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Conference Report

2018 International Symposium on Chemical Biology of the NCCR Chemical Biology Campus Biotech, Geneva 10.–12.01.2018

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Since it was launched on December 1, 2010, the National Centre of Competence in Research (NCCR) Chemical Biology 'Visualisation and Control of Biological Processes Using Chemistry' has succeeded in creating a center of excellence in Chemical Biology by Lake Geneva. The network represents a joint effort between the University of Geneva and the École polytechnique fédérale de Lausanne (EPFL), involving 24 professors and over 100 researchers coming from different areas of expertise: chemistry, biochemistry, physics and cell biology. This dynamic environment aims to develop novel chemical tools and innovative techniques based on small molecules and proteins to obtain new information about cellular processes and control them *in situ*. The second edition of the International Symposium on Chemical Biology was organized by a scientific committee led by ***Stefan Matile*** (UNIGE), which created a powerful programme with 14 international top speakers in the field. The great success of the symposium was highlighted by the over 200 participants from all over the world.

On Wednesday evening, the NCCR symposium on Chemical Biology opened with a fantastic lecture given by ***Pietro De Camilli*** (Yale University, USA). In his talk, he introduced a rapidly growing field of membrane contact sites. The endoplasmic reticulum (ER) is a type of organelle found in eukaryotic cells that forms an interconnected network of membrane-enclosed sacs (cisternae) or tube-like structures. Some of these elements touch the plasma membrane (PM) and are called ER-PM contact sites. At these sites, there are proteins that tether ER to PM. De Camilli group has found that two similar ER integral membrane proteins, oxysterol-binding protein (OSBP) - related protein 5 (ORP5) and ORP8, tethered the ER to the PM *via* the interaction of their pleckstrin homology domains with phosphatidylinositol 4-phosphate (PI4P) in this membrane. Their OSBP-related domains (ORDs) contained either PI4P or phosphatidylserine (PS) and exchanged these lipids between bilayers. Gain- and loss-of-function experiments showed that ORP5 and ORP8 could mediate PI4P/PS countertransport between the ER and the PM. This exchange helps to control plasma membrane PI4P levels and selectively enrich PS in the PM.[1] Very recently, his group has also shown that another ER protein, TMEM24, alters its localization at ER-PM contacts reversibly, governed by phosphorylation and dephosphorylation in response to oscillations in cytosolic calcium.[2] A lipid-binding module in TMEM24 transports the phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] precursor phosphatidylinositol between bilayers, allowing replenishment of PI(4,5)P2 hydrolyzed during signaling. He concluded his talk with a remark that the membrane contact sites are an emerging field, where even a new journal was created recently, with many open questions remaining, which provides many scientific opportunities for young researchers.

The second lecture of this first evening session was a Keynote lecture by ***Paul Wender*** (Stanford University, USA). A grand challenge in science and medicine is breaching biological barriers for the drug delivery, including plasma membranes, bacterial membranes, algal cell walls, mitochondrial membranes, skin, gastrointestinal tract, and blood brain barriers. The general golden rule is that a drug molecule has to have just the right polarity (not too polar, not too non-polar) to cross hydrophobic membranes. That is why RNA delivery is notoriously difficult; RNA has a high number of negative charges. This is the current bottleneck to its enormous clinical potential. To overcome this challenge, the Wender and Waymouth groups have synthesized new carrier molecules, Charge-Altering Releasable Transporters (CARTs), which are highly cationic and complex the anionic mRNA yet are designed to convert to neutral entities after crossing the membranes thereby releasing mRNA. This collaboration has shown the efficiency of CARTs by delivering luc-mRNA into mice using this delivery system, which presented luminescence emission peaking after 4 h.[3] They have employed CARTs also in cancer immunotherapy. In non-published work, he showed that the mouse immune system can be trained by injecting mice with mRNA-CART complexes, which dramatically improved the ability of mice to combat cancers that are implanted afterwards. This amazing new technology can be used as a cancer vaccination and also for treating established tumors. His group has also developed a scalable synthesis of a natural compound Bryostatin[4] and its analogs for latent HIV infections. Current HIV therapy is chronic with cost, compliance, resistance and chemo exposure issues because the active virus is re-supplied by 'reservoir cells' with replication competent genomically encoded virus. Elimination of these reservoir cells in conjunction with current antiretroviral therapy could lead to HIV/AIDS eradication. Bryostatin and the analogs Wender's group synthesized, activate these reservoir cells, allowing for their elimination through cytopathic or immunotoxin effects and possibly eradication of disease. Bryostatin is today in clinical trials for cancer immunotherapy, Alzheimer's disease and HIV eradication. These collaborative and translational efforts show how Chemical Biology can truly make a change in the world.

These first two excellent talks stimulated the open discussions during the following poster session, with some snacks and drinks.

Prof. Hendrik Dietz, Technische Universität München, Germany.

On Thursday morning, ***Hendrik Dietz*** (Technische Universität München, Germany) presented his recent progress on DNA origami. His group has been making enormous efforts over the last several years to advance DNA-based molecular assembly to the extent, where one can create, through designing, macromolecules with a desired function. Their specific goals start from encoding shapes, robust synthesis, mechanism and motion, higher-order assemblies, atomic accuracy, mass production, power and catalysis, and towards *in vivo*. Among the developments, an example was a pair of scissors based on DNA assembly, which moves in response to temperature change.[5] Another example is an impressive rotary unit with a rotating handle. Their challenge to make a bigger bottom–up constructions is currently already at the level of 1.3 gigadalton that consists of 220 viral genomes and 50,000 oligonucleotides in a spherical cage.[6] His group also foresees the potential of DNA origami technology in pharmaceutical drugs, which led them to develop a method for the DNA origami mass production based on biosynthesis that avoids chemical synthesis.[7] Finally, Dietz presented the application of these assembled macromolecules, in which the force between nucleosomes was measured by the statistical analysis of the geometrical change of a DNA force spectrometer.[8]

Rumiana Dimova (Max Planck Institute of Colloids and Interfaces, Potsdam, Germany) presented her diverse libraries of membrane characterization tools based on giant unilamellar vesicles (GUVs) for measuring different membrane properties. GUVs have an advantage compared to other synthetic lipid membrane systems due to their lack of high curvature, lack of contact to substrate (free-standing), and possibility to tune the lipid composition. Her group has developed and is employing *e.g.* the micropipette aspiration technique for estimating the membrane stretching elasticity, fluctuation spectroscopy for assessing the bending rigidity, and optical dynamometry for shear surface viscosity measurements. Towards this direction, one of the focuses in her group is to study the deformation of GUVs with electric fields.[9] GUVs exposed to AC field deform in a frequency-dependent manner. Interestingly, they have found that there is a threshold frequency, at which the direction of the deformation (either parallel or perpendicular to the electric field) switches. Their work elegantly proved that this switching frequency depends on the membrane capacitance. The bending rigidity of the membranes can also be measured with GUV electrodeformation, where they discovered that the incorporation of cholesterol does not necessarily always increase the membrane rigidity but the effect is lipid-dependent. On the other hand, DC pulses porate GUVs with pore sizes in the micrometer range. By measuring the pore lifetime with optical microscopy, the edge tension can be quantified. In addition, Dimova showed a variety of ways to exploit GUVs as a nanoreactor for quantum dot (QD) synthesis, for phase separation studies, and as a starting material to form lipid nanotubes by introducing membrane spontaneous curvature with bilayer asymmetry. The beautiful fluorescent microscopy images of lipid structures in different shapes[10] that she presented, clearly imply the importance of the spontaneous curvature in biological membrane shaping.

The development of membrane probes is one of the main focuses of NCCR Chemical Biology, highlighted by the push–pull probe created by the Matile Group (UNIGE). It is a notoriously cumbersome field, because the design of the molecules requires an extreme optimization due to the sensitivity of the probes to the lipid environment. ***Evan Miller*** (Berkeley University, USA) and his group develop voltage-sensitive dyes based on Photo-induced Electron Transfer (PeT) that have a higher sensitivity, longer lifetime, and operable in IR wavelength for two-photon microscopy applications. The first generation of PeT voltage sensors yielded 27% $\Delta F/F$ per 100 mV sensitivity, which is already above the standard voltage sensitive dyes (around 10% $\Delta F/F$). They have found that its efficiency can be further optimized by controlling the angle of the probe in the bilayer relative to the bilayer plane. By placing the probe more perpendicularly (θ = 19°) to the bilayer compared to the first generation dye (θ = 35°) thanks to a chemical modification of the structure, the sensitivity improved to 63% $\Delta F/F$ per 100 mV.[11] His next generation of probes (RVF5) have much higher photo-stability for longer imaging, which revealed the epileptic behavior of neurons, or operate in infrared (IR) wavelength for deep penetration in tissues with two-photon imaging. Miller and his group's next target is the long-time monitoring of the absolute membrane potential in non-neurons cells, because it may reveal important signatures for physiological phenomena such as cell growth.

Prof. Barbara Imperiali, MIT, USA.

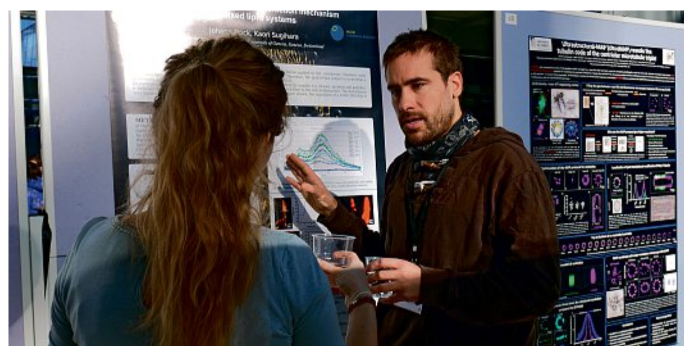
The last talk in the morning session discussed recent progress in our understanding of protein glycosylation by ***Barbara Imperiali*** (MIT, Cambridge MA, USA). Asparagine-linked (N-linked) glycosylation is a complex protein modification observed across all domains of life. Although the basic framework of the pathway is known to be similar from bacteria to man, interesting differences between the bacterial and human pathways suggest that enzymes in the pathways may be of considerable interest as targets for the development of anti-virulence agents. The talk focused on early enzymes in the N-linked pathway of *Campylobacter jejuni*, which is a serious Gram-negative pathogen that exploits glycans and glycoconjugates in the infection of human hosts. Phosphoglycosyl transferases (PGTs) initiate the biosynthesis of N-glycoproteins as well as many other glycoconjugates that play key physiological roles in bacterial survival and virulence, including peptidoglycan and O-antigen. The PGTs catalyze transfer of a phosphosugar moiety from a nucleotide diphosphate-activated donor to a polyprenol phosphate acceptor, to form a membrane-bound polyprenol diphosphosugar product in the first membrane-committed step in the pathway. Imperiali presented the detailed mechanistic analysis of PglC, a prototypic monotopic PGT from *C. concisus* revealing a ping-pong mechanism involving a covalent phosphosugar intermediate.[12] This is the

first evidence for a covalent phosphosugar intermediate and its existence shows that the monotopic PGTs differ fundamentally from the polytopic PGTs including MraY and WecA, which follow a ternary complex mechanisms of representative polytopic PGTs. In addition, Imperiali revealed the recently-solved structure of a prototypic monotopic PGT showing how the structure was expertly-purposed for catalysis at the membrane interface. With this knowledge, the Imperiali group is strategically positioned for the development of chemical tools that can be used to identify processes to target in the study of infectious diseases. N-linked glycans play critical structural and functional roles in living organisms. The dedication of her group to understand the biosynthetic pathways could provide new approaches to inhibit glycoprotein biosynthesis in antivirulence thus strategies for therapeutic applications.

After the lunch break with the second poster session, ***Atul N. Parikh*** (UC Davis, USA) gave a fantastic talk on membrane behaviors out of equilibrium. He presented how a simple effect like an osmotic pressure can create so many structural variances in membrane structures and even can induce dynamic membrane behavior, which inspired the audience to see the structure and the function of biological membranes from a new perspective. Lipid bilayer is a semipermeable membrane, which passes water but not ions or other bigger molecules such as sucrose. When GUVs have more sucrose inside than outside, the concentration difference attracts water to come inside of GUVs, resulting in swelling of the GUVs and increasing the surface tension. Based on the delicate balance between the osmotic pressure that tries to make a pore in GUVs and the line tension around the pore that tries to close the defect, under a certain condition, GUVs enter into a periodic cycle of 'swell and burst', where GUVs release the content inside in a short pulse over time. This phenomenon can be coupled to the phase separation in mixtures of lipids induced by increase in membrane surface tension. Parikh showed a breathtaking movie, where each time when GUVs swell microdomains appear, while once the swelling reached a critical point and GUVs released the content for a short period of time, the decrease in surface tension mixes the lipids and the microdomains disappear, which occurs repeatedly until GUVs run out of sucrose inside that is enough to drive this phenomenon.[13] Reversing the osmotic pressure (high sucrose concentration outside) deforms GUVs into *e.g.* tube-like shapes and induces Rayleigh instability, eventually breaking down the GUVs into small vesicles. By creating a spatial sucrose concentration gradient outside GUVs, the tube formation can be guided along the direction of the gradient.

Prof. Shiroh Futaki, Kyoto University, Japan.

Returning to the topic of delivering macromolecules into cells, ***Shiroh Futaki*** (Kyoto University, Japan) first reviewed his works on understanding the mechanism by which pyrenebutyrate (PyB) increases the efficiency of direct uptake of arginine-rich cell penetrating peptides. Simulations had been suggesting that membrane curvature loosens the lipid packing, which allows for a better insertion of arginine-rich peptide-parts into the lipid membrane. Experimental confirmation of this mechanism was obtained by employing a polarity sensitive fluorescent probe that can distinguish between different lipid packings. Direct uptake of arginine-rich cell penetrating peptides can thus be facilitated in the presence of PyB that induces membrane curvature and lipid packing defects. He then turned to a method by which larger proteins enter the cell through the cell's endocytic pathway. This process requires the peptide to lyse the endosomes once inside the cell. To achieve this, he introduced a new endosomolytic peptide (L17E).[14] Here, interactions with the membranes are modified by substituting leucine with glutamic acid. Instead of exploiting the acidic pH of endosomes, this peptide perturbs endosomal membranes in a specific manner. At the same time, it stimulates macropinocytosis, which in combination leads to a very efficient uptake of large molecules.



Poster session, during the 2018 International Symposium on Chemical Biology of the NCCR Chemical Biology in Geneva.

The second keynote lecture was given by ***Dennis Dougherty*** (Caltech, USA). In his very lively presentation, he reviewed his work on the nicotinic acetylcholine receptor and advocated the use of chemical biology to perform structure–function studies. The nicotinic acetylcholine receptor is a member of the Cys-loop ligand-gated ion channel superfamily and is essential for rapid synaptic transmission. He used heterologous expression of the channel in *Xenopous leavis* oocytes to study its properties. Since there are not enough possible variants from site-directed mutagenesis using the naturally occurring amino acids, he pioneered the application of unnatural amino acids.[15] Binding of the ligands occurs through interactions with three tyrosine and two tryptophan residues in the aromatic box of the receptor. Using fluorination, he found that acetylcholine and nicotine binding is through a cation-pi interaction with one tryptophan. The model further stipulated two hydrogen bonds for nicotine binding, which was then tested by mutating the drug and the receptor to suppress the hydrogen bonds. In a broader context, the specificity of some drugs for selected variants of the nicotinic acetylcholine receptor results from changes in the hydrogen bonds. In the final part of his presentation, he explained why nicotine is not potent at neuromuscular junctions. It turns out that a change of one lysine to a glycine outside of the aromatic box induces a shape-change in the latter that disrupts the cation-pi interaction between the nicotine and the receptor.

The last day of the symposium started with ***Nikta Fakhri*** (MIT, USA) who develops and employs new tools and concepts to characterize the non-equilibrium behavior of the cytoskeleton. In particular, she uses fluorescent single-wall carbon nanotubes to assess the mechanical properties of actin networks. In her presentation, she focused on how chemistry guides mechanics by studying symmetry breaking in a reconstituted actin cortex. Introducing drops of *Xenopus laevis* cell extract in oil and simultaneously directing the actin nucleation activating factor

ActA to the extract-oil interface results in well-defined actin cortices. It turned out that the connectivity of the actin network, which she tuned by varying the amount of the actin cross-linker alpha-actinin, is the crucial parameter for the cortex dynamic behavior and its mechanical properties.[16] At low connectivity, the cortex remains homogenous and quiescent, whereas for high connectivity, it contracts and forms a single macroscopic domain. Most interestingly, at intermediate connectivities, the cortex remained homogenous, but spontaneously developed a vertical cluster flow. In the last part of her presentation, Fakhri reported on spectacular contraction waves in the cortices of starfish oocytes. By deforming the oocytes into various shapes, her lab showed that the waves were initiated at points of high curvature and thus revealed a clear influence of geometry on cortex stability.

Prof. Aranzazu del Campo, Leibniz-Institute for New Materials, Germany.

The second talk of the morning session was given by ***Aranzazu del Campo*** (Leibniz-Institute for New Materials, Germany). She reviewed her work on fabricating synthetic microenvironments with optically regulated functions for studying cellular processes (photoactivatable extracellular matrix peptidomimetics). To understand cell interactions with the extracellular matrix, she adds photoactivatable ligands to synthetic hydrogels. The method is material independent and allows for a free choice of ligands. As the ligand is in place from the beginning, illuminating the material allows her to activate ligand–receptor interactions with high temporal and spatial resolution. She illustrated this method with a chemical scratch assay to study the escape of cells from tissues and the role of the escape channels' geometry for this process as well as with an *in situ* control of neuron polarity. By using two-photon activatable extracellular matrix peptidomimetics, structured activation in three dimensions is possible as was demonstrated with light-directed angiogenesis. This method can be applied in a medical context to tune an organism's response to implants. In this way, the fibrotic response to -and thus rejection of an implant- can be significantly reduced.[17] Further variants of the method include the embedding of optogenetically controlled bacteria into the environment or the use of different layers of gels that are endowed with distinct ligands for disentangling different aspects of cell-cell interactions on cell differentiation.[18] In an exciting perspective, del Campo indicated how this technology can be used to locally apply forces to a cell by using a light-driven molecular motor.

In the first of two talks given by former junior members of the NCCR Chemical Biology, ***Manuel Derivery*** (MRC Laboratory of Molecular Biology, UK) presented his work on polarized traffic during asymmetric cell division. Specifically, he discussed how cell fate determining endosomes are asymmetrically segregated in drosophila SOP precursors between the pIIa and the pIIb cell.[19] Automated analysis of thousands of endosome trajectories revealed that kinesis-driven endosome transport in the central spindle is bidirectional with a bias towards the pIIa cell. The underlying asymmetry in the central spindle results from asymmetric microtubule depolymerization through a Kin-13 motor and patronin that protects microtubule end from depolymerization. By using nanobodies for patronin, he managed to revert spindle asymmetry and thus exchanged the roles of the pIIA and the pIIb cells in developing flies. A theoretical analysis revealed an exponential overlap asymmetry amplification, which was quantitatively confirmed by experiments. Suspecting that this mechanism is present also in other cell types and organisms, he now uses quantum dots as replacements for endosomes to be able to disentangle the effects of different motor molecules on endosome transport. In the second part of his presentation, he presented his progress on delivering quantum dots to cells and dramatically reduce their toxicity. High-yield delivery was achieved by coupling the quantum dots to a cell penetrating poly-disulfide polymer, which self-hydrolyzes once delivered due to the reducing properties of the cytosol.[20] In turn, a novel zwitterionic coating of the quantum dot's core and shell dramatically reduces toxicity[21] and also allows coupling of the quantum dots with nanobodies. The methods presented offer most interesting perspectives for delivering various functionalized particles to cells.

In the last talk before the lunch break, ***Javier Montenegro*** (CIQUS research center, University of Santiago de Compostela, Spain), who had also been part of the NCCR Chemical Biology, shared with the audience his recent advances in using supramolecular peptides for cell delivery. He focused on the delivery of small interfering RNAs to cells, which have a large therapeutic potential. The use of viral vectors for this purpose comes with a significant biosafety risk. As an alternative, polymers are used. Currently, the identification of suitable polymers is costly and time consuming because purification and isolation steps are necessary even for candidates that eventually turn out to be inactive. Montenegro reported on the synthesis of poly(acryloyl hydrazide)s, which can be turned into amphiphilic polymers through a straightforward functionalization with aldehydes.[22] These polymers can be directly screened for their ability to transport nucleotides across lipid membranes under aqueous conditions and without purification steps. In this way, he could rapidly identify a polymer for siRNA delivery in HeLa cells with comparable efficiency to the current commercial gold standards. He then went on to show that the hydrazone linkage can also be applied in the modification of linear peptide sequences with helical amphiphilic character. The resulting hydrazone-modulated peptides afforded excellent molecular scaffolds for the delivery of circular DNA plasmids.[23] Finally, he showed recent results of his group regarding the application of these peptides to the delivery of large proteins such as the Cas9 ribonucleoprotein (~170 kDa). This technology allowed the cellular internalization and endosomal escape of Cas9 for CRISPR genome editing.[24]

The final talk of the symposium was given by ***Jason Chin*** (MRC Laboratory of Molecular Biology, UK). He reported on his recent advances in expanding and reprogramming the genetic code.[25] Together with specific chemical modifications, expanding the genetic code provides a powerful tool for characterizing the structural and biochemical consequences of post-translational modifications like phosphorylation. An engineered SepRS–$tRNA_{CUA}$ pair enabled efficient encoding of phosphoserine in *E. coli*. The incorporation of phosphoserine into proteins was then used to show that Aurora A-mediated phosphorylation of TACC3 stabilizes kinetochore fibers in mitosis. Chin then introduced a method based on parallel selection and deep sequencing to discover orthogonal aminoacyl-tRNA synthetase-tRNA pairs and applied this method to discover a phosphothreonyl-tRNA synthetase–$tRNA_{CUA}$ pair. He described stochastic orthogonal recording of translation (SORT) for cell-type-specific tagging of proteomes to brain slices and the brains of live mice.[26] In this

method, an orthogonal pyrrolysyl-tRNA synthetase–tRNA pair is used to selectively tag the proteome of neurons or glia cells by introducing a substrate containing a bioorthogonal cyclopropene group (CypK) or alkyne group (AlkK) stochastically and at low level into the respective proteins. The proteome tagged in this way can then either be labeled or enriched and identified.

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