is such potential molecule that can be developed as a new antibiotic agent for treatment of MRSA associated chronic infection in CF respiratory tract.

P174

New 4-Aminopyridine derivatives containing peptide fragment designed for the treatment of Alzheimer disease and multiple sclerosis

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New 4-Aminopyridine derivatives containing peptide fragment designed for the treatment of Alzheimer disease and multiple sclerosis L. Vezenkov, B. Anchev, K. Fidanov, D. S. Tsekova Alzheimer's disease (AD) and Multiple sclerosis (MS) are neurodegenerative diseases. The AD process is associated with plaques and tangles in the brain. MS is a disease that causes inability of the CNS cells to comunnicate each to other. Neurodegeneration leeds to problems with cognitive function (dementias) and / or movement. According the literature data, no cure for AD and MS, but the treatments can help for the changing of the diseases progresion. Among the most effective medicals used for both diseases treatment are 4-aminopyridine (4-AP) and galanthamin. Based on the literature, we proposed an approach to produce new hybrid molecules between 4-aminopyridine and different peptide fragments, thus we expect to combine important pharmacological effects to influence AD and MS:- Inhibition of β -secretase due to peptide fragments; - Blocking of potassium channels leads to an increase in

the level of acetylcholine in the brain as well as an anti-inflammatory action due to 4-aminopyridine; - Easier passage through BBB, that due to 4-AP. 4-AP is of very high toxicity, limiting its use in the treatment of neurodegenerative diseases. By introducing non-toxic peptide fragments into the molecules of these compounds, we expect a significant reduction in toxicity, which is reflected in an increase in LD50 values. Here we report the synthesis of nine new hybride compounds containing 4-AP and different peptide fragments. The syntheses were performed in solution by sequentially joining the protected amino acids. The condensation was carried out by TBTU method, with the reagents being dissolved in a minimal amount of DMF. The resulting crude products were recrystallized to chromatographically pure products. The compounds were characterized by m.p. thin layer chromatography and NMR. Performing tests for citotoxicity, LD50 was found as IC50>100μM (micromoles), which is much higher compared to 4-AP. Cell toxicity screening was performed towards the following three types cell cultures and cultivation 1. Human hepatocellular cancer HEP-G2; 2. Mice neuroblastoma cell Neuro 2a; 3. Human chronic myeloid leukemia - BV-173. In order to determine whether the new 4-aminopyridine (4-AP) derivatives have a protective effect against AD and MS a variotions of studies are still in progress, which results will be reported in the Symposium presentation. This includes: - Tests for learning and memory; - Tests for antiinflamatory activity – in-vitro and in-vivo; - Tests for β-secretase inhibition activity, which due to the peptide segment of the molecule. Acknowledgement: This study is supported by the Scientific Research Fund of Bulgaria through the project DH 03/8, "Galanthamine's and 4-aminopyridine's derivatives containing peptide motif with expected effect on the Alzheimer's disease and multiple sclerosis".

P175

GLP-1 and GIP receptors co-agonists

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It is predicted that in 2030, more than 15% of the United States population will be diabetic and consequently, in time, suffer from additional complications: kidney failure, vision loss, nerve damage, leg amputation and others. Incretin-based therapies for the treatment of type 2 diabetes exploit insulinotropic actions of two gastrointestinal hormones: glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) - both hormones increase insulin secretion from pancreatic beta-cell following food ingestion. Previously, several classes of dual agonists at GLP-1 R and GIP R have been shown to possess better antihyperglycemic and insulinotropic efficacy than mono agonist peptides.

Here we report new, dual incretin co-agonists with balanced activity at the human GLP-1 R and GIP R; potential candidates for the development of the treatment for type 2 diabetes, obesity, and associated comorbidities. Structural similarities between GLP-1 and GIP sequences guided our rational design of GLP-1 derived peptides and consequently yielded compounds of high, balanced agonism at both receptors. Selective changes to the GLP-1 sequence also allowed us to improve compounds' resistance to proteolytic enzymes. Further enhancement of pharmacokinetic profile of those peptides was achieved by lipidation; which subsequently resulted in candidates which required less frequent dosing.

P176

Identification of an Arg-Leu-Arg motif that contributes to the binding interface between the cytokine MIF and the chemokine receptor CXCR4

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Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine that is involved in the pathogenesis of inflammatory and cardiovascular disorders. MIF triggers atherogenic leukocyte recruitment by interacting with the classical CXC chemokine receptors CXCR2 and CXCR4 (Bernhagen et al., Nat. Med. (2007)) and has been defined as an atypical chemokine (ACK). While the residues and motifs contributing to the MIF/CXCR2 interface have been identified (summarized in Tillmann et al., Front Immunol 2013)), the molecular determinants governing the interaction between MIF and CXCR4 are incompletely understood. We recently showed that the conserved proline-2 residue of MIF, its N-like loop that also supports MIF/CXCR2 binding, and the extracellular loops (ECL) 1 and 2 of CXCR4 contribute to MIF/CXCR4 binding (Rajasekaran et al., J Biol Chem (2016)), but a complete understanding of the MIF/CXCR4 interface including differences to classical CXCL12/CXCR4 binding events is lacking. Here, we identified the Arg-Leu-Arg (RLR) region at position 87-89 of MIF as a novel hotspot of the MIF/CXCR4 interface. 'RLR' represents an apparent 3D extension of N-like loop of MIF and thus might contribute to Site 1 interaction with CXCR4. This notion was experimentally validated by peptide spot-array technology, structure-activity relationships and molecular docking experiments. Importantly, a triple alanine mutant of MIF (R87A-L88A-R89A-MIF) in which the 'RLR' motif was substituted by Ala residues was expressed in E. coli BL21/DE3, purified and biophysically characterized. Although the mutated MIF shares a similar secondary structure with the WT-MIF, it could not bind to the N-terminal of CXCR4. Furthermore, we analyzed the activity of the triple alanine mutated MIF with a focus on MIF/CXCR4-driven (patho)physiologic effects. R87A-L88A-R89A-MIF failed to activate CXCR4 in a cell system of genetically modified Saccharomyces cerevisiae that measures CXCR4-mediated cell signaling responses. Moreover, we tested the effect of the triple alanine mutation on CXCR4-mediated chemotactic activity of MIF using the human B cell line JVM-3. Wildtype MIF but not R87A-L88A-R89A-MIF elicited the chemotactic migration of these B cells, confirming that the 'RLR' sequence is an indispensable region of the MIF/CXCR4 interface (Lacy*, Kontos* et al., Sci Reports 2018). Our results offer novel information on MIF/CXCR4-specific inhibition strategies in inflammatory and cardiovascular diseases.

P177

Chemical synthesis and investigation of the native form and an improved gamma-core analogue of Neosartorya fischeri antifungal protein 2 (NFAP2)

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NFAP2 is a novel cysteine-rich antifungal protein from Neosartorya fischeri NRRR 1811. The Minimum Inhibitory Concentration (MIC) values on clinically relevant Candida species vary between 0.391 and 1.563 μg/mL. Beside isolation from natural source and preparation by recombinant technology, NFAP2 was synthesized by chemical method. Native chemical ligation of thioester of the N-terminal 22-mer and the C-terminal 30-mer provided the reduced form of the protein containing six free cysteine thiols2,3. Glutathione redox buffer at pH 7.5 has been applied for the formation of natural disulfide bond pattern (abcabc). For functional mapping, fragments of NFAP2 were synthesized and tested. Antifungal susceptibility test revealed that not the highly-conserved gamma-core motif, but the mid-N-terminal part had impact on antifungal activity. Our investigations proved the importance of physico-chemical properties, such as net charge, on the antifungal effect. Based on these findings, an improved gamma-core peptide was designed and synthesized. The lower MIC values of this peptide could be explained by its greater net positive charge. An NFAP2 analogue containing the improved gamma-core in place of the original one was synthesized. Preparation was performed by native chemical ligation of two fragments of the protein. Different oxidation conditions were tried for the formation of disulfide bridges: oxygen of air and glutathione redox buffer. Both methods led to the same disulfide bond pattern. Despite of the fact that the improved gamma-core protein exhibits greater net positive charge than the native form, it proved to be less effective against Candida albicans.

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P178

Novel peptide-based control measures against the rice fungal pathogen Pyricularia oryzae

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The filamentous fungus Pyricularia oryzae is the main causal agent of the rice blast disease, which accounts for 10-30% yield losses per year globally. New antimicrobial peptides, analogs of the natural peptaibol trichogin GA IV, have been synthesized and tested in vitro against several P. oryzae strains from different geographic origin. Trichogin GA IV is the main component of the mixture of peptide congeners produced by Trichoderma longibrachiatum as part of its defence mechanism against other microorganisms. Its primary sequence is as follows: Oct-Aib-Gly-Leu-Aib-Gly-Gly-Leu-Aib-Gly-Ile-Lol (where Aib is alpha-aminoisobutyric acid, Oct is the n-octanoyl group and Lol is the 1,2-aminoalcohol leucinol). All analogs were synthesized by SPPS using a cost-effective strategy. In this presentation, the synthesis and conformational analysis of the peptides are presented, together with the results obtained with our in vitro screening, that has allowed the identification of several peptide sequences very effective in inhibiting spore germination and fungal growth. Those sequences are currently being tested in vivo to confirm their efficacy in protecting rice from the blast disease. This work is part of the Scientific and Technological Cooperation Agreement between the Italian Ministry of Foreign Affairs and International Cooperation and the Department of International Cooperation of the Ministry of Science and Technology of Vietnam.

P179

Novel Oligopeptides with angiotensin I-converting enzyme inhibitory activity found in an elastase-treated hydrolysate of porcine aortic elastin

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[Objective] Hypertension is one of the main risk factors causing cerebro- and cardiovascular diseases. Angiotensin I-converting enzyme (ACE) catalyzes the production of a vasoconstrictive peptide, angiotensin II from angiotensin I. Therefore, the inhibition of ACE activity is an essential target for antihypertension. Elastin is an important macromolecular protein which exists widely in elastic tissues such as arteris, ligaments, lung, skin, etc. and exerts its elasticity to such elastic tissues. It was recently shown that elastin is the protein with ACE inhibitory activity. The peptides with ACE inhibitory activity were found in a thermolysin-treated hydrolysate of bovine ligamentum nuchae elastin[1] and an elastase-treated hydrolysate of porcine aortic elastin[2]. We are particularly interested in novel ACE inhibitory peptides derived from porcine aortic elastin. Here, we investigated to isolate the ACE inhibitory peptides from a pancreatic elastase-treated hydrolysate of porcine aortic elastin. [Methods] Porcine aortic elastin was treated with pancreatic elastase and the hydrolysate was separated by the use of HPLC. The ACE inhibitory activity of fractions obtained was analyzed by ACE inhibition assay using hippuryl-L-His-L-Leu as a substrate (ACE inhibition assay). The peptides in ACE inhibitory fractions were separated and their sequences were identified by means of LC/MS/MS and chemically synthesized by solid-phase method. ACE inhibitory activity of synthetic peptides was examined by ACE inhibition assay. [Results] The novel ACE inhibitory peptides derived from pancreatic elastase-treated hydrolysate of porcine aortic elastin were oligo peptides in the crosslinked regions. [Conclusion] The resulting ACE inhibitory peptides may be beneficial as ingradients of functional foods for preventing hypertension.

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P180

Cell-penetrating peptides restricting oligomerization of G protein-coupled receptors: the CB1R-5HT2AR dimer

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G protein-coupled receptors (GPCRs) have been classically described as monomeric transmembrane (TM) receptors that form a ternary complex between a ligand, the GPCR and its associated G protein. However, it is now well accepted that many GPCRs form, in addition to functional monomeric structures, higher-order oligomeric complexes constituted by a number of equal (homo) or different (hetero) monomers (Figure 1A). GPCR heteromers are defined as novel signaling units with functional properties different from homomers and represent a completely new field of study. For instance, when coexpressed in specific brain regions, CB1R and 5HT2AR form heteromeric structures (Figure 1B) that have been related to (tetrahydrocannabinol-linked) effects such as memory impairment, anxiety and dependence.1 In the present study, in order to identify the functional properties of the CB1R-5HT2AR heterodimer, we used synthetic peptides with the amino acid sequence of the TM domains of CB1R, fused to a cell-penetrating sequence derived from HIV TAT, to disrupt the formation of the heteromer. These peptides were tested in bimolecular fluorescence complementation assays in cells expressing receptors fused to two complementary halves of YFP, and by their ability to modify cAMP and p-ERK1/2 signaling when both receptors are co-activated with ligands. In addition, we used molecular dynamics simulations of the CB1R-5HT2AR heterodimer to identify hot-spots on the dimer interface. This has led to a series of designed peptides fused to a CPP vector that effectively interfere with this predicted CB1R-5HT2AR interface.

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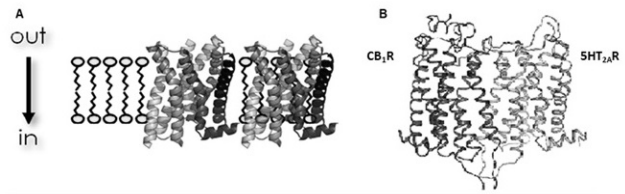

Figure 1. Representation of GPCRs oligomers in biological bilayers (A). Crystal structure of CB1R and 5HT2AR heteromers (B).

P181

The synthesis, oxidation and characterization of GLP-1 peptide receptor fragments

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Glucagon is a 29-amino acid peptide hormone that is produced by the post-translational cleavage of proglucagon, which is a 160-amino acid precursor polypeptide expressed in pancreatic α-cells, intestinal L cells and brain cells. In the intestine and the brain, proglucagon cleavage is catalysed by the PC1/3 enzyme, leading to the formation of glucagon-like peptide-1 (GLP-1), glucagonlike peptide-2 (GLP-2), glicentin and oxintomodulin. GLP-1 is a 31-amino acid peptide hormone that has approximately 50% amino acid sequence homology with glucagon, which is secreted mainly by the intestinal L-cells in response to nutrient ingestion. Despite this high degree of sequence homology, glucagon and GLP-1 perform opposing actions in glucose homeostasis. The main physiological role of glucagon is to protect the organism against the damaging effects of hypoglycaemia. [1] The mechanisms of action of GLP-1 go beyond just the augmentation of glucose-induced insulin secretion and include increasing insulin expression, inhibiting beta-cell apoptosis and promoting beta-cell neogenesis reducing glucagon secretion delaying gastric emptying, promoting satiety and increasing peripheral glucose disposal. It is clear from these varied actions that GLP-1 plays a central role in controlling postprandial blood sugar levels. These multiple effects have generated a great deal of interest in the discovery of long-lasting agonists of the GLP-1 receptor (GLP-1R) in order to treat type 2 diabetes. The receptors of GLP-1 (GLP-1R) formed a new branch of the GPCR superfamily, named 'Family B' which to date includes 15 members. Family B GPCRs bind their peptide ligands via a common mechanism known as the 'two-domain model' in which the NTD first binds to the C-terminal helical region of the ligand, thereby enabling a second interaction between the N-terminal region of the ligand and the core domain of the receptor. The latter interaction is essential for enabling agonist-induced receptor activation. [2] Our aims were the chemical synthesis, oxidation and characterization of GLP-1 peptide receptor fragments and the investigation of the peptide-ligand (GLP-1, liraglutide, exendin) interaction using NMR spectroscopy. Due to the difficulty and the length of the sequence we have decided to synthesize the 108 amino acid containing GLP-1 peptide receptor by native chemical ligation procedure. The designed fragments compatible with native chemical ligation was synthesized using solid phase peptide synthesis applying Fmoc/tBu strategy and the synthesis was carried out using a CEM® microwave assisted fully automated peptide synthesizer. The oxidation of the synthesized GLP-1 peptide receptor and the investigation of the peptide-ligand interaction is still in progress.

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P182

Influence of Arginine Mimetics on the Biological Effects of NT(8-13) Analogues

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Neurotensin (NT, pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) is a neuropeptide found in the periphery and in the central nervous system. The physiological and biochemical actions of NT are mediated through binding to NT receptors (NTRs). It is well-known that the active fragment of neurotensin is 8-13 (RRPYIL). The main drawback in the use of NT or any other endogenous peptide as a drug is extremely short half-life as a result of their rapid degradation by the action of peptidases. To overcome this problem, various neurotensin analogues were synthesized, which include linear peptides, cyclic peptides and non-peptide molecules. Additionally, incorporation of unnatural, guanidino containing and hydrophobic amino acids in bioactive peptides, and as we already reported, in the NT[8-13], increases both biological activity and resistance toward enzyme degradation. These observations prompted us to develop new analogues containing shorter homologues of Arg (having a chain of 5 carbons, α-amino-4-guanidino-butyric acid (Agb), and 4 carbon chain, α-amino-3-guanidino-propionic acid (Agp) at the N-terminal end of the NT[8-13]. In the present study, we thus investigated the effects of Agp/Agb residue incorporation at the positions 8 and 9 of the NT[8-13] fragment on biological activity (cytoxicity and central nervous activity).

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P183

Antistaphylococcal activity of the KR-12 alanine scan

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Staphylococcus aureus bacteria are still the most frequently isolated pathogens, responsible for mild as well as life threatening infections. Despite the constant progress in pharmaceutical sciences, the therapy of staphylococcal infections is sometimes ineffective, mainly because of an increasing resistance to antibiotics. Skin infections caused by staphylococci are associated with many interplaying factors, including genotypic background, immunological abnormalities, and skin barrier defects. As a matter of fact, patients with those diseases are characterized by an exceptional predisposition to be colonized, mostly by S. aureus. This can be also due to synthesis dysregulation of endogenous antimicrobial peptides (AMPs) such as LL-37. For this reason, the use of endogenous AMPs or their analogues in therapy may be the solution for treatment of staphylococcal skin infections. KR-12 (KRIVQRIKDFLR) is a 12-meric α-helical antimicrobial peptide derived from human cathelicidin LL-37. It exhibits anti-inflammatory and antimicrobial activity against broad spectrum of pathogens, including S. aureus. However, some staphylococcal enzymes such as metalloproteinase which disactivates the activity of LL-37 may also affect the peptide. The aim of this study was to learn whether or not the alanine scan of KR-12 could lead the compounds with improved activity of S. aureus and/or resistant to proteolytic activity. The biological studies included determination of the minimum inhibitory concentration and activity against biofilm for reference as well as clinical isolates of S. aureus. Moreover, to evaluate the cytotoxicity, the hemolytic activity against human RBCs was determined. The results confirmed the validity of the hypothesis. Some compounds exhibited the improved to KR12activity. The hemolysis rate was referred to individual amino acid substitution. Acknowledgments This study was supported by a grant from the Polish National Science Centre (Project No. 2016/23/B/NZ7/02919)

P184

Synthesis and in vitro Assessment of the Cytotoxic Effects of Novel RGD Analogues and Conjugates

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Delivering peptides with antitumour action directly into cancer cells is one of the most important strategies in recent decades. The amino acid sequence L-arginyl-glycyl-L-aspartic acid (RGD) plays role in interaction of many adhesion protens by connecting with $\alpha v\beta 3$ and $\alpha v\beta 5$ integrin receptors that are overexpressed on various cancer and angiogenic endothelial cells. Synthetic RGD peptides may affect adhesion, and tumour metastais, or directly induce apoptosis by activating caspases. Drug design based on the RGD structure may provide opportunity for targeted drug delivery and imaging, as well as a new chemotherapeutic treatment for cancer. We synthesized a series of short RGD mimetics and RGD conjugates and examined their cytotoxic activity against three cancer cell lines. RGD-modified peptides by shorter homologues of Arg (having a chain of 5 carbons, α-amino-4-guanidino-butyric acid (Agb), and 4 carbon chain, α-amino-3-guanidino-propionic acid (Agp), show an increase in the cytotoxic effect compared to the parent RGD peptide.

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P185

Computational Approaches to Peptide Permeability

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Resurgence of peptide drug discovery has taken place over recent years because the peptides can target the large interface of protein-protein interaction. However, there are some challenges - such as low membrane permeability that cause low oral bioavailability and difficult access to the intracellular targets. In this poster, we will discuss on the following computational tools established at Merck to assess the passive permeability of a peptide and a process of designing a peptide library guided by the design principles that led to identification of the permeable peptides. • Free

energy of Transfer (DG) • QSAR models of permeability assays • Rule of physicochemical parameters for passive permeability

P186

Novel hybrid reversed-phase packing material for high productivity of insulin purification

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Reversed-phase chromatography has an important role in purification of high value–added insulin pharmaceutical. It's very important to improve productivity in insulin industrial purification and packing materials which have long life time and high separation performance are strongly required. In order to meet such demands, we have developed novel reversed phase material based on hybrid silica, "Triart-SIL". It's commonly known that the choice of appropriate pore size and hydrophobicity is important for purification of peptides and proteins, and the packing materials bonded C8 ligand with 200 Å pore size increase efficiency for insulin purification. To enhance separation performance and stability for high productivity of insulin, we have optimized the pore size distribution, the density of C8 functional group and chemical bonding method. In this poster, we will show characteristics and improvements of these novel hybrid based packing material, and the chromatographic performance and advantages of them for use in purification of insulin.

P187

Improving uptake and metabolic stability of the therapeutic iCAL peptide in the context of cystic fibrosis.

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Cystic fibrosis (CF) is the most common lethal genetic disorder in populations of European descent. CF is due to loss-of-function mutations in CFTR (cystic fibrosis transmembrane conductance regulator) (1), an epithelial ion channel strongly involved in cell homeostasis and airway mucociliary clearance. Thus, CF patients suffer from airway obstruction and chronic infection. One of the three known functional defects associated with the most prevalent mutation, p.Phe508del-CFTR, addresses its stability at the apical membrane of endothelial cells. In 2010, our group reported the development of inhibiting peptides (iCAL) which are able to decouple the deleterious interaction between the CFTR-associated protein (CAL) and the C-terminal region of the p.Phe508del-CFTR, thereby rescuing CFTR activity at the apical membrane (11% increase in chloride efflux) (2-3). To further improve this therapeutic effect, we decided to optimize the ADME properties of the iCAL peptides, especially their intracellular delivery and their stability to the proteases encountered in CF lungs. In a first part, we will show that iCAL internalization can be dramatically improved by covalent coupling to cationic cell-penetrating peptides (CPP) (4). Internalization efficiency, subcellular localization and internalization mechanism on Caco-2 cells will be depicted and presented. In a second part, we report the optimization of the iCAL peptide sequence in terms of affinity and stability using the PIPEPLUS method (5), a variant of the SPOT synthesis (6) allowing the screening of peptide libraries. In this context, we will show that the introduction of non-natural amino acids within iCAL sequences could increase affinity or selectivity of the peptides for the CAL protein as well as their stability. Altogether, the presented results will push forward the development of more efficient and specific iCAL inhibitors as new therapeutics to treat the symptoms of CF patients.

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P188

Cytoplasmic delivery of a peptide modified with a sonosensitizer by using ultrasound

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In this study, we developed an ultrasound (US)-sensitive molecule for cytoplasmic delivery. As the molecule, we designed a peptide consisting of a functional peptide, a cell-penetrating peptide (CPP) and a sonosensitizer. We chose Bim peptide (apoptosis inducing peptide; the BH3 domain derived from Bim apoptosis-inducing protein) as the functional peptide. We also used Tat peptide derived from the HIV-1 transactivator of transcription protein as the CPP. CPPs have been widely used for intracellular delivery of various cargos. The conjugate of Bim peptide with Tat, TatBim was synthesized by conventional Fmoc-based solid-phase peptide synthesis. The TatBim was contained Cys residue at the C-terminus for connecting with rose bengal (RB)-maleimide as the sonosensitizer. The sequence of obtained US-sensitive molecule, TatBim-RB was RKKRRQRRR-EIWAQELRRIGDEFNAYYARG-C(RB). To confirm cellular delivery of TatBim-RB, Chinese hamster ovary (CHO) cells were incubated with TatBim-RB and we observed the intracellular internalization of TatBim-RB using fluorescence microscopy. TatBim-RB was detected in the cells with a dotted localization pattern, indicating that the TatBim-RB was entrapped in endocytotic compartments. CPP-fused molecules tend to become entrapped in endosomes. TatBim-RB needs to escape from the endosomes because a target of the Bim peptide exists in cytoplasm. To promote escape of the entrapped Tat-Bim-RB molecules, the CHO cells were irradiated with US. After US irradiation, stronger and diffuse fluorescence of TatBim-RB was observed in the cells, indicating that TatBim-RB molecules had escaped from the endocytotic compartments and diffused into the cytoplasm. To confirm apoptosis inducing by Bim peptide in the US-sensitive molecule, the CHO cells were stained using apoptosis detecting kit and the apoptotic cells stained were visualized using fluorescence microscopy. We successfully detected apoptosis inducing by the molecule irradiated with US. In the case of irradiation without US and treatment of TatBim without RB, apoptosis inducing was not detected. These results indicate that TatBim-RB induced apoptosis US-dependently. A fundamental question related to this study is the means by which US induces the endosomal escape of TatBim-RB. A minimal answer may be that US induces reactive oxygen species (ROS) generation in the presence of TatBim-RB and the endosomal membrane might be disrupted by the attack of ROS generated from the endosomally entrapped TatBim-RB. We confirmed the US-dependent generation of ROS in the presence of rose bengal using 2',7'-dichlorodihydrofluoresein, fluorescent ROS indicator. Our results suggest that this peptide-sonosensitizer conjugate strategy may facilitate numerous kinds of medicinal chemistry studies, and furthermore, this specific conjugate may exhibit potential as a novel therapeutic agent for the promotion of apoptosis.

P189

Development of murine model of Fungal Keratitis to test the antifungal efficacy of CPP conjugated Natamycin

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Corneal diseases including microbial keratitis are among the major causes of visual impairment and blindness worldwide. About 50% of the total microbial keratitis is caused by various fungal species. Fusarium and Aspergillus species are found to be the most common isolates in India. Natamycin is the only FDA approved drug which is used as a first line of treatment for fungal keratitis. Drawbacks associated with the use of natamycin is poor intraocular penetration and poor water solubility. Cell penetrating peptides (CPPs) are positively charged short peptides that can translocate across the cell membrane without damaging the cell. In our study, one of the CPP i.e. TAT is used as a nanocarrier to deliver an antifungal compound, Natamycin inside the corneal cells. Recently, we have successfully shown the increased uptake of TAT dimer conjugated natamycin by corneal cells in vitro. Also, conjugated natamycin showed increased water solubility as well as antifungal activity in comparison to natamycin alone. To further investigate the antifungal activity of CPP conjugated natamycin (TAT2-Natamycin) in vivo, we have developed a murine model of Fusarium keratitis. Immunocompromised female BALB/c mice were inoculated with different concentrations of Fusarium sp. spores and were clinically graded (0 to 4 based on the severity of the disease) after three days of infection. Based on clinical grading and microbiological examination, 105 spores/5μl was found to be the optimum concentration for the establishment of fungal keratitis. We are currently testing TAT2-Natamycin on this animal model for its antifungal activity and the results will be compared with the marketed formulation of natamycin (Natamycin 5% suspension).

P190

Design of amphiphilic helical inhibitor peptides for the membrane interaction of pathological TDP-43

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The TAR DNA-binding protein 43 kDa (TDP-43) is an aggregation prone protein with a high association propensity towards membrane surfaces. Mutations in TDP-43 result in formation of insoluble aggregates, which are suspected of causing dysregulation of RNA processing, mislocalization of TDP-43 and formation of proteinaceous inclusions, whereby membrane association plays a crucial role.[1] Pathological TDP-43 is found in many neurodegenerative diseases, for instance Alzheimer's disease, frontotemporal dementia (FTD), Parkinson's disease and amyotrophic lateral sclerosis (ALS), with hosting most of the mutations in the prion-like domain.[2,3] The membrane association of TDP-43 is mostly mediated by the membrane-interacting subdomain of the prion-like domain (MIS), which consists of two structural parts; a membrane-adhering α-helix and a membrane-inserting Ω-loop. The membrane interaction of MIS is discussed as a potential drug target to slow down the progression of these diseases.[1] We work on the design of synthetic inhibitor peptides targeting this interaction. The general concept involves a two-component system with an amphiphilic α-helical peptide (AHP), to interact with the helical part of MIS, and an aromatic N-terminal head group (aHG), to disturb the loop conformation. As proof-of-concept initial studies with de novo designed AHPs were performed, which revealed the potential of our design as an interference with the MIS-membrane interaction was detected by circular dichroism (CD) spectroscopy, Förster resonance energy transfer (FRET)-measurements and reflectometric interference spectroscopy (RIfS). Currently, we are synthesizing a library of AHPs with different aHGs to increase the efficiency of the inhibition of the MIS-membrane interaction.

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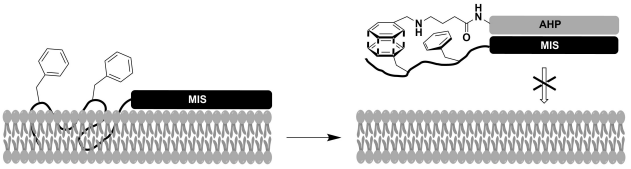

P191

Apelin peptide analogues as novel cardioprotective drugs

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Cardiovascular diseases including heart attack, stroke and ischemic reperfusion injury are the top killers worldwide accounting for almost 30% of global deaths.(1) Apelin is the natural peptide-substrate for the apelinergic system (APJ receptor) – an endogenous peptide hormone system that mediates a cascade of other cardio-metabolic processes ultimately fine-regulating the cardiovascular output. Apelin gets rapidly degraded in plasma owing to the activity of various proteases including Angiotensin converting enzyme 2 (ACE-2) and Neprilysin (NEP), limiting its half-life to under 2 minutes.(2,3) Apelin analogues including N-terminal extended isoforms and peptide isosteres reflect a promising alternative to minimize proteolytic degradation. The presentation will focus on the synthesis, metabolic stability and physiological activity of such Apelin analogues. These ACE2- and NEP-resistant drug candidates show potent blood pressure lowering and ischemic reperfusion recovery effects. They have considerable potential for their development as new agents for treatment of cardiovascular diseases.(4)

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P192

PI3King Apart a Protein-Protein Interaction: A Peptide Approach

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The phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) enzymes function at the plasma membrane, transducing cell surface receptor activation into internal cell signalling pathways. The PI3K/AKT/mTOR pathway is a central regulator of cell growth and migration, both of which are hallmarks of cancer. The PI3Kβ and PI3Kγ isoforms are activated by multiple mechanisms, one of which is a protein-protein interaction with G protein $\beta\gamma$ subunits released upon G protein coupled receptor (GPCR) activation. The PI3Kβ isoform, expressed in all cells and tissues, and the PI3Kγ isoform, expressed in immune cells, are targets for anti-cancer therapeutic development. Currently the function of these GPCR-linked PI3K enzymes is studied using small molecules that block ATP substrate binding and enzyme activity, or genetic methods that prevent the formation of the G protein-PI3K enzyme complex. The many roles of the PI3Kβ and PI3Kγ isoforms are not fully understood and evaluation of the up-stream activators using current tools remains challenging. This project aims to develop a chemical tool to target the protein-protein interaction between G$\beta\gamma$ and the PI3Kβ and PI3Kγ enzymes as an opportunity for isoform selective PI3K inhibition. This tool, comprising a peptide scaffold, will allow the chemical dissection of the G-protein related signalling pathways that activate the PI3K lipid kinase pathway. Protein-protein interactions are typically difficult to target with small molecules because of the extended interaction surfaces, although recently, peptides and peptidomimetic compounds have been developed to target these. Previous investigations of the binding interaction between PI3Kγ and G$\beta\gamma$ identified a crucial binding motif on one face of PI3Kγ. Small (10-24 residue) peptides derived from this PI3Kγ binding region have been synthesised and evaluated in a biochemical assay to assess their inhibitory activity at the protein-protein interface. Computational analysis of the hotspot on G$\beta\gamma$ has been aided by a previously discovered 15 residue peptide bound to G$\beta\gamma$, guiding the design of inhibitors.

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P193

Treatment with fatty acid modified analogues of apelin-13 improves glycaemic control and lipid profiles in diet induced obese diabetic mice

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Background: Apelin-13 is an adipokine which has promising metabolic effects but is rapidly degraded in plasma. We have shown that modified apelin analogues exhibited enzyme resistance in plasma and improved circulating half-life compared to apelin-13. The antidiabetic effects of chronic administration (28 days) of stable long acting fatty acid modified apelin analogues, namely, (Lys8GluPAL)apelin-13 amide and pGlu(Lys8GluPAL)apelin-13 amide, were investigated in diet induced obese-diabetic mice. Methods: Male adult (8 week old) NIH Swiss mice (groups n=8) were maintained either on a high-fat diet (45% fat) for 20 weeks, or control mice were fed a normal rodent chow diet (10% fat). When diet induced obesity-diabetes was established after high-fat feeding, mice were injected i.p. once daily with apelin analogues, liraglutide (25 nmol/kg) or saline (controls). Results: Administration of (Lys8GluPAL)apelin-13 amide and pGlu(Lys8GluPAL)apelin-13 amide for 28 days significantly reduced food intake and decreased body weight. Non-fasting glucose was reduced ($p<0.01$ to $p<0.001$) and circulating insulin concentrations elevated ($p<0.01$ to $p<0.001$). This was accompanied by enhanced insulin responses ($p<0.01$ to $p<0.001$) and significant reductions in glucose excursion after both oral ($p<0.01$) or i.p. ($p<0.01$) glucose challenges and feeding. Apelin analogues also significantly improved HbA1c ($p<0.01$), enhanced insulin sensitivity ($p<0.01$), reduced triglycerides ($p<0.001$), increased HDL-cholesterol ($p<0.01$) and decreased LDL-cholesterol ($p<0.01$), compared to saline treated diet induced obese mice. Cholesterol levels were decreased ($p<0.01$) by pGlu(Lys8GluPAL)apelin-13 amide and apelin treated mice showed improved bone mineral content, reduced fat deposits and increased plasma GLP-1 concentrations. Daily treatment with liraglutide mirrored many of these changes but not on bone or adipose tissue. Consumption of O2, production of CO2, respiratory exchange ratio and energy expenditure were improved by apelin analogues. These data show that long-term treatment with acylated analogues (Lys8GluPAL)apelin-13 amide and particularly pGlu(Lys8GluPAL)apelin-13 amide resulted in similar or enhanced therapeutic responses compared to liraglutide in high-fat fed diet induced obese mice. Conclusion: Stable fatty acid modified apelin-13 analogues represent a new and exciting therapeutic development which can ameliorate the effect of diet induced obesity diabetes.

P194

A non-stereogenic imidazolidinyl proline as a pH-sensitive and functionalizable probe to tune the collagen triple helix stability

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Numerous compounds have been developed to study the conformational properties of proline-containing peptides and proteins as well as for peptide functionalization [1]. Most proline derivatives possess substituents at the C4-position, similar to the naturally occurring hydroxy group of proline [2,3]. Notably, 4S/4R aminoprolines produce a number of different conformational effects, but also provide sites for functionalization and pH response of peptides [4-7]. We recently developed a minimal 4-azaproline analogue based on a non-stereogenic pseudoproline-scaffold which can be prepared in a single synthetic step, named Imp. The conformational properties of this proline analogue were investigated through NMR spectroscopic studies of model compounds and DFT calculations, and confirm that both Imp and functionalized Imp effectively mimic proline and other proline-based residues. Incorporation of Imp into a collagen model peptide (CMP) produces pH responsive CMPs and CMPs that can be functionalized through the Imp side-chain with sterically bulky groups. Thermal denaturation studies of the CMPs corroborate computational insight and suggest that Imp possesses a dynamic nature in the context of a peptide where the lack of a stereogenic center at the 4-position of the ring can alleviate steric constraints of bulky substituents that would otherwise be created by a mismatched 4S/4R proline stereoisomer. We envision that Imp will provide a useful alternative for the synthesis of functionalized peptides, particularly in examples where a dynamic residue can benefit the overall stability.

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P195

Fluorocarbon-peptide conjugates (FPC): new concept to increase the metabolic stability of peptides for therapeutic applications

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Over the past decade, peptides have shown an increasing interest for therapeutic applications as they are selective and efficacious signaling molecules [1]. However, they are often not directly suitable for use as convenient therapeutics because they have intrinsic weaknesses, including poor chemical and physical stability, and a short in vivo half-life due to rapid enzymatic degradation [2]. To address peptide instability issue for therapeutic applications, we propose a strategy based on the grafting of fluorocarbon chains (F-chains) onto peptides. Thereby, the hypothesis was to induce the self-organization of fluoropeptides in aqueous solution, resulting in the protection of the native peptide from enzymatic degradation. To demonstrate the efficacy of our approach, apelin peptide that presents a short plasma half-life has been selected as model [3, 4]. Apelin is a neurovasoactive peptide with potential interesting therapeutic applications for the treatment of cardiovascular diseases. However, its therapeutic development has been hampered by its low plasma stability. Different F-chains were then grafted onto apelin following a solid-phase approach. The most stable fluoroapelin (LIT01-196) was then evaluated in rat model demonstrating the positive impact of F-chain to greatly improve the in vivo efficacy of native apelin [5]. In this communication, we will present also the mechanism leading to the increase of human plasma stability of fluoroapelin using original fluorescent tools recently developed in our group [6]. Altogether, these promising results should open the route to a convenient, safe and general approach to greatly increase the metabolic stability of numerous peptides for their in vivo use as pharmacological tools and/or therapeutic agents.

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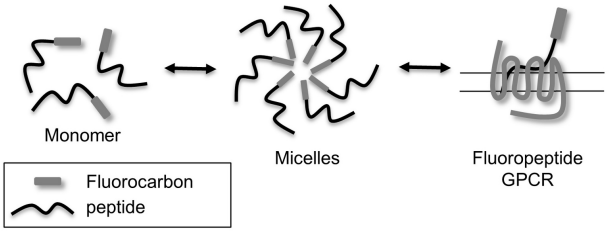

P196

PaMAP 1.9 and PaMAP 2: efficient multi-resistant antimicrobial peptides show evidence of anticancer activity

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The current healthcare scenario is bringing new challenges to the medical field, due to the new threats that are becoming responsible for high mortality ratios. Multi-resistant pathogens are one of them, with reports of increased numbers of severe infections on patients that current available drugs cannot attend. The World Health Organization has already pointed out the urgency in finding new molecules against different pathogens, named the ESKAPE group (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa and Enterobacter species). Furthermore, the number of drug resistance cases in cancer patients has also increased in recent years, associated with the occurrence of hospital infections that debilitate patients' health. Antimicrobial peptides (AMPs) are pointed out as a future alternative to conventional drugs in these contexts. AMPs are usually small, highly hydrophobic and with a global positive charge, which promotes the interaction with the pathogens or cancer cells membranes (with negative surface charges). Their mechanism of action is independent of intermediators, transporters or protein channels, being active mostly by peptide-membrane interaction, against bacteria, viruses or cancer cells (anticancer peptides, ACPs). Even so, these mechanisms are not yet fully understood. In our work, we focused in two AMPs (PaMAP 1.9 and 2), synthetically designed using a natural AMP as template. After initial promising results against bacteria, complemented by biocomputational studies, peptide-membrane interactions were extensively studied, using lipid vesicles and bacterial cells (including clinical multi-resistant strains). Different biophysical techniques, including fluorescence spectroscopy and microscopy, flow cytometry, dynamic light scattering, zeta-potential, circular dichroism and atomic force microscopy allowed to infer about the mechanism and efficiency in promoting bacteria cell death. Data obtained were confirmed and further extend by in vivo studies. Recently, we hypothesized if any of these AMPs could be good candidates for cancer therapies. Using the same approach, we demonstrated that PaMAP 1.9 has anticancer activity, but not PaMAP 2.

P197

Electric Field Assisted Inhibition of Aggregation in Amyloidogenic Proteins.

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Impeding the toxic aggregation of tau protein, a central pathological agent involved in Alzheimer's disease (AD), can serve as a straightforward therapeutic strategy. This present study presents a hybrid approach employing physical (electric/magnetic fields) as well as molecular (structure based designed peptides) perturbants for retarding the aggregation kinetics of core peptide segments of tau protein (VQIVYK and VQIINK). Herein, we use Thioflavin t staining, tyrosine fluorescence assay, dynamic and static light scattering, infrared spectroscopy, electron microscopy, as well as toxicity tests, to verify the effect of proposed self-assembly modulators on the aggregation kinetics, morphology, conformational state and cellular toxi-

city of the chosen model peptide systems. Our observations reveal that these physical as well as molecular perturbants not only significantly arrest the self-assembly of VQIVYK and VQIINK fibrils but also reduce the neurotoxicity instigated by them in human neuroblastoma SH-SY5Y cells. Our results utilizing field as aggregation inhibitor are in good agreement with theoretical (References 1,2) (based on molecular dynamics) and experimental (References 3-6) studies reported earlier on amyloid forming molecular systems. Our work demonstrates that efficacy of hybrid approach to inhibit aggregation of tau aggregation and can be further developed to serve as a useful therapeutic option, for treating Alzheimer's disease.

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P198

RGD Bicycles: High-Affinity Ligands for Rapid Integrin-Mediated Cell Adhesion

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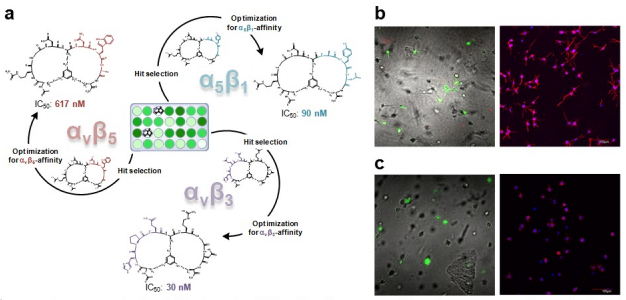
Integrins mediate cell-cell and cell-extracellular matrix interactions via interaction with proteins such as fibronectin and vitronectin. Ligands that mimic the function of these proteins can improve the cell adhesion and proliferation properties of biomaterials. Bicyclic peptides recently attracted interest as a powerful platform for novel therapeutics because of their high binding affinities and proteolytic stability. We therefore screened hundreds of different bicyclic peptides for binding to integrins $\alpha v\beta 3$, $\alpha v\beta 5$ and $\alpha 5\beta 1$, and gradually improved the affinities and selectivities. The best IC50 values were, for example, 30 nM for $\alpha v\beta 3$ (GRGDS: 5 μM, knottin-RGD: 38 nM), and 90 nM for $\alpha 5\beta 1$ (GRGDS: >10 μM, knottin-RGD: 114 nM). We also studied integrin-binding on cells via confocal microscopy with Cy5-functionalized bicycles. Finally, cell behavior studies with peptide-functionalized soft 3D hydrogels and elastin-like recombinamers (ELRs) revealed superior cell adhesion and proliferation of the bicycles compared with conventional RGD-peptides.



a) Screening approach for high integrin-affinity bicyclic RGD-peptides; hADSCs on soft 3D hydrogel functionalized with **b**) an $\alpha_5\beta_1$-binding bicycle, and **c**) *cyclo*-KRGDf. Brightfield images (left) showing live (green) and dead cells (red) were taken after 3d. Confocal images (right, z-stack projection 20x) showing nuclei (blue) and actin (red) were taken after 2d.

P199

Design and evaluation of dual GLP-1 and PYY receptor agonists

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Diabetes and obesity represent a severe global health epidemic with associated metabolic problems resulting in significant mortality and increased risk of cardiovascular disease, stroke or other complications. In the last decade, dipeptidyl peptidase-4 inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists have drastically enhanced the management of diabetes, providing greater benefits than glucose control alone, such as improvements in blood pressure, beta-cell function and positive effects on body weight. Other naturally occurring gut hormones have the potential to enhance the action of GLP-1 agonists in the clinic and focus within the pharmaceutical industry is now directed towards the delivery of such gut peptide hormone combinations for treatment of type 2 diabetes mellitus and obesity.

One naturally occurring peptide that has the potential to enhance the action of GLP-1 agonists is the anorectic peptide YY (PYY3-36); its actions, in tandem with GLP-1, result in superior reductions of food intake and body weight when compared to GLP-1 alone. Thus, the aim of this study was to secure dual pharmacology peptides which could simultaneously deliver glucose-lowering effects of GLP-1 and appetite-suppressing effects of PYY(3-36.)

The PYY(3-36) and GLP-1 peptides represent different families of peptide hormones, with unrelated peptide sequences and vastly different secondary structures. The N-terminal part of GLP-1 is known to be important for activity, whereas in PYY(3-36), the C terminal part is a crucial pharmacophore. This provides the opportunity for peptide engineering to produce a dual GLP-1/PYY(3-36) receptor agonist by combining these two key fragments.

Herein, a library is presented of >100 peptides with dual agonistic properties at both the hGLP-1 and hY2R (PYY) receptors; the library includes lipidated compounds and those modified to increase resistance to proteolysis. New peptides were tested in vitro in cAMP based assays using cell lines expressing human GLP-1 or Y2R (PYY) receptors and EC50 values were determined. Individual peptides displayed a broad range of agonistic potency and receptor selectivity. A selection of compounds that showed potent activity at both the GLP-1 and PYY receptors is discussed. To our knowledge, these GLP-1 based compounds are the first lipidated chimeric peptides that display dual agonism at Y2R and GLP-1R.

P200

Triple-negative breast cancer: new potential therapeutics derived from SOCS3 protein

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Suppressor of cytokine signaling (SOCS) proteins are a family of negative feedback regulators of cytokine signaling mediated by the JAK-STAT pathway (Carow, et al. 2014). Only two members of this family, SOCS1 and SOCS3, contain a kinase inhibitory region (KIR) crucial for the inactivation of JAKs, leading to suppression of inflammatory cytokines (Yoshimura, et al. 2012). It has been suggested that SOCS proteins can play pivotal roles in development and progression of cancers. Further, in triple negative breast cancer (TNBC) subtype the proteolytic degradation of SOCS3 protein causes the activation of inflammatory cytokines and, as a whole, recombinant SOCS3 demonstrated able to prevent TNBC tumour growth and metastasis by suppressing inflammatory cytokines (Kim, et al. 2015). In this study we designed several SOCS3'mimetics derived from the N-terminal region of SOCS3 encompasses KIR and ESS domain that interface the complex with JAK2. These peptides were characterized by Circular Dichroism and Surface Plasmon Resonance spectroscopies. Moreover, the activity of one sequence, named KIRESS, which contained crucial residues for the complex SOCS3/JAK2 was investigated in vivo in mouse xenografts of MDA-MB-231-luci tumours as model of human TNBC subtype. KIRESS peptide demonstrated capable to eliminate pulmonary metastasis and showed a significant reduction of primary tumour growth. KIRESS peptide can be considered as a starting point to create, through structural and chemical modifications, compounds with high affinity and stability as potential therapeutics in TNBC (La Manna, et al. 2018).

P201

Unraveling the Mechanism of Action of the Analgesic Peptdie DD04107. Synaptotagmin-1 as a Putative Target

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DD04107 (Palmitoyl-EEMQRR-NH2) is a peptide derived from the N-terminal sequence of SNAP25, a protein component of the SNARE complex. It blocks the inflammatory recruitment of TRPV1 ion channels to the plasma membrane of nociceptors and inhibits the release of calcitonin gene-related peptide (CGRP) from primary sensory neurons.1,2 DD04107 produces dose-dependent, long-lasting in vivo antihyperalgesic and antiallodynic activities in chronic models of inflammatory and neuropathic pain, but to date its specific target(s) is(are) not known.3 In order to get insight in the importance of the different side-chains for the inhibition of CGRP release, we first performed an Ala scan, which indicated that E1, M3, Q4 and R6 are essential residues. Curiously, they resemble the residues of SNAP25 involved in the SNARE/synaptotagmin-1 (Syt-1) complex, 4,5 namely E37, K40 residues from one copy of SNAP25 and the spatially close N159, M163 and D166 residues of the second SNAP25 copy, within the SNARE complex. This led us to hypothesize that DD04107 could exert its action through the direct interaction with Syt-1, probably competing with the SNARE complex formation. To prove this hypothesis we studied the possible interaction of Syt-1 protein to DD04107 and linear and cyclic analogues using ITC techniques, while molecular modeling studies allowed the proposal of a plausible mode of interaction. Additionally, the conformational behavior of these peptides in solution (NMR) could be considered as an approach to the bioactive conformation. In this communication we will describe the main results of all these studies.

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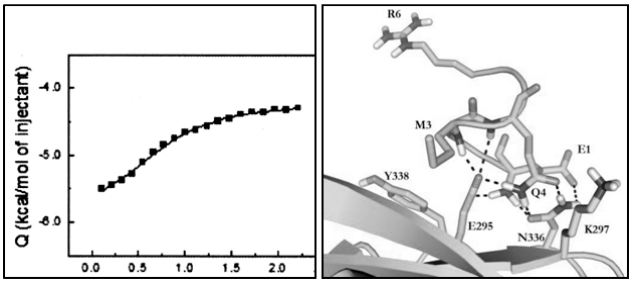

P202

Antimicrobial activity and action mechanism of chemokine CXCL14-derived antimicrobial peptide, CXCL14-C17 and its analogs

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CXCL14 is a CXC chemokine family that exhibits antimicrobial activity and contains an amphipathic cationic alpha-helical region in the C-terminus, a characteristic structure of antimicrobial peptides (AMPs). In this study, we designed three analogs of CXCL14(59-75)(named CXCL14-C17) corresponding to the C-terminal alpha-helix of CXCL14, which displayed potential antimicrobial activity against a wide variety of gram-negative and gram-positive bacteria with minimum inhibitory concentrations (MICs) of 4-16 microM without mammalian cell toxicity. Furthermore, two CXCL14-C17 analogs (CXCL14-C17-a1 and CXCL14-C17-a3) with improved cell selectivity were engineered by introducing Lys, Arg, or Trp in CXCL14-C17. Additionally, CXCL14-C17 analogs showed much greater synergistic effect (fractional inhibitory concentration index [FICI]: 0.3125–0.375) with chloramphenicol and ciprofloxacin against multidrug-resistant Pseudomonas aeruginosa (MDRPA) than LL-37 did (FICI: 0.75–1.125). CXCL14-C17 analogs were more active against antibiotic-resistant bacteria including methicillin-resistant Staphylococcus aureus (MRSA), MDRPA, and vancomycin-resistant Enterococcus faecium (VREF) than LL-37 and melittin. In particular, CXCL14-C17-a2 and CXCL14-C17-a3 completely inhibited the biofilm formation at sub-

MIC. Membrane depolarization, sytox green uptake, ONPG hydrolysis, flow cytometry, and confocal microscopy revealed the possible target of the native peptide (CXCL14-C17) to likely be intracellular, and the amphipathic designed analogs targeted the bacterial membrane. CXCL14-C17 also showed DNA binding characteristic activity similar to buforin-2. Interestingly, CXCL14-C17-a2 and CXCL14-C17-a3 effectively inhibited the production and expression of nitric oxide (NO), tumor necrosis factor (TNF)-alpha, interleukin (IL)-6, and monocyte chemoattractant protein (MCP)-1 from lipopolysaccharide (LPS)-stimulated RAW264.7 cells, suggesting that these peptides could be promising anti-inflammatory and antimicrobial agents.

P203

Combinatorially Screeened Peptide as Targeted Covalent Binder

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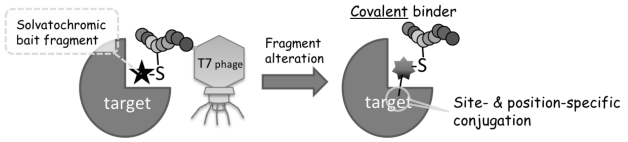
Finding targeted covalent binders (also known as covalent drugs) is one of the cutting-edge disciplines such as biomedical sciences / chemical biology / pharmaceuticals fields. As shown in the figure, we present here a novel concept for finding a peptide covalent binder from fluoroprobe-modified peptide library [1] on T7 bacteriophage constructed via the 10BASEd-T [2], followed by a structure alteration into a reactive warhead [3]. Site- and position-specific conjugation toward the target protein and target selectivity were confirmed. This is the first demonstration for finding peptide-type targeted covalent binder by a combinatorial screening, instead of a rational designing. We believe the discovery technology would be generally applied to the discovery of novel covalent drugs for clinically-important proteins (e.g., cancer-related cell-surface proteins). [1] Taki et al., Anal. Chem. 2016, 88, 1096; Uematsu et al., AIP Conf. Proc. 2017, 1807, 020028. [2] Fukunaga et al., Chem. Commun. 2014, 50, 3921; inside cover article. [3] Uematsu and Tabuchi et al., Bioconj. Chem. 2018, in press.



P204

Factor XIIIA-Inhibitor Tridegin: On the role of disulfide bonds for folding, stability and function

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Tridegin is a 66mer peptide isolated from the salivary gland of the giant leech Haementeria ghilianii in 1997.[1] So far, it is the only known peptide inhibitor with remarkable potency and specificity against the blood coagulation factor XIIIa.[1,2] Tridegin inhibits the catalytic activity of FXIIIa by impairing the cross-linking of fibrin in the final step of the blood coagulation cascade.[3] Consequently, tridegin represents an interesting tool for thrombolytic therapy in future medical applications. However, tridegin is a cysteine-rich peptide containing six cysteines linked by three disulfide bonds. This renders the selective synthesis of tridegin a challenging process.[4,2] Hence, a simplification of tridegin's structural complexity is highly desirable. Former studies have shown that the truncation of tridegin and the removal of all disulfide bonds leads to a significant loss of activity. Additionally, only three of fifteen possible disulfide-bridged isomers were identified as a result of an oxidative self-folding approach.[2] Interestingly, these three isomers share a common disulfide bond between cysteine 19 and 25. This raises the question on the role of this specific disulfide bond in the folding, stability and function of tridegin. We have thus selectively synthesized analogs of the three isomers lacking the disulfide bond Cys19-Cys25. The results are presented in three different parts resulting in a comprehensive analysis of 2-disulfide bonded tridegin analogs: 1) Synthesis and disulfide-bond analysis, 2) bioactivity studies, and 3) insights into structure-function relationships using molecular dynamics and docking studies. The peptides were synthesized by solid-phase peptide synthesis and a directed protecting group strategy with Acm-, and Trt-protected cysteines. Subsequently, the disulfide connectivity was elucidated by enzymatic digestion and subsequent MS/MS analysis. The inhibitory potential of these analogs on FXIIIa was tested in an in vitro activity assay and compared with bioactive synthetic and recombinant tridegin from former studies.[2] The molecular dynamics studies of the three different tridegin isomers were performed to elucidate the impact of the disulfide bond Cys19-Cys25 on the structure and the analogs conformational stability. In addition, molecular docking studies with the 2-disulfide-bonded analogs docked on FXIIIa added insights into the binding modes of the individual peptides. Our studies present three novel tridegin analogs retaining inhibitory activity while alleviating the synthesis effort. In conclusion, this work opens interesting new directions for the scientific community in the quest toward the development of novel FXIIIa inhibitors.

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P205

Deciphering specificity determinants for FR900359-derived Gαq inhibitors based on computational and structure-activity studies

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Heterotrimeric G proteins represent interesting targets in pharmacological studies and for developing novel therapeutic approaches due to their importance in signal transduction mediated by G protein-coupled receptors. Nevertheless, direct targeting of intracellular Gα subunits by chemical tools is still challenging and only few compounds are known for this purpose. One of these modulators is the Gq-inhibiting depsipeptide FR900359 (FR). The high potency of FR and the structurally related compound YM-254890 (YM) brought these substances in the focus of several research groups. These compounds represent small to medium-sized depsipeptides with a high degree of chemical complexity, specific three-dimensional conformations, and consequently precise orientation of backbone and side chain atoms through intrinsic rigidity. In a comprehensive structure-activity relationship study novel FR-based analogs from natural sources, synthetic cyclic peptides as well as all so-far known Gq inhibitors were analyzed.

We grouped 14 new FR- and YM-derived compounds as well as all 23 published analogs according to their potency to identify tolerated, partially tolerated, non-tolerated minor, and non-tolerated major modifications in the original sequence(s).[1–6] Concomitant application of 2D NMR spectroscopy and molecular docking allowed us to identify unique features in the macrocyclic structures important for binding to the target protein correlating with inhibitory activity.[1] Ultimately, a strategy is presented for the elucidation of characteristics that determine interaction with and inhibition of Gq in the specific FR/YM binding pocket.[1] It is revealed how changes in three distinct categories affect inhibitory potency compared to the lead compounds, namely I) backbone conformation, II) backbone conformation influencing the intramolecular hydrogen bond network, and III) side chain constitution and orientation.[1] Unfortunately, none of the analogs surpassed the biological activity of FR on Gq nor did they show an effect on Gi or Gs.[1] As a consequence the question remains whether depsipeptides such as FR already represent valuable tools for specific inhibition of Gq and if they are suitable natural lead structures for the development of novel compounds to target other Gα subunits.

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P206

Impact of cysteine pairing on disulfide-bond assignment and structure elucidation in different conformational isomers of the μ-conotoxin PIIIA

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The elucidation of structural and functional properties of oligopeptides containing more than four cysteine residues is a main topic in peptide research.[1] In order to get fundamental insights into the structure of a cysteine-rich peptide the determination of its disulfide connectivity is critical. Due to conformational restrictions, disulfide bonds confer stability and support formation of a defined 3D structure which is crucial for the bioactivity of such peptides. Depending on their disulfide connectivities, cysteine-rich peptides and proteins can adopt various 3D structures. The unambiguous identification of such conformational isomers harboring more than two disulfide bonds is particularly challenging and experimental approaches for unequivocal structural analysis are largely lacking. Cysteine-rich peptides isolated from the venom duct of different cone snails are known to have therapeutic potential in e.g. pain therapy and are therefore interesting research targets.[2] Previously, our group synthesized the 22mer conopeptide μ-PIIIA in an oxidative self-folding approach from which we identified three different conformational isomers.[3] As a direct consequence of these results, we now synthesized all 15 possible disulfide isomers of μ-PIIIA by means of a protecting-group strategy.[4] Subsequently, 2D NMR spectroscopy and MS/MS were applied in order to elucidate the structure of the individual disulfide isomers. This study allows for crucial insights in how disulfide connectivity alters the global fold of a toxin. In addition, HPLC coelution experiments were carried out with the intention of investigating the chromatographic separation performance on different disulfide isomers of the same peptide. In total, this study revealed the complexity of targeted synthesis and chemical characterization of a cysteine-rich conotoxin. The comprehensive analysis of the 15 different isomers using NMR and MS/MS techniques provided evidence that the unambiguous assignment of disulfides in cysteine-rich peptides and proteins requires extensive analysis procedures combining the methods mentioned. In addition, we conclude that standard compounds are of eminent importance for elucidating disulfide bonds and 3D structures of cysteine-rich peptides and proteins.

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P207

Metabolic effects of Adrenomedullin in combination with glucagon-like peptide-1 in lean NMRI mice

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Adrenomedullin (ADM) is a vasoactive peptide expressed in several peripheral organs and known primarily for its beneficial vasoactive effects. However, ADM is also known to inhibit insulin secretion, and central administration of ADM has been shown to elicit anorexigenic effects. Here, we investigated if peripheral co-administration of ADM and glucagon-like peptide 1 (GLP-1) could subdue the hypoglycaemic effects of ADM while enhancing its anorectic properties. The effects of mono- and combination therapy of ADM and GLP-1 on appetite regulation and glucose homeostasis were assessed acutely in male NMRI mice for 18 hrs, while effects on glucose homeostasis were assessed by oral glucose tolerance tests (OGTT). While the monotherapy with GLP-1 and ADM resulted in modest anorexigenic effects, co-administration of the two peptides led to a marked synergistic reduction in food intake. Moreover, while post-prandial blood glucose excursions were significantly increased by ADM monotherapy, co-administration of ADM with a lower dose of GLP-1 normalized glucose excursions. In conclusion, we demonstrate, for the first time an anorectic synergy between ADM and the pharmaceutically relevant incretin GLP-1. Additionally, we show that a low dose of GLP-1 can reverse ADM-induced impairment of the glucose tolerance. Consequentially, ADM could be interesting as a potential target for the treatment of obesity if combined with GLP-1.

P208

Small Synthetic, Multivalent Bicyclic Peptides That Activate T Cell Costimulatory Protein CD137

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CD137 agonism is a promising immunotherapeutic approach and there are currently two agonistic antibodies in clinical trials. CD137 receptors on immune cells form trimeric complexes in the activated state. Peptides binding to human CD137 ligand-binding site were identified by phage screening using proprietary Bicycle technology. Further chemical optimisation al-

lowed systematic generation of a matrix of dimeric, trimeric and tetrameric CD137 synthetic agonists with a broad range of cell-activity properties. CD137 synthetic multimers were shown to be stable in plasma and to have in vivo half-life of approximately 30 minutes. A trimer and tetramer using the Lysine 3 attachment point were efficacious in preventing syngeneic tumour growth in the hCD137 mouse model.

P209

Gold Nanoparticles Coated with Antimicrobial Peptides for Biological Activity Enhancement against Multidrug Resistant Bacteria

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Nowadays, the growing of multidrug resistant bacteria has brought severe problems to public health over the world. Thus, there is an urgency need for the development of new strategies or discoveries to tackle this alarming situation. Gold nanoparticles (AuNPs) have been widely applied in many areas of Medicine and Biotechnology, such as biosensors, diagnostics, immunoassays, imaging, and therapeutics. Considering gold's inertness, it is known to be a safe and biocompatible to the organism. On this way, the integration of the promising antimicrobial peptides (AMPs) with the AuNPs seems to be very interesting and relevant for the improvement to fight against multidrug resistant bacteria. In this study, the objective is to functionalize AuNPs (13 nm) with two AMPs: Ctx(Ile21)-Ha and Pantinin-1, both isolated from the skin secretion of an arboreal South American frog, Hypsiboas albopunctatus and the scorpion venon Pandinus imperator, also with a Europium probe for able diagnostical essays via fluorescence studies. The primary sequences were modified, adding a cysteine residue in the amidated C-terminus to facilitate the peptides' attachment. Firstly, the AuNPs were precoated with PEG-SH and sonicated for 10 min to avoid aggregation. AuNP-PEG-SH were centrifuged at 11.8 rpm and 13.3 g for 30 min. This proceeding yielded approximately 10 mL of AuNP in a concentration of 20 nM. The pH was raised to 9 with NaOH. After, the solution was added in glass flasks of 1 mL 500 uL of water and 500 uL AuNPs at 20 nM and add the peptides until 12 uL, in increments of 3 uL. Then, were added the Eu probe (stock solution of 0.2 mM) in a volume of 15 uL (increments of 3 uL for each addition). Finally, add 1:5 of 1 M NaCl solution, stirring with a magnet bar, which stabilize the final conjugated yielded (red coloured). After that, the peptides and the conjugated were tested in multidrug bacteria strains: Pseudomonas aeruginosa (regular and PA02 – clinical resistant), Acinetobacter baumannii, Klebsiella pneumoniae and Staphylococcus aureus. In conclusions, after several attempts, we synthesized the gold nanoparticles tethered with the suggested peptides, also containing an europium fluorescence probe. Although, it could see biological activity against the multidrug resistant bacteria strains for the pure peptides, in the results were not observed any biological activity for the AuNP/AMP/Eu conjugated. More experiments need to be done in order to improve the quality, characterization and the biological activity of these news compounds.

P210

Site-specific chemical modification of IgG antibody using Z34C affinity peptide

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We have developed a conjugation method of human IgG through an affinity peptide composed of 17 amino acids (IgG-BP: GPDCAYHKGELVWCTFH, whose two cysteines formed the disulfide bond)1. We call this chemical conjugation method CCAP (Chemical Conjugation by Affinity Peptide). This method enables to label Lys248 (or Lys 246) residue on IgG-Fc with a cross-linker reagent like DSG (disuccinimidyl glutarate), which is attached to the amino groups of the side chain of Lys8 on the peptide. This CCAP can generate ADC (antibody-drug conjugate) and IgG diagnostic drug for PET imaging, by using IgG-BP which was attached to drug or metal chelator like DOTA. As this IgG-BP can bind to human IgG1, 2, 4 and rabbit IgG, CCAP method using this peptide can be applied for labeling for these antibodies, but not for rodent antibodies, because of its lack of the binding abilities toward them. To overcome this limitation, we developed a new CCAP method using Z43C peptide, which is well known as a Fc-binding peptide originated from Protein A, with broad specificities toward rodent antibodies as well as human antibodies2. We designed the labeling reagent of Z34C whose N-terminal region was designed for cross-linking. The Fc of murine and rat IgG as well as human (IgG1, 2 and 4) and rabbit IgG antibodies was efficiently and site-specifically labeled with this peptide though a cross-linker. The CCAP method with broad specificities using Z34C peptide can be applied for labeling/conjugation of murine and rat antibodies, which are widely used as diagnostic and research reagents.

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P211

Hybrid peptidomimetics for the use in neuropathic pain

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Neuropathic pain is defined as a type of chronic pain that occurs when nerves in the central nervous system become injured or damaged. It is estimated that approximately 7.7% of people in European countries suffer from neuropathic pain. This kind of pain is difficult to treat because it responds poorly to standard treatments, including those used to treat acute pain. The pain sensation associated with neuropathic pain significantly affects the quality of life of a patient. A new approach to the neuropathic pain treatment and the search for new, effective drugs and their combinations are needed. In this work we present the synthesis and in vivo assay results of novel hybrid peptidomimetics for the use in neuropathic pain, comprising two ligands: an opioid receptors agonist (OP) and an MC4 receptor antagonist. Ligands were connected by spacers (linkers) of different lengths and rigidities. Some of the hybrid compounds showed high activity in preclinical studies in a mouse and rat model of neuropathic pain (ED50 0.004-0.0002 nM). Acknowledgment This work was supported by

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P212

Design, synthesis and evaluation of new peptidotriazole peptidomimetics as specific modulators of the Aβ42 peptide oligomerization process

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The misfolding and aggregation of the Amyloid-β 1-42 (Aβ42) peptide plays a central role in the pathogenesis of Alzheimer's disease (AD. [1] Targeting the highly cytotoxic oligomeric species that form during its aggregation process represents a promising therapeutic strategy to reduce the toxicity associated with Aβ42.[2] Exploiting our knowledge in Aβ42 peptide oligomerization, we developed a new and easy real time BODIPY fluorescence assay for screening inhibitors of the early Aβ42 oligomerization. This new BODIPY-binding assay could be routinely used for screening larger compound libraries, demonstrating reproducibility and statistical robustness of the results. We synthesized new peptidotriazoles as specific inhibitors of Aβ42 oligomerization. These peptidomimetics were designed on basis of the two hot-spot sequences of Aβ42: the hydrophobic nucleation site KLVFF and the C-terminal part (GVVIA). The structure-activity relationship study demonstrated the importance of the triazole ring position in the sequence and the possibility to obtain new peptidomimetics that are able to reduce the toxicity of Aβ42, by specifically targeting the oligomerization process instead of the fibrillization process.

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P213

A Bifunctional Biased Mu Opioid Agonist - Neuropeptide FF Receptor Antagonist as Analgesic with Improved Acute and Chronic Side Effects

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Opioid analgesics, such as morphine, oxycodone and fentanyl are established standard treatments for moderate to severe pain. However, upon chronic administration, their use leads to several side effects such as analgesic tolerance, dependence liability, respiratory depression or constipation.[1],[2] In order to achieve safer treatment of pain, two main strategies have recently emerged: the development of G protein-biased mu opioid receptor (MOPr) agonists, and the production of designed multiple ligands (DMLs), combining opioid and non-opioid activities.[3],[4]

We designed multitarget peptidomimetics combining high affinity binding to MOPr and neuropeptide receptors (NPFF1R and NPFF2R). Indeed, neuropeptide FF and its receptors have been identified as a pronociceptive system involved in opioid-induced hyperalgesia and analgesic tolerance.[5],[6] In vitro characterization of our peptidomimetics allowed us to identify two lead sequences KGFF03 and KGFF09 as G protein-biased MOPr agonists with full agonist or antagonist activity at the NPFFRs, respectively.[7] In vivo experiments with mice confirmed the benefit of G protein-biased MOPr agonist activity, since KGFF03/09 showed reduced respiratory depression compared to the unbiased parent opioid agonist KGOP01. Interestingly, only the opioid agonist - NPFFRs antagonist, KGFF09, exhibits a favorable profile upon chronic administration. It gave way to reduction of hyperalgesia and analgesic tolerance after chronic subcutaneous administration in mice. In addition, KGFF09 displays reduced withdrawal syndrome compared to KGOP01 and KGFF03, indicating that it induced less physical dependence.

Altogether, our data establish that combining, within a single molecule, G protein-biased MOPr agonism and NPFFR antagonism, has beneficial effects on both acute and chronic side effects of conventional opioid analgesics.

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P214

Peptide-Oligonucleotide Conjugates as Nanoscale Building Blocks

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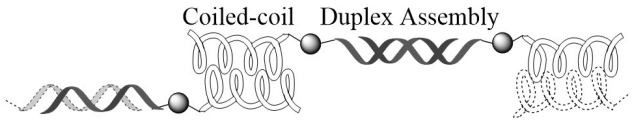
Directed self-assembly, using two bioorthogonal principles, offers the prospect of creating nanoscale building blocks and synthetic peptides. Delivery of therapeutics is often the most challenging part of drug development. Designed peptide-oligonucleotide conjugates (POCs) could attain a vital role in the delivery of drugs, as vehicles for drug transport. Here we synthesized an 8.9 kDa peptide-oligonucleotide conjugate, where the oligonucleotide forms an antiparallel duplex and the peptide has a high propensity to form an antiparallel dimer. It can thus form large nanoassemblies. Dynamic light scattering and circular dichroism were used to investigate one such peptide-oligonucleotide conjugate. Both methods indicated the formation of nanoscale assemblies, many times larger than the starting materials, through the formation of peptide coiled coils and hybridized oligonucleotide duplexes.



P215

Design, synthesis and biological evaluation of stapled helical peptides targeting the myddosome

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Protein-protein interactions (PPI) are involved in the regulation of a large variety of biological processes and their malfunctioning may lead to the development of diseases. These targets, considered as largely undruggable less than a decade ago, are receiving an increased interest in the discovery of novel therapeutic agents.1 An important challenge originates from the extended contact surfaces of interaction, to be targeted by small molecule modulators. Peptides, as small protein segments, can cover larger surface areas while three-dimensional stability can be preserved via chemical modifications, such as peptide macrocyclization or helical constraints.2,3

The target of this study is the intracellular adapter protein MyD88, which is involved in the signaling pathways of the Toll-like receptor (TLRs) and Interleukin-1 receptor (IL-1), both playing a crucial role in the innate immune and inflammatory response. The MyD88 protein consists of two domains: a TIR domain that interacts with TLRs and IL-1R, and a death domain which multimerizes with Interleukin-1 receptor-associated kinases (IRAKs) to form the myddosome.4 An X-ray structure of this challenging PPI target shows a left-handed helical oligomer that consists of several MyD88 proteins, IRAK4 and IRAK2 death domains, resulting in highly complex interactions.5 Three types of interfaces (type I-III) are revealed when the multimeric protein is unfolded along the helical axis. The type I interface, composed by a seven amino acid long helical epitope from MyD88, is particularly interesting as it shows several interaction points with IRAK4. Moreover, a non-occupied hydrophobic pocket on the IRAK4 side is found close to key side chains in MyD88.

Supported by molecular modeling, rationally designed stapled peptides were synthesized as helix mimetics of the type I epitope, to be evaluated as potential inhibitors of this PPI. Therefore, macrocyclizations were performed via lactamisation, ring-closing metathesis and copper-catalyzed azide-alkyne click chemistry (CuAAC). Currently, ten stapled peptides were identified to bind the IRAK4 domain in a surface plasmon resonance (SPR) assay. Circular dichroism (CD) spectroscopy confirmed that macrocyclization successfully induces helicity in several of the short sequence peptides. The optimized synthetic pathways, as well as structural peptide information and binding data will be discussed.

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P216

Sequence-activity relationship analysis for peptide optimization using Machine Learning techniques

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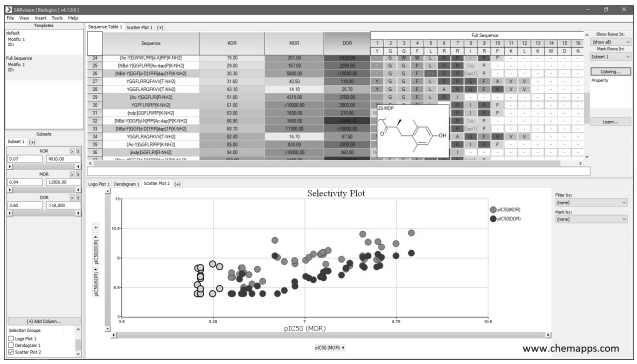
Optimization of therapeutic biologics has traditionally followed a series of steps in which activity is maximized, followed by target selectivity, and then subsequently multiple other pharmacokinetic and toxicological properties. A significant degree of efficiency could be achieved if multiple properties could be optimized simultaneously. Machine learning techniques could be used to generate sequence-biological activity relationships to be optimized. The first step is to develop methods to facilitate the analysis of structure activity relationships for peptides. We present several approaches to facilitate the identification of such trends and their analysis. The next challenge resides in that the most appropriate methods for each property can be different and require some expertise in their use. We have created a computer system that automatically examines the utility of different machine learning techniques for a dataset and selects the most predictive methods for different properties of clinical interest for peptides. The combination of these best in class algorithms provides an avenue to solve the multifactorial problem of biologics optimization. We illustrate that a wide range of machine learning techniques are needed to make accurate prediction for the full range of preclinical properties of interest. To that end we analyzed large datasets that revealed that while properties such as half-life or affinity towards a target can be modeled using Bayesian regression techniques, in other cases techniques such as support vector machines are needed to predict amyloid aggregation, when using positional physicochemical descriptors. The models can be used to predict peptides most likely to have the desired activity from within the combinatorial expansion of amino acids, natural, unnatural including modified peptides. Our ultimate goal is to develop a decision support system that guides the optimization of peptides towards the definition of a clinical candidate minimizing the number of peptides that need to be evaluated that can be used by non-experts



P217

Development of the Stapled Peptide Targeting the NCOA1/STAT6 Protein-Protein Interaction

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Aberrantly activated STAT6 transcriptional activity is implicated in various human diseases, and thus inhibition of STAT6 signaling has been proposed as a promising therapeutic strategy. Here we report the identification of a cell-permeable, proteolytically stable, stabilized α-helical peptide directly targeting NCOA1, a coactivator required for STAT6 transcriptional activity. This stapled peptide disrupted the NCOA1/STAT6 interaction, thereby inhibiting STAT6-mediated signaling. Furthermore, we solved the first crystal structure of a stapled peptide in complex with NCOA1. Importantly, this stapled peptide is able to specifically inhibit the NCOA1/STAT6 interaction, while current STAT6 inhibitors and biological methods (e.g., siRNA knockdown) suppress the entire functions of STAT6. The stapled peptide there-fore represents a highly useful chemical probe for dissecting the precise role of the NCOA1/STAT6 interaction and an excellent starting point for the development of a novel class of therapeutic agents.

P218

Designed macrocyclic peptides as a novel class of nanomolar inhibitors of amyloid self-assembly

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Amyloid self-assembly is linked to more than 25 devastating cell-degenerative diseases including Alzheimer's disease (AD) and type 2 diabetes (T2D). The design of inhibitors of amyloid self-assembly is, however, a difficult task; major reasons are the conformational flexibility of many amyloidogenic polypeptides, the high affinity of self-assembly mediating interactions and the dynamic nature and large size of involved surfaces. The key components of the amyloid plaques in AD and T2D are the intrinsically disordered polypeptides β-amyloid peptide (Aβ) and islet amyloid polypeptide (IAPP). Notably, increasing amounts of evidence suggest a link between the pathogenesis of the two diseases. A large number of peptide-based inhibitors of Aβ or IAPP amyloidogenesis has been derived from the sequences of their targets. However, full-length IAPP analogs have been also shown to inhibit amyloid self-assembly of Aβ as well (Yan et al., Angew. Chem. Int. Ed. (2007) & (2013)). More recently, we have designed linear peptides derived from the partial IAPP sequence IAPP(8-28) as mimics of IAPP self-/cross-interaction surfaces (Andreetto et al., Angew. Chem. Int. Ed. (2015)). These peptides were termed interaction surface mimics (ISMs) and have been shown to be highly potent inhibitors of amyloid self-assembly and related cell toxicity of Aβ, IAPP or both polypeptides. Here we will present designed macrocyclic peptides as a novel class of nanomolar inhibitors of amyloid self-assembly of both Aβ and IAPP or of Aβ alone. These peptides were termed macrocyclic inhibitory peptides (MCIPs) and were designed using minimal IAPP-derived recognition elements (Spanopoulou et al., Angew. Chem. Int. Ed. (2018)). Most importantly, sequence optimization yielded an Aβ-selective MCIP exhibiting high proteolytic stability in human plasma and human blood-brain-barrier (BBB) crossing ability in a cell model. Based on their favourable properties, MCIPs are promising leads for anti-amyloid drugs and useful templates for the design of small molecules as modulators of pathogenic protein aggregation in AD or in both AD and T2D.

P219

Synthesis of Novel Amino Acids Incorporating Zinc-Binding Groups as Inhibitors of HDAC1:MTA1 Co-repressor Complex

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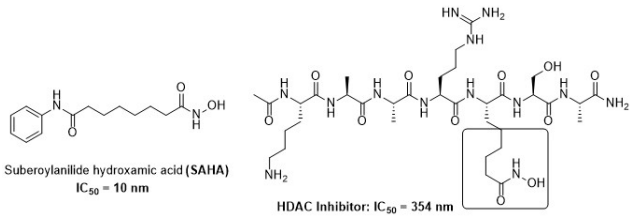
Epigenetic regulation of the human genome is vital for understanding both the etiology and fundamental mechanisms of diseases. Molecular regulation of gene expression is controlled in part by two distinct classes of lysine-modifying enzymes, the histone deacetylases (HDACs) and the histone acetyltransferases (HATs). HDACs have attracted considerable attention as therapeutic targets, especially due to their ability to modify the landscape of post-translational modifications (PTMs) on histone proteins in chromatin. Modifications in HDAC function have been linked to neurological disorders, muscular dystrophy, cardiac hypertrophy, cancer, HIV infection, and many other diseases. Class I, II and IV HDACs are zinc dependent enzymes. These isozymes are inhibited by molecules having zinc binding groups (ZBGs) such as SAHA (Figure). Here, we describe the synthesis of novel amino acids containing ZBGs and their incorporation into H3K27 histone tail. The foremost advantage of these amino acids (AAs) is they can be incorporated at any position in the peptide sequence without modification of standard Fmoc SPPS protocols. This synthetic strategy is facilitating the rapid generation of peptide inhibitors. Initial data demonstrates that these peptides are potent inhibitors of the HDAC1:MTA1 co-repressor complex and provide critical structure information for the development of next generation of HDAC inhibitors.



P220

Potent inhibition of CYP3A4 by the endomorphin-2 analogues

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Endomorphins (endomorphin-1, EM-1, Tyr-Pro-Trp-Phe-NH2, endomorphin-2, EM-2, Tyr-Pro-Phe-Phe-NH2) are endogenous peptides, which are very potent and highly selective μ-opioid receptor agonists. Moreover, they possess a potent analgesic activity comparable to that of morphine but are devoided of its undesirable effects. However, their use as therapeutic agents is limited due to their pharmacokinetic features, such as stability and permeability via blood-brain barrier (BBB). In our previous developments, modifications in the structure of endomorphine-2 have shown an improvement in stability and permeability through the membranes. In the current study, the goal was to investigate another pharmacokinetic feature, namely the risk of drug interactions at the level of cytochrome enzymes. CYP3A4 is the most important drug metabolizing enzyme in humans. It is highly expressed in liver and gastrointestinal mucosa and is involved in the metabolism of more than 50% of the used drugs. Therefore, the risk of drug interactions is greatest in drugs modifying the activity of CYP3A4. We used four endomorphin-2 analogues, two of them were modified at the third position - Phe(pF) (1) and conjugated at forth position with 1,2-ethylenediamine (2). The other two, were conjugated at first position with deoxycholic acid and modified at the third position - Phe(pF) (3) and Phe (pCl) (4). The four endomorphin-2 analogs used in concentrations of 25 to 100 μM showed potent and concentration-dependent inhibition of CYP3A4. On two of them (1 and 2), their effect was close to that of the classical inhibitor of CYP3A4 - ketoconazole. The results have shown some possible drug interactions of endomorphin-2 analogues which should be considered if they are used in clinical practice.

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P221

Injectable Peptide Hydrogels for Controlled Drug Release

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Currently, most drugs are given to patients, via oral or parenteral routes. In order to get the desired therapeutic effect, high doses are often required due to substantial clearance and biodegradation of the drug prior to interaction with the biological target, which may lead to adverse effects. To overcome this problem, hydrogels have been reported as suitable controlled drug-delivery systems. More specifically, peptide hydrogels loaded with active ingredients can liquefy during injection, followed by a quick hydrogel reformation once injected. These systems present several advantages including drug protection from enzymatic degradation through encapsulation in the hydrogel network, while maintaining the therapeutic plasma drug concentration over a long period of time. Consequently, lower dosage and frequency of administration are possible, resulting in improved drug efficacy while lowering the risk of side effects. Due to their biocompatibility, low toxicity and physically crosslinked network structure, peptide-based hydrogels represent an important class of injectable hydrogels suited to be matrices for controlled and slow drug release. In this work, a new family of hydrogel-forming peptides was designed starting from a short, tunable and amphipathic hexapeptide hydrogelator. After characterization, and aiming for the evaluation of their therapeutic potential, new hydrogels have been used for entrapment and sustained release of opioid drugs. The peptide-based hydrogelators were co-formulated with morphine, the gold-standard opioid analgesic.[1,2] Due to the well-known deleterious effects produced by morphine and other commonly used opioid analgesics, the development of alternative therapeutics is of utmost importance. Peptidomimetics of endogenous opioid peptides, especially ligands binding at the μ-opioid receptor, were designed and subsequently co-formulated with hydrogelators, and assessed in a mouse model of acute thermal nociception. We have demonstrated an extended antinociceptive effect up to 96 h after subcutaneous injection in mice. These promising results open an avenue for new therapies to treat chronic pain.

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P222

Peptide carriers for retinal drug delivery

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In ophthalmology retinal drug therapy is one of the major challenges, as it is hampered by ineffective and/or short-acting drug delivery to the targets, often located in intracellular compartments. Thus, there is a need for drug delivery systems able to transfer pharmaceutical cargo into retinal cells. Many retinal therapeutics are based on proteins and oligonucleotides with poor cell permeability. The development of cell-penetrating carriers can noticeably enhance their therapeutic effect. From this point of view membrane-active trichogin analogs are very promising candidates as carriers in retinal drug-delivery systems. In order to increase water solubility, we introduced lysine residues into the trichogin native sequence at different positions. The secondary structure of the corresponding peptides was studied by CD spectroscopy and 2D-NMR. Cell viability experiments showed low cytotoxicity up to 0.8 μM concentration. At the same time the peptides were efficiently internalized into retinal pigment epithelium cells. Thus, water-soluble trichogin analogs described herein can be very effective membrane-permeating molecules that can be used for drug delivery in the treatment of various diseases of the eye. This work has been performed in the framework of the project Megagrant 14.W03.31.0025.

P223

Targeting the coiled-coil domain of BCR-ABL1 kinase with new weapons

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The Philadelphia chromosome (Ph) encoded oncogenic tyrosine kinase BCR-ABL1 is the underlying cause of around 95% of chronic myeloid leukemia (CML) and 25% of adult acute lymphoblastic leukemia (Ph+ ALL). Current treatments for CML patients rely on ATP-competitive tyrosine kinase inhibitors. Nevertheless, 20−30% of patients manifest drug resistance associated with point mutations within the drug-binding site of BCR-ABL1. To overcome the drug resistance, discovery of inhibitors targeting the coiled-coil region involved in the activation of BCR-ABL1 kinase could be an alternative approach to generate drug leads with durable therapeutic response and to prevent or delay the emergence of resistance. In the current study, the coiled-coil domain BCR1-72 was synthesised chemically and screened against a thioether-macrocyclic peptide library generated using a cutting-edge technique named RaPID (Random nonstandard Peptides Integrated Discovery). Fourteen lead molecules discovered by the RaPID system showed significant binding affinities to the BCR1-72 in surface plasmon resonance and three of them further displayed growth inhibitory effects on K562 cells in the low micromolar range but did not show toxicity against human peripheral blood mononuclear cells. Taken together, the macrocyclic peptides discovered using RaPID system exhibited inhibitory activity towards the CML blast crisis cell line but not to the non-malignant human cells, indicating that they are promising candidates for therapeutic agent development.

P224

Entanglement: self-assembling peptide nanofibers that entrap and kill bacteria

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We are facing an era where the antibiotic cornerstone of modern medicine is under threat due to bacterial resistance. Deadly superbugs are not only resistant to current treatments, but can also evade the body's own defense strategy – our immune system. Adding to this crisis is that no new antibiotics have reached the pharmacy shelves in over 20 years. Antibiotics' standard tactic is straightforward: annihilation. But the strategy we are developing does this and more: by self-assembling into nanofibers this treatment entraps and kills bacteria. This mimics the body's own defense tactic of neutrophil extracellular traps (NETs), an important part of the innate immune system arsenal. Just like NETs, these nanofibers are decorated with peptides that kill bacteria, in this case the glycopeptide antibiotic vancomycin.

These fibers form by use the propensity of lipidated tri-beta-peptides self-assemble into nanofibers. To decorate these fibers with antibiotics we directly linked a beta3-peptide to vancomycin. Mixing these two beta3-peptides in different ratios resulted in vancomycin incorporated into fibers with different structures that have been visualized using electron microscopy, atomic force microscopy and stimulated emission depletion microscopy. These distinct fibers have different antimicrobial activity against the antibiotic resistant Staphylococcus aureus.

P225

Multicyclic Peptides via Templated Tandem CLIPS/CuAAC Cyclizations

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Multicyclic peptides provide a very attractive molecular format for the design of novel therapeutics,[1] Therefore, novel routes for synthesis and HTS-screening of this fascinating class of compounds are desperately needed. A decade ago, we launched a novel scaffold-assisted peptide-cyclization technology platform, termed "CLIPS", to generate in a one-steps procedure a new class of mono- and bicyclic peptides able to act as potent inhibitors of hitherto undruggable therapeutics targets.[2,3]

Following this, we now present a next-generation technology that combines both CLIPS and CuAAC chemistry into a one-pot methodology that enables the manufacturing of structurally complex peptide multicycles (tri-,[5] tetra-, penta- and hexacyclic constructs) by using either linear or backbone-cyclized[4] peptides. We present the chemical synthesis of two different types of CLIPS/CuAAC-scaffolds (T4 and T6) and show how these scaffolds behave in the synthesis of highly complex peptide constructs.

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P226

Hyaluronan based scaffold for cardiomyocyte adhesion

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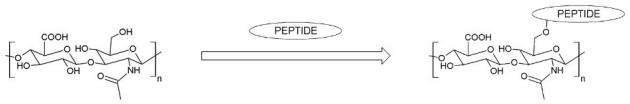
Hyaluronic acid (HA) is a biocompatible and biodegradable glycosaminoglycan which has a potential to be used in tissue engineering as a scaffold for cell or drug delivery. Since HA is highly soluble in aqueous solutions and is biologically inert, it is worth to modify the surface of material by functional groups or biologically active compounds which also stabilize the structure [1]. The modification of HA scaffold can be achieved by covalent bonding of peptides to primary hydroxyl group of N-acetyl-D-glucosamine unit in terms of Solid Phase Peptide Synthesis (SPPS) [2]. Our approach made possible to use HA in the form of fiber or non-woven textile as a carrier for direct preparation or binding of peptides. This construct can be directly employed for the study of certain chemical and physical properties and basic biological effects without need of peptide detachment. Different short peptides specifically enabling adhesion of cardiomyocytes were chosen for this study. A linker between HA and peptide containing two 6-aminohexanoyl units (Ahx-Ahx) was used to make adhesive motif more available for cell surface receptors. One of the advantages of this procedure is the water insolubility of the peptide-HA conjugate. Moreover, higher stability of this material is achieved in the presence of the Ahx-Ahx linker. Hyaluronan non-woven textiles bearing these specific peptides could be advantageously employed in various biomedicine applications, for example, as a patch in cardiac tissue repair or for wound healing. Its biodegradable composition is the main advantage because it eliminates the need of removal after the treatment. Preliminary results of the biological effects of prepared HA-peptide models confirmed the good direction of our approach. [1] A.M. Abdel-Mohsen et al. Carbohydr. Polym. 92 (2013) 1177–1187. [2] S. Karel et al. J. Pep. Sci. 22 (2016) S64.



P227

Novel HIV-1 protease inhibitor prodrugs using a degradation reaction of N-amidino-peptides

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The selective cleavage of an amide bond at room temperature or physiological conditions will be a powerful tool for life science and medicinal chemistry. However, the cleavage of amide bonds requires considerable energy, whereas ester bonds can be easily cleaved. The amide bond cleavage reactions are known in nature; for example, a protein, intein, undergoes protein splicing that involves an amide bond cleavage. To develop an amide bond cleavage reaction, we investigated the decomposition reaction of arginine methyl ester, which was reported by Photaki et al. In this reaction, the guanidino group of an arginine methyl ester attacks the ester carbonyl carbon of another arginine methyl ester, forming an arginine dimer. Next, the N-terminal amino group of the dimer attacks the guanidino-carbon within the molecule, forming a heterocyclic compound and ornithine methyl ester. We assumed that the driving force of this decomposition reaction is the release of the heterocyclic compound that is stabilized because of a conjugate structure. Previously, we reported a novel N-terminal degradation reaction of peptides via N-amidination. We designed and synthesized a series of N-amidino-peptides. Surprisingly, these peptides released N-degraded peptides at room temperature. Furthermore, we reported novel prodrugs of sparingly-soluble drugs, such as phenytoin and sulfa drug, using this degradation reaction. In this study, we designed and synthesized water-soluble prodrugs of HIV-1 protease inhibitor, ritonavir. The ritonavir prodrugs were stable in water and acidic media such as gastric fluid, and could release its parent drug rapidly and spontaneously under physiological condition such as gastrointestinal fluids. The ritonavir prodrugs appear to be suitable for an orally administered drug.

P228

Imaging Evaluation of an In-vivo Long-acting Neuropeptide(NPY) Analogue for Multimodality Breast Tumor Therapy

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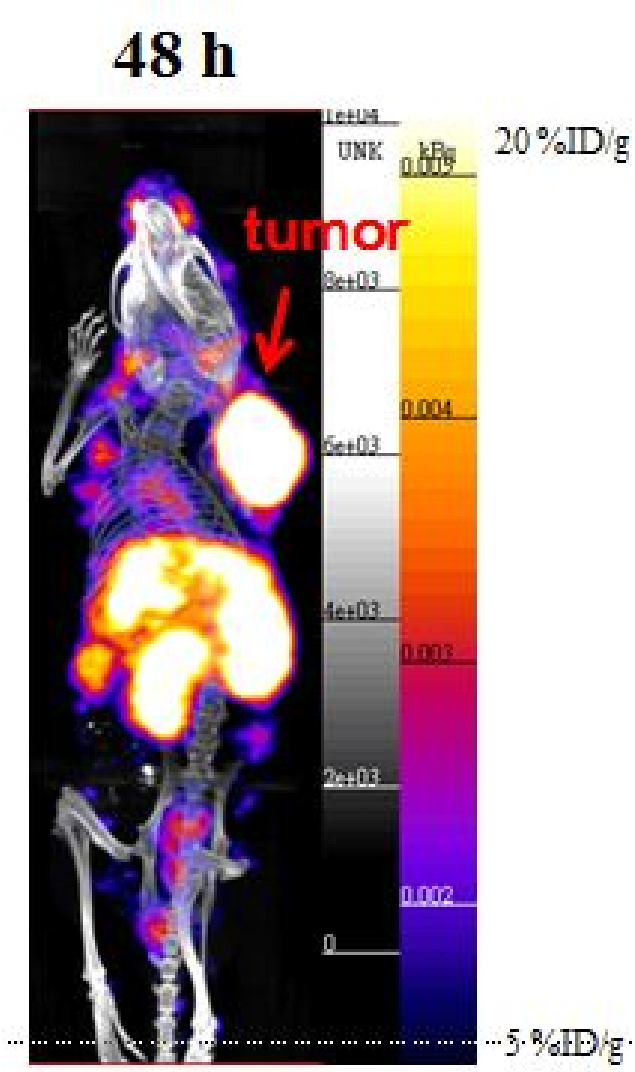
Neuropeptide Y (NPY) is a 36-amino acid peptide and regulates in various physiological functions through its four receptors. Recently, NPY receptor- Y1R has been found to be overexpressed in breast carcinomas. Although the truncated NPY analog has high affinity with Y1R, it is rapidly metabolized, resulting in low tumor uptake. Circulation time of NPY could be prolonged by conjugation with EB to enhance the tumor uptake in the body. The aims of this study were to synthesize a long-circulated NPY analog and evaluate tumor image of the 4T1 animal model as a candidate for breast cancer therapy. We synthesized DOTA-Boron-ENPY, which is a 16-amino acid NPY analog. Its structure consists of four parts-(1)tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid (DOTA), a chelate for radiolabeling(2)m-Carborane-1,7-dicarboxylic acid(C4H12B10O4) for high-density boron source(3)truncated Evans Blue as a good human serum albumin binder (4)truncated NPY analogue for high affinity with breast cancer. In the radiolabeling procedure, the amount of DOTA-Boron-ENPY was dissolved in sodium acetate buffer, followed by addition of (3-6mCi) In-111 or Lu-177, and incubated for 15-30 min at 95° C. The labeling efficiency was determined by Radio-TLC. The Radio-HPLC are used for analyzing labeling purity. In animal study, tumor xenografts were performed in 6-wk-old female BALB/c mice by subcutaneous injection of2*106 4T1 cells, and nanoSPECT/CT imaging was performed at 0.5 h to 48h after injecting of the111In- DOTA-Boron-ENPY. We can get the high labeling efficiency (>95%) of 177Lu- DOTA-Boron-ENPY after 30min labeling with Lu 177. However, the labeling efficiency of 111In- DOTA-Boron-ENPY is greater than 90% after reacting 15min with 111In. In vivo study, the nanoSPECT/CT image of the 4T1 animal model revealed that 111In- DOTA-Boron-ENPY has high tumor uptake value (ID%/g>10)and high tumor to muscle (T/M>9) ratio in 48 hours after tail vein injection. The result shows the DOTA-Boron-ENPY is easy to radiolabel with Lu177 and In111. The two compounds are high labeling efficiency and high resolution of the image. From the in vivo study, we consider the DOTA-Boron-ENPY as a long circulation radiopharmaceutical candidate for companion diagnostics (CDx). It could be long-acting in 48 hours after tail vein injection and has high tumor uptake value (ID%/g>10) and high tumor to muscle ratio (T/M>9). Furthermore, we apply such DOTA-Boron-ENPY for the design of boron carriers. Peptide Receptor Radionuclide Therapy (PRRT) is a widely known molecular targeted therapy. PRRT is performed by using a small peptide which is combined with ra-

dionuclides. DOTA-Boron-ENPY is a small peptide combined with boron and Evens Blue, and thus it can circulate longer in the animal model. It is not only a good tracer for BNCT but also a therapeutic drug by labeling with Lu177. The capabilities of DOTA-Boron-ENPY make it a potential drug for multimodality breast tumor therapy.



P229

NGR-DAU Conjugates, a Favorable Tumor-Homing Motif with Potential Dual-Targeting

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Cancer is currently one of the major health-related issues world-wide. Strategies to target and defeat tumor are limited due to the access to tumor, side effects and absence of selectivity to the tumor tissue. Peptides containing the Asn-Gly-Arg (NGR) motif can act as tumor-homing compounds that are recognized by Aminopeptidase N (APN/CD13) which is receiving an accurate attention due to its role in tumor growth, metastasis and immune regulation, generally expressed on the monolayer cell surface of malignant cells [1]. As stated in the literature the Asn-Gly-Arg sequence is inclined to Asn deamidation bearing the formation of a succinimide intermediate, generating isoDGR derivative which can bind to integrins [2]. This tendency of the NGR motif provides an ideal starting point for the development of conjugates with a selective dual acting anti-tumor effect. We recently described that the Dau=Aoa-GFLGK(c[KNGRE]-GG-)-NH2 conjugate has a significant anti-tumor activity against both CD13 positive HT-1080 human fibrosarcoma and CD13 negative but integrin positive HT-29 human colon adenocarcinoma cells [3]. However, it seems that the free ε-amino group of Lys in the cycle is not necessary for the biological activity. Therefore, we developed novel cyclic NGR peptide – daunomycin conjugates in which Lys was replaced by different amino acids [4]. From these investigations the Nle conjugate as the most efficient compound was selected for additional studies. The cytotoxic effect of the novel cyclic NGR peptide Dau conjugates were evaluated in vitro on HT-1080 (human fibrosarcoma), HT-29 (human colon adenocarcinoma) and KS (Kaposi Sarcoma) cell lines. The stability in cell culture medium and the lysosomal degradation in presence of rat liver homogenate were determined by LC-MS. As daunomycin present fluorescent properties, internalization of both peptides was also detected by flow cytometry. The achieved results from these studies confirm that the Lys - Nle replacement provides not only an easier synthetic route but also enhance the anti-tumor activity of the conjugate.

This project had received fundings from the European Union's Horizon 2020 research and Innovation program under the Marie Sklodowska-Curie grant agreement No 642004, and from the National Research, Development and Innovation Office (NKFIH K119552), Hungary. ____ [1] F. Curnis, G. Arrigoni, A. Sacchi, L. Fischetti, W. Arap, R. Pasqualini, A. Corti, Cancer Res 2002, 62, 867-74. [2] Y. C. Meinwald, E. R. Stimson, H. A. Scheraga, Int J Pept Protein Res 1986, 28, 79-84. [3] K. N. Enyedi, S. Toth, G. Szakacs, G. Mezo, PLoS One 2017, 12, e0178632. [4] A. A. P. Tripodi, S. Toth, K. N. Enyedi, G. Schlosser, G. Szakacs, G. Mezo, Beilstein J. Org. Chem. 2018, 14, 911-918.

P230

Design of multifunctional peptide-based nanoparticles for specific cell-delivery of siRNAs

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Small interfering RNAs (siRNAs) present a strong therapeutic potential because of their ability to inhibit the expression of any desired protein. However, siRNAs show a very weak propensity to cross the plasma membrane on their own. We recently developed a series of new cell-penetrating peptides able to form stable peptide-based nanoparticles (PBNs) once incubated with a given ratio of siRNA.1 With regard to future in vivo applications, we also studied recently the influence of the polyethylene glycol (PEG) grafting onto the PBNs on their in vitro and in vivo siRNA delivery properties. 2 We also planed to address specifically PBNs to tumor sites upon the incorporation of peptide targeting sequences on PBNs. Thanks to strategies offered by peptide chemistry, we designed, prepared and studied new PBNs made of several different peptides blocks (siRNA complexation, siRNA cellular transfer, targeting and prolonged blood-circulation) in order to improve significantly the cell-specific delivery of siRNAs.

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P231

Peptides specifically target bacteriophage MS2, filled with an apoptosis-inducing agent, to tumour

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Bacteriophages, especially MS2, are often used recently as containers for delivery (including the targeted one) of drugs, nucleic acids, etc. into cells. We employed bacteriophages MS2 for the tar-geted delivery of an apoptosis-inducing agent into tumour tissue. The targeted delivery was provid-ed by peptides of iRGD type, that specifically bound integrins presumably located on the surface of endotheliocytes of a novel vasculature formed in tumour tissues. Peptides were obtained by SPPS and cyclized via formation of S-S bridge. The peptides contained a spacer, through which they were conjugated to phage capsid proteins. Tl+ ions in the form of TlNO3 were used as apoptosis-induced agents, since it was known that multiple drug-resistant cancer cells could not eliminate Tl+. Tl+ was shown to penetrate into phage particles and to bind tightly to phage RNA, with the drop in Tl+ concentration in the media up to 30 times compared to the initial one. Peptide-modified MS2 preparations filled with Tl+ were tested on several solid tumour cell cultures. Filling of peptide-modified MS2 with 3.5 mkg of TlNO3 resulted in 80-85% cell death, while no cell death was observed in control experiments. When peptide-modified MS2 filled with Tl+ was in-jected to MCF7- and DA-MB-231-xenograft nude mice, it resulted in tumour mass loss up to 2.5 times compared to control. Overall concentration of TlNO3 in peptide-modified MS2 filled with Tl+ was 500000 times lower than TlNO3 LD50, and therapeutic index of the phage-based preparation was about 15000. The results show the perspective of the use of bacteriophage MS2 filled with Tl+ and containing a target delivery system as a lead substance for solid tumour therapy. Peptides repre-sent a convenient target delivery system for such preparations because of their easy and well-defined conjugation to phage capsid proteins. The research was supported by the State contract with the Russian Ministry of Education and Sci-ence No. 14.N08.11.0188 (27.11.2017) (unique project ID 17177105391357710010010030807219241). Peptides were synthesized with the use of the equip-ment of "Human Proteome" Core Facility of the Institute of Biomedical Chemstry (Moscow, Russia) supported by Ministry of Education and Science of the Russian Federation (unique project ID RMEFI62117X0017).

P232

Antibacterial coatings produced by chemoselective grafting of Dhvar-5 onto chitosan

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Bacterial colonization and establishment of antibiotic-resistant biofilms represent a significant healthcare problem. Also, the quick decline in the effectiveness of current antibiotics is boosting the search for new and effective strategies [1], among which antimicrobial peptides (AMP)-related approaches have been steadily gaining prominence; one example of a highly promising field of research in this area is development of AMP-based materials [2]. In this connection, the main goal of our work was to evaluate the effect of surface immobilization of Dhvar-5, an AMP with head-to-tail amphipathicity, on its antibacterial activity. To this end, Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) "click" reactions were explored for covalent immobilization of the peptide onto chitosan, a biopolymer with intrinsic antibacterial and bioadhesive properties. Dhvar-5 grafting onto chitosan by CuAAC was carried out either on (i) ground chitosan powder next used to prepare thin films (type 1 coatings), or (ii) pre-formed chitosan thin films (type 2 coatings). Also, peptide tethering was evaluated in both possible orientations, i.e., the peptide was covalently immobilized via either its N- or its C-terminus [3,4]. Antimicrobial activity assays were conducted using a combination dye of the LIVE/DEAD® Bacterial Viability Kit (Baclight™) for quantifying the viability of adherent bacteria. Results demonstrated that the mode of action and antibacterial efficacy of immobilized Dhvar-5 depended on both the strategy of immobilization and peptide orientation. As such, type 1 coatings displayed bactericidal effects, whereas anti-adhesive properties were exhibited by type 2 coatings. Moreover, higher antimicrobial activity was observed when Dhvar-5 was immobilized through its cationic C-terminus, i.e., exposing its hydrophobic domain. Altogether, findings made thus far suggest that CuAAC "click" chemistry is an attractive chemoselective approach to graft AMP onto chitosan, yielding bulk materials that are suitable to produce tailored biomaterials with excellent prospects for application as antimicrobial coatings.

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Synthesis and in vivo optical imaging of peptide tools for targeted renal drug delivery

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The treatment of chronic kidney diseases like ADPKD can benefit from drugs that specifically target the kidney. This can potentially reduce side effects and provide higher drug efficacy within renal tissue. In this context, peptide sequences (KKEEE)nK were reported to target the renal cortex1. In order to be able to assess the renal targeting ability of this family of peptides by in vivo optical imaging, we synthesised peptides (KKEEE)nK (n=2 and n=3) bearing the near IR Vivo-tag 680 XL dye at the N-terminus. These syntheses can be challenging due to the high cost of the Vivo-tag 680 XL dye and the use of a large excess of reagent typical of SPPS. Instead, an inverse approach was applied in which 0.3-0.5 eq of dye were used and the corresponding tagged and untagged peptides were easily separated by preparative HPLC. These peptides, when injected IV into normal mice, accumulated in the cortex of the kidney, specifically in the proximal tubules, and uptake by other organs was not observed.

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P234

Intranasal Administration of Diazepam-binding Inhibitor Derived Peptides for Brain Delivery

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Due to their poor pharmacodynamic and pharmacokinetic properties, experimental and therapeutic delivery of neuropeptides or their synthetic analogues to human brain has been hampered. Indeed, when administered systemically, these compounds are rapidly degraded, do not readily pass the blood-brain barrier (BBB), and often evoke potent hormone-like side effects when circulating in the blood. Thus, for their potential future clinical applications, it is mandatory to find an alternative route of neuropeptide administration to reach their parenchyma targets. Since more than a decade, the intranasal (i.n.) route has received considerable attention because of low proteolytic activity compared to the oral route, high vascularization and large absorptive surface, resulting in improved absorption. In addition, the intranasal route can offer a direct access to the brain to peculiar compounds. The diazepam-binding inhibitor-derived peptide ODN acts as a potent anorexigenic compound in rodents when injected intracerebroventricularly (i.c.v.). However, intravenous injection of 200-fold the minimal i.c.v. effective dose does not modify feeding behaviour suggesting that ODN does not comply to systemic administration. Hence, we have evaluated the ability of ODN, its isoactive C-terminal fragment OP and a potent and constrained analogue cyclo(1-8)OP to induce anorexigenic effects in mice via i.n. administration. Preliminary investigations revealed that i.n. administration (1 μg/twice a day/7d) of OP induces a significant reduction in body weight in mice, while ODN and cyclo(1-8)OP i.n. applications do not modify body weight compared to the vehicle injected group. The effect of OP was settled after 3 days of treatment and lasted until the end of treatment. Neither rebound hyperphagia nor gaining weight was observed after treatment cessation. Investigation performed using qPCR revealed that i.n. administration of OP induces an increase in UCP1 transcript expression in brown adipose tissue, suggesting that OP stimulates energy metabolism via sympathetic activation. Additionally, our investigation also revealed that i.n. administration of OP causes liver lipid metabolism modifications. Indeed, OP increased mRNA levels of gene related to both lipid oxidation (PPAR-γ, CPT-1α) and lipid uptake (FABP-1, FATP-1, CD-36), suggesting that OP i.n. administration enhances fatty acid β-oxidation in the liver. In conclusion, our data show that OP injected by intranasal route can reach the central nervous system by skirting around the BBB and then activates central anorexigenic pathways. Moreover, they provide evidence that the physicochemical characteristics of the compound govern its brain delivery.

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Antimicrobial activities of chimera peptides composed of human neutrophil peptide 1 (HNP-1) truncated analogues and bovine lactoferrampin

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Many pathogenic bacteria and fungi strains have become resistant to the most of commercially available antibiotics as the result of their common and excessive use in medical practice. One of the most promising strategy for searching for new compounds with antimicrobial activity is bioconjugation, covalent or non-covalent connection, of at least two chemical molecules of compounds with different activity that in the end display a different mechanism of action than the parent compounds. The main aim of our work was to obtain a new class of compounds with antimicrobial activity. These compounds are peptidic hybrids composed of two peptides with antimicrobial activity. First peptide is a modified 15-29 fragment of human neutrophil peptide 1 (HNP-1), a member of α-defensins [1]. This peptide displays antimicrobial activity against a broad spectrum of pathogens, the closest to that of full length HNP-1. Second peptide is bovine lactoferrampin (LFampB) which is the fragment 268-284 of bovine lactoferrin [2]. This peptide also exhibits broad antimicrobial action against several gram-positive and gram-negative bacteriaas well as candidacidal activity [3,4]. In the present study, we synthesized series of compounds consisting of two peptide chains conjugated by isopeptide bond and disulfide bridge. All peptides were screened against the wide range of microorganisms, including Gram-negative, Gram-positive bacteria and fungi from Candida species. Broth microdilution method for MIC determination was used as a standard procedure. As a result, we obtained three peptidic hybrids that effectively inhibited the growth of selected bacterial strains, but none of the chimeric peptides was able to inhibit fungi growth. Cytotoxicity assays on tumor and healthy cells show that tested chimera peptides induce a relatively low cytotoxicity in reference to antimicrobial drugs widely used in clinic. More interestingly, these compounds displayed a higher helical content and more potent antibacterial activities than the constituent peptides individually or their equimolar mixtures. These results indicate a significant influence of the linker used in chimera peptides on their biological activity and secondary structure of the studied compounds. We proved that a covalent linkage of two antimicrobial peptides significantly improves antibacterial activity, which makes such hybrids good lead structure for designing antimicrobial drugs.

Acknowledgement:This work was supported by the National Science Centre (NCN) under grant No UMO-2016/21/B/ST5/00101.

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P236

Design, Chemical Synthesis and Evaluation of Antimicrobial Activity Peptide Conjugates of Lactoferricin Analogues and Antibiotics

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Despite the increasing need for antibiotics to fight infectious diseases, fewer new antibiotics are available on the market. Unfortunately, developing a new class of antibiotics is associated with high commercial risk. Therefore, modification or combination of existing antibiotics to improve their efficacy is a promising strategy. In this broad field, peptide–drug conjugates linked by non-cleavable or intracellular cleavable structures have evolved as highly promising agents. Here we report synthesis and biological investigations a series of peptide conjugates composed of modified bovine lactoferricin truncated analogues (LFcinB) and three antibiotics, ciprofloxacin (CIP), levofloxacin (LVX) and fluconazole (FLC) commonly used in medicinal practice. The first two belong to the class of fluoroquinolone displaying broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria. They inhibit DNA gyrase and topoisomerases. FLC, similarly to other azole drugs, inhibits the lanosterol 14α-demethylase and decreases the level of ergosterol required for the integrity of cell membrane [1]. LFcinB displays a broad spectrum of antibacterial and antifungal activity and also cooperative effect with azole types of antifungal agents [2]. Our intention was to design a new class of antimicrobial compounds. Series of 8 new peptide conjugates containing 3 above mentioned antibiotics were obtained. Four different approaches were apply to couple a peptide and the antimicrobial agent. We developed an optimized conditions for coupling of levofloxacin via its carboxylic group, ciprofloxacin via its carboxylic and amino group to peptide chain using solid phase peptide synthesis. Also intermolecular disulfide bridge was used as a linker between peptide and CIP. In case of fluconazole-based conjugates, a "click chemistry" method was used to attach this antifungal agent derivative to a peptidic component. All studied peptides exhibited antimicrobial activity showing preferences to Gram-positive. Similar to the constituent peptides, synthesized conjugates exerted highest activity against S. epidermidis. The most active ones were conjugates containing CIP attached to the peptide by the redox-sensitive disulfide bridge. They displayed not only higher efficacy than constituent peptides, but also broader activity, inhibiting other experimental strains. Microscopic observations showed that such conjugate as well as its constituent peptide penetrate well into S. epidermidis cells. The obtained results indicate an important role of linker between antibiotic and peptide. Cytotoxicity assays on cancer and non-cancer cell lines show that tested conjugates induce a relatively low cytotoxic effect (especially to the later) in reference to antibiotics widely used in clinic, thus they can be considered as good candidates for drug development.

Acknowledgement: This work was supported by the National Science Centre (NCN) under grant No UMO-2016/21/B/ST5/00101.

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P237

Peptide conjugates of transportan 10 with antimicrobial and antifungal antibiotics

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Conjugates, in which two molecules differing by chemical and physical properties are connected, are a promising group of compounds. Conjugates consist of a cell penetrating peptide (CPP) and a molecule with therapeutic properties are among most frequently studied compounds. CPPs can deliver to eukaryotic and prokaryotic cells a vast range of different biologically active compounds, including chemotherapeutic agents (e.g. antibiotics). Transportan (TP) reported by Langel group, is a chimeric peptide composed of the first 12 amino acid residues of neuropeptide galanin and 14 amino acid residues-long wasp venom peptide, mastoparan, connected via a lysine residue. A short variant, named TP10, with deletion of the N-terminal hexapeptide, retains the efficient cell penetration property of the parent compound with significantly less potential side effects [1]. Taking into the consideration the above mentioned literature data, we decided to design, synthesize and determine antimicrobial (antibacterial and antifungal) activity of peptide conjugates composed of TP10 and antibiotics: levofloxacin, ciprofloxacin and fluconazole. Three different linkers (methylene carbonyl, amide and disulfide bridge) were used to connect both components. TP10 may not only help to transport antibiotic across the microbial cell membranes, but having intrinsic antimicrobial activity, it can potentiate and even change the molecular mechanism of conjugated antibiotic. In order to determine the cellular uptake of TP10 and its conjugate with ciprofloxacin, their fluorescently-labeled analogues were also synthesized (TP10-F and CIP-TP10-F). The synthesized conjugates, TP10, levofloxacin and ciprofloxacin were tested for growth inhibitory activity against several fungal stains. TP10, levofloxacin and ciprofloxacin did not exhibit antifungal activity at concentrations up to 100 μg/mL; however, two conjugates with ciprofloxacin were active against C. albicans, C. krusei and S. cerevisiae. Antibacterial in vitro activity of studied compounds was determined against four bacterial strains, S. aureus, S. epidermidis, E.coli and P. aeruginosa. TP10 did not display antimicrobial activity at concentrations up to 200 μg/ mL, whereas its conjugates with ciprofloxacin inhibited growth of all studied bacteria. To assess the cytotoxic effect of TP10, ciprofloxacin and its two conjugates, we evaluated IC50 values (using MTT assay) for two human cell lines: liver cancer cells (HepG2) and cell line of embryonic kidney (HEK 293), as well as a cell line isolated from kidney of male pig (LLC-PK1). In case of LLC-PK1 cells, TP10 and two conjugates were 4 times less toxic than ciprofloxacin. Using carboxyfluorescein-labeled TP10 and its conjugate with ciprofloxacin, we we shown that both compounds penetrate well into C. albicans SC 5314, and C. albicans ATCC 10231. Acknowledgement: This work was supported by the National Science Centre (NCN) under grant No UMO-2016/21/B/ST5/00101. References: 1. Soomets U., Lindgren M., Gallet X., Hällbrink M., Elmquist A., Balaspiri L., Zorko M., Pooga M., Brasseur R., Langel Ü., BBA, 1467 (2000) 165-176

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Host cell targeting with peptide candidates derived from a HSV-1 entry protein

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The Herpes simplex virus-1 (HSV-1) infects host cells via surface receptors such as nectin1 or Herpes virus entry mediator (HVEM)2, the virus particle uses its envelope glycoproteins to effect the entry. Unlike with other herpes viruses, in case of the HSV the gD glycoprotein has an important role in the mechanism of entry. In this study regions of the HSV-1 gD were chosen based on the known tertiary structure of the complex of the HSV-1 gD and the nectin-1 and HVEM receptors, respectively, for cellular internalisation studies. The peptides were prepared with Fmoc/tBu chemistry by solid phase peptide synthesis, with or without 5(6)-carboxyfluorescein attached to their N-termini. The RP-HPLC purified peptides were characterised chemically by ESI-MS and their fluorescence spectra. Their in vitro cytostatic properties were studied with tetrazolium test3, and then their cellular uptake by SH-SY5Y4 (CRL-2266) neuroblastoma cell culture was investigated by flow cytometry in a peptide concentration range of 0,4 – 50 μM. The secondary structure of selected peptides was studied by electronic circular dichroism. We have found a sequence in the C-terminal region of the gD glycoprotein showing high efficiency in the cellular entry, and further peptides showed low to moderate entry in a concentration dependent manner. Inhibition of internalisation revealed that the entry is (partially) effected via receptor mediated endocytosis. ECD studies revealed that the peptides with internalising ability partially retained their helix like secondary structure adopted within the protein even in ECD circumstances when using the ordered structure promoting solvent TFE or TFE-water mixture. The marked difference in the cellular entry of the peptides demonstrates that the peptide sequences corresponding to the most effectively internalising peptides are located in the nectin-1 binding region of HSV-1 gD glycoprotein.

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P239

Tumor Targeting of Cryptophycin based Conjugates Using Octreotide as Delivery Vehicle

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Cryptophycins are potent microtubule destabilizers that can not be used as traditional chemotherapeutic due to their lack of tumor preference, which causes neurotoxic side effects and lack of efficacy in vivo. However, their high potency, which is 100- to 1000- fold greater than paclitaxel or other tubulin binders, and the lack of drug resistance makes them a very promising agent to be used in targeted therapy.[1,2] The parent compound cryptophycin-52 lacks an addressable group that would allow the conjugation to a homing device. For this reason, the discovery of novel analogues that maintain the cytotoxicity of the parental compound and presents a functional group has received much attention.[3,4] Cryptophycin-55 glycinate has emerged as potential payload to be used in tumor targeted therapy. This synthetic analogue retains the cytotoxicity and includes a functional group for conjugation to an antibody or small molecule. The conjugation of cryptophycin-55 glycinate to octreotide using different linkers lead to small molecule-drug conjugates targeting the somatostatin receptors (SSTRs). The binding affinity to the SSTRs, the cytotoxic effect on different tumor cell lines and the plasma stability will be reported. Moreover, a conjugate bearing an infrared dye instead of the drug was used to test the specificity of octreotide towards SSTRs and was evaluated in vitro and in vivo.

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P240

Biochemical-activity studies of NGR-peptide-drug conjugates for targeted tumour therapy

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Based on statistical data, it is known that cancer is the second most common cause of death worldwide. Chemotherapy has a great importance in the treatment of cancer patients. However, the vast majority of chemotherapeutic drugs used today can cause serious side effects. Targeted tumour therapy may offer an opportunity to overcome this problem by conjugating chemotherapeutic drugs to tumour specific peptides. NGR (Asn-Gly-Arg)

peptides received particular interest when phage display libraries were used to identify non-RGD integrin binding motifs. Among the non-RGD peptides the NGR motif was the most frequent that showed integrin binding properties [1]. However, peptides containing the NGR motif are not integrin ligands, but specifically recognize specific CD13/aminopeptidase N isoforms that are selectively overexpressed in tumour neovasculature [2]. In addition, these sequences prone to spontaneous decomposition through a succinimide-ring formation, forming isoAsp and Asp derivatives which is strongly influenced by the structure. The resulting isoaspartyl (isoDGR) derivatives are recognized by RGD-integrins that are essential for tumour metastasis [3]. Hence NGR peptides are of high interest, due to their simultaneous and selective targeting of CD13 and RGD-integrin receptors, therefore potential applications in dual drug targeting Based on the literature and our earlier work, we have chosen seven small cyclic NGR peptides as our targeting molecules: c[KNGRE]-NH2, c[NleNGRE]-NH2, c[GNSarRGK], Ac-c[CNGRC]-NH2 and H-c[CNGRC]-NH2, and also the thioether bond containing c[CH2-CO-KNGRC]-NH2 and c[CH2-CO-NGRC]-NH2 [4,5,6]. Using these small cyclic NGR derivatives as a homing moiety, we designed, synthesised and characterized novel cyclic NGR peptide-drug conjugates with a well-known chemotherapeutic agent, daunomycin (Dau). Since sequences of certain peptides allowed the conjugation of daunomycin in different positions, where possible, not only the C-terminal elongated, but also branched variants were prepared. To ensure selective drug release for targeted tumor therapy we have incorporated an extracellular MMP-2 enzyme labile spacer into the conjugates. The cytotoxicity effect of the novel cyclic NGR peptide-Dau conjugates were examined in vitro on CD13 positive HT1080 (human fibrosarcoma) and KS (Kaposi's sarcoma) and, as a CD13 negative control, on HT-29 (human adenocarcinoma) cell lines. The results were compared with the data acquired from chemostability, and degradation measurements.

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P241

Synthesis and in vitro Biological Effect of GNRH-Protoporphyrin IX Conjugates

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Photodynamic therapy (PDT) combines non-toxic components, a photosensitizer, light and oxygen. The photosensitizer can be activated to its excited states by irradiation with visible light, which produces reactive oxygen species after crashing with molecular oxygen in the tissue. In most cases a singlet oxygen is generated that has a short intracellular life time (3 μs) and a very small intracellular diffusion distance that makes PDT highly selective [1]. Conjugation of the photosensitizer to a carrier peptide that itself has anti-tumor effect can increase the efficacy and selectivity of the treatment. Gonadotropin-releasing hormone (GnRH) is a decapeptide (<EHWSYGLRPG-NH2) that plays a central role in the vertebrate reproduction by regulating gonadal activity. Its receptor was found on several tumor cells, which makes this peptide a proper targeting moiety for targeted tumor therapy.[2] In this work GnRH-protoporphyrin IX conjugates were synthesized. Various GnRH analogues were used, in most cases the 4Ser in the sequence was replaced by a butyric acid modified lysine (Lys(Bu)) since this change increases the receptor binding affinity and the stability of the molecule against enzymes [3]. In the sequences of GnRH-I and GnRH-II analogues 6Gly was replaced by D-Lys to have a proper conjugation site for the photosensitizer [4], while in case of the GnRH-III derivative the Protoporphyrin IX (Pp) was attached to the side chain of the 8Lys. In GnRH-III the side chain of 6Asp was blocked as methyl ester because in this case the carboxyl group of Asp cannot react under the conditions used for amide bond formation. Since the solubility of these conjugates was limited, Pp was derivatized with PEG-NH2 and a PEG-Pp-GnRH-I analogue was prepared, as well. The in vitro biological assays were performed on Detroit-562 human pharynx carcinoma cells that highly express GnRH receptors [5]. We found that all GnRH-Pp conjugates showed excellent anti-tumour effect at low concentrations (1 μM) with short irradiation time (10 min), and we also observed that the PEGylation highly increased the solubility.

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Structure-activity relationship of HER2 receptor targeting peptide and its derivatives in targeted tumor therapy

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Breast cancer is one of the most leading causes of death worldwide. In 20% of all cases human epidermal growth factor receptor 2 (HER2/ErbB2) is found on breast cancer tissues. The overexpression of HER2 leads to aggressive course of disease. Recently, targeted tumor therapy is a promising research area based on the increased selectivity of antitumor drug attached to a targeting molecule, which binds to tumor specific antigens/receptors. Since HER2 might be a good target to prevent tumor growth, current researches are directed to discover HER2 targeting moieties for drug delivery. Kadcyla® as an antibody−drug conjugate (ADC) is on market now. In spite of benefits of ADCs (long half-life in circulation and high selectivity) there are some disadvantages of ADCs (low tissue penetration and high costs). Therefore, small molecule−drug conjugates (SMDCs) based on peptides as targeting moieties might have advantages over ADCs. Appropriate homing peptides can be selected by phage displays or molecular dynamic simulations. In our research KCCYSL peptide selected by phage display [1] as well as GYYNPT peptide that bind selectively to HER2 according to MD modelling [2] were chosen to study their in vitro cellular uptake by HER2+ cancer cells. Furthermore, the cysteine residues were replaced by alanine or serine in KCCYSL peptide to investigate the role of cysteines. The two different peptide sequence were combined (KAAYSLGYYNPT, KSCYSLGYYNPT) too, to increase the biological activity. All compounds were labelled with 5(6)-carboxyfluorescein (CF) on their N-terminus. The in vitro cellular uptake was measured by flow cytometry on MDA-MB-453 HER2+ cancer cells. The results indicated that CF-KCCYSL-NH2 and its modified analogs were taken up by cells with low intensity while CF-GYYNPT-NH2 did not internalized. The amino acid replacements in the KCCYSL sequence did not show significant influence on internalization. In contrast to the CF-labelled hexapeptides, the recognition of the combined peptides by cells was high also at low concentrations. Interestingly, the localization of CF-labelled combined peptides was detected rather on the cell membrane than intracellularly. The extracellular localization was also indicated by trypsinization resulted in

significantly lower fluorescence intensity. This observation suggested that the combined peptide suitable for receptor binding with high affinity but the internalization of receptor conjugate complex is low. Therefore, these homing peptides may be applied for tumor diagnostic (PET) or selective delivery of radiotracers with therapeutic activity. In addition, the application of extracellular enzyme (elastase, MMPs) cleavable spacers between the homing peptide and an antitumor agent might be a good choice for the development of conjugates as drug delivery systems. This project was founded by the National Research, Development and Innovation Office (NKFIH K119552, NVKP_16-1-2016-0036), Hungary.

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P243

Synthesis and in vitro Evaluation of Tetrameric RGD-Cryptophycin Conjugates

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Cryptophycins are novel and compelling antimitotic payloads for tumor targeting applications, owing to their high potency as tubulin polymerization inhibitors and retention of activity against MDR cell lines [1]. Different cryptophycin analogues have been designed and synthetized to elucidate what moieties are essential for the biological activity. In addition, the functionalization of the drug for conjugation to tumor targeting peptides or antibodies has been attempted [2,3]. The synthetic cryptophycin-55-glycinate that has been reported to be active in the low nM range, contains a functional group providing the possibility of conjugation, as well as shows increased stability of the macrocycle compared to the parent compound. Therefore, it has the potential to be used as payload in the construction of drug conjugates. In this study, cryptophycin-55-glycinate was conjugated to monomeric or tetrameric cyclic[RGDfK] peptide ligands targeting integrin $\alpha v\beta 3$ [4]. The protease cleavable dipeptide Val-Cit and the PABC self-immolative spacer were inserted between the drug and the tumor targeting ligand [5]. The in vitro cytotoxicity of the conjugates was evaluated on M21, M21-L human melanoma cells, showing increased antitumor activity of the tetrameric conjugate against the antigen-positive M21 cell line. The in vitro plasma metabolic stability of the monomeric conjugate and the lysosomal drug release will be also reported.

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Cell-penetrating peptide sequences for high precision targeting, imaging and sensing in live cells

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The recent development of fluorescent probes has allowed biologists to visualize subcellular components in vivo, both structurally and functionally. The observation of many biological processes relies on the ability to identify and locate subcellular environments. There have been many reports of Ru(II) metal complexes as imaging and sensing probes, but require the use of detergents as well as requiring high concentrations in order to get them to cross the cell membrane. Once in the cell, there is no control as to where the probes will localize. We have demonstrated that the use of cell-penetrating peptide (CPP) sequences can overcome these drawbacks and achieve high precision targeting of our probes, selectively to specific organelles in the cell. CPPs are short, positively charged peptide sequences, and function to promote and facilitate cellular uptake of molecules at low concentrations. To date, however, there have been few reports on the use of CPPs to direct metal complexes into live cells. Here we present the CPP sequences we have successfully exploited for cell imaging and sensing. We have examined simple oligoarginine sequences, as well as more complicated signal peptide sequences. In particular, we have exploited the mitochondrial penetrating peptide (MPP) sequence FrFKFrFK to selectively target the mitochondria and direct [Ru(bpy)2(phen)-MPP]7+, an O2 sensing probe, exclusively to the mitochondria. Here we were able to measure O2 and ROS in vivo based on the luminescent lifetime of the probe using Fluorescence Lifetime Imaging Microscopy (FLIM). We have also conjugated a nuclear-localizing signal peptide, from the transcription factor NFkB, and an endoplasmic reticulum (ER) peptide sequence, to direct [Ru(dppz)(bpy)2-NLS]6+ and [Ru(bpy)2(phen)-ER]9+ to the nucleus and ER of live cells. This allowed us to carry out super-resolution STED imaging of chromosomal DNA at the stages of mitosis, as well as achieve high-resolution images of the ER. Using this NLS peptide, we reported the first example of [Ru(tap)2(bpy)-NLS]6+ to the nucleus of live cells for photo-induced DNA damage. In all cases, without the CPP present, the parent complexes did not enter the cells. This, along with their targeting capabilities, demonstrates the value of using CPPs for imaging and sensing probes.

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Development of a New Tetrafunctional Hybrid to Target Cancer Cells

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Dysregulation of apoptotic pathways that enable apoptosis escape is a main feature of cancer cells that is an overwhelming problem for conventional cancer therapy. Another problem is still unselective activity of drugs over both cancer and normal cells. Herein we designed a hybrid containing Smac peptide fragment along with RGD targeting unit, a unit for conjugation of drugs/dyes and Canavanine in order to lower down the apoptotic thresholds and trigger apoptosis in cancer cells. AVPI (Ala-Val-Pro-Ile) sequence is the functionally essential tetrapeptide sequence of the N-

terminus of pro-apoptotic regulator Smac (Second mitochondria derived activator of caspases). Smac is shown to help proceeding of extrinsic and intrinsic apoptotic pathways by inhibiting IAP proteins (Inhibitor of Apoptosis Proteins). Furthermore it is reported an inverse correlation between Smac and IAP protein levels in a wide range of cancer cells, defining these regulators interesting for targeted drug design. We combined a modified AVPI sequence with RGD (Arg-Gly-Asp), known to bind molecules overexpressed on cancer cell membrane. Cell permeability predictions led us to the attachment of a canavanine-tail of three canavanines. Canavanine is a non-proteinogenic arginine analogue. The peptide was synthesized by SPPS (solid phase protein synthesis) stepwise method. The purity of peptide was defined by HPLC, and Mass-spectrometry. The cytotoxic potential of the peptide was tested over A549 lung cancer cell line by MTT assay.

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VirD2 Derived Cell Penetrating Peptide Exhibits Efficient Cellular Translocation and Cargo Delivery in Plants

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Cell penetrating peptides (CPP) are relatively short peptides, with the inherent ability to traverse impermeable biological membrane. They are extensively studied as efficient tool in field of drug delivery. Amino acid sequences of VirD2 of three different Agrobacterium strains revealed that these sequences are well conserved. VirD2 protein of Agrobacterium tumefaceins play significant role in nuclear localization and integration of T-DNA in plant genome. In present study, the membrane translocability of VirD2 derived peptides namely Vir, Vir1, Vir2 and Vir3 has been investigated. Vir peptide directs the β-galactosidase to the nuclei of wheat cell and tissues. Interestingly Vir2 formed by substitution of arginine with alanine from N terminus enhances the β-galactosidase delivery ability. Thus, our results show the importance of Cterminus arginine in macromolecule delivery. Additionally, circular dichroism was performed where the increase in α-helical percentage of Vir2 - β-galactosidase complex in the presence of membrane mimicking environment was clearly observed. Slight unfolding with change in conformation of β-galactosidase was observed which enable the complex to translocate across the cell membrane with the help of CPPs. For the first time we have conclusively demonstrated that cargo protein is transported by CPP in its relaxed/ unfolded state.

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Development of Helix-Stabilized Amphipathic Cell-Penetrating Peptides for siRNA Delivery

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Cell-penetrating peptides (CPPs) are receiving much attention as an intracellular delivery tool for hydrophilic cargo molecules such as drugs, proteins, and nucleic acids. It has been reported that CPPs contain a high relative abundance of positively charged amino acids such as arginine (Arg), and the interaction between cationic side chains of arginines and acidic groups existing on the cell surface is essential for cell membrane permeability. On the other hand, the relationship between secondary structures of CPPs and their cell membrane permeability still remains unclear. Previously we reported that amphipathic Arg rich helical peptides whose helical structures were stabilized with hydrophobic 2-aminoisobutyric acid (Aib) residues, penetrated cells more effectively than non-helical Arg-rich peptides. However, these helical peptides showed lower cell permeability than nonaarginine (R9) because replacement of cationic Arg with hydrophobic Aib reduced in the number of arginine and/or change distribution of arginine in peptide. We also reported that (Leu-Leu-Aib)n-peptides (1) are capable of acting as a useful helical promoter. Based on this knowledge, we hypothesized that conjugation of helical peptide 1 with R9 could stabilize the helical structure and enhance cell membrane permeability. Herein, we designed and synthesized a helix-stabilized R9-based peptide [Block3: FAM-bAla-(Leu-Leu-Aib)3-(Gly)3-(Arg)9]. At first, we performed preferred secondary structural analysis using CD spectra. As a result, Block3 showed negative maxima around 208 and 222nm indicating Block3 formed a stable helical structure whereas R9 did random structures. Furthermore, we evaluated effects of the peptides on delivery of the plasmid DNA or siRNA into cells. MCF-7 cells were treated with the complex of peptide and pDNA or siRNA, and the target gene expression were evaluated by reporter gene luciferase assay and western blotting analysis, respectively. As a result, Block3 efficiently deliver the pDNA and siRNA into cells. These results suggest that conjugation of helical promoter peptide 1 with R9 enhanced cell-penetrating ability and the Block3 could be a candidate for a novel intracellular delivery tool.

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PPMO's as Therapeutic Modulators for Myotonic Dystrophy Type 1

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Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy in adults. DM1 is caused by the pathological microsatellite (CTG) repeat expansion in the 3'untranslated region of the DMPK gene. Mutant RNAs containing the repeat CUG expansions are retained in the nucleus as foci and sequester proteins required for the regulation of mRNA splicing and translation. These perturbations result in a multisystemic disorder characterised by myotonia, progressive muscle weakness, cardiac arrhythmias, cataracts and impaired endocrine and nervous system function. To date there is no cure for DM1. Antisense oligonucleotides (ASOs) are a promising genetic therapy for RNA gain-of function diseases like DM1. A leading strategy for an enhanced delivery system is modulation of ASO chemistry though peptide conjugation. This allows for tissue-specific delivery while directly targeting CUG repeat expansions that can interfere with abnormal sequestration and binding of RNA-binding proteins like MBNL1. This study investigates the therapeutic ability of an arginine-rich cell-penetrating peptide, pip6a, conjugated to an ASO targeting the CUG repeat in DM1 patient cells and in the HSALR mouse model of DM1. Pip6a-PMO treatment in vitro can correct molecular abnormalities by liberating MBNL1 and normalising splicing profiles in patient cells. Single intravenous administration of pip6a-PMO can partially correct mis-splicing events in gastrocnemius muscle. Repeat administration of PPMO successfully normalises mis-splicing events in skeletal muscle, has reduced foci, illustrates redistribution of MBNL1 and fully corrects myotonia phenotype. This treatment also has positive long lasting effects, up to 6 months, in HSALR mouse skeletal muscle. This work provides insights into the application of arginine-rich cell-penetrating peptide coupled to PMOs for treatment of myotonic dystrophy type 1. Pip6a-PMO

demonstrates an encouraging therapeutic potential for DM1 patients.

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Homology Modelling and Evaluation of the Cannabinoid Receptor Type 2

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The human cannabinoid receptor type 2 (CB2) is linked to a variety of immune functional events which are found primarily in brain and tissues of immune and hematopoietic systems like spleen, tonsil, and thymus. In the absence of crystal structure of CB2 receptor, 3D homology patterns with different templates are built. The aims of the present study are: (1) to choose within the recently published crystallographic structures templates for homology modelling of the CB2 receptor; (2) to evaluate the models with different computational tools; and (3) to precise the most reliable model basing on the correlation between data from molecular docking and the values of the biological activity of the cannabinoid ligands. A new model of the CB2 was generated and evaluated by different approaches. The scoring function correlates (Pearson r = -0.945, p-value = 0.0013) with the biological activity of a series of cannabinoid ligands known from literature. Therefore, the current investigation allows suggesting a reliable model of CB2. The model of CB2 receptor obtained by homology modelling could be used further for in silico experiments, structure-activity relationship evaluations and it will give possibility for the design of selective and effective ligands for the CB2 receptor.

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Software-aided workflow for predicting sites of cleavage using molecular descriptors of the natural and synthetic amino acids in peptide-based drug discovery

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Predicting possible sites of cleavage for individual proteases is an important task to be completed during drug-design process of peptide therapeutics to improve their stability and availability as a promising drug. In this study we presented a new approach in WebMetabase that helps to predict cleavage sites for the specific peptide family or for specific experimental condition (i.e. individual protease). One of the main advantages of this approach is that it generates a searchable database for the information coming from LC-MS based experimental data or from external sources such as MEROPS database. In this database each amino acid is described as a vector of physicochemical properties, Volsurf molecular descriptors, and/or pharmacophoric properties, SHOP descriptors. This way to store the data can be utilized to perform frequency analysis to discover the most frequent scissile bonds within the generated database. These results can be used to train predictive models for individual proteases or complex matrices. This database enables the user to combine the external and internal sources of information. We used database that contained data extracted from MEROPS and experimental data to train several models using different classifier learning approaches for eighteen proteases from four protease families: serine, cysteine, aspartic and matrix metalloproteases. In the training dataset each sequence pattern around the potential cleavage site and actual site of cleavage was represented as a combination of Volsurf descriptors that characterized the physicochemical properties of the residues in the sequence of the site of cleavage. In this case there would be no limitations related to the amino acids used for the training. We compared the predictive performance of the models trained with different learning approaches applying 5-fold cross validation test and more importantly prediction results on an external dataset. Moreover, we examined the influence of the local window sequence size around the site of cleavage by comparing the models trained for P1-P1' and P4-P4' range. We revealed that the logistic regression and random forest classification models trained using window P4-P4' outperformed other machine learning methods or the models trained using the P1-P1' window. We demonstrated that predictive performance of the models trained with different learning approaches was comparable to the other publicly available tools (PROSPERous and SitePredicting). In contrary to the state-of-the art tool, developed tool enables the user to build the private models for internal use. This knowledge can be applied during the design-make-test drug discovery cycle.

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