2016 International Symposium on CHEMICAL BIOLOGY

PROGRAMME //
PROGRAME // Wednesday January 13th

15:45 Opening of registration desk - main entrance

16:25 Introductory remarks by NCCR directors

16:30 Keynote lecture

Activity-based proteomics – applications for protein and ligand discovery
Prof. Ben Cravatt - Dept. of Chemical Physiology, The Scripps Research Institute, California / USA

17:30 Targeting prenylation to inhibit hepatitis delta virus infections
Prof. Jeffrey Glenn - Stanford School of Medicine, California / USA

18:30 Poster session (Group A #1 to 34) | Wine & cheese
ProgrAMME // Thursday January 14th

8:30 Nontraditional chemical approaches for drugging traditional targets: kinases and GTPases
Prof. Kevan Shokat - Cellular and Molecular Pharmacology, University of California San Francisco / USA

9:30 Advances in super-resolution STED microscopy: prospects for studying membrane bioactivity
Dr. Christian Eggeling - MRC Human Immunology Unit & Wolfson Imaging Centre Oxford, Weatherall Institute of Molecular Medicine, University of Oxford / UK

10:30 Coffee break

11:00 Neuronal disease modeling with all-optical electrophysiology
Prof. Adam Cohen – Dept. of Chemistry, Chemical Biology and Physics, Harvard University, Massachusetts / USA

12:00 Hunting the targets of natural product inspired compounds
Prof. Herbert Waldmann - Dept. of Chemical Biology, Max-Planck-Institut für molekulare Physiologie / Germany

13:00 Lunch + Poster session (Group A #1 to 34) + Academic speed dating with the speakers

14:30 Moving chemical information through the plasma membrane
Prof. Alanna Schepartz – Dept. of Chemistry, Yale University, Connecticut / USA

15:30 A chemical approach to understanding cell division
Prof. Ulrike Eggert - Department of Chemistry/ Randall Division of Cell and Molecular Biophysics, King’s College London / UK

16:30 Coffee break

17:00 Developing covalent inhibitors of Her3, KRAS and CDK7
Prof. Nathaniel Gray - Dana-Farber Cancer Institute & Harvard Medical School, Massachusetts / USA

18:00 Publishing with eLife
Prof. Ben Cravatt – Reviewing Editor, eLife - Dept. of Chemical Physiology, The Scripps Research Institute, California / USA

18:10 Poster session (Group B #35 to 68) | Beer & cheese

8:30 Sugars and proteins
Prof. Ben Davis - Chemistry Department, University of Oxford / UK

9:30 Integrating chemistry and evolution to illuminate biology and enable next-generation therapeutics
Prof. David Liu – Dept. of Chemistry and Chemical Biology, Harvard University & Howard Hughes Medical Institute, Massachusetts / USA

10:30 Coffee break

11:00 Chemical biology studies of new antiviral targets
Prof. Priscilla Yang – Dept. of Microbiology and Immunobiology, Harvard Medical School, Massachusetts / USA

12:00 Lunch + Poster session (Group B #35 to 68) + Academic speed dating with the speakers

14:00 Photopharmacology and the restoration of vision
Prof. Dirk Trauner - Dept. of Chemistry, Ludwig-Maximilians-Universität / Germany

15:00 Chemical biology in drug discovery: a pharma perspective
Prof. Mark Bunnage – Pfizer & University of Oxford / UK

16:00 Coffee break

16:30 Towards understanding and controlling cell rounding in mitosis
Prof. Daniel Müller - NCCR Molecular Systems Engineering, ETH Basel / Switzerland

17:30 Concluding remarks by NCCR directors
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The diamide class of insecticides are one of the front-line defences in crop protection with regards to insects control. However, cases of resistance against these insecticides have started emerging. To this end, we have synthesised a range of chemical probes to identify the targets, particularly P450 enzymes which may be implicated in the rapid detoxification processes. Our strategy involves chemical modification of a model diamide insecticide to install a warhead and a click handle. The probes synthesised possess excellent in vitro activity, in the nM range, and are metabolised in a similar way to the parent diamide. Overall, this work will hopefully lead to a better understanding of the P450-mediated metabolism of insecticides and help design more robust analogues to circumvent metabolic resistance, thereby ensuring food security for the additionally growing world population.

References
(4) Harvey, M.; Pilgrim, S. Food Policy 2011, 36, 540.

TOR is an essential kinase conserved across Eukaryota. S. cerevisiae has TOR-1/2 proteins nucleating 2 complexes: TORC1 & TORC2 that regulate cell growth. Cerevisiae TORC2 localizes at Plasma Membrane (PM) in MCTs (Membrane Compartment containing TORC2) that are adjacent to esosomes. Increased PM tension activates TORC2 which involves release & relocalization of Slm1/2 (Synthetic Lethal with Mss4) proteins from esosomes to MCTs. Activated TORC2 triggers sphingolipids synthesis which reduces PM tension. Mechanistic details of this pathway remain elusive. To understand how PM tension regulates TORC2 signaling, we took a structural-biochemistry approach. We purified functional Slm proteins that are used in crystal trials & biochemical assays.

References
Lipids serve not only as sources of energy and integral components of cellular membranes, but are ubiquitously involved in intercellular communication. Usually localized on the plasma membrane, they participate in a wide range of signaling cascades through interaction with transmembrane and membrane-associated proteins. Fatty acids are lipids that are composed of a long linear carbon chain, and are components of a variety of more elaborate lipids. Here, we present a series of 8 photoswitchable fatty acids (FAAzos), which contain an azobenzene photoswitch at varying positions along the carbon chain. The FAAzos can be used as modular building blocks for the construction of other photoswitchable lipids, now coined photolipids.

By modifying the FAAzos to resemble capsaicin, we prepared a series of photolipids targeting the Vanilloid Receptor 1 (TRPV1), a non-selective cation channel known for its role in nociception. Several azo-capsaicin derivatives (AzCAs) emerged as photoswitchable agonists of TRPV1 that are relatively inactive in the dark and become active on irradiation with UV-A light. This effect can be rapidly reversed by irradiation with blue light, and permits the robust optical control of dorsal root ganglion neurons and C-fiber nociceptors with precision timing and kinetics not available with any other technique.

More recently our work has focused on the incorporation of the FAAzos into the scaffold of glycerolipids; including diacylglycerol. We have also prepared photoswitchable sphingolipids that permit optical control of lipid rafts in supported membrane systems. These studies direct photopharmacology towards the plasma membrane, and we expect that photolipids will find many applications in controlling cellular function.

Reference
This communication will report the design, synthesis and application of the first functional photocrosslinkable affinity probes for sensory sodium channel NaV1.7, based on the cystine knot scaffold of spider venom peptide Huwentoxin-IV. Analysis of the folding of these probes, as well as data demonstrating their low nM potency at the NaV1.7 channel through electrophysiological assays will be presented. Additionally, I will discuss our preliminary chemical proteomic analyses in engineered cell lines. The successful application of these photo-probes to identify complexes of NaV1.7 points the way to future work on patient-derived neurons.

A chemical biology approach to understand binding partners of sensory sodium channels

Foteini Tzakoniati (1), James Bilsland (2), Sarah Skerratt (2), Ian Storer (2) and Edward Tate (1)

(1) Department of Chemistry, Imperial College London, UK. (2) Pfizer Neusentis, Granta Park, UK.

The insertion of [18F] into peptides and proteins is extremely useful for in vivo imaging purposes as well as radiopharmaceutical chemistry, and there are several reported methods for achieving this. However, many of these methods have drawbacks (i.e. harsh radiolabelling conditions, using fluorinated prosthetic groups, special handling).

Herein, we describe a novel method for the direct C-F bond formation onto annexin V, a valuable biomarker for apoptosis detection, under mild conditions and straightforward handling. Dehydroalanine (Dha), a chemically modified protein tag, is formed on a single cysteine (C316) by reaction with 2,5-dibromohexadiazole (DBHDA), and is then treated with [18F]-Selectfluor bis(triflate) to give the [18F]-radiolabelled annexin V (C316Dha). Subsequently, the resulting radiotracer was used in an animal model study monitored by positron emission tomography (PET), demonstrating the effectiveness of this new method for the [18F]-radiolabelling of proteins.

Anuchit Phanumartwiwath (1), Matthew Schombs (1), Omar Boutureira (2), Anna Kirjavainen (3), SANTA FORSBACK (3), Olaf Solin (3), Merja Haaparanta-Solin (4), Alex M. Dickens (4)(5), Gonçalo J. L. Bernardes (6), Daniel C. Anthony (5) and Benjamin G. Davis (1)

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Cellular RNAs carry more than 140 post-transcriptional modifications that help them to fulfill their highly diverse cellular functions. They are chemically and structurally highly diverse and can be found in all RNA classes. Recent studies suggest that some RNA modifications can be dynamic, thus opening the possibility that reversible modification of RNA might serve as another layer for biological regulation in addition to the well-studied epigenetic regulation.

We use metabolic labeling of RNA to probe the scope and mechanisms of dynamic RNA modifications. We also record changes in the abundance of modified ribonucleosides after exposure to external stimuli to gain insight into how they may help an organism to adapt to environmental challenges.
Sphingolipids are major eukaryotic membrane lipids which have been shown to play important signaling roles during cellular stress. Using non-targeted and targeted lipidomics we found a connection between 1-deoxy dihydroceramide and resistance to anoxia in the nematode *Caenorhabditis elegans*. Animals that produce more 1-deoxy sphingolipids show lower survival during anoxia and suppression of 1-deoxy sphingolipid production can extend survival without oxygen. We found that anoxia changes first the amino acid balance in the cell increasing alanine and decreasing serine as a consequence of upregulated glycolysis. Alterations in the amino acids cause changes in sphingoid base production leading to a decrease in sphinganine and an increase in 1-deoxy sphinganine, the precursor of toxic 1-deoxy dihydroceramides. Genetic screens in yeast and worms to identify the target of toxic 1-deoxysphingolipids might help to not only extend survival of cells during lack of oxygen like in stroke or heart attack but also to reduce lipotoxicity during metabolic syndrome and diabetes where 1-deoxysphingolipids have been found to have increased.

**1-deoxy dihydroceramides in anoxia survival**

J. Thomas Hannich (1)(5), Augustinus Galih (1)(5), Denia Mellal (2)(5), Fabrice David (3), Nicolas Guex (4), Andreas Zumbühl (2)(5), Jean-Claude Martinou (1) and Howard Riezman (1)(5)

(1) Biochemistry Department, Geneva University, (2) Organic Chemistry, Fribourg University, (3) EPFL, (4) Swiss Institute of Bioinformatics, (5)NCCR Chemical Biology

**Diubiquitin-based probes reveal DUB specificity mediated by S1-S2 ubiquitin-binding pockets**

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Ubiquitin (Ub) post-translationally modifies target proteins on either a lysine residue or on its N-terminus. Ub can also form poly-Ub chains, the nature of which determines the transduction of Ub signaling. Deubiquitylating enzymes (DUBs) can reverse ubiquitylation by cleaving the (iso)-peptide bond between Ub and the target protein. Targeting different binding regions in DUBs has been useful in determining their mode of action (see Fig. A). Here we report the development of a novel set of probes and substrates based on a diUb motive (see Fig. B and C). We show that these probes are excellent tools to study the binding modus and pockets involved in recognition of specific Ub-chain topologies in proteases from the ovarian tumor DUB family (see Fig. D).
Azobenzenes are versatile photoswitches that can be cycled between their trans- and cis-configuration with light. The wavelengths for this isomerization are substantially shifted from the UV to the visible range through tetra-ortho chlorination. However, the installation of this substitution pattern remains limited and inefficient. Herein, a new general method for the synthesis of tetra-ortho-chloro azobenzenes is described using a direct palladium-catalyzed C-H tetra-chlorination of pre-existing azobenzenes. This method was used to prepare red-AzCA-4, a photoswitchable vanilloid that enables optical control of the cation channel TRPV1.

Target identification of bioactive molecules is a challenge faced by the academic and pharmaceutical drug discovery industries. Understanding the mode-of-action of drug candidates is especially important with respect to drug safety, drug efficacy, patient selection and eventually drug approval. To achieve robust target ID of these compounds, we are developing a workflow that can be combined with label free ID techniques using photoaffinity probes. We will describe the synthesis and application of photoprobes of AZ001, a compound developed by AstraZeneca against GPR119 which showed significant blood glucose lowering in vivo, but displayed the same phenotype in a GPR119-knockout mouse model.[1] The successful implementation of this workflow may elucidate new targets for type 2 diabetes.

Reference

Reference
A chemical biology approach to the target identification of bioactive molecules with unknown modes-of-action

Kate Hadavizadeh (1), Ed Tate (1) and Jamie Scott (2)
(1) Department of Chemistry, Imperial College London, SW7 2AZ. (2) AstraZeneca, Cambridge, CB4 0Fz
The influenza A virus protein hemagglutinin (HA) recognizes sialic acid (SA), a plasma membrane glycan that functions as the primary virus attachment factor. Recently, the epidermal growth factor receptor (EGFR) was shown to be activated and involved in virus entry. Using quantitative super-resolution microscopy, we show that SA and EGFR are organized in clusters that co-localize, eventually allowing SA-mediated virus-EGFR activation. Our analysis enabled us to simulate 2D virus trajectories, which could be confirmed by live cell single-virus tracking. We provide a functional model for the initial events of influenza virus infection.
We demonstrate the development of a new probe for bioluminescent imaging of nitroreductase (NTR) activity—the enzyme used in gene-directed enzyme prodrug therapy (GDEPT) of cancer. The probe consists of an NTR-sensitive moiety linked to a bioluminescent reporter which results in "caging" of luciferin. The probe is selectively uncaged by NTR, followed by luciferin release and light emission. We report successful application of this probe for imaging of NTR in vitro, in bacteria and cancer cells, as well as in vivo in mouse models of bacterial infection and NTR-expressing tumor xenografts.
Multiple myeloma (MM) continues to claim the lives of a majority of patients. This unfavorable prognosis is attributed to cancer stem cells (CSCs). CSCs are characterized with resistance, self-renewal and differentiation. Thus, compounds capable of eradicating MM-CSCs may significantly improve the prognosis of MM patients.

Withaferin A (WFA), a steroidal lactone derived from Withania somnifera, is known to exhibit anticancer activities in several cancer cell lines, including osteosarcoma, colon, leukemia and myeloma. Here we report that WFA showed a promising activity against MM-CSCs. Highly tumorigenic MM-CSCs derived from a patient with MM and RPMI 8226 myeloma cells were used. WFA inhibited the growth of MM cells, with an IC50 of 650 nM in MM-CSCs and 225 nM in RPMI 8226 cells. WFA also induced cell death and apoptosis in a dose-dependent manner in both MM-CSCs and RPMI 8226 cells. WFA treatment resulted in apparent differences in MM-CSCs morphology as demonstrated by cytoskeletal phalloidin stainings.

In order to identify a possible mechanism of action, the expression of stem cell-specific genes was measured. Quantitative real time PCR analyses revealed alterations in gene products involved in stem cell maintenance and differentiation in response to WFA treatment in MM-CSCs. These results warrant further investigation of WFA in other MM-CSCs models and potentially in in vivo models.

Mechanistic insights into post-translational epimerizations in proteusin peptide natural products

Tailoring enzymes from ribosomally synthesized and post-translationally modified peptide (RiPP) natural product pathways catalyze an extraordinary diversity of amino acid transformations. Perhaps the most heavily modified RiPPs known to date are the polytheonamides, potent pore-forming cytotoxins isolated from the Japanese marine sponge Theonella swinhoei and produced by one of its endosymbionts, ‘Candidatus Entotheonella factor’.

Of the ~50 modifications which decorate the 49-residue polytheonamides, 18 post-translational L- to D-amino acid epimerizations are attributed to a radical S-adenosylmethionine (rSAM) superfamily member PoyD. Whereas most known epimerizations are catalyzed by racemases that form a mixture of L- and D-configured products, PoyD and its homologs act irreversibly to site-specifically install diverse patterns of D-amino acids into the core regions of precursor protein substrates. Deuterium labeling and semi-synthetic substrate experiments afforded mechanistic insights into the radical-based catalysis. Since inclusion of D-amino acids can drastically alter the stability, structure, and activity of peptides, rSAM epimerases could have substantial applications in custom protein and peptide engineering.
Nanopore-based measurements enable investigating single biomolecules at ultrahigh precision. The precision relies on the dimension of the nanopore and our single-layer MoS$_2$ nanopores could offer sub-nm resolution due to its atomic geometry\[1\]. We have realized for the first time with solid-state nanopores, identification of all four types of nucleotides by introducing an ionic liquids gradient system to control the translocation dynamics\[2\]. I will present the latest results towards solid-state nanopore sequencing and other understandings of biophysics based on nanopore-experiments.

References
Membrane forces play pivotal roles in cells. Currently used characterization methods allow for controlled force application to bilayers but not to detection of these. We are developing a mechanosensitive membrane probe that enables direct measurement of molecular forces applied within lipid bilayers. As a first step, we demonstrate a vesicle assay that detects peptide adsorption and conformation change in the bilayer. We employ a mechanochromic polymer polydiacetylene (PDA) that changes color and fluorescence intensity upon force application. The probe was calibrated by correlating the spectral shift to the concentrations of melittin peptide. The developed assay revealed unexpected slow interaction kinetics between melittin and the PDA vesicles.

Reference:
Herbal extract containing sesquiterpene lactones have been extensively used in traditional medicine and are known to be rich in α,β-unsaturated functionalities that can covalently engage target protein(s). The sesquiterpene lactone deoxyelephantopin is the most active ingredient in extracts of *Elephantopus scaber*. [1] Biological investigations have demonstrated cytotoxicity against several human cancer cell lines, and an activity superior to that of Paclitaxel in breast cancer models.[2] While diverse targets have been proposed,[3-5] the covalent interactome of deoxyelephantopin has not been investigated and requires a tagged derivative. A divergent synthesis of deoxyelephantopin analogues and their biological evaluation will be presented, including the identified pharmacophores, novel potential drug targets and the binding mode with PPARγ.

References
Peptidic Nucleic Acids (PNAs) are synthetic DNA mimics based on a peptidic backbone. They hybridize to DNA and RNA with higher affinity and better sequence specificity than their natural counterparts. By combining the hybridization properties of DNA with the modularity of peptides, PNA is an attractive platform for the design of assemblies with emergent properties or functions and has been used to display ligands with controlled geometry or in templated reactions. The properties of PNA can be tuned by stereochemically defined substitutions on the backbone[2] and diverse nucleobases extending beyond the five canonical ones, however the combinatorial potential of the diverse modifications is limited by the synthetic accessibility of these different combinations. We developed a reiterative strategy based on the Ugi 4 component reaction[3] to access α, β or γ modified PNAs from readily available fragments, thus alleviating upfront work in order to tap into the potential of diverse PNA modifications.

References

The endocytic pathway plays a central role in cell life. During this process membranes dynamic is regulated by many factors including the control of lipid composition. The aim of the project is to find new chemical tool to decipher the regulation factors of two lipids: cholesterol and LBPA. A first screen reveals one particularly interesting compound able to increase specifically the endosomal level of LBPA. The compound issued from an FDA approved library is known to antagonize the Histamine 3 receptor. By using other compounds, siRNA or antibodies we are able to confirm the target. With NPC patient cells we also demonstrate that the compounds can rescue the cholesterol phenotype. Now we are investigating the pathway involved and we are running in vivo experiment on NPC mice.
With 795 million people suffering from chronic hunger in 2015[1], global food security is at risk. Persistent low-level infection of plants by pathogenic fungi has been estimated to result in food crop production losses that, if mitigated, could feed up to 8.5% of the world population[2]. Consequently, it is imperative to increase understanding of crop protection against pathogenic fungi.

Infections by plant pathogenic fungi are combated through the use of fungicides, but fungi evolve detoxification pathways to diminish the effects of these exogenous molecules, rendering the fungicides less effective than would be ideal. Many fungicides lose efficacy through genetic modifications in target organisms, and most of these detoxification pathways are well-studied[3]. However, our understanding of metabolic resistance – that is through rapid oxidative clearance of the exogenous molecule from the fungus – remains very incomplete.

We have designed, synthesised, and evaluated a suite of fenpropimorph-derived chemical probes for application in chemoproteomic profiling. The probes remain potent inhibitors of ergosterol biosynthesis and display comparable rates of oxidative clearance to fenpropimorph in the fenpropimorph-resistant fungus Zymoseptoria tritici (or Mycosphaerella graminicola), a critical wheat-infecting pathogen. Therefore, proteomics experiments employing these probes are expected to shine light on the enzymes responsible for metabolic resistance in these fungi. Such knowledge shall be used to develop long-lasting fungicides that will, ultimately, enhance food security.

References

27
Chemical probes to explore the fungal metabolome

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With 795 million people suffering from chronic hunger in 2015[1], global food security is at risk. Persistent low-level infection of plants by pathogenic fungi has been estimated to result in food crop production losses that, if mitigated, could feed up to 8.5% of the world population[2]. Consequently, it is imperative to increase understanding of crop protection against pathogenic fungi.

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References

28
Thiazolidine-protected β-thiol asparagine: applications in one-pot ligation-desulfurization chemistry

Jessica Sayers, Robert E. Thompson, Kristen J. Perry, Lara R. Malins and Richard J. Payne

School of Chemistry, The University of Sydney, NSW 2006, Australia

The first example of ligation chemistry at native asparagine has been enabled by the development of an efficient synthetic route to a β-thiol asparagine (Asn) derivative, bearing a novel 2,4,6-trimethoxyphenyl-thiazolidine protecting group[1]. The efficient incorporation of the amino acid into the N-termini of peptides is demonstrated, as well as the utility of the β-thiol Asn moiety in rapid, high-yielding ligation reactions with a range of peptide thioesters. Radical-based desulfurization of the β-thiol auxiliary is also demonstrated to yield the corresponding native peptide products. The utility of this one-pot ligation-chemoselective desulfurization chemistry at Asn is highlighted by a one-pot assembly of the HIV entry inhibitor enfuvirtide.

Reference

Thiazolidine-protected β-thiol asparagine: applications in one-pot ligation-desulfurization chemistry
An azobenzene-containing cyclic dipeptide PAP-DKP-Lys is a photoresponsive low-MW hydrogelator. The gelation process can be triggered with temperature, pH, light, and ionic strength. The resulting self-healing gels can encapsulate dsDNA or an anticancer drug doxorubicin, and release them in a light-dependent manner. Such behavior can be explored in the future to design systems for light-induced delivery of drugs or therapeutic oligonucleotides.

To address membrane order changes surrounding the insulin receptor during signaling, we observed the insulin receptor covalently linked to Nile red, an environment-sensitive dye, using SNAP-tag system. Our system suggests that the membrane environment surrounding the SNAP-tagged insulin receptor changes upon insulin stimulation and during endocytosis of the insulin receptor. However, there is no obvious change in the general plasma membrane environment in cells labeled with a Nile red derivative, NR12S which stains the entire plasma membrane. These results indicate that our SNAP-tagged insulin receptor system reflects the local membrane environment surrounding insulin receptor in insulin signaling.
The emergence of robust conjugation methodologies has facilitated access to glycoconjugates, most notably protein or oligonucleotide conjugates. Inspired by the advent of Shoda’s activation of unprotected glycans, we report a novel reagent to functionalise unprotected glycans with a picolyl group at the anomeric position for chelation-assisted CuAAC glycoconjugation. We show that glycans functionalised with this moiety react with rate constant of $40 \text{M}^{-1}\text{s}^{-1}$ and can be used to functionalize biomolecules bearing alkynes introduced through metabolic labelling, including in live cells.

References

Voltage sensitive dyes (VSDs) are powerful tools for membrane potential monitoring. In this work, we demonstrate a new approach for the characterization of VSDs based on VSDs incorporated free-standing lipid bilayers spanning on highly ordered 1μm-sized pore arrays in Si3N4. The developed platform, after being mounted in a home-made optical compatible electrochemical cell, enables the possibility 1) to apply any voltage sequences, 2) to modify bilayer composition freely, and 3) to acquire two-dimensional mapping of the VSD activities, allowing more detailed studies of the most famous VSDs (e.g. di-4-ANNEPS).
Inspired by the excellent translocation efficiency of cell-penetrating poly(disulfide)s (siCPDs), we proposed and demonstrate the existence of a thiol-mediated uptake that takes place when the polymer undergoes disulfide exchange with the free thiols exposed on the cellular membrane.

In order to explore further this newly discovered uptake mechanism, we decided to decorate vesicles with strained disulfides to enhance their delivery inside cells. By adding also positive charges as present in usual cell-penetrating peptides, we plan to deliver liposomes and polymersomes by thiol-counterion mediated uptake.

Lucía Aimo (1), Robin Liechti (2), Nevila Hyka-Nouspikel (1), Anne Niknejad (2), Lou Gótz (2), Dmitry Kuznetsov (2), Fabrice P.A. David (3,4), Gisou van der Gout (5), Howard Riezman (6,7), Lydie Bougueleret (1), Ioannis Xenarios (1,2,6,8) and Alan Bridge (1)

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Lipids are a diverse group of essential molecules with roles in energy metabolism, membrane structure, and cell signaling. A more complete understanding of the roles of lipids in human health requires the integration of lipidomic data with biological knowledge. To facilitate this task we have developed a knowledge resource for lipids – SwissLipids. SwissLipids provides a hierarchical classification that links mass spectrometry analytical outputs to over 300,000 lipid structures and expert-curated knowledge on enzymatic reactions, interacting molecules, tissue and subcellular localization, etc., sourced from over 900 peer-reviewed articles. SwissLipids is updated daily with new knowledge and freely available at http://www.swisslipids.org.
High-affinity scaffolds for protein-protein interactions, such as monobodies and DARPin, can be engineered in vitro to bind to protein targets. We speculate that the affinity for the target protein can be modulated by incorporating these evolved scaffolds into semi-synthetic protein switches. We have attempted to alter the affinity of a monobody towards the N-SH2 domain of the Shp2 phosphatase. The monobody was initially expressed in a fusion protein between the self-labeling SNAP-tag and circular permuted dihydrofolate reductase (DHFR). An intramolecular tether with trimethoprim, a ligand for DHFR, was covalently attached to the SNAP protein.

The affinity of the monobody for the N-SH2 target was higher (Kd = 1 ± 0.5 nM) when the intramolecular tether was present. The increase in affinity could be reversed with free trimethoprim (Kd = 9 ± 1 nM). In a second attempt, the intramolecular ligand with trimethoprim, a ligand for DHFR, was covalently attached to the SNAP protein.

We are now investigating the mechanisms by which the protein-protein affinity is modulated and looking to generalize the method.
The prospect of exploiting the high data storage density and longevity of DNA for storing binary information are fuelling current efforts to design systems that enable dynamic control over the information associated to a nucleic acid strand. To this end, we devised a strategy that exploits chemical transformations for the manipulation of information encoded on synthetic DNA libraries. As a result, we demonstrated that (i) multiple layers of information can be recovered from a single DNA template and (ii) by reversibly altering the chemical identity of nucleobases these layers can be dynamically recovered over multiple cycles.

Dynamic multilayer DNA-based data storage

Clemens Mayer, Gordon R. McNroy, Pierre Murat, Pieter van Delft and Shankar Balasubramanian
Department of Chemistry, University of Cambridge, United Kingdom.

Despite years of study, the brain remains one of the most misunderstood organs. Its function can be monitored by a plethora of methods but more potent or complementary ones remain necessary. We are developing a neuronal signal integrator named CaProLa for Calcium dependent Protein Labelling. CaProLa is composed of a self-labelling protein, which is active only in presence of calcium and reacts with an orthogonal substrate carrying a click moiety. Leaving a permanent mark in the animal brain during behavioral tests, CaProLa is revealed post-mortem by reacting with an orthogonal fluorophore, allowing to image the neuronal circuits.

Development of a neuronal signal integrator based on self-labelling proteins

Julien Hiblot, Iulia A. Karpenko, Ruud Hovius and Kai Johnsson
Ecole Polytechnique Fédérale de Lausanne (EPFL), Institute of Chemical Sciences and Engineering (ISIC), Institute of Bioengineering, National Center of Competence in Research (NCCR) in Chemical Biology, Lausanne, Switzerland.
The current therapeutic agents are not effective enough to treat patients with type 2 diabetes satisfactorily. Also, there are many side effects associated with them. Herein, we reported the design, syntheses, and SAR studies of some novel small-molecule sugar mimics, namely carbocyclic sodium-dependent glucose cotransporter 2 (SGLT2) inhibitors, as potential antidiabetic agents.

A glycoside-based SGLT2 inhibitor is metabolically unstable. To address this shortcoming, we replaced its endocyclic oxygen atom with a methylene unit to render the molecule free from glycosidase degradation, resulted in a more long-lasting blood glucose lowering effect.

Reference
Po-Chang Shih, Geoff Wells and Gary Parkinson
UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX.

STAT3 is a transcription factor over-expressed in more than 70% of cancers\(^1\); however there is no marketed STAT3 anti-cancer drug. We aimed to inhibit the STAT3-DNA interaction as a new binding pocket has been identified by co-crystallising with a 4’-substituted benzylamine derivative and validated fluorescence polarisation (FP) assays. This has informed our design of new, higher affinity ligands that occupy this pocket to make DNA unbound. FP Results show that 4’-substituted benzylamine derivatives (MW ~250) compete with the STAT3-DNA interaction 2 to 11 fold more readily than the STAT3-STAT3 dimerisation which many groups are focusing on, with IC\(_{50}\)s in the range of 100-600 \(\mu\)M. The derivatives with polar groups and a phenyl substituent show better results.

A novel methodology for the construction of high-quality DNA-templated libraries of macrocyclic molecules was established. An orthogonal set of codons capable of encoding more than a quarter of a million library members was identified. The structural and stereochemical parameters of the building blocks were carefully designed in order to obtain a balanced and diverse space of molecules with optimized parameters of druglikeness. A second-generation 256,000-membered DNA-templated library of macrocycles was prepared via an unprecedentedly convenient and reliable assembly scheme.

Dmitry L. Usanov and David R. Liu
Department of Chemistry and Chemical Biology, Harvard University, USA.
Chromatin-associated effector proteins that regulate chromatin-templated transactions often exist in homo- or heterooligomeric complexes with multiple domains targeting post-translationally modified histones. How this potential multivalent recognition influences kinetics towards target chromatin is not well understood.

Here we developed a method allowing us to directly visualize the interaction of a chromatin-associated effector protein (HP1α) with chromatin at the single-molecule level. This allowed us to obtain quantitative information on the kinetics of recruitment and retention towards chromatin. Based on this method we show that the multivalency of the effector critically contributes to the recruitment and retention of HP1α.

Multivalency of HP1α promotes recruitment and prolongs retention towards heterochromatin

Carolin C. Lechner, Ninad D. Agashe and Beat Fierz
Laboratory of Biophysical Chemistry of Macromolecules, Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland.

Nucleosomes carry complex patterns of post-translational modifications (PTMs) that are involved in epigenetic signaling. The two copies of histones co-existing in a nucleosome may not carry the same PTMs but can be differently (asymmetrically) modified. A key example of such asymmetry is ‘bivalent’ chromatin where K4me3 and K27me3 are found on different H3 copies in one nucleosome.

We developed a facile and traceless method to produce a library of asymmetric bivalent nucleosomes which allowed investigating the intra-nucleosomal crosstalk between H3K4me3 and H3K27me3 in the regulation of the histone methyltransferase PRC2 in bivalent chromatin.

Traceless synthesis of asymmetrically modified bivalent nucleosomes

Sinan Kilic, Andreas L. Bachmann, Louise C. Bryan and Beat Fierz
Laboratory of Biophysical Chemistry of Macromolecules, ISIC, EPFL, 1015 Lausanne, Switzerland.

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The willing of unravelling the secrets of life has fed the progress of humanity. In this line, our group contributes by developing novel fluorescent stains to measure the properties of membranes. These innovative membrane stains are based on the coupling of fluorophore planarization and fluorophore polarization. This mechanism has not been largely explored although it is commonly found in several biological processes. This combination enables to create planarizable push-pull fluorescent probes to image not only lateral organization of membranes but also membrane potentials as well as the poorly detectable membrane tension.

Planarizable push-pull fluorescent probes

Thiophene-based probe (1) and ithienothiophene-based probe (2)

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Monobodies are small antibody mimics based on a diversified fibronectin scaffold that have successfully been used to target protein-protein interactions. In collaboration with the Koide lab (University of Chicago), high affinity binders were selected targeting the SH2 domains of the nine Src family kinases and the E3 ubiquitin ligase Cbl, which play important roles in cancer signaling. We exploited biophysical assays to determine affinity and specificity of the monobodies and confirmed interaction with full-length target proteins in cancer cells by tandem affinity purification in combination with mass spectrometry analysis. Currently, we are setting up a screening platform within the NCCR framework to screen for novel monobodies.

Monobodies - versatile antibody mimics as tools for chemical biology

Tim Kükenshöner (1), Nadine Schmit (1), Akiko Koide (2), Fern Sha (2), Shohei Koide (2) and Oliver Hantschel (1)
(1) Swiss Institute for Experimental Cancer Research (ISREC), Ecole polytechnique fédérale de Lausanne (EPFL). (2) Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL 60637.

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Chromatin effectors dynamically interact with post-translationally modified histone proteins via multivalent interactions, thereby interpreting the combinatorial histone modification language into biological function. Here we use single-molecule imaging to reveal the effect of chromatin fiber conformation on multivalent binding of HP1α to its cognate methyl mark at H3K9. By controlling chromatin conformation by acetylation of H4 (decondenses fibers) or inclusion of linker histones H1 (resulting in a compacted state), we find that HP1α more loosely interacts with extended fibers but stably binds compacted chromatin.

Novel candidates for the formation of intraluminal vesicles in mammalian cells

Cargo such as activated signaling receptors are quickly endocytosed to the early endosome where a second invagination process from the limiting membrane forms IntraLumenal Vesicles (ILVs) laden with cargo. The exact role of these ILVs and the mechanisms by which they can fuse to and from the limiting membrane is not well established. Using a forward screen via proximal biotinylation, new candidates will be validated by high resolution microscopy and functional assays.
Targeting and stabilizing distinct kinase conformations is an instrumental strategy for dissecting conformation-dependent signaling of protein kinases. Herein the structure-based design, synthesis, and evaluation of pleckstrin homology (PH) domain-dependent covalent-allosteric inhibitors (CAIs) of the kinase Akt is reported. These inhibitors bind covalently to a distinct cysteine of the kinase and thereby stabilize the inactive kinase conformation. These modulators exhibit high potency and selectivity, and represent an innovative approach for chemical biology and medicinal chemistry research.

The enzyme iridoid synthase plays a crucial role in the biosynthesis of a large class of ecologically and pharmacologically important plant natural products, the iridoids. Iridoid synthase generates the core of iridoid natural products by cyclizing a monoterpene precursor in a reductive mode fundamentally different from canonical, cationic monoterpene cyclization. Recently, the first gene of an iridoid synthase has been described in the medicinal plant *Catharanthus roseus* [1]. Here, we report crystal structures of this enzyme with and without ligands and inhibitors bound that provide more detailed information on the mechanism of iridoid biosynthesis [2].

References
Cancer patients with EGFR-mutant NSCLC show a significant clinical response (50-80%) to first-generation reversible EGFR inhibitors. Acquired resistance, mediated by a secondary point mutation at the gatekeeper T790, to these inhibitors is a bottleneck in modern targeted cancer therapy. Covalent-irreversible inhibitors represent a powerful approach in overcoming T790M drug resistance. The covalent-irreversible mode of action requires the precise characterization of binding kinetics and target residence time to foster the design of next generation drugs. Here we present the structure-based design and detailed biochemical characterization of novel covalent-irreversible EGFR inhibitors.
Targeting drug resistance in EGFR with covalent inhibitors: a structure-based design approach

Jonas Lategahn (1), Julian Engel (1), André Richters (1), Matthäus Getlik (2), Stefano Tomassi (1), Marina Keul (1), Martin Termathe (2), Christian Becker (1), Svenja Mayer-Wrangowski (1), Christian Grütter (1), Niklas Uhlenbrock (1), Jasmin Krüll (1), Niklas Schaumann (1), Simone Eppmann (1), Carsten Schultz-Fademrecht (3) and Daniel Rauh (1,2)

(1) Faculty of Chemistry and Chemical Biology, TU Dortmund University, Dortmund, Germany. (2) Chemical Genomics Centre of the Max-Planck Society, Dortmund, Germany. (3) Lead Discovery Center GmbH, Dortmund, Germany.

Aberrant activity of the epidermal growth factor receptor kinase (EGFR) plays an important role in the onset and progression of non small cell lung cancer (NSCLC). Here, we present a structure-based approach to design novel and irreversible EGFR inhibitors based on a screening hit that was identified in a phenotype screen of 80 NSCLC cell lines against approximately 1500 compounds. Protein X-ray crystallography was utilized to decipher the binding mode, which constituted the basis for further rational design approaches. Chemical synthesis led to further compound collections that revealed increased potency and selectivity toward mutated vs wild-type EGFR.

Optimization of TRPV6 calcium channel inhibitors using a 3D ligand-based virtual screening method

Céline Simonin (1), Mahendra Awale (1), Michael Brand (1), Ruud van Deursen (1), Julian Schwartz (1), Michael Fine (2), Gergely Kovacs (2), Pascal Halliger, Gergely Gyimesi (2), Ablashan Sithampari (2), Roch-Philippe Charles (2), Matthias A. Hediger (2) and Jean-Louis Reymond (1)

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TRPV6 belongs to the vanilloid family of the transient receptor potential channel (TRP) superfamily. TRPV6 is highly Ca²⁺-selective channel and is known to be upregulated in breast and prostate cancer tissue [1, 2]. Herein, we report the discovery of the first potent and selective inhibitor of TRPV6 and its use to test the effect of blocking TRPV6-mediated Ca²⁺ influx on cell growth. An in-house developed 3D ligand based virtual screening method called “xLOS” was used in combination with chemical synthesis to transform weekly active inhibitor into the novel selective molecule with 0.3 μM inhibition of TRPV6 [3], which can be used as tool compound to further study TRPV6 channel. The Scaffold hopping ability of xLOS method may be generally useful to develop tool compounds for poorly characterized targets.

References

Here, we present the structure-based synthesis of 2 arylquinazolines, aiming to optimize the biochemical properties and to rule out possible shortcomings of a initially identified lead structure binding to the so called lipid pocket in the C terminus if the in p38α MAPK. The exact biological role of this binding site is yet unknown and might be involved in allosteric regulation of kinase activity, mediating protein-protein interactions or even functions beyond the catalytic phosphotransfer - a scientific question we aim to address with our library of putative probe molecules.
Chloromethyl triazoles (CMTs) are readily accessed in only two chemical steps, thus enabling rapid optimization of pharmacological properties of these inhibitors. We demonstrate the tunability of CMTs towards a specific biological target by synthesizing AA-CW236 as inhibitor of O\textsubscript{6}-alkylguanine DNA alkyltransferase (MGMT), a protein of major clinical significance for treatment of several severe cancer forms. Using quantitative proteomics profiling techniques, we show that AA-CW236 exhibits high degree of selectivity towards MGMT. Finally, we validate the effectiveness of our MGMT inhibitor in combination with the DNA alkylating drug temozolomide in breast and colon cancer cells using fluorescence imaging and cell viability assay.

There is currently no straightforward way for measuring the surface tension of a membrane. Therefore the Matile-group Fluorescent Flippers are an attractive new tool to easily access to this particular property. We have studied the Fluorescent Flipper in monolayers at the air/water-interface under fluorescent microscopy and grazing incidence angle X-ray diffraction synchrotron irradiation. We found a long-range ordering phenomenon at high surface-pressure and an excellent correlation between monolayer phase changes and Fluorescent Flipper response. Overall, we confirm the potential of the Flipper molecules for surface tension measurements.
Small hydrophobic binders of hSULT1A1 (i.e. 2-naphthol) are able to induce conformational changes leading to the reduction of the pocket volume into a much smaller size by hydrophobically interacting with relevant residues of the active site. In the case of bigger binders (i.e. ethinylestradiol), Phe76 and Phe81 flexibility contributes to opening an extra pocket and extend the hydrophobic system allowing stacking interactions with the ligand. The MD combined with pocket size analysis suggests that there is a relation between the volume of the ligand and its substrate/inhibitor characteristics and thus specificity and selectivity.

Covalent protein modification is a gateway technology for the production of protein-based therapeutics, diagnostics or biomaterials. Formylglycine-generating enzymes (FGE) mediate site-specific insertion of aldehyde functions into proteins. Aldehyde containing proteins are substrate to a broad range of specific labelling chemistries. In the past, this technology has been limited due to poor in vitro activity of FGEs. We found that reconstitution of recombinant FGE with CuI, coupled with a number of point mutations increases the catalytic activity of this enzyme by nearly 200-fold. In this presentation we will discuss applications of this improved in vitro FGE technology to construct complex biomaterials.

References
Organosulfur compounds are a highly important class of molecules, which is underscored by their vital presence in biological mechanisms, through reactive cysteine residues. Due to the versatility of acetylene chemistry, methods that selectively transfer alkynes on sulfurs into multi-functionalized proteins are highly sought after in chemical biology. Thus, we reported a highly chemoselective thioalkynylation reaction, which served as platform for study post-translational modifications and bioconjugation.

Since their discovery, knotted proteins have attracted considerable attention, as the reason underlying knot formation and their function remain unexplained. The mechanisms involved in the spontaneous threading through a loop are not well understood but it has been hypothesized that the hydrophobic effect could be the main driving force. Here we present a molecular system that mimics this folding mechanism: building block amphiphiles composed of hydrophobic aromatics and hydrophilic cysteines spontaneously fold into knots upon the influence of the hydrophobic effect. This discovery enabled us to produce a variety of knots and may provide a first step towards understanding the interactions that drive the formation of knots in biomolecules.

References
Monobodies are small, antibody mimics that can be selected to bind to intracellular target proteins. Several monobodies have been validated in our lab, using retroviral gene transfer to express them. We are now developing technologies for direct cytosolic delivery of monobody proteins targeting intracellular proteins. We are coupling the monobodies to cell-penetrating peptides or to polydisulfides via a disulfide bond that is cleaved in the cytosol. Furthermore, we are evaluating the binding to on- and off-targets of monobodies selected against members of the Src-family kinases as well as the E3 ubiquitin ligase Cbl.

Characterization and cytosolic delivery of monobody proteins

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Phospholipids in intraluminal vesicle formation

Ubiquitinated signaling receptors are sorted for downregulation into the intraluminal vesicles (ILVs) of multivesicular endosomes by ESCRTs, which also mediate membrane deformation and the ILV formation process. These vesicles are transported to late endosomes and fuse with lysosomes where their cargo is degraded. Evidence shows that other mechanisms of ILV formation might exist. While PI3P is necessary for ESCRT function and ILV formation in early endosomes, LBPA is found only in late endosomes and it plays a key role in ILV formation within late endosomes. I investigate the precise role of these lipids and their effectors in ILV formation.

Phospholipids in intraluminal vesicle formation

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The centrosome is a key microtubule organizing center in animal cells. It is known to regulate cell cycle progression, spindle orientation and symmetry, cytokinesis, and checkpoint signaling. Defects in centrosome arrangement or number can lead to loss of cell polarity, defective cell division, and abnormal chromosome segregation. Thus, full understanding of the centrosome duplication dynamics is of great interest. Herein we present the project which focuses in developing live-cell imaging probes for centrosome visualization based on recently reported silicon rhodamine (SiR) derivatives. The key goal of the project is finding compounds which specifically bind to centrosome. Three approaches are currently undertaken to indentify centrosome targeting motives; i) Peptide binders; ii) Known small molecule binders; iii) Screening. The obtained candidates are conjugated to SiR and evaluated in live-cell context. We present the current results of tested SiR conjugates of PARP-3, Aurora A and Plk-4 inhibitors. Also, we show results of performed PNA encoded screen for human Centrin-2 binders, which was done in collaboration with Prof. Winssinger’s group.

Using a DNA display of PNA-encoded fragments libraries, we have discovered a new molecular entity capable of inhibiting, in an irreversible manner, a subset of Bromodomain-containing proteins[1]. This family of proteins is known to be tightly linked to the development of inflammatory disorders and, most importantly, to several types of cancer.

Reference
Polydiacetylenes (PDAs), a family of conjugated polymer, have been investigated due to their environment sensitive chromic and fluorescent properties. Many biosensors developed so far focus on the blue to red transition of PDA to detect target molecules or environmental stress. Our project aims to investigate the electrical properties of a carboxylated PDA. The polymer phase transitions are therefore characterized with advanced electrochemistry methods, such as impedance spectroscopy and cyclic voltammetry, showing the correlation between chromic phase change and polymer conductivity. The long term purpose is to develop accurate sensors based on well characterized electrical behavior.

Tryptophan metabolites in the kynurenine pathway are up-regulated by pro-inflammatory cytokines or glucocorticoids, and are linked to anti-inflammatory and immunosuppressive activities. In addition, they are up-regulated in pathologies such as cancer, autoimmune diseases, and psychiatric disorders. The molecular mechanisms of how kynurenine pathway metabolites cause these effects are incompletely understood. On the other hand, pro-inflammatory cytokines also up-regulate the amounts of tetrahydrobiopterin (BH4), an enzyme cofactor essential for the synthesis of several neurotransmitter and nitric oxide species. Here we show that xanthurenic acid is a potent inhibitor of sepiapterin reductase (SPR), the final enzyme in de novo BH4 synthesis. The crystal structure of xanthurenic acid bound to the active site of SPR reveals why among all kynurenine pathway metabolites xanthurenic acid is the most potent SPR inhibitor. Our findings suggest that increased xanthurenic acid levels resulting from up-regulation of the kynurenine pathway could attenuate BH4 biosynthesis and BH4-dependent enzymatic reactions, linking two major metabolic pathways known to be highly up-regulated in inflammation.
visualising and controlling biological processes using chemistry

Developing chemistry-based tools to manipulate and visualize biochemical activities in living cells

Addition of long-lasting, chemical-reactive tags to membrane components for imaging and diagnostics

Making fluorescent membrane probes that change color like shrimps during cooking

Lighting up the membrane!

Organic synthesis of artificial phospholipid probes

Making fluorescent membrane probes that change color like shrimps during cooking

Inhibition of epigenetic targets using organometallic chemistry for the treatment of malignant diseases.

Development of bicyclic peptides inhibiting Notch signaling – towards the development of an anti-cancer therapy

Programming protein ligands using artificial nucleic acid templates

Overexpressing chemically-bonded RNA, mRNA, and artificial nucleic acid templates

Using site-specific labeling of cell membranes for precision targeting in supramolecular chemistry

Membrane traffic: membrane transport affects cell function and division

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