2016 EMBO Chemical Biology Conference

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From August 31st- September 3rd the biennual EMBO Chemical Biology conference, hosted by the European Molecular Biology Laboratory (EMBL) in Heidelberg took place. The conference series has earned a well-deserved reputation as one of the finest meetings representing this discipline of life science research. A feature of this EMBO meeting is the consistency of the venue (EMBL is in a delightful location after all) and the organisers (Maja Kohn, John Overington and Carsten Schultz). This ensures familiarity and high complementarity with prior meetings. Indeed, a policy of the organising committee is that no speaker can be talk more than once so one should choose their opportunity wisely.

The typical format was adopted where the conference is divided into sections based on topic and talks were a mixture of 4 keynote speakers and 38 speakers, the latter being invited or selected from abstracts. Almost 200 posters were also displayed throughout the meeting.

Innovation in approaches to drug discovery

The first talk of this introductory session was delivered by Gitte Neubauer (Cellzome). Cellzome is a successful EMBL spinout company which is now integrated within Glaxosmithkline (GSK). The founding principal of Cellzome is to employ mass proteomics and chemical biology to drive the drug discovery process, but also to establish target engagement thereby providing much needed insight into drug efficacy and toxicity. Gitte discussed a number of examples employing this strategy leading to the discovery of phosphoinositide 3-kinase γ inhibitors, and their underappreciated off-targets[1]. The next speaker was Jason Gestwicki (UCSF). Jason discussed work that led to the successful identification of pharmacological chaperones that demonstrate potential for treating cataracts[2]. Cataracts arise from amyloid fibril formation in the lens of the eye. The solubility of the lens proteins (crystalins) is maintained by the presence of molecular chaperones known as αA-crystallin (cryAA) and αB-crystallin (cryAB). A disease model for cataracts and differential scanning fluorimetry were used as the basis of a screen for molecules that might stabilize the aggregation-prone mutant cryAB dimer. A class of sterol compounds were found to have stabilizing activity and NMR experiments revealed that the compounds targeted the cryAB dimer interface, thus are likely to stabilize the dimeric state and maintain its solubility. Excitingly, cataract resolution was achieved in mice and in human ex vivo samples. The study demonstrates that the discovery of therapeutic agents against seemingly undruggable targets can be accelerated by the development of innovative screening approaches. Felix Hausch next presented recent work on the discovery of FK506-binding protein 51 (FKBP51) inhibitors that were selective over the highly homologous counterpart FKBP52[3]. Selectivity was imparted via an unanticipated induced fit mechanism. As FKBP51 is an established risk factor for stress-related psychiatric disorders, this discovery potentially opens new avenues for novel antidepressant development. The following talk was delivered by Derek Tan (Memorial Sloan

Kettering Cancer Center). Derek gave an overview of his labs work on the use of sulfonyladenosine-based inhibitors of adenylation enzymes. Adenylation enzymes are involved in a broad repertoire of cellular processes and are promising antibacterial and anticancer targets. Using mechanism- and structure-based design, 5'-O-(N-salicylsulfamoyl)adenosine (salicyl-AMS) was shown to inhibit a key adenylation enzyme involved in siderophore biosynthesis in *Mycobacterium tuberculosis,* with demonstrated efficacy in mouse models of tuberculosis[4]. Olli Pentikainen (University of Jyväskylä) described virtual screening approaches that were associated with rapid execution and excellent success rate for the systems under study[5]. The next speaker was Stephanie Heinzlmeir from Bernhard Kuster's lab (Technical University Munich). Stephanie reported the application of chemical proteomic approaches based on Kinobead technology for assessing the selectivity of kinase inhibitors[6]. Using this approach, Dasatinib was found to have high off-target activity towards the receptor tyrosine kinase EPHA2[7]. Structural biology enabled the elaboration of chemical analogues that were subsequently repurposed by profiling for enhanced EPHA2 selectivity, again using the Kinobead platform. This revealed that certain analogues had enhanced EPHA2 selectivity whilst retaining potency. This work showcased the described strategy as a viable approach for repurposing promiscuous kinase inhibitors and enhancing their selectivity via structure-based design.

The first keynote talk rounded off the session which was delivered by Frances Arnold (California Institute of Technology). She delivered an engaging talk on her pioneering work on directed evolution. A goal of France's lab is to create new enzymes and increase the repertoire of chemical transformations that can be genetically encoded. As an example, the ability of heme proteins to catalyze otherwise difficult to perform carbene- and nitrene-transfer reactions was demonstrated, together with enhancement of their activity via directed evolution[8].

DNA/RNA/Chemical Epigenetics

A new session began the following morning where Chuan He (University of Chicago) described his discovery of a novel class of RNA modifying enzymes. The enzyme demethylases FTO and ALKBH5 were characterized and it was shown that reversible RNA methylation is a prevalent process that could serve a biological regulatory role analogous to epigenetic chromatin modification[9]. Indeed, it was shown that one such role is mRNA transcript synchronization and intriguingly, demethylation of ribosomal and transfer RNA are also observed. Andreas Marx next discussed the evolution of enzymes with new function such as DNA polymerases with reverse transcriptase activity[10]. Innovative chemical biology approaches for preparing proteins bearing site-specific post-translational modifications (PTMs) such as such poly(ADP)ribosylation were also discussed, together with methods for their imaging in cells[11].

Sophisticated semisynthetic strategies have been developed for the preparation of selectively modified chromatin thus serving as a powerful toolkit for addressing the histone code hypothesis[12]. However, the nucleosome building blocks of chromatin consist of dimers of the 4 core histone proteins. Contrary to physiological chromatin, conventional semisynthetic approaches furnish "symmetrically" modified octomeric nucleosomes containing the introduced modification on both of the protomers of a given histone. Beat Fierz (EPFL) described a novel approach to address this caveat by employing latent histone dimer fusions which could be tracelessly converted into native asymmetrically modified

histones via protease treatment[13]. Andres Jaschke (Heidelberg University) introduced the unanticipated presence of 5'-modified RNA in bacteria and characterized a prokaryotic decapping enzyme[14]. Hening Lin (Cornell University) gave a talk on the unappreciated activity of the sirtuin family of deacetylases, their archetypal substrate being acetyl-lysine. However, Hening revealed that certain sirtuin family members, with negligible activity towards acetyl-lysine, were highly active towards succinylated and fatty acylated lysine[15]. This led to the identification of these previously unidentified PTMs. Next, Masafumi Minoshima (Osaka University) spoke on probes of histone deacetylase activity[16], and Richard Hartley (University of Glasgow) presented his work on probes for detecting the reactive oxygen species, superoxide[17].

Protein profiling and target validation

A fitting talk by Stephan Sieber (Technical University Munich) on activity-based protein profiling (ABPP) kicked off the next session. Stephan emphasized that currently only 3 major antibacterial targets are addressed pharmacologically, thus placing emphasis on the need to explore novel target space. Using the established fluorophosphonate-based ABPP platform for serine hydrolases, inhibitors of a novel bacterial target, the ClpP virulence regulator, were identified and selectivity profiled, leading to the discovery of series of phenyl ester inhibitors with improved selectivity and potency over previous beta-lactone-based scaffolds[18]. The following talk was delivered by Edward Tate (Imperial College London) describing the development and application of lipid-based chemical probes, such as "clickable" myristic acid analogues, for the identification of targets of myristoylation. Using these tools, proteome-wide changes in lipidation in response to drug treatment could be assessed. Next, Satpal Virdee (University of Dundee) reported the development of a novel class of activity-based probes for profiling ubiquitin E3 ligases[19]. The utility of these probes was demonstrated by addressing the requirements, and their hierarchy, for the activation of the Parkinson's disease (PD)-associated E3 ligase parkin. Biomarker potential of the probe technology for disease predisposition was also described. Next, Robin Bon (University of Leeds) presented work on the brain-penetrable compound KHS101 which was found to induce self-destruction of gliobastoma muliforme cells. KHS101 derivatives bearing benzophenone photocrosslinking groups were subsequently employed to identify the target of KHS101 via chemical proteomic methods.

The evening was rounded off with a second keynote talk delivered by Horst Kessler (Technical University Munich). Horst discussed his pioneering work on the development of peptide-based inhibitors and methods for enhancing their activity though methods such as cyclisation and modification of guanidine groups (N-methylation, N-alkylation, or N-acylation)[20]. Selective inhibitors against distinct integrin subtypes were also presented and how these tools can be used to study the development of diseases that may involve specific integrin family members[21]. Horst also talked about combination therapies involving peptide inhibitors and the use of peptides in PET-based imaging[22].

Peptide and protein engineering

This new topic began with a talk from Christian Heinis (EPFL). This talk focused on an innovative fusion of phage display and peptide modification to create bicyclic peptides. The technology involves phage-based selection of peptides which are rendered bicyclic through

covalent modification with a small molecular aromatic core[23]. This produces chemical entities which demonstrate the potency and specificity of biologics but with pharmacokinetic properties more akin to small molecules. Christian described selection methods to derive highly potent inhibitors for a number of extracellular targets and approaches to optimise the technology were also discussed. Next, Hiroaki Suga (The University of Tokyo) presented an alternative platform for generating libraries of peptide-based inhibitors termed Random non-standard Peptide Integrated Discovery (RaPID). The technology involves in vitro translation of mRNA libraries which produces peptides containing non-proteinogenic amino acids that undergo spontaneous posttranslational cyclisation. As with Christians' strategy, the peptides can be screened for binding against a protein target (e.g. the ubiquitin E3 ligase E6AP) and in this case the genotype-phenotype link is retained via tethering of the mRNA sequence to an obligate amino acid present in the peptide[24]. The following speaker was Yongjoo Bill Kim (Harvard University) from David Liu's lab. The therapeutic potential of genome engineering approaches such as CRISPR/Cas9 technology is currently hampered by the off-target effects associated with the editing process. Conventional CRSIPR/Cas9 technology requires cutting of the DNA which can give rise to random insertions and deletions. The strategy presented by Yongjoo involved the use of catalytically inactive Cas9 fused to a cytidine deaminase. This enabled site-specific "base editing" where cytidine was converted to thymidine and this strategy was exemplified by correcting a number of disease-relevant point mutations[25]. Edward Lemke (EMBL, Germany) described approaches for the biorthogonal, dual-colour labelling of proteins in cells[26]. These were enabled by a combination of genetic code expansion and ultrafast "tetrazine ligation" methods. The utility of these approaches for super-resolution microscopy was also discussed[27]. Next, Anna Mapp spoke on strategies for targeting transient protein-protein interactions (PPIs). Taking transcriptional activator-coactivator interactions as a system, Anna reported a strategy where particular conformations of transcriptional coactivators are engaged thereby modulating the assembly of active transactivator-coactivator complexes[28]. The next speaker was Wesley Robertson (Yale University) from Alanna Schepartz's lab. Despite significant progress made in the area of genetic code expansion techniques, all non-canonical amino acids that can currently be incorporated into proteins typically retain a standard backbone. The ability to incorporate variants, such as β-amino acids, could grant access to biological macromolecules with desirable properties such as enhanced proteolytic stability. Wesley presented work on evolved ribosomes that could incorporate β-phenylalanine analogues into full-length protein thereby demonstrating the potential to prepare backbone perturbed polymeric materials with new function[29].

The next talk by Sanne Schoffelen (University of Copenhagen) described a novel method for site-selective protein labelling. This involved the introduction of azide functionality into proteins via a chemical diazotransfer reaction, which could be performed under aqueous and near-neutral pH conditions[30]. Intriguingly, the reaction appeared to be sensitive to the reduced pK_a of N-terminal amino groups thereby rendering the approach site-specific. This presents a valuable addition to protein-labelling toolkit by broadening the applicability of Click-based bioconjugations.

Natural products/second messengers

The first talk of this session was by Christopher Schofield (University of Oxford). An aspect of the talk was on prolyl hydroxylation carried out by prolyl hydrolase isoforms 1-3

(PHD1-3) under normoxic conditions. PHD's are attractive therapeutic targets and non-isoform selective inhibitors are in clinical trials for the treatment of anaemia. The substrate of PHD's are the N- and C-terminal oxygen-dependent degradation domains (NODD and CODD) present in HIFα isoforms. Chris reported PHD2 variants in erythrocytosis and cancer that were highly specific for NODD or CODD and the basis for this specificity was characterized by biophysical and cellular methods[31] . Next, Dorothea Fiedler (Princeton University) spoke on the establishment of an enrichment and subsequent proteomic strategy for detecting substrates for the posttranslational modification, pyrophosphorylation[32]. Pyrophosphorylation occurs through enzyme-independent modification of existing Ser-phosphorylated residues by reaction with inositol pyrophosphates. The importance of the enzymes involved in the biosynthesis of inositol pyrophosphates was highlighted thereby placing a need to determine what the substrates of pyrophosphorylation are and what cellular function this process might mediate. Next, Dowine de Bruijn (University of Groningen) introduced another strategy for chemoselective protein modification. The method involved the introduction of a terminal dehydroalanine residue into recombinant protein, which was then modified by oxidative Heck reaction. Kalie Mix (UW-Masison) from Ronald Raines' lab reported a novel approach for decorating the surface of proteins with ester-linked bioreversible cell penetrating tags by O-alkylation of carboxylic acids with diazo compounds[33]. The high esterase activity of mammalian cells subsequently hydrolyses the tags thereby proving valuable for cases where irreversible protein modification might compromise biological activity of the cargo. The following talk was by Miriam Fontanillo (EMBL Heidelberg) from Maja Köhn's lab. Miriam highlighted the roles of phosphatases in diseases such as cancer and Alzheimer's and how imparting inhibitor selectivity to one of the two main phosphatases (PP1 and PP2A) has remained challenging. A series of synthetic analogues of naturally occurring microcystins were prepared by peptide synthesis, in good yields, producing the first microcystin analogues with high selectivity for PP2A (282-fold)[34].

A thought-provoking third keynote talk followed, which was delivered by Giulio Superti-Furga (CeMM Center for Molecular Biology of the Austrian Academy of Sciences). This talk introduced the importance of solute carrier proteins (SLC's) - transmembrane proteins responsible for the cellular import of the majority of chemical matter. A result of Giulio's studies was the discovery that SLC's are responsible for the uptake of a number of cytotoxic drugs. This was realized through screening haploid cell line libraries containing individual gene disruptions[35]. A particular example was the discovery of SLC35F2 as the SLC responsible for the uptake of the clinically evaluated anticancer compound YM155[36]. These discoveries place emphasis on the importance of identifying SLCs responsible for compound uptake in the early stages of drug discovery (e.g. to assess limitations that might arise from SLC heterogeneity).

Light-based tools

To start off the session, Robert Tampé (Johann Wolfgang Goethe-University Frankfurt) presented a new optochemical toolbox for the controlled assembly of receptor networks[37]. Additionally, specific labelling approaches, conducive to super-resolution live cell imaging, were also described. These involved the introduction of a fluorescent *tris*NTA probe which selectively labelled proteins bearing polyhistidine tags[38]. Jennifer Prescher (University of California, Irvine) next spoke on new tools for imaging cellular interactions. As examples, both caged luciferins and split luciferase were used to form the basis of cellular proximity

sensors[39]. Jon Beck (University of Nebraska-Lincoln), a student in Cliff Stain's lab, presented his work on sensors of tyrosine phosphatase activity with picomolar limits of detection[40] and Manuela Zaccolo (University of Oxford) discussed novel FRET-based reporters that detect compartmentalised cAMP with unprecedented spatial resolution. Dmytro Yushchenko (EMBL Heidelberg) from Carsten Schultz's lab introduced tools for studying lipid signaling such as caged arachidonic acid derivatives and photoswitchable diacylglycerols (DAGs)[41]. The next talk was presented by James frank (LMU Munich) from Dirk Trauner's lab. Therein, application of the previously described photoswitchable DAGs was employed which upon UV irradiation promote translocation of C1-domain-containing proteins to the plasma membrane. The ability to reverse the processes enabled the generation of oscillation patterns of DAG activity. This enabled the proteins protein kinase C and Munc13 to be placed under optical control thereby controlling vesicle release[41b]. The next talk was presented by Zbigniew Pianowski (KIT Karlsruhe) which described the use of photoswitchable hydrogels that had potential as a light-inducible delivery system for drugs or therapeutic oligonucleotides[42].

The closing talk was a keynote from Paul Wender (Stanford University). Paul's talk summarized a large body of his inspirational work which was abound with translational implications. The first part described work towards eradicating HIV/AIDS. One aspect sought to address the challenges in eliminating persistent HIV reservoirs in latently infected immune cells. One strategy towards this was to induce HIV expression in these cells allowing their depletion through cytopathic effects[43]. This was achieved by synthesizing a series of prostratin analogs which had significantly higher potency over the promising existing preclinical lead.

The excellent posters should also not be forgotten with nearly 200 being displayed throughout the conference. Numerous prizes were awarded which were sponsored by ChemBioChem (winners: Fleur Kleinpenning (Radboud University) and Christian Martin H (Institute for Scientific Research and Technology Services, Panama)), ACS Chemical Biology (winner: Joseph Rogers (University of Tokyo)), Royal Society of Chemistry (winner: Ulrik Bering Keiding (Aarhus University)) and Journal of Biological Chemistry (winner: Sabine Studer (ETH Zurich)).

Summary

Overall the quality of the chemical biology presented at the EMBO 2016 Chemical Biology conference was exceptional. Innovative approaches are enabling the targeting of previously considered undruggable proteins with small molecules. The perceived notion of many targets as being undruggable will surely continue to be unfounded as the field of chemical biology continues to flourish, thereby furnishing novel therapeutic targets for the future. However, the very nature of certain targets such as PPIs certainly do pose unique challenges but technologies such as the reported cyclic peptidic systems are perhaps one route to address this. The activity-based protein profiling platform continues to be a mainstay of many chemical biology programmes and extension to microorganisms and novel enzyme families, as described, will further its utility in identify novel enzyme family members and therapeutic targets. What was also clearly apparent was the level of precision with which we can now perturb cellular signaling pathways with innocuous methods such as light exposure. In particular, the use of diazobenzenes as reversible photoswitches is proving to be particularly valuable technology which is now routinely being used in creative ways. Such

methods are proving to be broadly applicable and so should continue to reveal insight into many biological systems.

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