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Conference Report

7th SCS – Syngenta Symposium 2024

'Unlocking Innovation through Chemical Biology'

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The 7th edition of the SCS-Syngenta Symposium, titled *Unlocking Innovation through Chemical Biology*, took place on October 24th 2024 at the Syngenta Research Site in Stein AG.

The program featured 8 lectures from a diverse lineup of academic and industrial speakers, featuring latest advances in Chemical Biology, covering drug target validation and mode of action elucidation, chemical genetics, chemical inducers of proximity for protein degradation and beyond, mechanistic investigations of cellular processes and drug discovery success stories enabled by Chemical Biology.

Invited Speakers' Talk Summaries

Chemically Induced Proximity as an Enabling Concept in Chemistry and Biology

Prof. ***Dennis Gillingham***, University of Basel

Prof. Gillingham kicked off the morning session with a fascinating demonstration of induced proximity exploited to reprogram biological processes and enable new function. The presentation detailed the group's contributions to advancing the development of DNA encoded libraries (DELs).

DELSTAR is a unique, solution-phase DEL selection platform developed in the Gillingham Lab that overcomes many of the open challenges in DEL screening. It directly records information about binding events onto the pendant DNA of the DEL through hijacking terminal deoxynucleotidyl transferase (TdT), an unusual DNA polymerase that adds untemplated dNTPs to 3′-ends of DNA. If a target protein is expressed as a TdT fusion and incubated with a DEL in the presence of dATP, the binders of the target induce proximity between TdT and the DNA, promoting the synthesis of a polyadenine tail. The polyA tail length is proportional to the binding affinity, effectively serving as a stable molecular record of binding events. An important feature of the method is that it uses more than 100-fold less protein than a typical affinity selection, meaning that small-scale protein production can support a selection campaign.

Ligands spanning nanomolar to double-digit micromolar binding could be cleanly identified by TdT extension, whereas only the tightest binding ligands are identified by classical affinity selection. The method is simple to implement and can function on any DEL that bears a free 3′-end.

Bioactivity Annotation for Small Molecules Using Cell Painting

Dr. ***Slava Ziegler***, MPI Dortmund

Dr. Ziegler's research focuses on the identification of small-molecule modulators of processes that contribute to tumorigenesis, using phenotypic screening. This is tightly coupled to the identification of the molecular targets and the mode of action of hit compounds.

In her presentation, Dr. Ziegler provided compelling evidence that morphological profiling technologies, such as the cell painting assay, are best suited to provide an unbiased view on the targeted bioactivity space of small molecules in cells. Cell painting employs high-content imaging and analysis of six stained cellular components and compartments to extract hundreds of morphological features. The profiling of reference compounds with known activities is prerequisite for the generation of target hypotheses, as profile similarity stems from sharing the same MoA. However, morphological profiles may be caused by an unanticipated target rather than the nominal target.

Dr. Ziegler's team defined several bioactivity clusters, thereby enabling rapid bioactivity annotation of compound collections. They used Cell Painting to map the bioactivity of both reference compounds and uncharacterized small molecules. The obtained morphological profiles allowed uncovering previously unknown off-target activity for reference compounds as well as assigning a mode of action to novel bioactive small molecules.

Exploring the Endocannabinoid System Pharmacology through Chemical Biology Approaches

Dr. ***Thais Gazzi***, F. Hoffmann-La Roche

The second session started with Dr. Gazzi illustrating the central role that chemical biology tools play across all stages of pharmaceutical research. The talk explored the endocannabinoid system, a complex signalling network found in vertebrates which is vital in neurophysiological processes. It includes cannabinoid receptors CB1R and CB2R, endogenous ligands, and regulatory enzymes like monoacylglycerol lipase (MAGL). Despite its importance, poor characterization hinders therapeutic progress.

The presentation focused on the development of fluorescent probes designed to localize and study CB2R. While classic SAR

studies guided the identification of optimal linker attachment handles, a reverse-design approach from known validated small molecules was employed to generate high affinity and selective fluorescent probes over CB1R. The importance of these probes within biochemical assays was demonstrated monitoring intracellular CB2R distribution by live-cell imaging and by determining on-target ligand binding kinetics *via* TR-FRET. A second-generation of matched molecular pairs of agonist and inverse agonist probes were developed to selectively localize in real-time the active and inactive states of CB2R.

In the second part, the talk explored chemical probes targeting MAGL for potential therapeutic applications. Initial screening hits from a campaign to find MAGL inhibitors were utilized as the starting point to develop a novel fluorescent probe to assess MAGL inhibition potency in living cells. A NanoBRET cellular assay was established to measure target engagement *via* an optimized fluorescent MAGL tracer, facilitating efficient identification of promising MAGL inhibitors. This presentation demonstrated how smart probe design can support various stages of drug discovery by providing insights into receptor pharmacology, facilitating the development of new therapeutic agents.

Screening Approaches for the Identification of Covalent Peptide Inhibitors

Dr. ***Scott Lovell***, University of Bath

The Lovell Lab is interested in developing chemical modalities and technology platforms that enable the discovery of inhibitors for difficult-to-drug proteins in cancer and antibiotic-resistant bacteria. During his talk, Dr. Lovell covered two innovative projects showcasing the potential of high-throughput screening technologies for the identification of covalent peptide inhibitors.

In the first part, Dr. Lovell described a family of isoform-selective activity-based probes (ABPs) and inhibitors for dissecting the specificity and function of kallikrein-related peptidases (KLKs), a family of 15 serine proteases which orchestrate a cross-activation network mis-regulated in oncologic malignancies, like pancreatic ductal adenocarcinoma (PDAC). The probes optimized from a peptidomimetic scaffold enabled a detailed investigation of the KLK activome, supporting the hypothesis that KLK6 inhibition reduces the invasiveness and migration of PDAC Cells.

The second part of the talk focused on a phage display screening platform that enables the ultra-high throughput screening of covalent peptides. Libraries of targeted covalent macrocycles (TCMs) were created by cyclising billions of phage-displayed linear peptides with reactive linkers possessing latent electrophilic moieties. An emerging chemical modality, TCMs have the combined properties of a macrocyclic peptide (high affinity and specificity) and a covalent inhibitor (durable target engagement) and are particularly effective at engaging challenging targets such as the FphF hydrolase from *Staphylococcus aureus.*

Profiling the Proteome-Wide Selectivity of Diverse Electrophiles

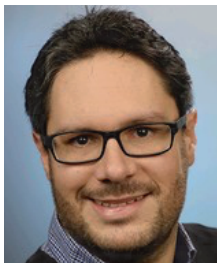
Prof. ***Stephan Hacker***, University of Leiden

Closing the morning program, Prof. Hacker described his group's recent research on targeted covalent inhibition as a valuable concept to expand the druggable proteome. It can offer advantages such as increased binding affinity, improved selectivity between closely related proteins, or enhanced pharmacodynamic properties. However, prerequisite proteome-wide selectivity assessments by competitive residue-specific proteomics have thus far been limited to covalent inhibitors bearing cysteine-directed electrophiles and a minority of selected probes targeting other amino acids, while a comprehensive comparison of more diverse probes was still completely lacking.

To this end, a generally applicable workflow was presented, that relies on a modification of the isotopic tandem orthogonal proteolysis activity-based protein profiling (isoTOP-ABPP) method by using isotopically labelled desthiobiotin azide tags (isoDTB) to streamline the experimental procedure, as well as on several tailored extensions of the MSFragger-based FragPipe computational platform to analyse the results. These extensions include new features for filtering open searches, analysing offset searches, and quantifying data from stable isotopic labelling-based MS experiments. The workflow was subsequently used to investigate the proteome-wide reactivity and selectivity of 54 electrophilic alkyne probes.

Overall, the new workflow allowed the verification or new discovery of 17 selective probes for nine amino acids as well as the *N*-terminus. Amongst them are the first probes to globally monitor arginine, histidine, and tryptophane, as well as newly tailored probes for aspartate, glutamate, and methionine.

Genetic Reprogramming to Map Target Exons Amenable to Splicing Modulation



Dr. ***Philipp Ottis***, Novartis Biomedical Research

The afternoon session started with the second industrial lecture. Dr. Ottis presented an innovative approach by his team to control protein translation through splicing modulation. Their research program investigates alternatives to small molecule modulators like branaplam and risdiplam, which stabilize U1-spliceosome interactions at non-canonical splice sites.

Dr. Ottis and his team developed a novel method using mutant U1-snRNA expression constructs to stabilize these splice sites, effectively mimicking the action of small molecule modulators. The study involved creating a comprehensive collection of U1-snRNA constructs, covering all 16 possible nucleotide combinations of the two exonic nucleotides closest to the 5' splice site. This approach successfully replicated and validated known targets susceptible to low molecular weight splicing modulation. More importantly, it opened new avenues for identifying potential targets in splicing modulator drug discovery projects. To demonstrate the practical applications of their method, the team evaluated MYB as a splicing target. This showcased the potential of their approach in identifying and validating new opportunities for therapeutic intervention through splicing modulation. The research represents a significant advancement in the field of genetic reprogramming and splicing modulation, offering a powerful tool for mapping target exons and potentially accelerating the development of new treatments for various genetic disorders.

Mechanistically Driven Prediction and Design of GPCR Signalling for Precision Medicine

Prof. ***Patrick Barth***, EPF Lausanne.

Prof. Barth's presentation shifted the focus to the most recent advances in *in silico* tools which are developed in the Laboratory of Protein and Cell Engineering at EPFL. His team has pioneered a novel *in silico* method that integrates molecular dynamic simulations with computational protein design to engineer specific amino acid 'microswitches' at allosteric sites in G protein-coupled receptors. These microswitches can fine-tune receptor stability and long-range coupling, effectively reprogramming signalling properties. To validate this method, Prof. Barth's team designed and tested 36 variants of the dopamine D2 receptor. These engineered receptors demonstrated significantly enhanced potency and signalling responses to both the native agonist dopamine and the non-native ligand serotonin, showcasing the method's ability to predict and engineer both constitutive and ligand-induced signalling. This method has potential applications in the *de novo* design of allosteric biosensors and signalling receptors for reprogramming cellular functions. The potential applications span across synthetic biology and cancer immunotherapy, offering new avenues for manipulating cellular behaviour through precisely engineered protein switches.

Covalent Inhibitors in Drug Discovery: From Serendipity to Rational Design

Dr. ***Sebastian Essig***, Bayer Life Science

The symposium concluded with a tour de force from Dr. Essig of Bayer. His plenary presentation offered a comprehensive journey through the evolution of covalent drug discovery, from the early days dominated by serendipity to the modern era of rational design and targeted covalent inhibitors (TCIs). The talk also provided a detailed account of the innovative approaches employed at Bayer to validate new targets amenable to covalent inhibition and to discover TCIs modulating these targets, explaining their mechanisms and advantages over reversible inhibitors.

During the lecture, special attention was dedicated to the Vividion chemoproteomics platform for identifying and characterizing covalent probes, which is then complemented by a MALDI high-throughput peptide reactivity assay and cell painting for early toxicology evaluation. Further, Dr. Essig introduced Bayer's Next Generation Library Initiative (NGLI). This initiative aims to go beyond the initial chemical space explored by the Vividion library, to include a wider range of chemical properties for covalent inhibition. A unique aspect was 'Forced Serendipity', Bayer's strategy to uncover hidden covalent motifs in their vast compound library. This involved reverse engineering chemo-informatics tools to identify potential covalent compounds from over 5.1 million entries, with ongoing profiling of 50,000 compounds to discover novel covalent chemistries. The presentation concluded by discussing the expansion into non-cysteine targeting warheads, broadening the scope of covalent drug discovery.

Poster Session

During the lunch break, attendees explored a diverse poster session showcasing a wide range of chemical biology topics. The Helvetica Best Poster Prize, presented by Richard J. Smith on behalf of *Helvetica Chimica Acta*, recognized two outstanding presentations. Céline Noemi Weller (ETH Zurich) was honored for her work on '*Rational design of RNA-PROTAC against FUS*' and Leon Rebhan (University of Bern) for his work on '*Synthesis of Chiral Tricyclic Piperazine-like Scaffolds from the GDB Database*'. These posters exemplified the high-quality research presented during the session.

Acknowledgement

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