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Conference Report

2016 International Symposium on Chemical Biology of the NCCR Chemical Biology

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In the last five years, the members of the National Centre of Competence in Research (NCCR) Chemical Biology 'Visualisation and Control of Biological Processes Using Chemistry' established an excellent environment for research at the interface of chemistry and biology at the arc lémanique, encompassing researchers from the University of Geneva and the École polytechnique fédérale de Lausanne. The NCCR Chemical Biology provides a dynamic mix of established and junior groups that are collaborating on six large interdisciplinary projects to develop novel chemical tools and pursue exciting biological questions. This year's international symposium on Chemical Biology marked a high point in this collaboration between groups from the University of Geneva, EPFL and Fribourg. The organizing committee led by ***Nicolas Winssinger*** (UniGE) gathered 15 international leaders in the field and the resulting high-powered program attracted over 250 participants from all over Europe to Geneva. The participants thus spent three days in the light-suffused glass buildings of the Campus Biotech, learning about the newest and hottest developments in chemical biology and exchanging ideas in front of posters and over glasses of local beer and wine.

Main building of the Campus Biotech in Geneva. Photo by © Brigitte Djajasasmita; Licence Creative Commons BY-NC-ND 4.0.

On Wednesday evening the NCCR symposium on Chemical Biology opened with a fascinating keynote lecture given by ***Ben F. Cravatt*** (Scripps Research Institute). In his talk, Dr. Cravatt described novel and exciting developments in activity-based proteomic profiling (ABPP) of enzymes. This chemical proteomics strategy allows profiling the activity of enzyme classes in their biological environment, and to screen for specific inhibitors.[1] Focusing on the diverse family of serine hydrolases, Dr. Cravatt presented the development of pharmaceutical approaches to modulate their activity in specific disease settings. A neurodegenerative disease called PHARC results from mutations in a specific serine hydrolase gene, ABHD12. Lipidomic profiling revealed the function of the enzyme: The degradation of lysophosphatidylserine (lysoPS), which in high concentrations proved to be neuroinflammatory. Importantly, lysoPS is produced from the lipid phosphatidylserine by a lipase called BAT5. The Cravatt group then employed ABPP and discovered a potent BAT5 inhibitor (KC01) – similar compounds may thus lead to promising avenues for treatment of PHARC.[2] In final part of his talk, Dr. Cravatt then outlined extended applications of ABPP to target functional cysteines across the proteome, with applications in discovering covalent inhibitor for previously undruggable disease targets. Together, this lecture perfectly outlined key goals of chemical biology, namely combining discovery of novel activities and molecular pathways in living systems with the development of specific small-molecule chemical probes, and eventually drugs, to modulate biological function in disease.

The second lecture of this first evening session was held by ***Jeffrey Glenn*** (Stanford University). The Glenn lab is running an innovative research program that aims at identifying novel antiviral targets of either the virus itself or the host cells. In his talk, he provided an exciting overview on new approaches on targeting the Hepatitis Delta Virus (HDV). This represents an urgent medical problem given the large number of infections (~15 million) worldwide, the inadequate HDV therapies and possible progression of an HDV infection to liver cirrhosis.[3] During virus maturation, a genome editing event leads to the production of a larger variant of the delta virus antigen that adds a CAAX-box on its longer C-terminus, which becomes farnesylated and thus membrane anchored. Farnesylation of the delta antigen is functionally critical for virus particle maturation and thus provides a good target for pharmacological intervention. Importantly, several potent farnesyltransferase inhibitors, including lonafarnib, have been in clinical trial for several oncology indications, as H- and K-Ras oncoprotein signaling are critically dependent on farnesylation. Treatment of HDV-infected cells with lonafarnib prevents farnesylation of the large delta antigen and decreased virus particle maturation in a humanized mouse model. Dr. Glenn subsequently presented highly encouraging results of different clinical trials that showed a strong reduction in viral load in HDV-infected patients.[4] A common side-effect of farnesyltransferase inhibitors at higher doses is gastrointestinal toxicity, which is dose-limiting and needs to be balanced when choosing the appropriate dose for HDV treatment in humans. Overall, this interesting example how detailed studies of biological mechanisms can pave the way for re-purposing of a drug.

These first two stimulating talks opened the rest of the evening, where all participants and speakers enjoy the first round of poster presentations, some snacks and drinks.

On Thursday morning, ***Kevan Shokat*** (University of California, San Francisco) presented exciting new approaches to

target the mTOR kinase and the Ras GTPase, both of which are among the most challenging drug targets in oncology. mTOR can be targeted by the natural product rapamycin and its derivatives (rapalogues), which act as allosteric inhibitors. More recently, ATP-competitive inhibitors that directly target the active site of the mTOR kinase domain were developed. Both types of drugs may easily lose their potency by single point mutations in the respective binding domains. Based on the recently solved crystal structures of mTOR fragments, the Shokat lab has synthesized a bivalent inhibitor that targets both sites on mTOR simultaneously. Intriguingly, even mTOR mutants in which both drug binding sites were mutated were sensitive to the bivalent inhibitor. Dr. Shokat also presented two strategies to target Ras signaling in cancer cells. First, a compound that reacts with and blocks the Ras farnesylation site was delineated: It is generally difficult to make specific cysteine-reactive compounds that are not in an active site of an enzyme, such as the cysteine residue in the Ras C-terminus that gets farnesylated. The Shokat lab produced farnesyl-competitive inhibitors that act as neo-substrate and block Ras farnesylation. Compounds with a reasonable GC50 in the low micromolar range were presented and are awaiting further characterization. Pioneering work of the Shokat lab has recently identified direct inhibitors of the oncogenic G12C mutant form of Ras from a fragment-based tethered compound screen.[5] Structural analysis revealed that these compounds exploit a novel pocket that is formed in the GDP-bound state of Ras. By using the novel compound ARS-853, Shokat showed that the nucleotide state of the RAS G12C mutant is in a state of dynamic flux and represents a hyper-excitable rather than a constitutively active state.[6] These finding provides strong evidence that targeting the inactive, GDP-bound form of RAS is a promising new approach for generating novel RAS inhibitors.

A major aim of chemical biology approaches is to observe biological processes while they proceed in living systems. In his presentation, ***Christian Eggeling*** (Oxford University) outlined the formidable problem of directly observing processes in living cells using optical microscopy, as the involved spatial (nm to μm) and temporal (μs to hours) scales are vast and span many orders of magnitude. Recent developments in microscopy techniques, including super-resolution or lattice light-sheet microscopy, fill specific roles by either providing high spatial or temporal resolution, high tissue penetration or allowing long-time observations. There is, however, a great need to develop novel imaging modalities which combine several of these advantages to gain insight into complex biology of living cells. Indeed, Dr. Eggeling established together with Stefan Hell (MPI Göttingen) a method to parallelize super-resolution nanoscopy by the RESOLFT method (based on switchable fluorescent proteins), which allows to speed up imaging by over five orders of magnitude. This enabled the imaging of whole cells with sub-second temporal resolution.[7] In a second part of the lecture, Dr. Eggeling then focused on a major problem in biophysics of biological membranes: The detection of lipid rafts, proposed nanoscopic domains of proteins and lipids which organize cellular signaling clusters on the surface of living cells. Using a novel technique, combining super-resolution STED microscopy with fluorescence correlation spectroscopy (FCS), Dr. Eggeling and his team were able to measure the diffusional behavior of fluorescently labeled lipids on the nanoscale, thereby demonstrating local heterogeneity, *i.e.* raft formation, in lipid membranes as a function of the lipid environment.[8] Combined, these experimental systems now pave the way for in-depth molecular investigations of cellular signaling in complex biological systems, in particular in the field of immunity where Eggeling and his group are currently active.

Visualizing biological processes does not only require novel instrumentation, but also the development of highly sensitive sensors that can be introduced into biological systems. In his exciting presentation, ***Adam Cohen*** (Harvard University) showcased how protein engineering can provide a window into neuronal signaling itself. Channelrhodopsins, membrane ion-channels that can be opened and closed by light, are the key workhorses for optogenetics and allow controlling neural activity by light. Dr. Cohen realized that some members of this family, in particular specific archaerhodopsins, exhibit fluorescence emission that is dependent on the local membrane potential.[9] This makes them excellent optical reporters of neuronal signaling, and he thus coined the term 'optopatch' as an optical equivalent for the classical method, the invasive 'patch-clamp' method used for direct measurements of electrical currents. The Cohen group then managed to combine channelrhodopsins (for local switching of membrane potential) with optopatch (for a real-time readout of membrane depolarization) in single neurons.[10] With a specially constructed microscope that allows highly accurate light stimulation of specific areas in neuronal cells (*e.g.* the axons or single dendrites) Cohen and his team managed to directly measure signal propagation in single and multiple connected neurons. Cohen then described how this methodology can be applied to study human disease, in particular amyotrophic lateral sclerosis (ALS), where motor neurons progressively die resulting in paralysis and death. In neurons generated from ALS patient tissues Dr. Cohen's team could detect irregular signaling, that could be alleviated by addition of drugs which are now in clinical trials.

The talk of ***Herbert Waldmann*** (Max-Planck-Institut für molekulare Physiologie, Dortmund) was focused around natural products, which are very successful, potent and common drugs, but suffer from their structural complexity that make them challenging for synthetic chemistry and difficult to build large compound collections for screening. The Waldmann lab aims to systematically analyze the chemical structure and map the chemical space of natural products to derive a classification and logic of their core structures to derive natural product-inspired compound collections with reduced chemical complexity but retained biological activity. This pioneering work has led to a 'Periodic Table' of natural products.[11] Dr. Waldmann then presented several elegant examples of natural-product inspired scaffolds that are easily amenable to chemical modification so that libraries of a few dozen compounds can be synthesized readily. These were shown to give rise to reasonably potent and diverse biological activities, such as a Hedgehog inhibitor, a modulator of autophagy and an inhibitor of cell proliferation.[12] The last part of the talk focused on a critical bottleneck in drug discovery, in particular when using natural products, is the identification of the biologically relevant target(s) from phenotypes observed in cell-based screens. Often a combination of intuition (educated guesses), chemoinformatics approaches, as well as systematic experimental methods, such as chemical proteomics can be used. Dr. Waldman presented the intriguing case of a modulator of centrosome integrity that target nucleophosmin and the nuclear export factor CRM1.

After lunch in the lobby of the Campus Biotech building and simulating discussions in front of an excellent selection of posters, ***Alanna Schepartz*** (Yale University) opened the afternoon session with an intriguing lecture on the molecular mechanism of cellular signaling. The EGFR is a key receptor tyrosine kinase controlling cell growth and differentiation and EGFR mutations are critically implicated in various types of cancer. An intracellular juxtamembrane (JM) peptide segment plays a key role in information transfer through the membrane as its structure changes upon receptor activation. Dr. Schepartz and her team used an elegant set of experiment to characterize structural transitions in the JM. To this end, they employed a dye (ReAsH) that only becomes fluorescent when interacting with a two pairs of cysteine residues that are specifically arranged

Keynote speaker Dr. Ben Cravatt at one of the poster sessions.

Dr. Ulrike Eggert answering questions from the audience after her talk.

in three dimensional space (a method called bipartite tetracysteine display).[13] Indeed, upon binding to its cognate ligand (EGF), the JM transitioned into an antiparallel coiled-coil state. Surprisingly, a different ligand (TNFα), involved in suppressing EGFR signalling, induced a different conformation (an 'inside-out' coil-coil), resulting in a different downstream signaling outcome. Importantly, EGFR-carrying oncogenic mutations can lose the ability to adopt either one or the other conformations in a ligand-dependent fashion.[14] Investigating the contribution of coiled-coil identity to the oncogenic activity of mutant EGFR might guide the development of selective EGFR inhibitors for cancer therapy.

The beautiful but mind-blowingly complex cell division process has fascinated biologists for more than 100 years. ***Ulrike Eggert*** (King's College London) highlighted the innovative approaches that her lab uses to study cell division using a variety of chemical biology strategies. First, an in-depth study that identified an off-target action of Prazosin, a commonly used drug to treat hypertension, mediated by the G protein-coupled receptor dopamine receptor D(3) (DRD3) was presented.[15] They hypothesized that Prazosin potently inhibits cytokinesis by stabilizing a normally transient interaction between DRD3 and the membrane coating protein COPI. This complex may be involved in membrane transport, and dramatically shifts the morphology of endocytic vesicles. This work established prazosin as a powerful tool for the rapid and reversible perturbation of endocytic dynamics and further highlighted the close and underappreciated connection of endocytosis and cytokinesis. In the second part of her talk, Eggert presented exciting recent data in which her lab used unbiased lipidomics to map lipids that are differentially regulated during cell division.[16] A dozen lipid species were shown to accumulate in dividing cells and to be responsible for differences in the mechanical properties as opposed to non-dividing cells. Excitingly, it could be shown that cells regulate the localization of lipids to midbodies, which are the membrane-based structures where cell cleavage occurs.

Effective strategies to specifically target tumor growth are urgently needed but are still challenging to establish. Addressing this problem, ***Nathanael Gray*** (Dana-Farber Cancer Institute/ Harvard University) presented his pioneering approaches for the development of covalent inhibitors targeting different oncoproteins, which often display superior selectivity.[17] In his lecture, Gray described methodologies exploiting high throughput screening strategies and structural analysis to develop and test small molecules. One promising candidate compound (THZ1), which was previously discovered in Gray's laboratory, is targeting the cyclin-dependent kinase 7 (CDK7), which is involved in transcriptional regulation. THZ1 uses a cysteine residue in the C-terminus of CDK7 that folds back to the active site and with which the drug reacts. The wide range of sensitivities of cancer cell lines to THZ1 was explained by the regulation of super-enhancer dependent target genes by CDK7 that include the major cancer driver MYC.[18] Treatment of mice with THZ1 in a small cell lung cancer (SCLC) xenograft model resulted in a remarkable reduction of tumor growth. Complementary to the presentation of Kevan Shokat, Gray also presented progress on targeting K-Ras G12C using innovative covalent compound libraries with acryl- and iodacetamide-electrophilic handles.

Presenting the full picture from biochemical analysis to mice models, Gray showed an intriguing and fascinating story of how to develop alternative inhibitors paving the way for new and modern cancer therapies.

On Friday morning, ***Ben Davis*** (University of Oxford) delved into the chemistry of oligosaccharides and its applications, thereby shifting our focus from proteins and lipids to this further fundamental family of bio-macromolecules. Bacteria form cell walls; some are waxy coats containing complex oligosaccharides linked to lipids. The cell wall of *Mycobacterium tuberculosis*, the causative agent of tuberculosis, contains lipid-modified forms of the disaccharide trehalose, which is not found in mammalian cells. Using a library of substituted trehalose molecules, the Davis team realized that the enzyme linking trehalose to lipids in the mycobacterial cell wall is quite tolerant in its substrates. Indeed, fluorescently labeled trehalose was readily accepted and incorporated into *M. tuberculosis*, rendering the bacteria fluorescent and straightforward to detect.[19] This opens the way for novel rapid tuberculosis screening technologies. To combat bacterial infections, new antibiotics are urgently needed as resistance against third- and fourth-line compounds is rapidly arising. Tunicamycins, fatty acyl nucleoside antibiotics, inhibit bacterial peptidoglycan synthesis but are toxic for mammalian cells, thus precluding their clinical application. In an effort to develop tunicamycins with reduced cytotoxicity against mammalian cells, Davis and his group deciphered the tunicamycin biosynthetic pathway resulting in their unusual carbon framework. Intriguingly, their work suggests that the key intermediate tunicamine is produced *via* an enzyme-catalyzed radical reaction from an exo-glycal precursor not previously observed in biology. Davis then showed that the antibacterial activity of tunicamycins is dependent on the fatty acid moieties, which may thus provide opportunities for increased specificity and the clinical development of tunicamycin-based antibiotics. In the final part of his lecture, Davis then discussed new strategies to target gram-negative bacteria by targeting polysaccharide transporters with glycomimetic pore blockers as well as by the development of novel vaccines against lipo-polysaccharides, the core component of the gram-negative cell wall.[20]

David Liu (Harvard University) started his impressive presentation by introducing DNA-templated synthesis, which has been pioneered in his lab.[21] Using this approach, a new compound (6bK) inhibiting the Insulin Degrading Enzyme (IDE) has been selected. IDE is a zinc protease for which no inhibitors existed and an important target in diabetes. 6bK improved the glucose tolerance in diet-induced obese (DIO) mice when glucose was administered orally but not upon intravenous injection of glucose. This led to the discovery that IDE also degrades glucagon and the development of 2nd and 3rd generation IDE inhibitors that are insulin-specific *in vitro* and *in vivo*. During the second part of his talk, Liu presented another ground-breaking technology that his lab has developed: phage-assisted continuous evolution (PACE). This system allows a very rapid and directed evolution of genes in a manner that is dependent on the activity of interest by linking it to the bacteriophage life cycle, as he showed on the examples of an RNA polymerase, TEV protease and even a widely used insecticide.[22] Liu's talk demonstrated the potency and reach of such innovative methods and how the development of new compounds can also lead to discoveries in basic research.

After a short coffee break, ***Priscilla Yang*** (Harvard University) presented her innovative work on viral pathogens and the identification of novel antiviral targets, in particular host proteins and lipids that are required for viral replication. Her work on Dengue virus, the most widespread mosquito-transmitted virus, using a kinase inhibitor libraries has identified several kinase inhibitors that interfere with the Dengue virus life cycle at several stages.[23] The allosteric Abl kinase inhibitor GNF-2 was discovered to inhibit Dengue virus through its kinase activity but also through inhibition of the Dengue virus E (envelope) protein. Structure–activity studies of GNF-2 allowed dissection of the critical molecular determinants involved in its anti-Dengue activity and enabled the synthesis of GNF-2 derivatives useful in establishing GNF-2's dual mechanisms of action. As highlighted in the talk of Dr. Eggert, lipid composition of cells may dramatically change upon virus infection. Dr. Yang's group has also used an untargeted lipidomics approach to study changes in lipid composition upon hepatitis C virus (HCV) infection.[24] Among the strongest deregulated lipids that were identified in this study was desmosterol, a close metabolite to cholesterol. Depletion of desmosterol reduced HCV replication, and addition of exogenous desmosterol, but not cholesterol, rescues HCV replication, further implicating desmosterol as specifically required by HCV.

The lunch break allowed another opportunity to interact with the various poster presenters and discuss their interesting projects. Back in the lecture hall, ***Dirk Trauner*** (Ludwig-Maximilians-University Munich) described his work on photopharmacology, which uses azobenzenes that are photoswitchable at various wavelength depending on its derivatization. Over the past decade, the Trauner lab has used these simple, yet extremely powerful photoswitches in the context of various biological systems. Impressive examples of photoswitchable ion channels and lipids that enable the control of ion flux and lipid phase transitions were developed in the Trauner lab. The first photoswitchable inhibitor of microtubule dynamics that allows to optically control mitosis and cell death was presented.[25] In the last part of his talk, Trauner presented the conversion of a G protein-coupled receptor into a photoreceptor. The Trauner group used photo-switchable orthogonal remotely tethered ligand (PORTL), which combines genetically encoded self-labeling tags, such as the SNAP tag, with photochromic ligands connected to a benzylguanine (SNAP-tag ligand) *via* a flexible linker.[26] This method enables efficient optical control over the neuronal function of GPCRs.

One of the biggest challenging in drug development by the pharmaceutical industry is the selection of drug targets. Providing an industry perspective, ***Mark Bunnage*** (Pfizer, Oxford) stressed the importance of appropriate target validation using chemical probes to improve drug discovery efforts. Based on an evaluation of drug development programs in Pfizer, Bunnage then outlined four pillars of cell-based target-validation using quality chemical probes: 1) Probes need to reach the targets in sufficient concentrations, 2) they should bind their protein targets specifically and efficiently, 3) they should show efficacy in biological assays and finally, 4) they should produce a perturbed cellular phenotype of relevance to human disease.[27] In the following, Bunnage discussed recent efforts to develop chemical probes against a particularly interesting class of proteins, epigenetic reader/writer/eraser proteins. Firstly, he focused on bromodomain proteins, widespread readers of histone lysine acetylation. SMARCA2/4 is a SWI/SNF chromatin remodeling complex, a large protein machine that alters the packing of the genetic material and is involved in cancer progression. A novel chemical probe was developed to specifically target the epigenetic reader domains of SMARCA2/4. Applying the probe in embryonic stem-cells revealed an important function the remodeler in maintaining the stem-cell properties and lineage determination.[28] Moving to protein methyltransferases, Bunnage then discussed the development of a novel probe against SETD7, an enzyme targeting histones as well as other protein targets. Intriguingly, using this compound it was found that SETD7 is not only involved in epigenetic regulation, but also modulates reactive oxygen species (ROS) homeostasis in cells, showcasing the power of chemical probes in elucidating complex biological pathways.

In the final lecture of this stimulating conference, ***Daniel Müller*** (ETH Zürich, Basel), demonstrated the role of physical chemistry and physics in observing the cellular machinery at work. Atomic force microscopy, where a thin cantilever is deflected by molecular interactions, allows direct binding energy measurements of ligands with their receptors by measuring the mechanical force required to dissociate the complex. Using this methodology, Müller and his team quantified the energy landscape of interactions of a variety of ligands with a G-protein coupled receptor to gain a deep understanding of its ligand selection mechanisms.[29] Cantilevers can however also be used to study the mechanics of whole cells. When cells undergo mitosis, they change shape and assume a rounded form. If cell rounding fails, mitosis is disturbed which can lead to apoptosis or abnormal phenotypes. Using force measurements combined with fluorescent microscopy, the Müller group measured the rounding force to be very strong – too strong to be produced by molecular motor proteins. Biochemical investigations then revealed that the actin cytoskeleton together with increased osmotic pressure provided the necessary force and shape change for cell rounding. Importantly, a feedback mechanism signals if cells fail to adopt the correct shape. Using microfabricated surface features – micropillars – Müller and his team tested how cells behave when space is restricted. Intriguingly, they found that cells try to escape from tight space, climbing the micropillars, which allows them to assume the correct shape for mitosis.[30] If this fails they however undergo apoptosis to prevent the development of deregulated cellular phenotypes.

After three days of excellent lectures from the forefront of chemical biology research worldwide, and stimulating discussions at posters of the international participants, the symposium drew to a close. The participants and members of the NCCR chemical biology left the Campus Biotech on Friday night inspired and with novel ideas to make an impact in this exciting field of research.

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