2nd International Conference on Peptide Materials for Biomedicine and Nanotechnology

PepMat 2016

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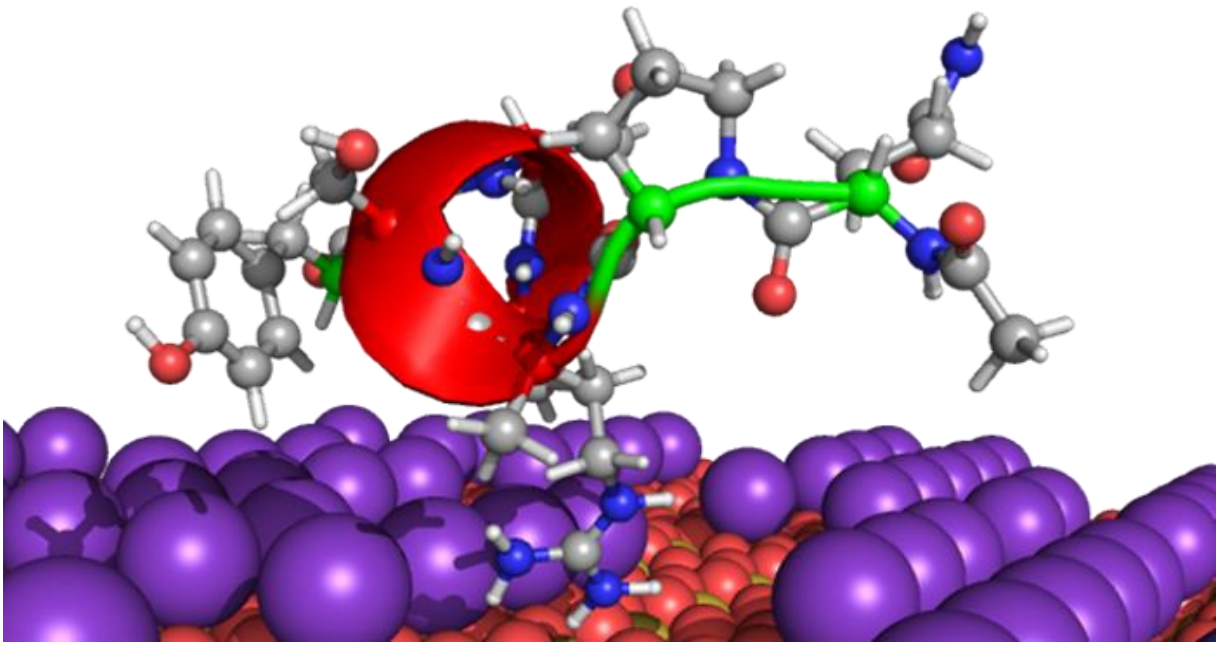
BOOK OF ABSTRACTS



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B|BRAUN SHARING EXPERTISE

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ORAL PRESENTATIONS

POSTER PRESENTATIONS

Activation or Suppression of Immune System with Using Peptide Assemblies

Shunsaku Kimura

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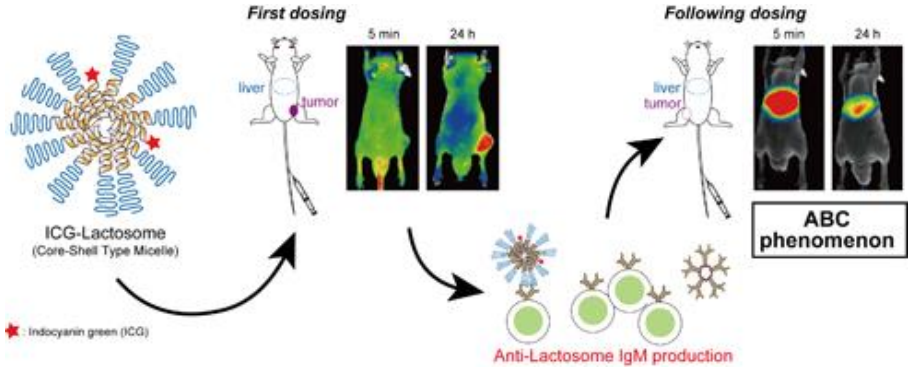
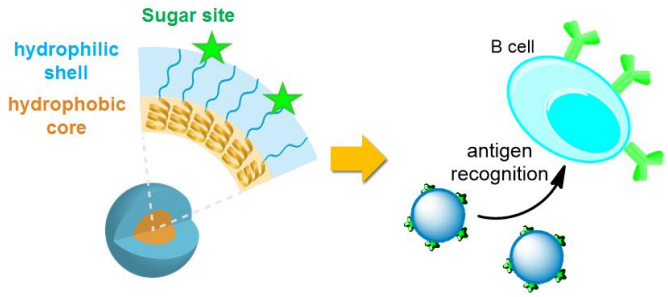
Nanoparticles are expected to be applicable for the theranostics medicine as a carrier vehicle of the diagnostic and therapeutic agents. We have prepared nanoparticles in a wide range of size, morphology, and surface character. As a result, we found that immune system is deeply involved in the *in vivo* disposition of nanoparticles upon multiple administration. The ideas how to suppress or activate the immune system with modifying nanoparticles will be shown[1,2].

Alteration of *in vivo* Disposition of Polymmeric Micelle



Scheme 1 Schematic illustration of the accelerated blood clearance of polymeric micelles.

Activation of Immune System with Raising anti-Lewis^y IgM Production



Scheme 2 Schematic illustration of triggering the immune system against Lewis^y.

[1] Hara E. et al., *J. Pept. Sci*. **2014**, 20, 570-577.
[2] Hara, E. et al., *ACS Med. Chem. Lett.* **2014**, 5, 873-877.

Interactions between Peptides and Inorganic Matter: From Basic Science to Applications

Meital Reches

The Institute of Chemistry and The Center for Nanoscience & Nanotechnology, The Hebrew University of Jerusalem

Several natural processes are mediated by the interactions between proteins and peptides with inorganic materials. The immune response towards an implant is mediated by proteins. Composite materials are formed by the interactions of organic materials (usually proteins) and minerals. Biofouling, the process in which organisms attached to surfaces, is also mediated mainly by proteins. Moreover, the ability to anchor peptides to inorganic surfaces is important for many areas of research. This includes the development of biosensors, coatings and thin films. Understanding the nature of interactions between peptides and proteins with inorganic materials will bring to the development of improved implants, new composites, antifouling materials and functional surfaces.

Using single-molecule force spectroscopy with AFM, we study the underlying forces between individual biomolecules (either amino acid residues or short peptides) and inorganic surfaces in aqueous solution. Using this method, we were able to measure low adhesion forces and could clearly determine the strength of interactions between individual amino acid residues and inorganic substrates.[1] Our results with peptides also shade light on the factors that control the interactions at the organic-inorganic interface.[2]

Based on our knowledge from single molecule experiments, we designed a tripeptide that can spontaneously form a coating that resists biofilm formation. Our results demonstrate the formation of a coating on various surfaces (glass, titanium, silicon oxide, metals and polymers). In addition, we showed that this coating prevents the first step of antifouling, which involves the adsorption of bioorganic molecules to the substrate. Moreover, the coating significantly reduced the attachment of various organisms to surfaces. This includes pathogenic bacteria and fungi.[3]

[1] Razvag, Y., Gutkin, V. & Reches, M. (2013) *Langmuir* 29, 10102–10109
[2] Maity, S., Zanuy, D., Razvag, Y., Aleman, C. & Reches, M. (2015) *Phys. Chem. Chem. Phys*. 17, 15305-15315.
[3] Maity, S., Nir,S., Zada, T.,Reches, M. (2014). *Chem. Commun*. 50, 11154-11157

Peptide Assemblies by Design

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Structurally defined materials on the nanometer length-scale have been historically the most challenging to rationally construct and the most difficult to structurally analyze. Sequence-specific biomolecules, such as proteins, have advantages as design elements for construction of these types of nano-scale materials in that correlations can be drawn between sequence and higher order structure, potentially affording ordered assemblies in which functional properties can be controlled through the progression of structural hierarchy encoded at the molecular level. However, the predictable design of self-assembled structures requires precise structural control of the interfaces between peptide subunits (protomers). In contrast to the robustness of protein tertiary structure, quaternary structure has been postulated to be labile with respect to mutagenesis of residues located at the protein-protein interface. We have employed simple self-assembling peptide systems to interrogate the concept of designability of interfaces within the structural context of nanotubes and nanosheets (see below).[1,2] These peptide systems provide significant evidence that the designability of protein interfaces is a critical consideration for control of supramolecular structure in self-assembling systems.

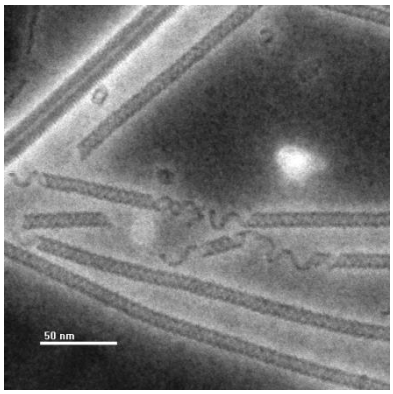

Figure 1. STEM image of nanotubes derived from self-assembly of a *de novo* designed, bi-faceted coiled-coil pèptide.

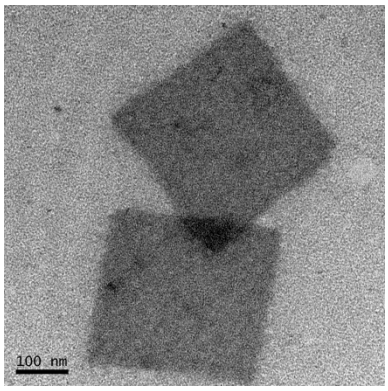

Figure 2. TEM image of two-dimensional nano-sheets derived from self-assembly of charge-complementary collagen peptides.

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[1] Egelman, E.; Xu, C.; DiMaio, F.; Magnotti, E.; Modlin, C.; Yu, X.; Wright, E.R.; Baker, D.; Conticello, V.P. *Structure* **2015**, *23*, 280-9. [2] Jiang, T.; Xu, C.; Zuo, X.; Conticello, V.P. *Angew. Chemie Int. Ed. Engl.* **2014**, *53*, 8367-71

β-sheet forming peptides: from self-assembly to functional biomaterials

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The use of non-covalent self-assembly to construct materials has become a prominent strategy in material science offering practical routes for the construction of increasingly functional materials for a variety of applications ranging from electronic to biotechnology. A variety of molecular building blocks can be used for this purpose, one such block that has attracted considerable attention in the last 20 years is de-novo designed peptides. Peptides offer a number of advantages to the material scientists. The library of 20 natural amino acids offers the ability to play with the intrinsic properties of the peptide such as structure, hydrophobicity, charge and functionality allowing the design of materials with a wide range of properties. Synthetic peptides are chemically fully defined and easy to purify through standard processes. Being build form natural amino acids they result usually in low toxicity and low immune response when used in-vivo and can be degraded and metabolised by the body. In the past 10 years our group has focussed on the development of a technological platform for the design of novel biofunctional materials, in particular hydrogels, exploiting the self-assembly of β-sheet forming peptides. The β-sheet motif is of increasing interest as short peptides can be designed to form β-sheet rich fibres that entangle and consequently form hydrogels. These hydrogels can be functionalised using specific biological signals and can also be made responsive through the use of enzymatic catalysis and/or conjugation with responsive polymers. Through the fundamental understanding of the self-assembly of these peptides we have been able to designed hydrogels with tailored properties for a range of applications including injectable systems for in-vivo cell delivery and sprayable systems for topical drug delivery.

Folding and self-assembly of peptides using OPEP coarse-grained simulations

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I shall present where OPEP coarse-grained simulations coupled to advanced sampling techniques stand in the context of fast and accurate peptide structure determination, and the self-assembly of amyloid proteins associated with Alzheimer's disease.[1,2]

The understanding of protein/RNA behavior in cell-like environments from computer simulations is still a challenge because of the size and the spread of length- and time-scales characterizing the systems, and I will show where we are in this context.

1. Nasica-Labouze J, Nguyen PH, Sterpone F, Berthoumieu O, Buchete NV, Coté S, De Simone A, Doig AJ, Faller P, Garcia A, Laio A, Li MS, Melchionna S, Mousseau N, Mu Y, Paravastu A, Pasquali S, Rosenman DJ, Strodel B, Tarus B, Viles JH, Zhang T, Wang C, **Derreumaux P**. Chem Rev. 2015 May 13;115(9):3518-63.
2. Sterpone F, Melchionna S, Tuffery P, Pasquali S, Mousseau N, Cragnolini T, Chebaro Y, St-Pierre JF, Kalimeri M, Barducci A, Laurin Y, Tek A, Baaden M, Nguyen PH, **Derreumaux P**. Chem Soc Rev. 2014 Jul 7;43(13):4871-93.

Peptide-based Self-assembled Hybrid Materials

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Since J.M. Lehn's pioneering contributions, the bottom-up self-assembly strategy has emerged as one of the most applicable approaches to drive small molecule arrangements. Following a hierarchical process, under appropriate conditions the combination of a variety of non-covalent interactions may allow properly designed molecules to form stable, ordered aggregates that can evolve into nano-, micro- and macroscale materials characterized by discrete 3D-architectures with specific shapes, dimensions and functions, exploitable in areas ranging from chemistry to materials science and medicine. In this connection, peptides represent powerful building blocks on the nanoscale because of their modularity, molecular diversity, which can be expanded beyond the repertoire of genetically coded amino acids, and secondary structure variety. More specifically, nanotubes and nanofibers predominate as molecular architectures among the self-assemblies formed by short peptides, while vesicles-like structure are commonly found for high molecular weight peptides. Notably, such architectures may adsorb or entrap or covalently link other molecules, thus generating ordered hybrid materials with interesting applications. In particular, we made use of short or long peptides (Figure 1) to organize at molecular levels fullerenes,[1] carbon nanotubes,[2] carbon quantum dots,[3] gold nanoparticles,[4] *azo*benzene derivatives,[5] and several other chromophores, and we explored novel applications of these peptide-based, self-assembled hybrid materials.

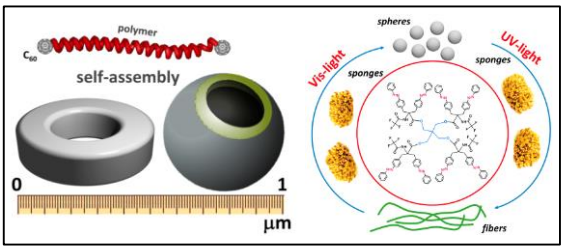

Figure 1. Two examples of self-assembled peptide-based nanostructure.Left: a C_{60}-poly-g-benzyl-L-glutamate hybrid polymer. Right: a reversible multistate hydrogel, based on a photo-responsive non-natural α-amino acid.

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[1] D.Mazzier, M. Mba, M. Zerbetto, A. Moretto Chem. Commun. (2014), 50, 4571. [2] M. Mba, A. I. Jiménez, A. Moretto Chem. Eur. J. (2014), 20, 3888. [3] D. Mazzier, M. Favaro, S. Agnoli, G. Granozzi, S. Silvestrini, M. Maggini, A. Moretto Chem. Commum. (2014), 50, 6592. [4] D. Mazzier, F. Carraro, M. Crisma, M. Rancan, C.Toniolo, A. Moretto Soft Matter (2016), 12, 238. [5] D. Mazzier, M. Maran, O. Polo Perruchin, M. Crisma, M. Zerbetto, V. Causin, C.Toniolo, A. Moretto Macromolecules (2014), 47, 7272

Self-assembly of phenylalanine oligopeptides

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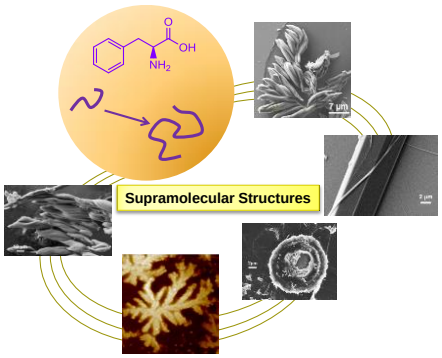
Self-assembled structures in the nano- and microscale have become essential for future technological applications in the nanoscience and biomaterials fields. Hence, since the pioneering work of Reches and Gazit in 2003,[1] in which diphenylalanine (FF) nanotubes were obtained in aqueous solution, relevant efforts have been oriented to develop a new generation of materials consisting in the self-assembly of aromatic peptides. However, research on self-assembled structures based on triphenylalanine (FFF) and tetraphenylalanine (FFFF) is very scarce. This contribution describes the self-assembly of a series of different phenylalanine-based oligopeptides that differ at the N- and C-termini. Hence, depending on environmental conditions such as solvent:co-solvent ratio, peptide concentration, and temperature, these oligopeptide derivatives self-organize into a wide variety of stable polymorphs. The overall of the results suggest that highly aromatic oligopeptides with four, or even more, phenylalanine residues should be considered as powerful building blocks for the fabrication of complex and relatively infrequent structures for advanced functional applications.



[1] Reches, M.; Gazit, E. *Science* **2003**, 300, 625-627

Protein control of soft and hard matter

Tobias Weidner

Max Planck Institute for Polymer Research, Mainz, Germany

Proteins can act as Nature's engineers at interfaces and manipulate both hard and soft tissue – they shape biominerals, manipulate cell membranes and nucleate materials. Despite the apparent importance for physicists and chemists working in the fields of biomineralization, surface engineering, drug delivery, or diagnostics, the molecular mechanisms behind interfacial protein action have remained largely elusive. In order to understand protein function our goal is to probe the structure and structural dynamics of such active proteins – in action at the surface.

Mineral proteins have the ability to control and steer the growth of biogenic hard tissue by binding specific mineral facets. They control the intricate mineral morphologies found in diatom cell walls, mollusk nacre, but also human teeth and bone. Inspired by diatom silification we used amphiphilic peptides consisting of leucine and lysine (LK peptides) to investigate biomineralization at surfaces. These peptides can adopt helical or beta sheet structures at the air-water interface. Upon addition of a silica precursor we obtained freestanding peptide-silica hybrid sheets with thicknesses of ~4 nm. We have followed the biomineral composition and interactions between peptides and silica at different early stages of biomineralization using a combination of surface spectroscopies and microscopies. Our experimental findings were complemented with molecular dynamics simulations. Our data shows that the peptide surface folding dictates the nanometer scale morphology of the prepared silica film.

A particularly fascinating example of protein driven nucleation and phase transitions are ice-nucleating proteins. These proteins are used by specific bacteria to attack plants and cause frost damage by growing ice crystals at temperatures that would otherwise not allow ice formation. A recent survey by the NASA found large amounts of biological ice nucleators in the troposphere where they may affect global precipitation patterns. We have followed the interaction of freeze proteins with surrounding water molecules – how specialized protein sites lock water molecules in place and manipulate the flow of energy within the surrounding layers of water.

Structure-Function Relationships in Self-Assembled Peptide Nanostructures: The case of Charge transport

Prof. Nurit Ashkenasy

The Ilse Katz Institute for Meso- and Nanoscale Science and Technology,
Ben Gurion University of the Negev, Beer-Sheva, Israel

The function of Proteins is determined by the spatial arrangement of amino acids, which in return depends on the sequence of amino acid in the proteins' chain. Inspired by these relationships, intensive effort is made to reveal the effect of peptides' sequence on their ability to fold and self-assemble into nanostructures. Naturally, the following challenge is to reveal the structure - function relationships of these asemblies. Such understanding will provide a predicting power that can be used for the design of bio-inspired functional materials. Our group focusses on understanding the structural factors that govern charge transport in self-assembled peptide nanostructures. In this talk, I will demonstrate that the sequence of the peptide affects charge transport both by controlling the morphology of the resulting nanostructures, and by direct involvement of the side chains in the charge transport process. The influence of these factors on both electron and proton transport will be demonstrated using several self-assembling peptide motifs. Finally, I will show how these understandings are used for the design of new sequences with improved electronic characteristics.

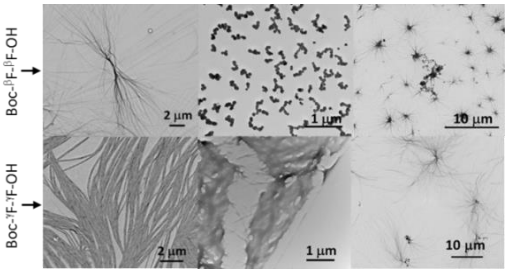
Self-assembly of diphenylalanine backbone homologues and their combination with carbon nanotubes

*Bhimareddy Dinesh, Cécilia Ménard-Moyon, Alberto Bianco**

CNRS, Institut de Biologie Moléculaire et Cellulaire, Laboratoire d'Immunopathologie et Chimie Thérapeutique, 67000 Strasbourg, France

The natural simple constituents of life - amino acids, sugars, lipids and nucleic acids - are intrinsically bioactive, biodegradable and biocompatible which makes them as perfect building blocks to generate new materials.[1] In particular, the research on peptide-based materials has intensified over the last few years, not only because of their synthetic versatility and their potential in biomedical applications, but also they are promising bio-based alternatives to synthetic materials.[1] The peptides composed of natural amino acids or in combination with other unnatural analogues are known to self-assemble or self-organise generating complex architectures, such as peptide nanowires,[2] nanotubes[3] and nanofibers.[4]

Herein, we studied the self-assembly of β and γ homologues of diphenylalanine peptides under different solvent and pH conditions. The backbone effected self-assemblies were shown to form nanofibers of different lengths and dimensions although possessing the same side chain.The pH variation transformed the nanofibers into spherical structures. Moreover, the co-assembly of β and γ peptides with carbon nanotubes (CNTs) covalently functionalized with the same peptide generated unique dendritic assemblies. This comparative study on self-assembly and co-assembly with CNT covalent conjugates is the first example exploring the capacity of β and γ peptides to adopt precise nanostructures, particularly in combination with CNTs *(Bhimareddy et.al. Nanoscale, **2015,** 7, 15873*).



[1]Alemán, C; Bianco, A; Venanzi, M *Peptide Materials: From Nanostuctures to Applications*, *John Wiley & Sons*, **2013**.
[2] Reches, M; Gazit, E *Science* **2003**, 300, 625-627.
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[4] Renliang, H; Rongxin, S; Wei, Q; Jun, Z; Zhimin, H *Nanotechnology* **2011**, 22, 245609.

Hydrogen bonding and lipid bilayer mediated aggregation of various peptide helix topologies

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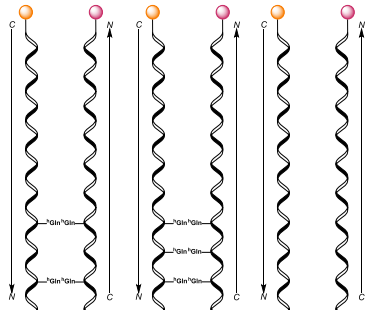
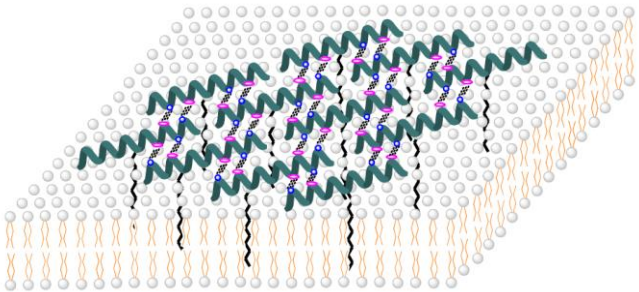
The influence of lipid bilayers on the organization of peptide helices is investigated by various helical peptide topologies, especially focusing on β-peptide helices. β-Peptides were designed to be incorporated into membranes and thereby organized as transmembrane helices or to be aggregated on the membrane surface.

Peptide nucleic acids based on a β-peptide 14-helix backbone (β-PNA) are ideally suited to function as helix architectural unit that can be further organized in two or even three dimensional space to build up supramolecular assemblies.[5,6] β-PNAs are especially well suited as architectural elements because of the rigid β-peptide 14-helix scaffold, exactly three amino acids forming one helical turn, and the defined base pair hydrogen bonding complementarity.

β-Peptide helices were designed as transmembrane helices and their incorporation in lipid bilayers and aggregation within the membrane will be presented depending on helix topology, lipid interaction, and hydrogen bonding recognition units (Figure left).





A second approach uses β-peptide aggregation on the membrane surface (Figure right) in order to provide a membrane supporting peptide layer with shape inducing properties. Alternatively, the aggregation of lipidated β-peptides on the membrane surface will be used to influence the membrane lipid composition, thereby, inducing phase separation and signaling.

[5] Brückner, A. M.; Chakraborty, P.; Gellman, S. H.; Diederichsen, U. *Angew. Chem. Int. Ed.* **2003**, 42, 4395-4399.
[6] Chakraborty, P.; Diederichsen, U. *Chemistry Eur. J.* **2005**, 11, 3207-3216.

Peptides and Peptide Conjugates: From Self-assembly Towards Applications in Biomedicine

Ian William Hamley

Department of Chemistry, University of Reading, United Kingdom

Self-assembling peptides and their conjugates offer exceptional potential in nanomedicine. I will present some of our recent work on nanoscale assembled peptides and their conjugates, focussing on lipopeptides[1,2] and PEG-peptide conjugates.[3] PEGylation is an important technique in the development of conjugates for applications in therapeutics. It is found to greatly influence self-assembly of peptides and proteins - one example from our own work is a peptide which itself forms twisted fibrils but when PEG is attached, self-assembly of the conjugate leads to spherical micelles.[4] The conjugate can be enzymatically degraded using alpha-chymotrypsin, releasing the peptide. This nanocontainer delivery and release system could be useful in therapeutic applications. Thermoresponsive telechelic PEG/peptides with hydrophobic dipeptide end groups (di-tyrosine or di-phenylalanine) were developed, one of which shows a de-gelation transition near body temperature and which may be useful in bioresponsive delivery systems.[5] Examples from our recent work on self-assembling lipopeptides will also be outlined. Our focus is to investigate potential relationships between self-assembly and bioactivity, in particular in the fields of regenerative medicine,[6-10] antimicrobial systems[11,12] and immune therapies.[13]

---

[1] Dehsorkhi A, Castelletto V, Hamley IW. Self-Assembling Amphiphilic Peptides. J. Pept. Sci. 2014;20:453-67. [2] Hamley IW. Self-Assembly of Amphiphilic Peptides. Soft Matter 2011;7:4122-38. [3] Hamley IW. PEG-Peptide Conjugates. Biomacromolecules 2014;15:1543-59. [4] Castelletto V, McKendrick JME, Hamley IW, Cenker C, Olsson U. PEGylated Amyloid Peptide Nanocontainer Delivery and Release System. Langmuir 2010;26:11624-27. [5] Hamley IW, Cheng G, Castelletto V. Self-Assembly of Telechelic PEG End-capped with Hydrophobic Dipeptides. Macromol. Biosci. 2011;11:1068-78. [6] Jones RR, Castelletto V, Connon CJ, Hamley IW. Collagen Stimulating Effect of Peptide Amphiphile C_{16}–KTTKS on Human Fibroblasts. Mol. Pharm. 2013;10:1063-69. [7] Castelletto V, Hamley IW, Whitehouse C, Matts P, Osborne R, Baker ES. Self-Assembly of Palmitoyl Lipopeptides Used in Skin Care Products. Langmuir 2013;29:9149-55. [8] Gouveia RJ, Castelletto V, Alcock SG, Hamley IW, Connon CJ. Bioactive films produced from self-assembling peptide amphiphiles as versatile substrates for tuning cell adhesion and tissue architecture in serum-free conditions. Journal of Materials Chemistry B 2013;1:6157-69. [9] Castelletto V, Gouveia RJ, Connon CJ, Hamley IW, Seitsonen J, Ruokolainen J, Longo E, Siligardi G. Influence of elastase on alanine-rich peptide hydrogels. Biomaterials Science 2014;2:867-74. [10] Gouveia RJ, Castelletto V, Connon CJ, Hamley IW. Submitted 2014. [11] Dehsorkhi A, Castelletto V, Hamley IW, Seitsonen J, Ruokolainen J. Interaction Between a Cationic Surfactant-Like Peptide and Lipid Vesicles and Its Relationship to Antimicrobial Activity. Langmuir 2013;29:14246-53. [12] Hamley IW, Dehsorkhi A, Castelletto V. Self-Assembled Arginine-Coated Peptide Nanosheets in Water. Chem. Comm. 2013;49:1850-52. [13] Hamley IW, Kirkham S, Dehsorkhi A, Castelletto V, Reza M, Ruokolainen J. Toll-like Receptor Agonist Lipopeptides Self-Assemble into Distinct Nanostructures. Chem. Comm. 2014; 50: 15948-51.

Self-assembling protein nanoparticles for targeting in colorectal cancer

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We have developed a protein engineering principle, based on the modular combination of cationic peptides and poly-histidines, to generate self-assembling building blocks of protein-only nanoparticles [1-3]. This principle, that exploits the cationic nature of several tumor homing peptides, has been applied to the *in vivo* targeting of primary tumor and metastatic foci in animal models of cancer [4-6]. We have recently found that nanoarchitecture and function of nanoparticles can be controlled at the cell factory level during biofabrication [7]. This fact impels the further development of this nano-architectonic principle towards the generation of true artificial viruses for highly specific and efficient drug delivery in colorectal cancer [8].

1 Vazquez,E. *et al.* Protein nanodisk assembling and intracellular trafficking powered by an arginine-rich (R9) peptide. (**2010**) *Nanomedicine. (Lond)* 5, 259-268.

2 Unzueta,U. *et al.* Non-amyloidogenic peptide tags for the regulatable self-assembling of protein-only nanoparticles. (**2012**) *Biomaterials* 33, 8714-8722.

3 Vazquez,E. *et al.* Internalization and kinetics of nuclear migration of protein-only, arginine-rich nanoparticles. (**2010**) *Biomaterials* 31, 9333-9339.

4 Cespedes,M.V. *et al.* In Vivo Architectonic Stability of Fully de Novo Designed Protein-Only Nanoparticles. (**2014**) *ACS Nano.* 8, 4166-4176.

5 Unzueta,U. *et al.* Intracellular CXCR4(+) cell targeting with T22-empowered protein-only nanoparticles. (**2012**) *Int. J. Nanomedicine.* 7, 4533-4544.

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Short peptide supramolecular hydrogels for crystal growth

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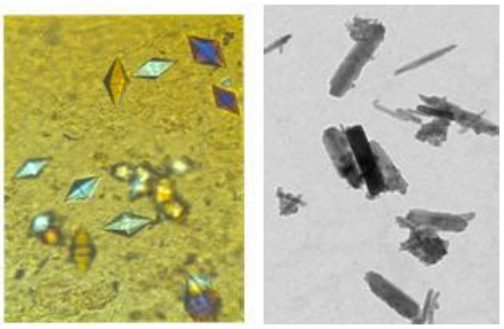
Short-peptides supramolecular gels are a subgroup of low molecular weight gelators that have found useful biotechnological applications thank to their biocompatible and biodegradable nature.[1] In this work we have studied for the first time the behavior of these types of gels as a medium for protein crystallization. Results of the crystallization behavior and crystal quality of these proteins in the hydrogels will be presented and discussed. The influence of the chirality in protein crystallogenesis is also studied.[2] Additionally, preliminary results of the influence of these hydrogels in magnetite growth will be commented.



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Peptides as Protein-Surface Interactors

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The breakthrough concept that proteins function as a contact network rather than as independent individuals is not only one of the most important advances in our comprehension of living systems, but also translates to a new era in drug discovery. The few reported examples of diseases caused by “impolite” protein social behavior certainly represent only the tip of the iceberg. Therapeutic intervention through molecules designed to selectively modulate the strength and specificity of protein-protein interactions (PPIs) is becoming a reality. This will not only feature molecules with inhibitory capacity: equally or even more interesting are those compounds which can rescue pre-established interactions or structures whose loss results in disease.

In this context, peptides are destined to play a major role as therapeutic agents. My laboratory is contributing to speeding up this process. On the one hand, we devote efforts to studying the molecular details and dynamics of the events that occur during molecular recognition at protein surfaces. We succeeded to design and synthesize peptides able to modulate these recognition events either permanently or in response to light. On the other hand, we are discovering and designing peptides able to cross biological barriers. Our aim is to use these peptides as shuttles for targeting therapeutic agents to organs, tissues, or cells, with a special emphasis on drug delivery to the brain.

PPIs are the result of an ensemble of exquisitely regulated molecular recognition events that take place at protein surfaces. Inspection of protein-protein interfaces allows distinguishing two categories of PPIs: domain-domain and peptide-mediated PPIs [1]. Relatively rigid peptides and peptidomimetics have proved to be very efficient inhibitors of this last class of interactions. In this presentation, recent results from our group related to the use of peptides to modulate PPIs will be discussed. This include, among others: *i)* the recent development of cell-permeable

photoswitchable PPI inhibitors, that opens the way to manipulating a specific PPI locally and in a time-controlled manner using illumination patterns [2,3]; *ii)* the application of the retro-enantio approach to obtain a peptide capable of overcoming the blood–brain barrier [3,4,5]; and *iii)* the use of peptides to modulate the dynamic behavior of prolyl oligopeptidase (POP), a large 80 kDa protease relevant as therapeutic target in schizophrenia [6].

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Peptide Nanofibers for Diagnostic and Therapeutic applications

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Aromatic peptides such as diphenylalanine (FF) or tetraphenylalanine (FFFF) are able to self-assemble into many ordered self assembled nanostructures from nanotubes to organogels. These nanostructures have been investigated for their mechanical, electrochemical and optical properties. Recently, few studies have been devoted to investigate their properties for biomedical and biotechnological applications, for example for regenerative nanomedicine, to fabricate stable drug-delivery systems with proteolytic resistance, to produce modern nanodevices, and to prepare ultrasensitive and selective sensors for toxin detection[1]. However, research of poly-phenylalanine based compounds as diagnostic tools, for example as MRI contrast agents, remains largely unexplored.

Magnetic Resonance Imaging (MRI) is one of the most powerful and non-invasive technique for medical diagnosis. Currently, stable Gd(III)-poly(aminocarboxylate) complexes are widely used as contrast agents (CAs) in MRI to produce high quality images.

In the present communication, novel peptide materials, based on poly-phenylalanine are discussed as a new class of supramolecular gadolinium based MRI contrast agents. These materials contain phenylalanine residues, Gd-DOTA or Gd-DTPA complexes and PEG spacers[2]. Their structural and relaxometric properties and their capacity to increase contrast in Magnetic Resonance Imaging are discussed.

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Computational studies on peptides in drug design: insights from molecular dynamics simulations

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The development of new peptides with therapeutic effects has gained interest within the pharmaceutical research during the last decade with around one hundred and forty peptides now being developed in clinical phase I, II or III[1,2]. Their high specificity along with their low toxicity profiles and the development of novel design and modification techniques are within the reasons that have promoted it, however, it is usually more difficult to fully understand their mechanism of action. Peptides are larger in size and more flexible compared to the common small molecules used as drugs, thus studying the way they interact with a receptor, for example, demands grater efforts than what usually is needed. With the implementation of supercomputers and the most recent development of algorithms and codes adapted to use GPUs, the use of computational techniques within the drug design process has received a major bump allowing for example the use of long timescale molecular dynamics simulations to study protein-ligand(peptide) complexes or the folding of small peptides or even small proteins. Here we will present two different cases where the use of molecular dynamics simulations helped in the development of peptides with therapeutic effects and to explain their possible mechanism of action[3,4].

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Intramolecular Backbone···Backbone Hydrogen Bonds in Peptide Conformations: The Other Way Around

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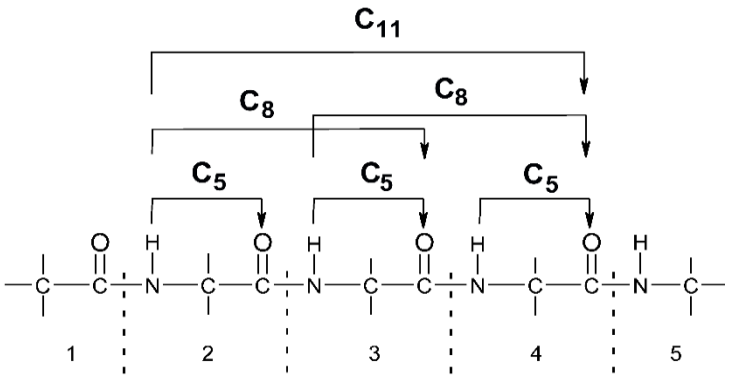
Folded conformations stabilized by intramolecular H-bonds between a main-chain amide N-H donor and a main-chain amide C=O acceptor are extremely common observations in solution studies and crystal-state analyses of peptides and proteins. Moreover, fully-extended, intramolecularly H-bonded 3D-structural motifs have also been reported, although to a very limited extent.

In particular, in a system of *four* linked peptide units the possible conformations intramolecularly H-bonded "the other way around" (as compared to the traditional and extensively investigated C_7, C_{10}, and C_{13} forms, where C stands for *cyclo* and the number indicates how many atoms are involved in the *pseudo*-ring closed by the intramolecular H-bond) are C_5, C_8 and C_{11} (see *Scheme* below).



In this lecture, I will examine the characteristic properties of: (i) the fully-extended (C_5) conformation and its related 2.0_5-helix, (ii) the C_8- (also called δ-) turn, and (iii) the C_{11}- (also called ε-) turn. In particular, the C_8 and C_{11} forms, that are either sterically forced to or may include a *cis*-amide disposition, have been only occasionally mentioned and rarely authenticated experimentally. Specifically, results from X-ray diffraction analyses, conformational energy calculations, and solution investigations will be discussed.

Bioinspired Visible Fluorescent Nanolabels for Photonic and Medical Applications

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Genetically encoded green fluorescent protein and its homologs opened the avenue for a wide applications of these intrinsic biological labels representing specific proteins. We report on a new visible blue/green fluorescent phenomenon found in β-sheet nanowires of ultrashort bionspired peptides[1].

Di- and tri-peptide nanostructures self-assembled from biomolecules of different compositions and origin such as aromatic and aliphatic (diphenylalanine, FF-; dileucine, LL-; triphenylalanine, FFF- monomers) have been studied. At elevated temperature 140-180°C these supramolecular structures of diverse morphologies undergone the same irreversible thermally-induced phase transformation. This reconstruction process is followed by deep modification at all levels: molecular, electronic, elementary symmetry, peptide secondary structure, displaying new nanowire morphology and completely new common physical properties. The thermally-induced nanowire supramolecular ensembles acquire β-sheet secondary structure. In this phase the β-sheet nanowires, irrespective of their native biomolecules origin, exhibit similar profound alteration of optoelectronic properties and visible (blue and green) fluorescence ascribed to hydrogen bonds. Observed visible fluorescent of peptide fiber nanostructures can be used as intrinsic optical labels in biomedicine as biocompatible markers and for a new generation of bio-nanophotonics such as bio-lasers and integrated optical devices.

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Mecwins: developing biomedicine applications using nanomechanical sensors

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Mecwins was founded in 2008 by Dr. Javier Tamayo and Dr. Montserrat Calleja, Bionanomechanics group leaders from the Institute of Microelectronics of Madrid (IMM - CSIC) [1]. Since then, we have been developing cutting edge technology for nanomechanical sensing. The technology, based on detecting variations in the deflection and resonance frequency of nanomechanical sensors, was the groundwork for the technical improvements that led us to our new ultrasensitive detection device, SCALA

SCALA (SCAnning Laser Analyzer) [2] is a commercial platform with high potential for the analysis of biomolecule interactions in human whole blood samples for biosensing applications. Cantilever arrays have been extensively explored as high-sensitive nanomechanical biosensors [3]. The molecular recognition on the surface of a biofunctionalized cantilever results in a nanomechanical response, that produces cantilever bending of a few nanometers (static mode) or changes in cantilever resonance frequency (dynamic mode) where the added mass due to selective molecular recognition decreases resonance frequency value.

In the present study, SCALA has been used for the successful detection of different protein biomarkers in clinical diagnosis, focusing our preliminary experiments on oncology, cardiac and infectious diseases biomarkers. SCALA combines mechanical detection technique (microcantilevers resonance frequency analysis) with a new optical detection technique that increases current levels of sensitivity in clinical practice a million times [4] in comparison with techniques currently used in hospitals and central laboratories and without increasing current cost per sample. The adoption of ultrasensitive detection equipment will enable screening for early detection of a wide range of diseases with established diagnostic biomarkers from a droplet of blood.

The adoption of ultrasensitive detection equipment will enable screening for early detection of a wide range of diseases with established diagnostic biomarkers from a droplet of blood. Moreover, we have developed our technology to be portable, in order to be introduced in the market as a POC device (Figure 1) that allows the implementation of fast and efficient clinical procedures for diagnosis, monitoring and prognosis that will cut down costs in healthcare expenditure, insurance or laboratory work.

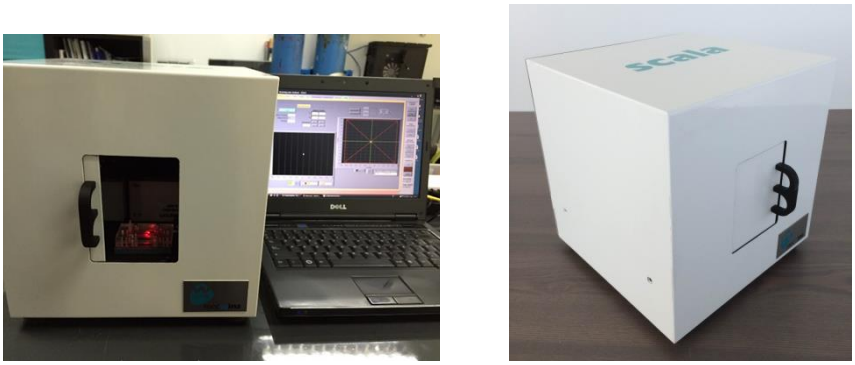

Figure 1. SCALA Platform

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Controlling Multicomponent Dipeptide Hydrogels

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Low molecular weight gelators (LMWG) self-assemble into one-dimensional fibrous structures; this self-assembly leads to the immobilisation of the solvent and the formation of a gel. These materials are attracting significant interest, for example in cell culturing, where the low LMWG concentration needed can be useful, as can the gel's reversibility as the cells grow and re-form their environment.

In the majority of cases, gels are formed from a single LMWG. Mixing different LMWG (all of which can form gels independently) can result in interesting new materials.[1-3] Depending on how these LMWG assemble, this method can adjust the properties of the final gels, or to prepare systems with higher information content by the selective positioning of specific functional groups in space. Here, we will describe a range of mixed dipeptide-based LMWG systems. We will show how fibrous structures form in these systems and show how we can control how different types of fibrous networks are built up in multicomponent systems. We describe both self-sorted and co-assembled networks and the effect of these different networks on the gel properties.

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Drug Delivery via Cell Membrane Fusion using Lipopeptide Modified Liposomes

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Efficient delivery of drugs to living cells is still a major challenge. Currently, most methods rely on the endocytotic pathway resulting in low delivery efficiency due to limited endosomal escape and/or degradation in lysosomes. Here, we report a new method for direct drug delivery into the cytosol of live cells *in vitro* and *in vivo* utilizing targeted membrane fusion between liposomes and live cells. A pair of complementary coiled coil lipopeptides was embedded in the lipid bilayer of liposomes and cell membranes respectively, resulting in targeted membrane fusion with concomitant release of liposome encapsulated cargo including fluorescent dyes, high molecular weight dextran, cytochrome c. Next, the cytotoxic drug doxorubicin was delivered into cells thereby increasing its therapeutic efficacy and cytosolic delivery in vivo using zebrafish embryos was proven. Using a wide spectrum of endocytosis inhibitors and endosome trackers we demonstrate that the major site of cargo release is at the plasma membrane. This method thus allows for the quick and efficient delivery of drugs and is expected to have many applications both *in vitro* and *in vivo*.

Nanoscale Molecular Junctions Incorporating Peptides and Peptidomimetics

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Peptides are involved in numerous biologically relevant processes and their broad range of functions results from ability to adopt variety of structural motifs. Therefore when suitably designed, peptides can provide desired properties that are useful in biosensing devices as well as in nanoscale electronic circuits. However, there are still numerous problems which need to be solved in order to design and build functional molecular devices such as molecular wires, logic gates, switches, or molecular-scale transistors. One of the crucial issues is to understand the fundamental mechanisms determining efficiency of charge transport through these molecules. Numerous experimental approaches can be utilized to probe electron transport efficiency through organic molecules. Among them, scanning probe microscopy (SPM) offers unique capability to investigate electric properties of individual molecules or molecular films at nanoscale. In general this method involves entrapment of the molecules between two metallic contacts established by metal support and SPM probe. The electric properties of the resulting metal–molecule–metal junction can be probed while the bias voltage is applied between the electrodes. The additional advantage of SPM-based method comes from the fact that it enables modulation of the efficiency of peptide mediated ET by controlling mechanical strain or stress of the molecules entrapped within the junction. Using such experimental approach, it is possible to probe electric properties of peptides as well as peptide-mimicking compounds. The latter can be represented by *N,N'*-substituted oligoureas and their derivatives. These compounds adopt very robust 2.5-helix in the solution and in the solid state. To the best of our knowledge, such unusual structural motif was not investigated so far in terms of the efficiency of electron transmission.

Perspectives for Stimuli-responsive peptide: from self-assembly to biomolecular spring

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Biomolecule-based materials that change properties in response to different local stimuli are increasingly being studied in the context of several applications, with particular attention to bioelectronics.

Peptides are ideally suited for this purpose because of the range of distinct physical properties available from the amino acids. This diversity allows several non-covalent interactions including electrostatic (acidic and basic amino acids), hydrophobic, p-stacking (aromatic amino acids), hydrogen bonding (polar amino acids) as well as covalent (disulfide) bonds and steric contributions (strand directing amino acids). Crucially, these interactions depend by different factors such as ionic strength, pH and temperature. The dynamic nature of these interactions then allows the molecular organisation to be altered in response to changes in the direct environment. Changing the nature of these contacts by introducing responsive amino acids can alter the stability of the conformation and provide a mechanism for control of dynamic materials. The use of acidic and basic amino acids that can be protonated or deprotonated by a change in pH allows dynamic control over the secondary structure of the peptides and can be used to control the assembly of coiled-coils.

This presentation will focus on the study of different peptides immobilized on surface and on their response by changing external stimuli. The loading and the conformational switching properties of the surface-bound peptides were investigated by means of several surface techniques. The self-organization process is shown to be severely affected by the type of peptide and surface properties, including specifically the charge state at a given pH. The results strongly support a self-assembly mechanism based onto a very specific organization processes at the liquid-solid interface, based onto the active role played by the surfaces to promote the orientation and organization of single molecules as the prime processes at nanometer scale, followed by the aggregation process at the mesoscopic scale.

Applications in the emerging field of the bio-inspired nanotechnology are suggested.

Ultrashort Self-assembled Peptides for Biomaterial Applications

Dr Garry Laverty, Alice McCloskey

Biofunctional Nanomaterials Group, School of Pharmacy, Queens University Belfast, N.Ireland

Antimicrobial resistance is one of the greatest threats facing society worldwide. Biomaterial infections, commonly caused by multi-drug resistant pathogens, are responsible for the majority of healthcare associated infections. The implant surface provides an optimum environment for microbial attachment and growth. Pathogens form an extracellular polymeric biofilm matrix, providing protection against the host immune response and standard therapies. This results in failure of treatment, increased spread of resistant pathogens, device removal, morbidity and increased mortality. Despite their high value to healthcare and the economy, infections of biomaterials remain unsolved and an ongoing burden. Our group has developed a library of ultrashort, cost-effective, diphenylalanine-based peptides (X_1-FF-X_2) which target (viability reduced >90%) the most resistant biofilm pathogens implicated in biomaterial infection including: methicillin resistant and sensitive *Staphyloccoccus aureus* and *Staphyloccoccus epidermidis*; *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Escherichia coli*. They demonstrate a reduced cell cytotoxic profile, possessing limited haemolysis.[7] These peptides respond form supramolecular hydrogel nanomaterials at low concentrations (~0.5%w/v) in response to subtle changes in pH associated with infection. Conjugation of variety of aromatic-based drugs at the X_1 position, for example non-steroidal anti-inflammatories (NSAIDs), confer further pharmacological properties enhancing their therapeutic potential. *In vivo* studies using waxworms (*Galleria mellonella*) demonstrate the low toxicity and high antimicrobial activity of these low molecular weight gelators in animal models. This work shows biofunctional peptide-based nanomaterials hold great promise for future translation to patients as biomaterial platforms.[2]

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Molecular dynamics simulations of intermolecular interactions involving peptides

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Peptides have a wide range of applications, going from drug design to innovative materials. This high versatility comes from their chemical and conformational flexibility. On the other hand, structural flexibility is also the main problem to face when studying this class of molecules. In this context, molecular dynamics (MD) simulations represent an essential tool to evaluate the structural and dynamic features of peptides in different environments. In the last years, we have applied a combined approach of MD and spectroscopic techniques for studying peptides under different conditions, by focusing both on topics of biological relevance and on aspects more related with a possible use in innovative materials.

In the former case, we have also investigated antimicrobial peptides (AMPs). AMPs are a promising class of antimicrobial drugs, which act as permeabilizing agents of the bacterial cell membrane. This nonspecific mechanism makes difficult for the pathogens to develop resistance. In this context, we have studied different structural features that influence the interaction with phospholipid bilayers. [1] Other biological problems involving peptides are related to their interactions with proteins. In this field, we have studied protein-peptide complexes involved in the onset of pathological conditions. [2]

With the aim to use peptides as building blocks for innovative materials, it is pivotal to understand the properties that influence the aggregation processes. In the last years, we have studied both self-aggregation processes of peptides and their ability to interact with ions. [3]

In the talk, an overview of these works will be presented, particularly focused on the contributions obtained by applying

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Peptide aggregation in 2D and 3D

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Peptide-based materials have been finding application in very different fields, from molecular electronics to tissue engineering.[1]

In this contribution, we will discuss on a molecular basis how the aggregation propensity of different peptide secondary structures may affect the morphology of nano- and mesoscopic peptide architectures. This effect is the result of hierarchical self-assembly, *i.e.* the self-organizing aggregation of molecular building blocks.

Following this principle, a suitable modification of the peptide sequence may propagate its effect from the secondary structure to the formation of nanostructures of controlled size and shape, to the assembly of mesoscopic structures. The understanding of the basic interactions governing this process will help to fix the conditions leading to structures of the desired morphology and function.

The strategy to prosecute this program from the very elementary constituents is to use oligopeptides predominantly formed by $C^{\alpha,\alpha}$-tetrasubstituted amino acids. The proper selection of these conformationally constrained residues allow to get oligopeptides attaining specific conformations, to be used as building blocks in the self-assembly process.

The conformational properties and physical interaction governing the formation of peptide aggregates in solution (fibrils, globules)[2,3] and as molecular films (self-assembled monolayers, Langmuir-Blodgett film) will be thoroughly discussed at the Conference.[4,5]

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Recombinamer-based injectable artificial ECM

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Introduction

Mimicking the natural extracellular matrix (ECM) has become the Holy Grail in the development of new scaffolds for regenerative medicine. However, that statement of intent turns out to be very elusive due to the complexity of the natural ECM as well as the complex relationship existing between cells and ECM. Properties of the ECM that must be taken into consideration range from mechanical properties[1] and microstructure to much more specific bioactivities[2]. In addition, such scaffold will have to cope with potential rejection by immune system of the host. Finally, the adequacy of its injectability is higly relevant. Therefore, the system that can fulfill all those requirements must rely on powerful self-assembling processes that ideally drive both the gelation of the system as well as the development of the proper microstructure.

This task could be at reach by exploiting the potential of recombinant technology and the use of recombinant protein polymers as the source of complex materials that contains all the functions required, including capacity to self-assemble and form stable gels upon injection.

Results

Among the different option within the area of recombinamers Elastin-like recombinamers (ELR) stand out[3]. In this work, different ELRs have been produced and used sowing different approaches to achieve injectable artificial ECM. The full range of ELRs designed and produced for this purpose include:

- Injectable two-component ELR for covalent cross-linking.
- Thermosensitive amphiphilic multiblocks for physical cross-links
- Advanced beta-sheet forming molecules for developing fibrous gels on setting.

The first group relies on catalyst-free click chemistry reactions. Those materials have proved exceptional performance in different applications, especially in the cardiovascular area. The second group relies on the thermal sensitiveness of the ELR. Due to their basic amphiphilic block nature and the ability to show a self-

aggregating effect induced by an increase in the temperature, those materials have the chance to be liquid below body temperature and make stable gels at BT. Those injectable scaffolds have proven their efficiency in musculoskeletal applications, particularly in the total regeneration of osteochondral injuries. That is also relevant its role in designing biomimicked mineralization templates and scaffolds. The last group is based on ELRs that, along with the elastin-like main contribution and all the epitopes rendering cell-adhesiveness, protease sensitiveness, etc., included epitopes taken from silk that endow the system with a capacity to self-organize in a regular microfibrilar structure resembling that of the natural ECM proteins such as collagen.

[1] J. Swift et al. Science, 341: 6149. 2013.
[2] Sudhir Khetan, et al. Nature Materials 12, 458–465, 2013
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Crystal Structures of the Halogenated Variants of the Amyloidogenic Core Sequences DFNKF and KLVFF

G. Cavallo[1], A. Bertolani[1], L. Gazzera[1], A. Pizzi[1], N. Demitri[2], P. Metrangolo[1], G. Resnati[1] and G. Terraneo[1]

[1]Laboratory of Nanostructured Fluorinated Materials (NFMLab), Dept. Chem., Mater., and Chem. Eng. "Giulio Natta", Politecnico di Milano, Milan, Italy.

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Amyloid fibril self-assembly is linked to pathological disorders such as type II diabetes mellitus, Alzheimer's, Parkinson's, Creutzfeldt–Jakob, and Huntington's diseases.[1] While some general details about the overall features of amyloid fibrils are well known, there still remains a lack of knowledge about exact oligomer conformations and details of the molecular mechanisms of aggregation. These may be addressed by elucidating high-resolution structures of amyloid conformations, but due to low solubility and crystallinity of these structures this remains a real challenge. Here, we describe that halogenation in the *para*-position of a phenylalanine residue (Phe5) of DFNKF (Figure 1)[2] or KLVFF induces their crystallization allowing us to determine for the first time the single crystal X-ray structure of DFNKF(I) along with those of KLVFF(I) and KLVFF(Br). Moreover, we demonstrate by molecular dynamics simulations that wild-type DFNKF and the iodinated peptide DFNKF(I) populate similar conformations therefore confirming that the crystal structure of DFNKF(I) is representative of the structure of DFNKF itself.

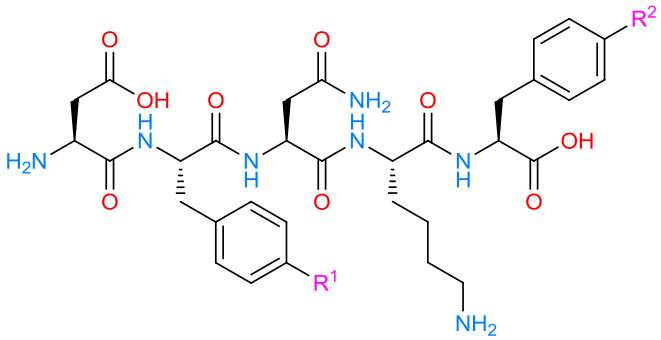

Figure 1: Chemical structure of the DFNKF (R1: H, R2: H) and DFNKF(I) (R1: H, R2:I) peptides.

[1] Dobson C.M. *Nature* **2003**, 426, 884-890.
[1] Bertolani A. *et al. Nat. Commun*. **2015**, 6, 7574, doi: 10.1038/ncomms857.

Bacterial Resistance to Silver: The Role of SilE Protein

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University of Fribourg, Switzerland

Silver has been used for hundreds of years for its antimicrobial properties. Since the emergence of many multi-resistant bacterial strains against classical antibiotics, the research of new silver compounds is now at its apogee. Nowadays, a lot of researches are focused on compounds with slow- and stimuli-responsive- release of Ag^+. While these drugs have been shown to be highly able to kill bacteria, some of these pathogens have developed a resistance to high concentrations of Ag^+.[1]

This resistance is provided by the plasmid pMG101, which encodes for eight proteins that act together in an efflux pump system to deal with silver ions. Among these, the SilE protein is the only one of which its mode of action is actually unknown.[2]

To identify the role of SilE in this bacterial machinery, two approaches have been intended in our group. While one way is to study the interaction of the whole protein with silver ions, the other is based on a bottom-up approach, investigating the interaction of silver ions with short peptide sequences of this protein.

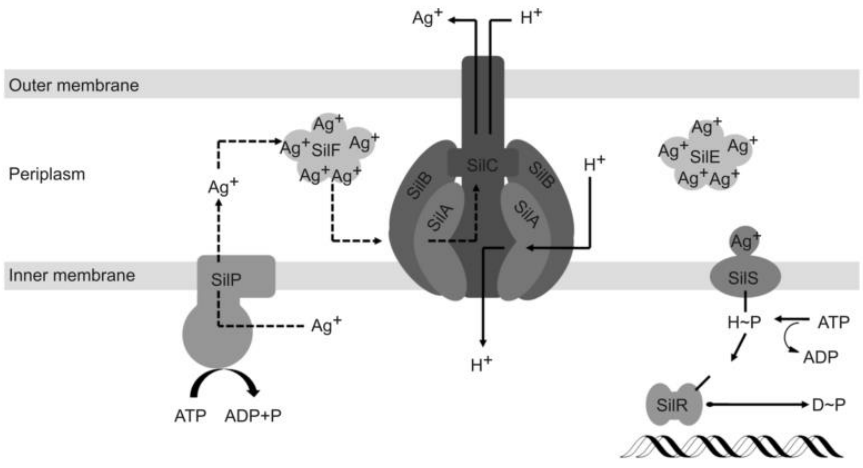

Figure 1: Proteins products of pMG101 silver resistance genes.

[1] Eckhardt, S. ; Brunetto, P. S. ; Gagnon, J. ; Priebe, M. ; Giese, B. ; Fromm, K. M. *Chem. Rev.* **2013**, 113, 4708-4754.
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Aqueous self-assembly of short hydrophobic peptides containing α,α-disubstituted amino acids

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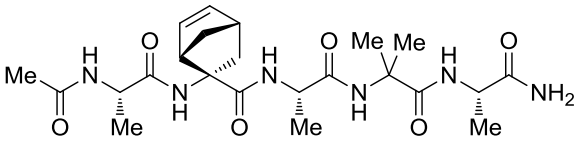
Università degli Studi di Milano, Dipartimento di Scienze Farmaceutiche, Milan, Italy

Here we report on the preparation of a series of short hydrophobic peptides and on their different ability to stabilize the formation of supramolecular assemblies in water. Pentapeptides AcAla-**X**-Ala-Aib-AlaNH$_2$ **1-6** containing unnatural constrained amino acids **X**, are insoluble in organic solvents but completely soluble in water despite the presence of hydrophobic non polar scaffolds.

NMR, CD, IR experiments were performed to gain insight on their secondary structure. Furthermore, the formation of supramolecular assemblies in water was studied by DLS.

In the case of **1** and **2**, containing the two enantiomers of the norbornene amino acid, DLS was performed either on the pure compounds or on their mixture.



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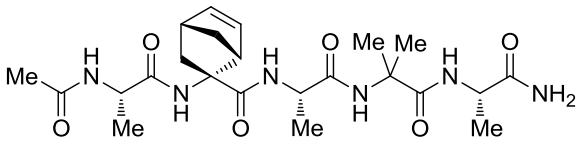

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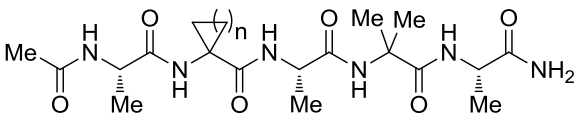
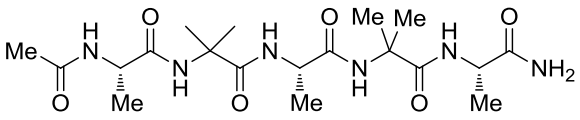


3-5 (n= 1,2,3)



6

Interestingly, each couple of the diastereoisomeric mixture of the oligopeptide gave monodisperse structures similar to that obtained by the single diastereoisomer. This result is of strong value because allows the availability of a large amount of the peptide material useful for several applications. The stability of the oligopeptide assemblies in serum at 37°C was tested showing unmodified dimension opening the way to further studies as candidates for drug delivery.

Gel synthesis of iron oxides nanoparticles

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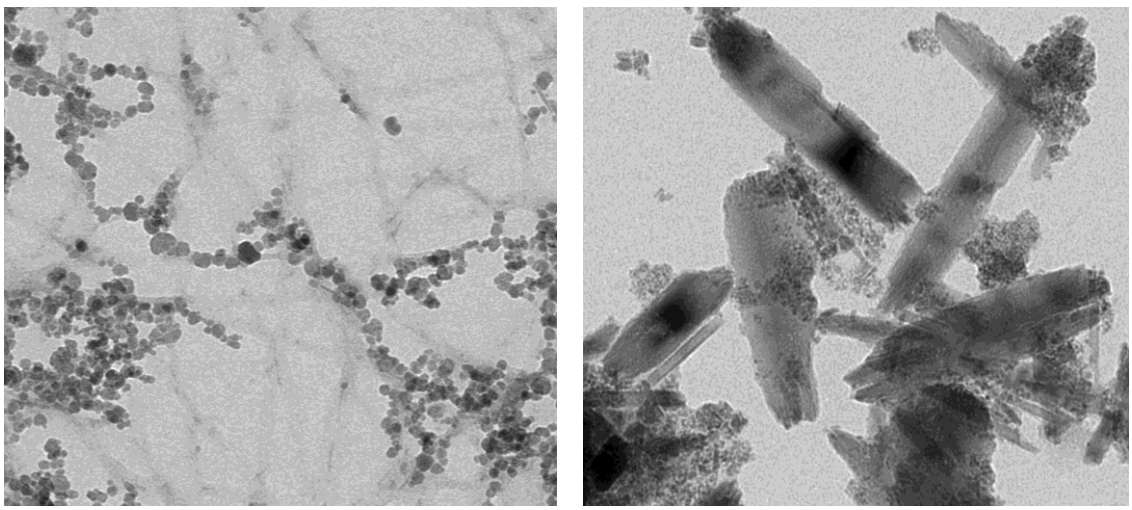
[1] *Facultad de Ciencias, Departamento de Química Orgánica, Universidad de Granada, 18071 Granada, Spain. lac@ugr.es.* [2] *Facultad de Ciencias, Departamento de Física Aplicada, Universidad de Granada, 18071 Granada, Spain.*

Short-peptides supramolecular gels are a subgroup of low molecular weight gelators that have found useful technological applications thank to their versatile nature.[1] In this work we have studied for the first time the behaviour of these types of gels as a medium to growth magnetite and goethite nanoparticles. The influence of the hydrogels and the protocols in the different kinds of structures obtained will be presented.



[1] Dasgupta, A.; Mondal, J. H.; Das, D. *RSC Adv*. **2013**, 3, 9117-9149.

Self-assembly of PEGylated tetra-phenylalanine derivatives: structural insights from solution and solid state studies

C. Diaferia,[1] F.A. Mercurio,[2] C. Giannini,[3] T. Sibillano,[3] G. Morelli,[1] M. Leone,[2] A. Accardo[1]

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Peptide nanostructures containing diphenylalanines (FF), which constitutes the core motif of Alzheimer's β-amyloid, have been investigated for their mechanical, electrochemical and optical properties. More recently FF aggregates have been also proposed for applications in nanomedicine.[1] Despite the growing literature about FF, only few examples of aggregates based on tetraphenylalanines (F4) or their polymeric analogues, chemically modified with PEG or PEO chains at the N-terminus, have been reported until now.[2,3] We developed PEGylated tetra-phenylalanine conjugates (DOTA(Gd)-L_6-F4 and DTPA(Gd)-L_6-F4) as potential Magnetic Resonance Imaging (MRI) contrast agents.[4] In these F4-conjugates the aromatic framework is derivatized at N-terminus with a PEG chain and with DOTA or DTPA chelating agents for achieving gadolinium coordination. The replacement of the Gd(III) with radiactive metal ions (^{111}In, $^{67/68}$Ga) could open novel perspective in diagnostic field. As alternative, chelating agent could be replaced with active pharmaceutical ingredient (API). Here we report a physicochemical characterization of supramolecular aggregates of L_6-F4 and its analogue containing DOTA chelating agent (DOTA-L_6-F4) using several techniques (CD, IR, NMR, WAXS and SAXS). Results of this characterization open new knowledge on the comprehension of the molecular interactions at the bases of the hierarchical organization of molecules.

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[1] Yan, X., Zhu, P, Li, J. *Chem. Soc. Rev.* **2010** 39(6), 1877-1890.
[2] Castelletto, V, Hamley, I. W. *Biophys. Chem.* **2009**, 141(2-3), 169-174.
[3] Tzokova, N., Fernyhough, C. M., Topham, P. D.; Sandon, N., Adams, D. J., Butler, M. F., Armes, S. P., Ryan, A. J. *Langmuir*, **2009**, 25(4), 2479-2785.
[4] Diaferia, C., Gianolio, E., Palladino, P., Arena, F., Boffa, C., Morelli, G., Accardo, A *Adv Funct. Mater*. **2015,** 25, 7003-7016.

Aligning Perylene Bisimides for Enhanced Photoconductive Properties

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[a] *University of Liverpool, UK.* [b] *University of Sheffield, UK.*

We have shown that a series of amino acid functionalised perylene bisimides (PBI) form aggregates in solution that exhibit photoconductive behaviour upon irradiation exclusively with UV light.[1] PBIs show promise for use as n-type materials in organic electronics such as photovoltaic devices. The PBI molecules form fibre-like aggregates, these are formed by self-assembled π-stacking of the molecules in aqueous solution at high pH due to their hydrophobicity. By aligning these aggregates in solution before drying into a thin film, the pathway of the electron is shorter. A shorter path length shows a greater conductivity. We show by drying PBI solutions in the presence of a magnetic field, we are able to form well defined aligned structures compared to when allowed to dry in air. We also are able to align solutions and gels using shear to give reproducibly aligned films (Fig. 1a and b). These films show great directional dependence with larger currents along the alignment of the fibres than against the alignment of the fibres. This ability to reproducibly align the structures is essential for their use in application such as p-n heterojunctions.

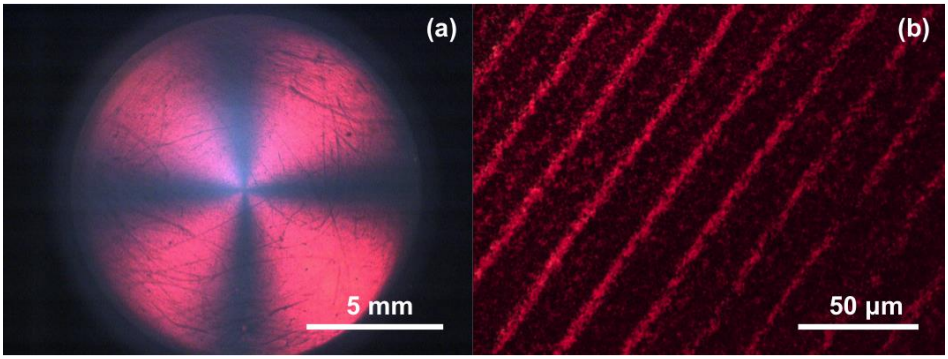

Figure 1. Opitical microscope images under cross-polarised light of (a) solution under shear and (b) a shear aligned dried film.

[1] E. R. Draper, J. J. Walsh, T. O. McDonald, M. A. Zwijnenburg, P. J. Cameron, A. J. Cowan and D. J. Adams, *J. Mat. Chem. C,* 2014, **2**, 5570-5575

HIV-1 peptide inhibitor derived from the E2 protein of GB virus C conjugated to nanosystems

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The use of nanocarriers for the targeted delivery of antiretroviral drugs significantly increases their therapeutic efficacy and safety, offering a large number of benefits such as protection of the drug against enzymatic degradation and their delivery to the specific site of action. They also provide conformational and physicochemical stability to the active molecule they transport, increase the cellular uptake of not very membrane-permeable drugs, reduce their cellular and tissue elimination, and maintain the prolonged release of active molecules at their site of action[1].

This work involves the anti-retroviral study of a synthetic peptide derived from the N-terminal E2 protein of GB virus C which was previously defined as an entry HIV-1 inhibitor[2] incorporated on lipid vesicles and polymeric nanoparticles. These nanosystems were obtained following procedures previously described[3,4]. The lipid vesicles mimicking the lipid composition of HIV-1 membrane (Large Unilamelar Vesicles, LUVs composed of POPC/DPPC/SM/CHOL) rendered an increase in the peptide anti-HIV-1 activity about one order of magnitude.

These results demonstrate that the liposomes composed of a lipid raft-mimicking mixture facilitate targeted delivery of the inhibitor peptide to membrane subdomains where the process of entry of the HIV-1 virus in the cell takes place.

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[1] Mallipeddi, R; Rohan, LC. . *Int. J. Nanomedicine* **2010**, 4, 533-547

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[3] Vasconcelos, A; Vega, E; Pérez, Y; Gómara, MJ; García, ML; Haro, I. *Int. J. Nanomedicine* **2015**, 10, 609-631

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Microarrays based on synthetic chimeric fibrin/filaggrin peptides for the diagnosis of Rheumatoid Arthritis

M. José Gómara[1], Javier Rodríguez[1], Pablo Salvador[2], M. José Bleda[1], Raimon Sanmartí[3] and Isabel Haro[1]

(1) Unit of Synthesis and Biomedical Applications of Peptides, IQAC-CSIC, Barcelona

(2) Nanobiotechnology for Diagnostics Group, IQAC-CSIC, Barcelona

(3) Unit of Arthritis, Hospital Clínic, Barcelona

Rheumatoid Arthritis (RA) is a chronic autoimmune disease that causes join inflammation and extra-articular manifestations. To prevent progressive and irreversible structural damage, early diagnosis of RA is of paramount importance. We have recently obtained and highlighted the application of chimeric peptide bearing different citrullinated protein domains for the design of improved RA diagnosis/prognosis systems[1-3]. In the present work, we pursue the transfer of immunological assays from microtiter plates to microarray formats in order to allow the simultaneous analysis of several peptide sequences and the reduction in the volume of serum required from patients. Peptides were covalently immobilized on the microarray slides previously derivatized with epoxide groups. The immobilization of peptides or printing was conducted using a Miniarrayer (BioOdisseyTM CalligrapherTM, BioRad) and the assay was carried out according to the protocol described previously for the ELISA plates[1], using as secondary antibody anti-human IgG labelled with Alexa Fluor 647. Fluorescence of the spots was quantified in a scanner (ScanArray GX Plus, Perkin Elmer). A comparison of the results obtained by ELISA and Microarrays methodologies working with two cohorts of sera (96 from RA patients and 96 from healthy blood donors) will be presented.

[1] Pérez, ML; Gómara, MJ; Ercilla, G; Sanmartí, R; Haro, I. *J.Med.Chem*. **2007**, 50, 3573-3584.
[2] Sanmartí, R; Graell, E; Pérez, ML; Ercilla, G; Viñas, O; Gómez-Puerta, JA; Gratacós, J; Balsa, A; Gómara, MJ; Larrosa, M; Cañete, JD; Haro, I. Arthritis Res. Ther. 2009, 11(5):R135.
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Structural chameleonic behaviour in peptides derived from choline binding repeats

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Choline-binding domains display a ββ-solenoid structure formed by choline binding repeats (CBRs). Each CBR comprises a β-hairpin (approx.14-residues) connected to the following repeat by a short linker sequence (about 6-residues). A choline molecule binds between consecutive CBRs. These domains are good models for understanding the folding and stability of repeat proteins, and also have applicability as affinity tags for protein purification and immobilisation. To find whether a minimal peptide comprising the β-hairpin core of a single CBR would conserve its native structure and its choline-binding capacity, we started the structural characterization of peptides derived from pneumococcal LytA autolysin. In these studies, we have found that a 14-mer peptide ($LytA_{239-252}$) corresponding to the third CBR of LytA forms a very stable native-like β-hairpin in aqueous solution and also in the presence of trifluoroethanol, but it converts into a stable amphipathic α-helix in the presence of detergent micelles.[1] Apart from its relevance in the fields of peptide design and protein folding, this result might be of potential applicability as a structure-switch biosensor. Hence, to get insights into the chemical-physical properties responsible for the unusual reversible β-hairpin to α-helix transition exhibited by $LytA_{239-252}$, we are analysing the effect of solvent conditions on the structural behaviour of designed $LytA_{239-252}$ variants, and of peptides corresponding to other CBR repeats of LytA by using CD and NMR.

[1]Zamora-Carreras, H.; Maestro, B.; Strandberg, E.; Ulrich, A. S.; Sanz, J. M.; Jiménez, M. Á. *Chem Eur J.* **2015**, 21, 8076-8089.

Halogenation promotes the self-assembly and gelation properties of DFNKF peptides

Luisa Lascialfari,[a] Arianna Bertolani,[a] Andrea Pizzi,[a] Alessandro Gori,[a] Nicola Demitri,[b] Giuseppe Resnati,[a] Pierangelo Metrangolo[a,c]

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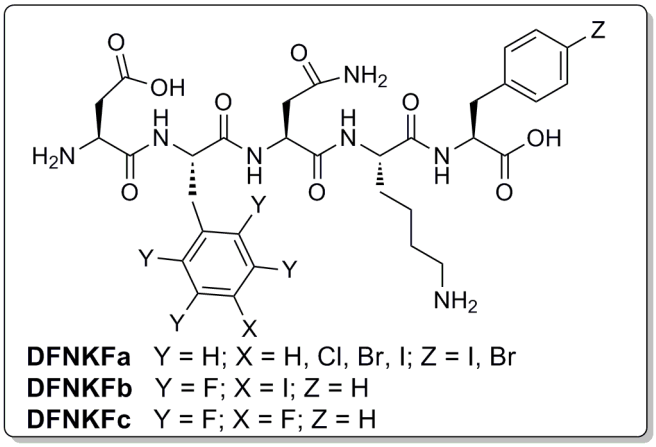
c) VTT – Technical Research Centre of Finland, FI-02044 VTT Espoo, Finland.

Amyloid fibrils, formed by the tight assembly of β-sheet-like structures, are widely studied because of their disease-related implications and because the robustness of their supramolecular architectures can be exploited in nanostructured bio-inspired materials.[1] It is at the same time true that, the halogenation of proteins is a consequence of oxidative-stress, which is often associated to misfolding and aggregation.[2] Choosing as a model fibrillation system the DFNKF core sequence of hCT, we have demonstrated that halogenation strongly promoted fibrils, and thus, hydrogels formation.[3] Considering that DFNKFa aggregation performances increase as the halogen-bond donor (XB-d) character of the halogen increases, we then synthesised and studied the properties of DFNKFb, containing a more effective XB-d aminoacid. Indeed, we found better gelation abilities of DFNKFb if compared to DFNKFa, while DFNKFc, lacking of XB-d residues, is a worse gelator. This point out a positive role of XB interactions in addition to common intermolecular interactions that are recognized as responsible in peptide systems aggregation**.**



DFNKFa Y = H; X = H, Cl, Br, I; Z = I, Br
DFNKFb Y = F; X = I; Z = H
DFNKFc Y = F; X = F; Z = H

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Protein nanomaterials from renewable resources

Christofer Lendel

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Proteins are Nature's own high-performance materials providing both extraordinary mechanical properties (e.g. muscles, silks) and sophisticated functionality (e.g. adhesion, biological signalling). The key to copy these properties to man-made materials lies in controlling the hierarchical material structure, from molecular- to macro level. From this perspective, the self-assembly of proteins and peptides into ordered nanofibers is of utmost interest. The use of protein nanofibers to create new materials is an expanding field of research. Much of the efforts in this field of research have, however, been made on recombinantly produced and highly purified starting material. For large-scale applications, methods for production of nanofibers and macroscopic materials from crude staring materials are needed. We explore the potential of producing the nanocomposites based on proteins from renewable source, e.g. plant proteins. The questions we address are:

1) How can we produce nanofibers from various crude starting materials?
2) How can we control the hierarchical assembly of nanofibers into macroscopic materials?
3) How can the nanofibers be used to create improved or new functionalities in bioplastics?

Injectable peptide hydrogels for controlled-release

C. Martin,[1] E. Oyen,[1] M. Bibian,[1] J. Gardiner,[2] B. Van Mele,[3] A. Madder,[4] R. Hoogenboom,[5] M. Spetea[6] and S. Ballet[1]

[1]Research Group of Organic Chemistry, Vrije Universiteit Brussel, Belgium. [2]CSIRO Materials Science & Engineering, Australia. [3]Vrije Universiteit Brussel, Physical Chemistry and Polymer Science, Belgium. [4]Organic and Biomimetic Chemistry Research Group, Ghent University, Belgium. [5]Supramolecular Chemistry Group, Ghent University, Belgium. [6]Institute of Pharmacy, University of Innsbruck, Austria.

Currently, most drugs are directly administered into patients orally or systemically, via parenteral routes. Therefore, to get the desired therapeutic effect, high doses are required due to substantial biodegradation of the drug prior to interaction with the biological target, resulting in the appearance of adverse effects. To overcome this, hydrogels have been reported as suitable controlled drug-delivery systems. These systems present several advantages such as the protection of the drug against the enzymatic degradation by 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 and result in an improvement of the drug efficacy while reducing the risk of side effects.

In this work, a new family of hydrogel-forming peptides was designed starting from a short, tunable and amphipathic hexapeptide hydrogelator.[1] After characterization, and in order to study their eventual therapeutic potential, the hydrogels have been used for entrapment and sustained release of opioid drugs. The *in vitro* drug release properties and hydrogel toxicity (cell viability experiments) were also determined. Based on the best physicochemical, mechanical, and noncytotoxic properties, selected hydrogels were investigated for *in vivo* release of opioids. Opioid administration by subcutaneous injection and subsequent testing in the tail-flick assay (acute pain model), showed sustained antinociceptive effects over longer periods of time, as compared to drug injections in saline solutions.[2]

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[2] Martin, C.; Oyen, O.; Mangelschots J.; Bibian, M.; Ben Haddou, T.; Andrade, J.; Gardiner, J.; Van Mele, B.; Madder, A.; Hoogenboom, R.; Spetea, M. and Ballet, S. *Med. Chem. Com.* DOI:10.1039/c5md00440c.

The evolution of surface-coating molecules in biomaterials science: integrin-binding peptidomimetics and multifunctional platforms

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2. Institute for Advanced Study and Center for Integrated Protein Science, Technical University of Munich, Garching, Germany

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The biofunctionalization of implant materials with integrin-binding peptides and proteins derived from the extracellular matrix has long been investigated to improve cell-material interactions. However, classical biomimetic strategies present some limitations and may prove insufficient to elicit the biological signals required in the process of tissue regeneration.[1]

To overcome these limitations, we have focused on the development of innovative biological coatings with enhanced integrin-specificity and/or multiple biological potential, aiming at designing cell-instructive and versatile biomaterials for tissue regeneration.

In this work two novel strategies will be presented: i) the use of two RGD peptidomimetics with high activity and selectivity for integrins αvβ3 or α5β1;[2] and ii) a peptide-based platform with the capacity to simultaneously present two distinct bioactive motifs in a chemically controlled fashion.[3]

The potential of these biomolecular tools to engineer biomaterials with bone regenerative properties has been proven *in vitro* using bone-like and mesenchymal stem cells, and *in vivo* in a calvarial defect model in rats.

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[1] Williams, D.F. *Biomaterials* **2011**, 32, 4195-4197.
[2] a) Rechenmacher, F.; et al. *Angew. Chem. Int. Ed.* **2013**, 52, 1572-157; b) Fraioli, R.; et al. *Colloid Surf. B-Biointerfaces* **2015**, 128, 191-200.
[3] Mas-Moruno, C.; et al. *ACS Appl. Mater. Interfaces* **2014**, 6, 6525-6536.

Hierarchical surface-mediated self-assembly of triphenylalanine with N- and C- terminal fluorenylmethyl groups

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The 9-fluorenylmethoxycarbonyl (Fmoc) N-capped oligophenylalanine derivatives (F_n, n=2,3,4) form a wide range of micro- and nano-assemblies that include fibrils, nanoplates and hydrogels depending on the preparation and incubation conditions. Particularly, morphological differences in the surface self-assembly are tested for Fmoc-FF by varying peptide concentration and the pH of the aquous media.[1]

In this work we have investigated the influence of the substrate in the formation of supramolecular assemblies by a highly aromatic FFF derivative capped with N- and C-terminal fluorenylmethyl groups. Surface-induced assemblies in 9 substrates that differ in its chemical nature were examined. Dendritic-like morphologies were obtained for glass and plasma treated polystyrene. Micrometric jellyfish-like structures were obtained onto silanized glass. Crystalline assemblies and birrefringent spherulites were observed for mica, whereas structures grown onto steel AISI 316, silicon wafer and evaporated carbon coatings consist on spherical aggregates arranged in necklaces. The reported structures point out that come from a complex hierarchical assembly of densely packed nano-assemblies.

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Halogen bonding: A new supramolecular tool in the molecular self-assembly of peptides

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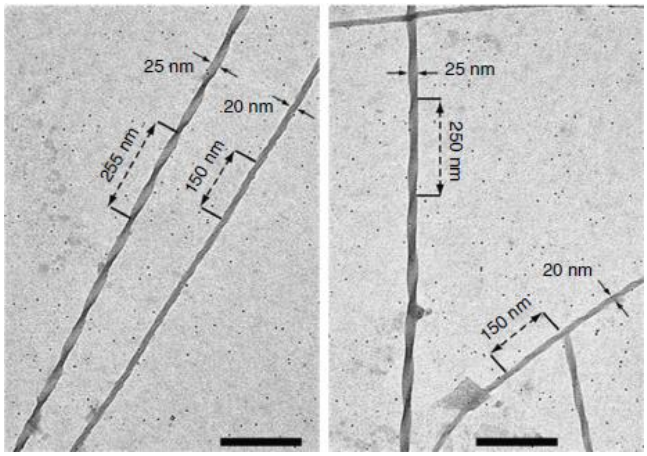
A halogen bond occurs when there is evidence of a net attractive interaction between an electrophilic region associated with a halogen atom in a molecular entity and a nucleophilic region in another, or the same, molecular entity.[1]



Although many modifications of amyloidogenic sequences have been utilized to tune their self-assembly behaviour, halogenation has rarely been pursued. The advantage of a strategy based on the introduction of halogen atoms on peptide motifs lies in the fact that halogenation is a minimal structural modification, which, on the other hand, may induce a large difference in the peptide supramolecular behaviour as a consequence of the rich variety of noncovalent interactions given by halogen atoms. [2] In this presentation we show how the halogen bond can be used to promote the molecular self-assembly of peptides. We have applied this new supramolecular concept to the augmented fibrillation of amyloidogenic peptides and proteins, such DFNKF,[3] KLVFF, and hCT. Implications of oxidative stress-induced halogen of proteins are discussed in terms of biomarkers of diseases such as Parkinson and Alzheimer's. The obtainment of a novel unnatural amino acid functioning as strong halogen-bond donor may pave the way to totally new design principles in peptide-based molecular self-assembly.

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Ultrashort peptides as bifunctional nanomaterials

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Advances in modern medicine have resulted in increased life expectancy and quality of life for patients. Biomaterials have contributed greatly to these advances in healthcare however, despite their many uses in replacing/ facilitating normal physiological functions, they provide an ideal surface for bacterial adhesion and subsequent development of biofilm infections.[1]

The presence of and sometimes trauma caused by insertion of a biomaterial, stimulates the host immune response system to upregulate inflammatory mediators. These can lead to destruction or impairment of the biomaterial. This in turn, prolongs illness and delays healing impacting negatively on the patient's recovery.[2]

Peptides are ideal candidates to help overcome these associated challenges due to their vast bioavailability in nature, biocompatibility and biodegradability. Ultrashort peptides are of increasing interest in novel research and development due to their economic advantages over longer counterparts benefiting their potential transition from the lab bench to the clinic.[3]

In this research an ultrashort diphenylalanine- dilysine peptide sequence has been conjugated to the clinically used non-steroidal anti-inflammatory drugs (NSAIDs)- naproxen, indomethacin and ibuprofen. These peptides have inherent dual antimicrobial and anti-inflammatory activity and at suitable concentrations NSAID conjugated peptides have been shown to possess the ability to self-assemble.[4]

Broad-spectrum antimicrobial activity was demonstrated against a range of Gram positive and Gram negative bacteria. Anti-inflammatory activity was tested in terms of inhibition of two key enzymes, cyclooxygenase-1 and -2 and conjugation of the NSAID to the peptide did not affect inhibition of these. An ability to form hydrogels was assessed in terms of a vial inversion assay, rheological analysis and cryo-SEM and TEM imaging to determine the presence of nanofibrous networks. Cytotoxicity and hemolytic activity assays showed that the peptide-NSAID conjugates were more selective for bacterial cells.

The findings of this research are very promising and indicate that peptide- drug conjugates such as these have great potential as bifunctional materials for biomedical applications.

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$\beta^{2,3}$ diaryl amino acids as molecular tools for the preparation of peptide nanotubes

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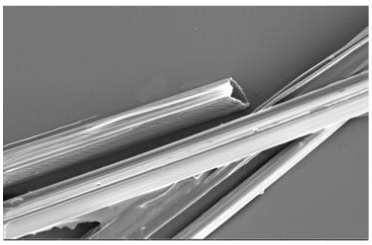
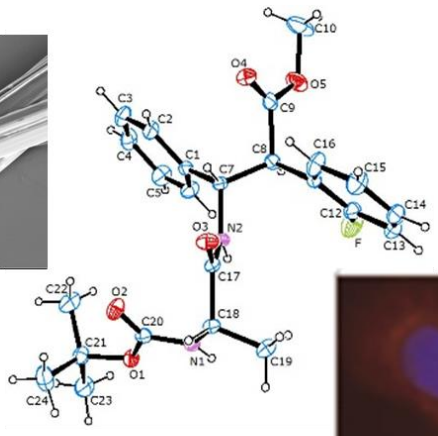
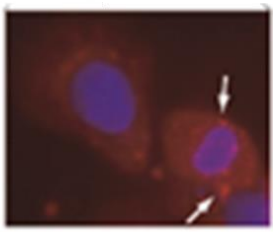
Peptide nanotubes are a class of organic nanotubes attracting great interest due to their potential applications in nanotechnology and biomedicine. On the other hand, one drawback of using peptides is their high susceptibility toward proteases and their short half-lives.

Here, we present the preparation of proteolytic stable nanotubes containing fluorine-substituted (*S*,*S*)-$\beta^{2,3}$-diaryl-amino acid and (*L*)-alanine. In the solid state, the supramolecular architecture is strengthen by intermolecular N-H…O hydrogen bonds, Cπ-H…O, Cπ-H…F and van der Waals interactions.[1] Furthermore, the nanotubes were able to incorporate small organic molecules, to enter the cell and to locate in the cytoplasmic/perinuclear region. Therefore they represent interesting candidates for biomedical applications.







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Elastin-like peptide nanoparticles for crossing the blood-brain barrier

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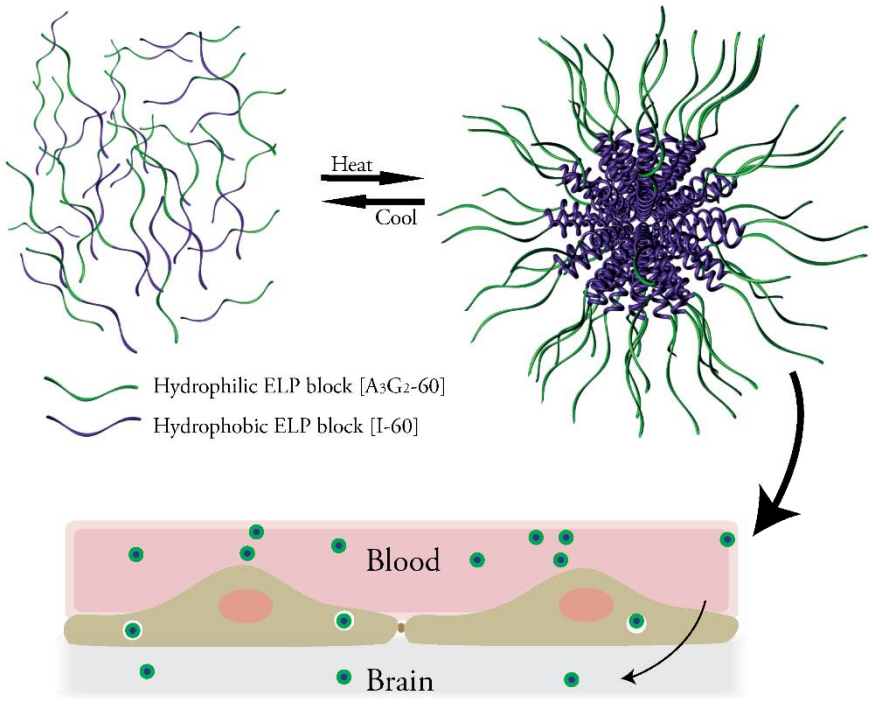
Treating neurological disorders demands overcoming the blood-brain barrier. Hijacking the active transport mechanisms of this tight, selective endothelial cell layer shows great promise in administering therapeutic agents to the brain. A potential carrier system needs to be well-defined, bio-compatible and able to display functional moieties for transcytosis. Here we develop elastin-like peptide (ELP) nanoparticles that are highly monodisperse, stable down to nanomolar concentrations and are able to display a range of functional peptides. The GM1b-binding peptide G23[1] is used to induce uptake and transcytosis of ELP nanoparticles by brain endothelial cells.



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Tuning amyloid peptide self-assembly by halogenation

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Alzheimer's disease (AD) is a pathology associated with the formation of β-sheet containing fibrils. The peptide associated with this disease, constituted by 40-42 aminoacids, is called Amyloid beta (Aβ). Different groups have investigated the fibrillation of several fragments of Aβ. The results suggest that the sequence Aβ (16-20) -KLVFF- (Lys-Leu-Val-Phe-Phe) is critical for fibrillation[1]. There is no current evidence of halogenation in AD; however, there is substantial evidence of oxidative stress – which may result in a halogenated biomolecule – playing an important role in this disease[2].

In this contribution we report on a study of various halogen-modified derivatives of KLVFF. We demonstrated by different techniques like AFM, TEM, and Congo Red staining that varying the halogen position in the sequence confers very different structural properties, as previously reported for another amyloidogenic sequence[3]. In general, the presence of a halogen atom in the 4-position (internal phenylalanine) of the peptide sequence increases the rate of fibrillation, since we obtained amyloid hydrogels using concentrations four time lower compared to the minimal gelation concentration of the wild type sequence. An unexpected behavior characterizes the fragments having just a halogen in the 5-position (terminal phenylalanine), since they form crystals rather than fibrils. Finally, di-iodination confers polymorphism to the sequence by varying concentration and pH.

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Fibrin Association at Hybrid Biointerfaces Made of Clot-Binding Peptides and Polythiophene

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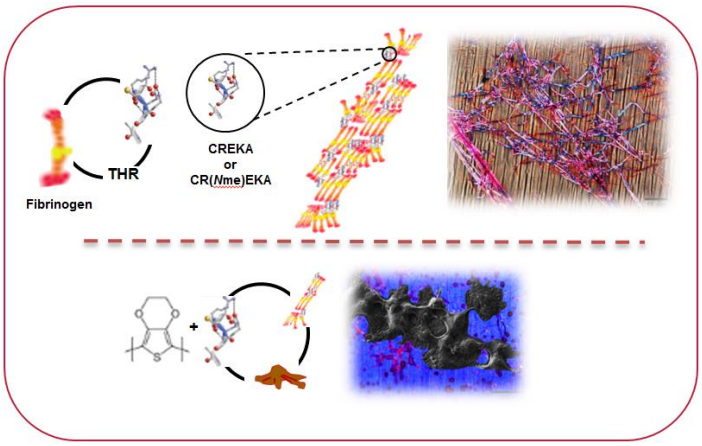

Scheme 1. Illustration of how peptides influenced fibrin polymerization and facilitate cell adhesion.

Electroactive conducting polymer-peptide biocomposites formed by poly(3,4- ethylenedioxythiophene) (PEDOT) and CREKA or CR(*N*Me)EKA (where Glu has been replaced by *N*-methyl-Glu) have been prepared and their properties compared (**Scheme 1**). The surface topology is considerably more leveled for PEDOT-CREKA than for PEDOT-CR(*N*Me)EKA. This feature suggests that CREKA molecules enter into the PEDOT matrix individually or forming small groups (dimers or trimers), whereas entrapped CR(*N*Me)EKA forms bigger aggregates. Furthermore, the electrochemical and electrical behaviors of PEDOT improve upon the incorporation of the peptides. Both CREKA and CR(*N*Me)EKA affect fibrinogen thrombin–catalyzed polymerization causing the immediate formation of fibrin, whereas in absence of thrombin this is only observed for CR(*N*Me)EKA. The fibrin-adsorption capacity of fibrin decreases as follows: PEDOT-CR(*N*Me)EKA > PEDOTCREKA ≈ bare steel > PEDOT. PEDOT-peptide films coated with fibrin promote the attachment of metastatic cells with respect to normal cells. However, this clearly manifested selectivity is lost in absence of surface adsorbed fibrin.

Engineering of single-chain polypeptides to self-assemble as BBB-crossing protein nanoparticles

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[3] *CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Bellaterra, 08193 Barcelona, Spain*

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The major challenge for the treatment of brain disorders is to develop optimal carriers to deliver therapeutic molecules, as chemicals or nucleic acids, to the Central Nervous System (CNS). Apart from the renal clearance that affects the retention of small molecules in the blood stream, the main obstacle to the targeted delivery is the blood–brain barrier (BBB) that acts as a filter between the bloodstream and the brain.

The aim of this work is the design, production and characterization of self-assembling protein nanoparticles directed to CNS, and to assess their biodistribution. Likewise, we have explored the potential influence of a supramolecular protein organization in BBB specific targeting.

Briefly, two different brain cell binding peptide domains, SEQ1 and Angiopep, where both engineered in order to increase their positive electrostatic charge to promote protein nanoparticle assembly. Each of these modified domains, fused to His-tagged GFP protein were produced in *E.coli* expression system and further characterized.

SEQ1charge8 peptide generates nanoparticles of about 30-40 nm. The nanoparticulate presentation dramatically enhances in vitro, LDLR-dependent cell penetrability of the protein compared to the parental monomeric version, but the assembled protein does not show any enhanced brain targeting upon systemic administration.

The presentation of protein chemicals in form of nanoparticles is advantageous when addressing non brain metastatic cancers, preventing renal clearance and ensuring re-circulation until cell binding and uptake. However, our data demonstrate that this principle might not apply to brain targeting, as the BBB imposes steric constraints different from those in systemic circulation. Irrespective of this fact, the fine engineering of proteins electrostatic profiles is revealed here as a powerful tool for the rational functional design of building blocks and of protein-only nanoparticles.

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Electroactive polymer modified with RGD-derivated peptide for cell adhesion

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Electroactive polymer–peptide conjugates have been synthesized by combining poly(3,4-ethylenedioxythiophene), a polythiophene derivative with outstanding properties, and an Arg-Gly-Asp (RGD)-based peptide in which Gly has been replaced by an exotic amino acid bearing a 3,4-ethylenedioxythiophene ring in the side chain. The incorporation of the peptide at the ends of preformed PEDOT chains has been corroborated by both FTIR and X-ray photoelectron spectroscopy. The effect of the incorporation of the peptide at the ends of PEDOT chains on the surface and electrochemical properties have been characterized. Density functional theory calculations have been used to ascertain the conformational preferences of the peptide. Electroactive surfaces prepared using the conjugates displayed the higher bioactivities in terms of cell adhesion, with the relative viabilities being dependent on the roughness, wettability and electrochemical activity of the conjugate. In addition to the influence of the peptide fragment in the initial cell attachment and subsequent cell spreading and survival, the results indicate that PEDOT promotes the exchange of ions at the conjugate–cell interface.

Solution-state NMR to understand peptide gel fibre surface chemistry and gelation

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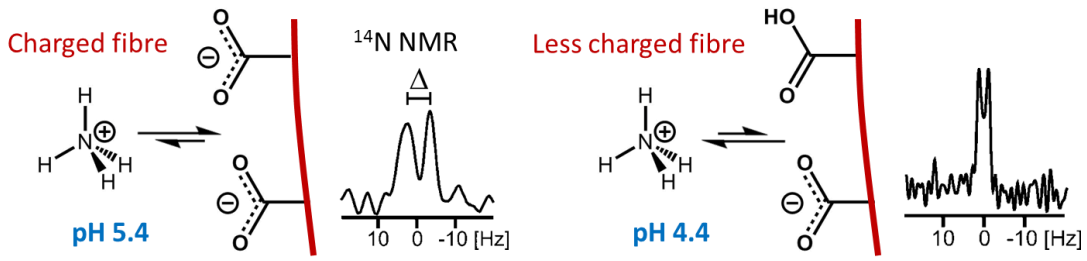
Supramolecular hydrogels can be formed *via* the self-assembly in solution of low molecular weight peptide-based gelators and have a range of applications as diverse as cell culturing and catalysis. A number of well-established analytical techniques exist for the study of such hydrogels at the molecular level, the focus being on how the gelator molecules are packed together in the self-assembled gel fibres.[1] Hydrogels, however, are hierarchical systems with the primary gel fibres further associating with one another to form larger-scale structures that constitute the bulk macroscopic gels. Techniques for probing the surface chemistry of the gel fibres are thus required in order to build a better understanding of both the final hydrogels and the processes by which they are formed; however, such techniques remain relatively underdeveloped. To this end, we are developing a range of techniques based on solution-state NMR spectroscopy to probe the surface charge, hydrophobicity and ion binding dynamics of hydrogel fibres. The fibres themselves are invisible by conventional solution-state NMR, but much valuable information can be obtained by studying instead their selective interaction with probe molecules dissolved in the bulk solution of the gel. For example, by studying the relative residual quadrupolar couplings, (Δ), of NH_4^+ and isopropanol-d_8 as the pH of the bulk solution is decreased, we are able to follow the loss of charge from the gel fibres which leads to a stiffening and contraction of the bulk gel.[2] In other systems, we are also able to observe the selective displacement of Na^+ ions by Mg^{2+} or Ca^{2+} from a peptide fibre.



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Probing interactions in β-sheet peptide / graphene derivatives hybrid hydrogels

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The exploitation of the self-assembling properties of β-sheet forming peptides has become a prominent strategy in material science for the construction of soft material (i.e.: hydrogels)[1,2]. The incorporation of nanofillers such as carbon nanotubes and graphene derivatives offers an effective route for the construction of increasingly complex functional materials for a variety of applications ranging from electronics to biomedical and tissue engineering[3,4]. Understanding the interactions between the β-sheet fibres and the nanofillers is key for the design of the materials. In this study the co-assembly of three peptides, FEFKFEFK (F8), FEFEFKFE (FE), VEVKVEVK (V8) (V: valine; F: phenylalanine; K: lysine; E: glutamic acid) with varying designs and charge states and graphene derivatives nanofillers (graphene oxide and reduced graphene oxide) was investigated using a variety of techniques including: oscillatory rheology, Raman spectroscopy and atomic force microscopy (AFM). Processes for the homogeneous dispersion of graphene oxide in the peptide hydrogels and its subsequent reduction to graphene were developed. Strong interactions between the graphene flakes and the peptides were observed that affected the overall mechanical properties of the hydrogels. In particular electrostatic interactions and π-π stacking, when phenylalanine residues are present, were shown to play a key role in determining the final properties of the material.

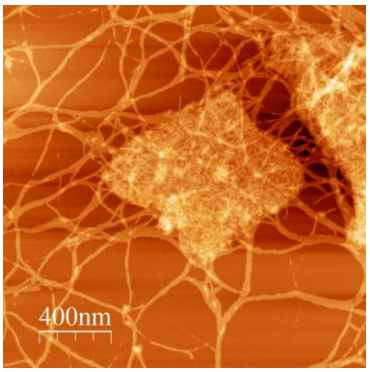

Figure 2. Network of F8 peptide fibres with Graphene Oxide flake.

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Bifunctional Surfaces: A Peptide-Based Coating that Resists Fouling and Promotes Cell Adhesion

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The interface between organic and inorganic matter is of high importance when designing an implant or a biomaterial. On one hand, since these materials should be integrated into the human body, their interface with tissues should encourage cell attachment. On the other hand, these materials can serve as a surface for bacterial colonization, termed biofilm formation, which can lead to an infection. Despite the preventive measures against infections, such as sterilization, bacterial infection at the site of implants is still of major concern for the health care system. The infection causes discomfort for the patient and may result in the removal of the implant and even patient death. Different approaches attempt to prevent biofilm formation; however, there are disadvantages to each approach. This includes low stability, high cost, complexity of the synthesis or fabrication method and the limitation to a specific substrate.

Recently, our lab designed a tripeptide that self-assembles into a coating that resists fouling. It contain: (i) two aromatic residues that direct the peptide self-assembly into an ordered coating, (ii) the amino acid DOPA which promotes the adsorption of the peptide onto different substrates and (iii) fluorine atoms as substituent on the phenyl groups that mimic the properties of Teflon© as a nonstick surface and therefore provide the antifouling activity. Our results demonstrated the formation of a coating which prevents protein adsorption and significantly reduces the number of bacteria adsorbed on the substrate.[1] Based on this tripeptide, we designed and synthesized a bi-functional peptide that both acts as an anti-biofilm coating and encourages cell attachment. We study the formation of coating by several derivatives of this peptide, their antifouling activity and their ability to attract cells. These peptides can be used as a coating on implants and potentially reduce the risk of infection and simultaneously increase the probability of the implant to well integrate in the tissue.

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Self-assembling peptide hydrogels cross-linked by disulfide bonds

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Self-assembling ionic complementary peptides (SAPs) have been receiving much interest from the scientific community as promising scaffolds for tissue-engineering. In order to investigate the effects of cross-linking on hydrogels characteristics, we have designed four SAP sequences: Cys-EAK (CAEAEAKAKAEAEAKAK), Cys-EAK-Cys (CAEAEAKAKAEAEAKAKC), Cys-EAK_{mod} (AEAEAKAKCEAEAKAK) and EAK (AEAEAKAKAEAEAKAK) as reference[1]. Atomic Force Microscopy analysis confirmed the presence of nanofibers that were longer and more interconnected in the oxidized Cys-EAK hydrogel. Small amplitude shear tests showed a typical gel-like behavior for all samples, oxidized or not. In the case of Cys-EAK hydrogel, the oxidation produced a stiffer hydrogel but this trend was not observed for Cys-EAK-Cys. All SAPs hydrogels (oxidized or not) were good scaffolds for mouse fibroblasts and osteoblasts at 24 h. Interestingly at 72, immunohistochemical analysis showed apoptotic round-shape cells in not oxidized Cys-EAK and Cys-EAK-Cys scaffolds (Figure 1).

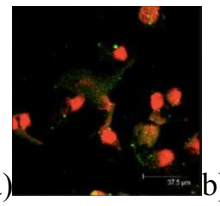
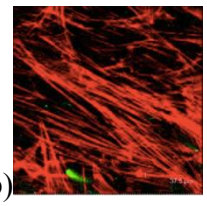
a)  b) 

Figure 1. Immunohistochemical analysis of mouse fibroblasts at 72 h of not oxidized Cys-EAK (a) and oxidized Cys-EAK (b).

[1]Gambaretto, R; Tonin, L; Biopolymers, **2008**, 89 (11), 906-915

Analysis of Gold-Tethered cationic RPAR Peptide

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The ability of the peptides to recognize organic surfaces has been renewed attention due to the interaction of proteins with inorganic surfaces, this being very useful in fields such as biomedicine and the development of new composite materials. Blood vessels are generally impermeable to macromolecules and supramolecular complexes.

The increased permeability of blood vessels is associated with angiogenesis and carcinogenic processes. The RPAR peptide interacts with neuropilin (NRP-1), which is a receptor that plays an essential role in these processes. Molecular dynamics simulations have been used to study the conformational preferences of RPAR in solution and tethered to biocompatible inorganic surfaces.

Self-assembled monolayers of redox peptides

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Biosensors based in protein-protein interaction plays an important role in the early diagnosis diseases. The development of redox capacitive biosensors, coupled with the electroanalytical techniques, has recently been introduced with great potential of low cost and fast method. However, there are disadvantages because of the high cost and low stability of the molecular recognition component. Peptides have proved to be a promising tool improve the affinity, specificity, and stability of molecular recognition components for the development of self-assembled monolayers. Herein, we described synthesis of redox-tagged peptides with self-assembling capability in electrochemically active capacitive surfaces, for the C Reactive Protein detection (biomarker of inflammation and cardiovascular disease). Peptides containing ferrocene (fc), redox group, was synthesized by solid phase peptide synthesis (SPPS), with the sequences: Fc-E-A-A-C-NH_2 and Fc-K-A-A-C-NH_2. To obtain the electrochemically capacitive interface, the side chain of Cys was covalently bound to the gold electrode (sulfur group) and the N-terminus group was used to attach the ferrocene in the peptide chain. The anti-CRP was attached to the peptide using the side chain of glutamate and lysine, respectively. The self-assembly and redox capability was characterized by cyclic voltammetry and electrochemical impedance-based capacitance spectroscopy techniques. Although, the affinity constant and the surface coverage of both peptide SAMs were similar, the limits of detection were different: 0.11 nmol L^{-1} and 0.31 nmol L^{-1} for the peptides Fc-E-A-A-C-NH_2 and Fc-K-A-A-C-NH_2, respectively. Results showed that, the bound of the anti-CRP to glutamate improved the detection. In addition, the design of redox active peptides self-assembly is predictably useful in the development of biosensor devices, making use of the electrochemical capacitance signal intrinsically existing in redox-active monolayers.[1]

[1] Santos, A. et al; Piccoli, J.P.; et al *Biossensors and Bioeletronics* **2014**, 68, 281-287.

Functional Peptide Assemblies on Surfaces

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Biofouling is an undesirable process in which a surface becomes encrusted with organisms and their by-products. This unwanted colonization has a serious impact on marine devices, as it lead to deterioration of the surfaces and can alter fluid flow rates leading to significant increase in cost of marine transportation. In the healthcare system, the attachment of bacteria and biofilm formation on medical devices may lead to a severe infection and consequently death. In the US alone, the American Centre for Disease Control and Prevention (CDC) reported that healthcare-associated infections account for an estimated 1.7 million infections and 100,000 deaths annually.

Many approaches to prevent biofouling have been suggested, however, they suffer from drawbacks such as release of toxic materials to the surroundings, low stability that limits their long-term application or complex and expensive synthesis.

We have recently designed a tripeptide that under different conditions self-assembles in an organized manner to form coatings that interfere with the attachment of organisms to the substrate and therefore act as an antifouling agent.[1]

The peptide contains three elements that enable i) its self-assembly, ii) its adsorption onto any substrate and iii) its antifouling activity. The peptide-based coating completely prevented the first stage of biofouling and abolished the adsorption of proteins to a substrate. Moreover, the coating significantly reduced the amount of different bacterial strains adsorbed on the substrate.

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[1] Maity S., Nir S., Zada T. and Reches M. *ChemCommun*, **2014,** 50, 11154-1157.

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