2024 International Conference on Molecular Systems Engineering

NCCR Molecular Systems Engineering

Abstract Book

2-4 September 2024

Kollegienhaus, University of Basel, Switzerland

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Plenary Talks

Imagine a World Where Anyone Can Make Molecules

Martin D. Burke, *University of Illinois, USA*

Toolmaking made us human, and over the last 2 million years we've gotten pretty good at it. But we've only been intentionally making tools on the molecular scale for about 200 years. And currently only a tiny fraction of a fraction of the world's population can meaningfully participate in the molecular innovation process. Consider that many of the most important challenges facing society today likely have molecular solutions that await discovery. Then imagine the impact we could achieve together if everybody could make molecules.

Small molecules in particular possess tremendous functional potential that remains largely untapped due to "the synthesis bottleneck". This limits both the efficiency with which small molecules can be made by chemists and the participation of non-specialists in the molecular innovation process. An automatable modular platform based on iterative coupling of iminodiacetic acid boronate building blocks is accelerating and expanding access to small molecule synthesis and functional discovery. This platform has specifically been leveraged to develop molecular prosthetics – small molecules that autonomously perform protein-like functions and thus have the potential to treat a wide range of currently incurable diseases, including cystic fibrosis and anemias. Recent advances in Csp3 cross-coupling are substantially expanding the scope of complex small molecules that are accessible. Interfacing this approach with frontier AI and automated functional testing methods has enabled closed-loop discovery of new molecular functions, including organic lasers and photovoltaics. A first of its kind "Molecule Maker Lab" has now been created at UIUC that has opened the door for non-specialists to enter the molecular innovation process. And we are dreaming about globally accessible innovation competitions that leverage such resources to capture so many brilliant imaginations that the field of chemistry has been missing out on. Continued advances in these directions have the potential to democratize molecular innovation and thereby broadly empower tomorrow's molecular innovators worldwide.

Exploring Chemical Complexity with Assembly Theory and Chemputation

Lee Cronin, *University of Glasgow, UK*

Recent advancements in automation and digitization of chemistry have opened new avenues for exploring chemical complexity. In this talk I will explain how Assembly Theory[1-2] and Chemputation[3-4] can be used to develop a new paradigm to understand and harness the principles of Assembly Theory in chemical synthesis. Assembly Theory provides a framework for quantifying molecular complexity and understanding the emergence of complex chemical systems. Chemputation, on the other hand, offers a standardized method for digitizing and automating chemical synthesis through modular robotic platforms and a chemical programming language (χDL). By combining these approaches, researchers can systematically explore vast chemical spaces, optimize reaction conditions, and potentially discover novel molecules and materials. The integration of these two methodologies enables a new approach to explore chemical space with autonomous experimentation and discovery. As these technologies continue to evolve, they promise to accelerate chemical research, improve reproducibility, provide new insights into the fundamental nature of chemical complexity, as well as an entirely new language.

References

1. M. Jirasek, A. Sharma, J. R. Bame, S. H. M. Mehr, N. Bell, S. M. Marshall, C. Mathis, A. MacLeod, G. J. T. Cooper, M. Swart, R. Mollfulleda, L. Cronin 'Investigation and quantifying molecular complexity using assembly theory and spectroscopy', *ACS Cent. Sci.*, **2024**, *10*, 1054-1064, https://doi.org/10.1021/acscentsci.4c00120.
2. A. Sharma, D. Czégel, M. Lachmann, C. P. Kempes, S. I. Walker, L. Cronin 'Assembly theory explains and quantifies selection and evolution', *Nature*, **2023**, https://doi.org/10.1038/s41586-023-06600-9.
3. Y. Jiang, D. Salley, A. Sharma, G. Keenan, M. Mullin, L. Cronin 'An artificial intelligence enabled chemical synthesis robot for exploration and optimization of nanomaterials', *Sci. Adv.*, **2022**, *8*, eabo2626, https://doi.org/10.1126/sciadv.abo2626.
4. S. Rohrbach, M. Siauciulis, G. Chisholm, P. –A. Privan, M. Saleeb, S. H. M. Mehr, E. Trushina, A. I. Leonov, G. Keenan, A. Khan, A. Hammer, L. Cronin 'Digitization and validation of a chemical synthesis literature database in the ChemPU', *Science*, **2022**, *377*, 172-180, https://10.1126/science.abo0058.

Hybrid Responsive Nanomaterials for Crossing Barriers and Selective Organ Uptake

Luisa de Cola, *University of Milan, IT*

Advancements in the use of nanoparticles for biomedical applications have clearly shown their potential for the preparation of improved imaging and drug-delivery systems. However, only a few successfully materials translate into clinical practice, because, of their incomplete elimination, difficulties to cross barriers and lack of slectivity. We have recently reported disulfide-bridged organosilica nanoparticles with cage-like morphology, and assessed in detail their bioaccumulation *in vivo*. [1,2] The fate of intravenously injected 20 nm nanocages was investigated in both healthy and tumor bearing mice. Interestingly, the nanoparticles exclusively co-localize with hepatic sinusoidal endothelial cells (LSECs), while avoiding Kupffer-cell uptake in both physiological and pathological condition. Our findings suggest that organosilica nanocages hold the potential to be used as nanotools for drug delivery and for crossing important body barriers.[3]

But how to target specific organs or cancer tissues?

To improve selective uptake, we have recently developed a technology based on the use of extracellular vesicles, EVs. Through a strategy we are able to separate the membrane of these vesicles and reconstruct it on top of our nanomaterials. The membrane contains all the original targeting proteins and receptors and depending from which tumor is isolated able to target specific organs or metastasis. We show that not only in vitro we have an excellent selectivity, but also in vivo we are able to demonstrate an excellent selectivity towards cancer cells vs normal cells of the same tissue. [4]

The use of melanoma EVs showed a tropism, of the hybrid materials, towards lungs and quantitative analysis on mice models suggested that the targeting behavior of the EVs can be indeed used as a strategy for the targeting of lungs and reduces dramatically the accumulation in liver.

Finally, silica nanoparticles containing single-stranded nucleic acids, that are covalently embedded in the silica network, have been reported [5]. The system can be programmed to be more dynamic and responsive by designing supramolecular organo-silica systems based on PNA- derivatives that can self-assemble through direct base paring or can be joined through a bridging functional nucleic acid, such as the ATP-binding aptamer [6].

These systems can be followed by confocal microscopy in different cell lines and their biological effect was measured in cells to assess the biological effect of the aptamer.

References

1. P. Picchetti *et al. ACS Nano* **2021,** *15,* 9701–9716
2. P. Picchetti, *et al. J. Am. Chem. Soc*. **2021**, *143*, 7681-7687.
3. M. Sancho Albero, *et al. Adv. Healthcare Mater.*, **2023**, *12*, 2202932
4. M. Sancho Albero, L De Cola, L. Terracciano *et al.* Submitted
5. P. Picchetti *et al. J. Am. Chem. Soc*. **2023**, *145*, 22896-22902
6. P. Picchetti *et al. J. Am. Chem. Soc*. **2023**, *145*, 22903-22912.

In Vitro Evolution and Molecular Engineering of RNA-Modifying Ribozymes

Claudia Höbartner, *University of Würzburg, DE*

Synthetic functional nucleic acids such as fluorogen-activating RNA aptamers and RNA-alkylating ribozymes have emerged as enabling tools for tagging and visualizing RNA in vitro and in cells. Novel activities of ribozymes and aptamers are generated by iterative cycles of selection and amplification from an initially random library. This presentation will focus on ribozymes that catalyze site-specific RNA modification using small molecule cofactors.

Natural RNA modifications such as site-specifically methylated nucleotides are conserved throughout evolution and expand the structural and functional diversity of RNA. Synthetic RNA modifications enable RNA labelling and visualization to study RNA localization, folding and structural dynamics. We discovered a methyltransferase ribozyme (MTR1) that catalyzes the installation of 1-methyl-adenosine (m^1A) in a target RNA. The ribozyme shows a broad RNA sequence scope, as exemplified by site-specific adenosine methylation in native tRNAs and synthetic mRNAs. The crystal structure of MTR1 revealed an active site reminiscent of natural guanine riboswitches and suggested the mechanistic involvement of a protonated cytidine. Further research revealed alkyltransferase ribozymes using established benzylguanine substrates for site-specific fluorescent labelling of RNA. Recently we found an alkyltransferase ribozyme that uses a synthetic, stabilized S-adenosyl-methionine (SAM) analogue and catalyses the transfer of a propargyl group to a specific adenosine in the target RNA. A genetically encoded version of the SAM analogue-utilizing ribozyme (SAMURI) was expressed in eukaroytic cells, and intracellular propargylation of the target adenosine was confirmed. The transferred alkyne group was efficiently conjugated to different azides, including fluorophores and biotin, to enable studies of RNA localization, folding and structural dynamics. Such engineered ribozymes provide attractive means for tracking RNA localization, folding and structural dynamics, and may be used as protein-free tools to modulate the cellular RNA modification landscape.

An Approach to the De-Novo Synthesis of Life

Sijbren Otto, *University of Groningen, NL*

How the immense complexity of living organisms has arisen is one of the most intriguing questions in contemporary science. We have started to explore experimentally how organization and function can emerge from complex molecular networks in aqueous solution.[1] We focus on networks of molecules that can interconvert, to give mixtures that can change their composition in response to external or internal stimuli (Figure 1). Noncovalent interactions *within* molecules in such mixtures can lead to the spontaneous formation of foldamers of remarkable structural complexity.[2,3] In contrast, molecular recognition *between* molecules in such mixtures leads to their mutual stabilization, which drives the synthesis of more of the privileged structures. As the assembly process drives the synthesis of the very molecules that assemble, the resulting materials can be considered to be self-synthesizing. In this process the assembling molecules are replicating themselves, where replication is driven by self-recognition of these molecules in the dynamic network.[4] We have witnessed spontaneous differentiation (a process akin to speciation as it occurs in biology) in a system made from a mixture of two building blocks.[5] When such systems are operated under out-of-equilibrium conditions, replicators can complexify.[6]

Replicators that are able to catalyse reactions other than their own formation have also been obtained, representing a first step towards metabolism.[7,8] Rudimentary Darwinian evolution of purely synthetic molecules has also been achieved [9,10] and the prospect of synthesizing life de-novo is becoming increasingly realistic.[11,12]

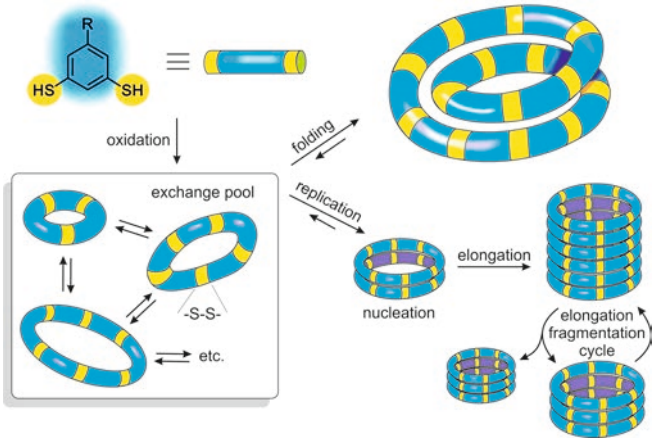

Figure 1. *Molecular recognition within molecules yields foldamers and between molecules drives self-replication.*

References

1. Li, J.; Nowak, P.; Otto, S. *J. Am. Chem. Soc.* **2013**, *135*, 25, 9222-9239.
2. Liu, B.; Pappas, C. G.; Zangrando, E.; Demitri, N.; Chmielewski, P. J.; Otto, S. *J. Am. Chem. Soc.* **2019**, *141*, 1685-1689.
3. Pappas, C. G.; Mandal, P. K. Liu, B.; Kauffmann, B.; Miao,X.; Komáromy, D.; Hoffmann, W.; Manz, C.; Chang, R.; Liu, K.; Pagel, K.; Huc, I.; Otto, S. *Nature Chem.* **2020**, *12,* 1180–1186.
4. Carnall, J. M. A.; Waudby, C. A.; Belenguer, A. M.; Stuart, M. C. A.; Peyralans, J. J.-P.; Otto, S. *Science* **2010**, *327*, 1502-1506.
5. Sadownik, J. W.; Mattia, E.; Nowak, P.; Otto, S. *Nature Chem.* **2016**, *8*, 264–269.
6. Yang, S.; Schaeffer, G.; Mattia, E.; Markovitch, O.; Liu, K.; Hussain, A. S.; Ottelé, J.; Sood, A.; Otto, S. *Angew. Chem. Int. Ed.* **2021**, *60*, 11344-11349.
7. Monreal Santiago, G.; Liu, K.; Browne, W. R.; Otto, S. *Nature Chem.* **2020**, *12*, 603-607.
8. Ottelé, J.; Hussain, A. S.; Mayer, C.; Otto, S. *Nature Catal*. **2020**, *3*, 547-553.
9. K. Liu, A. Blokhuis, C. van Ewijk, A. Kiani, J. Wu, .W.H. Roos, S. Otto *Nature Chem.* **2024**, *16*, 79–88.
10. Eleveld, M.; Geiger, Y.; Wu, J.; Kiani, A.; Schaeffer, G.; Otto, S. *Nature Chem. in press* **2024**, *ChemRxiv*, **2024**, https://doi.org/10.26434/chemrxiv-2024-w9k6w.
11. Adamski, P.; Eleveld, M.; Sood, A,; Kun, A.; Szilágyi, A.; Czárán, T.; Szathmáry, E.; Otto, S. *Nature Rev. Chem.* **2020**, *4*, 386-403.
12. Otto, S. *Acc. Chem. Res.* **2022**, *55*, 145-155.

Transcriptional Linkage Analysis with *In Vivo* AAV-Perturb-seq

Randall Platt, *ETH Zurich, CH*

The ever-growing compendium of genetic variants associated with human pathologies demands new methods to study genotype-phenotype relationships in complex tissues in a high-throughput manner. Here we introduce adeno-associated virus (AAV)-mediated direct in vivo single-cell CRISPR screening, termed AAV-Perturb-seq, a tuneable and broadly applicable method for transcriptional linkage analysis as well as high-throughput and high-resolution phenotyping of genetic perturbations in vivo. We applied AAV-Perturb-seq using gene editing and transcriptional inhibition to systematically dissect the phenotypic landscape underlying 22q11.2 deletion syndrome genes in the adult mouse brain prefrontal cortex. We identified three 22q11.2-linked genes involved in known and previously undescribed pathways orchestrating neuronal functions in vivo that explain approximately 40% of the transcriptional changes observed in a 22q11.2-deletion mouse model. Our findings suggest that the 22q11.2-deletion syndrome transcriptional phenotype found in mature neurons may in part be due to the broad dysregulation of a class of genes associated with disease susceptibility that are important for dysfunctional RNA processing and synaptic function. Our study establishes a flexible and scalable direct in vivo method to facilitate causal understanding of biological and disease mechanisms with potential applications to identify genetic interventions and therapeutic targets for treating disease.

Crisscross Polymerization of Single-Stranded and DNA-Origami Slats

William Shih, *Harvard University, USA*

DNA origami, in which a long scaffold strand is assembled with a large number of short staple strands into parallel arrays of double helices, has proven a powerful method for custom nanofabrication of shapes up to 100 nm in size. The scaffold represents about half the mass of an origami, therefore the origami size is restricted by the length of the scaffold. However, it is impractical and prohibitively expensive to scale the length of the scaffold. Here I will discuss a strategy, that we call crisscross polymerization, that combines all-or-nothing scaffold-dependent initiation of folding with scaffold-independent growth, therefore allowing for sizes unbounded by the length of the scaffold. Assembly using single-stranded DNA slats enables digital counting of molecular analytes, where each molecular detection event triggers growth of a single filament resolvable by low-cost microscopy. Assembly using DNA-origami slats, conversely, enables fabrication of fully addressable structures that are twice the mass of the E. coli genome, and that span an area of two microns by two microns.

Matter to Life: Bottom-Up Assembly of Synthetic Cells

Joachim Spatz, *MPI Medical Research, DE*

The evolution of cellular compartments for spatially and temporally controlled assembly of biological processes was an essential step in developing life by evolution. Synthetic approaches to cellular-like compartments are still lacking well-controlled functionalities, as would be needed for more complex synthetic cells. With the ultimate aim to construct life-like materials such as a living cell, matter-to-life strives to reconstitute cellular phenomena *in vitro* – disentangled from the complex environment of a cell. In recent years, working towards this ambitious goal gave new insights into the mechanisms governing life. With the fast-growing library of functional modules assembled for synthetic cells, their classification and integration become increasingly important. We will discuss strategies to reverse-engineer and recombine functional parts for synthetic eukaryotes, mimicking the characteristics of nature's own prototype. Particularly, we will focus on large outer compartments, complex endomembrane systems with organelles and versatile cytoskeletons as hallmarks of eukaryotic life. Moreover, we identify microfluidics and DNA nanotechnology as two highly promising technologies which can achieve the integration of these functional modules into sophisticated multifunctional synthetic cells.

Artificial Cells Interacting with Mammalian Cells

Brigitte Städler, *Aarhus University, DK*

Bottom-up synthetic biology aims to design life-like units (aka artificial cells) that can substitute for missing/lost cellular activity or to add non-native function to mammalian cells and tissue. Artificial cells are minimal, simplistic structures that imitate selected structural or functional aspects of living cells.

We focus our efforts on hydrogel-based artificial cells equipped with a specific liver-like function and their integration and communication with mammalian cells. Specifically, the artificial cells support their living counterpart in fighting reactive oxygen species either by direct conversion or by deploying supportive nano-units. Further, we showed that hepatic cell aggregates could be 3D bioprinted together with artificial cells to boost catalytic activity for at least 2 weeks. In addition, we illustrated that artificial cells can eavesdrop on a typical activity of a liver cell due to co-existence in a semi-synthetic tissue.

Our efforts illustrate the potential of nano-engineered artificial cells for tissue engineering purposes.

Combining Molecular and Process Systems Engineering (M&PSE) to Produce Cost-Effective Liquid Fuels from Renewable Feedstocks

Gregory Stephanopoulos, *MIT, USA*

The importance of liquid fuels in transportation is well established, yet, there are presently no viable options for their cost-effective production from renewable feedstocks. During the past 15 years we have been engineering the molecular and bioprocess system for the conversion of sugar substrates and gasses to oils and alkanes. Despite achieving near theoretical yields, production of liquid fuels from sugars in not economical, due mainly to the high substrate cost. Mixtures of gasses, on the other hand, like CO_2 and hydrogen (or CO) is more promising using a two-stage system comprising anaerobic fixation of CO_2 and conversion of the CO_2 fixation product (for example, acetate) to lipids, from which biodiesel can be produced. In another application, the CO_2 fixation product is converted to alkanes. Our work includes both the molecular engineering of the microbes *and* development of a process to achieve gas to liquid conversion in prototype systems. These systems are scalable, make no use of land (beyond what is needed for generating renewable electricity for hydrogen production), do not compete with food and are cost competitive based on high level cost analysis and TEA. I will present the essential features of this process in my talk; full details can be found in the 5 papers cited.

References

1. Peng Hu*, et al.*, **"**Integrated system for biological conversion of gaseous substrates to lipids," *Proceedings of the National Academy of Sciences,* **2016***,* 201516867, https://doi.org/10.1073/pnas.1516867113.
2. Jingyang Xu, *et al.,* "Application of metabolic controls for maximization of lipid production in oleaginous yeast," *Proceed. of the National Academy of Sciences,* **2017**, *114*, E5308-E5316, https://doi.org/10.1073/pnas.1703321114.
3. K.J. Qiao, *et al.*, "Rewiring metabolism to maximize lipid production in *Yarrowia lipolytica," Nature Biotechnology,* **2017**, *35*, 173-177, https://doi.org/10.1038/nbt.3763.
4. J.O. Park, *et al*, "Synergistic substrate co-feeding stimulates reductive metabolism," *Nature Metabolism*, **2019**, *1*, 643–651, https://doi.org/10.1038/s42255-019-0077-0.
5. Li, Jingbo, *et al.,* "Synthesis of High-Titer Alka(e)Nes in *Yarrowia Lipolytica* Is Enabled by a Discovered Mechanism." *Nature Communications*, **2020** *11*, 1-13, https://doi.org/10.1038/s41467-020-19995-0.

Understanding Human Organ Development with Single Cell and Organoid Technologies

Barbara Treutlein, *ETH Zurich, CH*

Pluripotent stem cell derived organoids are exciting, complex *in vitro* models to study human organ development. Integrative, multi-modal single-cell technologies are needed to understand the mechanisms underlying fate specification during human organoid development.

In my talk, I will present our efforts to develop and use single-cell technologies combined with genetic and environmental perturbations to dissect the mechanisms underlying patterning and fate specification during human organoid development with a focus on brain and vasculature. Further, I will highlight our attempts to further improve organoid development and maturation by introducing missing lineages. Together, our work highlights the power of single-cell and organoid technologies to understand cell fate and state specification during human organ development.

Spatiotemporal Control in Synthetic Cells Using Light

Seraphine Valeska Wegner, *University of Münster, DE*

Bottom-up synthetic biology aims to construct cell-like systems starting from molecular building blocks and give insight into principles that give rise to cell function. Many functions in cells arise directly from the spatial and temporal control of processes such as protein localization, cell migration, tissue assembly and cell-to-cell communication. In this talk, I will present strategies of how such spatiotemporal control over adhesions in synthetic cells can be achieved with visible light using photoswitchable proteins and functions that arise from these. The photoswitchable adhesions allow us to recapitulate cell migration, to self-assemble and self-sort synthetic cells into multicellular functional architectures with high precision. Moreover, the organization in these multicellular communities is of significance for their communication and the overall arising behaviors. These synthetic cell-mimetic systems, which reduce complexity and yet capture key features of natural cells, allow us to quantify and correlate cell behavior with molecular information. Further, complementary approaches pursued with synthetic minimal cells as well as bacterial and mammalian cells allow translating concepts between different systems and integration into hybrid structures. Overall, our work on one hand provides insight into underlying design principles of life and on the other hand engineer new synthetic cell biology.

Short Talks

Chemical Reservoir Computation in a Self-Organizing Reaction Network

Mathieu Baltussen, *Radboud University Nijmegen, NL*

Chemical reaction networks, such as those found in metabolism and signalling pathways, enable cells to process information from their environment[1]. Current approaches to molecular information processing and computation typically pursue digital computation models and require extensive molecular-level engineering[2]. These approaches demonstrate how molecular systems may perform computation, but do not achieve the information processing capabilities of living systems. Unlocking the full potential of molecular systems requires (1) moving beyond a strict adherence to reproducing digital computation principles and (2) finding an approach that overcomes the laborious nature of bottom-up 'molecule-by-molecule' design patterns.

We recently reported on the discovery of emergent information processing capabilities and implementation of chemical reservoir computation in the formose reaction[3]. This prebiotic, complex, self-organizing reaction network produces a rich diversity of possible chemical compositions that are non-linearly dependent on a small number of input chemicals. Under flow conditions, the distribution of chemical compositions can be modulated using changes in reactor input concentrations, allowing a range of complex reaction responses to be controlled with a relatively simple set of input parameters[4]. These properties, and the experimental tractability of the formose reaction, make it an excellent candidate system for exploring chemical information processing using the model of physical reservoir computation[5]. We demonstrate how the formose reaction can be used as a reservoir computer and investigate its capabilities for several computational tasks. We first show it can emulate all Boolean logic gates, and several, more advanced nonlinear classification tasks in parallel, achieving performance similar to machine learning algorithms. We next demonstrate its suitability for more complex computational tasks, by using the formose reservoir to predict the dynamics of an *E. coli* carbon-metabolism pathway in a fluctuating environment. Finally, we achieve time-series forecasting of chaotic changing environments, and evaluate the memory properties of the reservoir by quantifying the propagation of information through the formose network over time.

Our work shows how chemical reaction networks process information on the basis of self-organization, and, much like biological systems, can achieve a variety of powerful computational tasks using information from their environment, obviating the need for complex bottom-up design and creating new opportunities for scalable molecular computing. This in-chemico information processing system provides proof of principle for the emergent computational capabilities of complex chemical reaction networks, paving the way for a new class of biomimetic information processing systems.

References

1. Nurse, P. Life, logic and information. *Nature* **2008** *454*, 424–426.
2. Grozinger, L. *et al.* Pathways to cellular supremacy in biocomputing. *Nat. Commun.* **2019** *10*, 5250 (2019).
3. Baltussen, M.G., de Jong, T.J., Duez, Q. *et al.* Chemical reservoir computation in a self-organizing reaction network. *Nature* **2024**.
4. Robinson, W. E., Daines, E., Duppen, P. V., Jong, T. D. & Huck, W. T. S. Environmental conditions drive self-organisation of reaction pathways in complex prebiotic reaction networks. *Nat. Chem.* **2022** *14*, 623–631.
5. Nakajima, K. Physical reservoir computing – an introductory perspective. *Jpn. J. Appl. Phys.* **2020** *59*, 060501

A Multi-Epitope Protein-DNA Nanoswitch Platform for the Monitoring of Bioavailable Therapeutic Antibodies

Denise Di Lena, *University of Parma, IT*

Monoclonal antibodies (mAbs) represent a key therapeutic option for managing chronic conditions. Therapeutic drug monitoring (TDM) presents a promising method for personalized treatment plans, especially in chronic diseases where excessive treatment can result in significant adverse effects. To advance TDM strategies, the NanoHybrid (NH) platform developed by Ulisse BioMed has been redesigned into an innovative protein-DNA nanoswitch sensor. This new configuration utilizes entire protein binding units to detect mAbs by taking advantage of multiple identifiable epitopes. Nanoswitch probes, composed of specifically designed DNA strands conjugated with whole tumor necrosis factor α (TNFα) proteins, facilitated the one-step quantification of various mAbs such as Infliximab and Adalimumab in concentrations ranging from 2.4 µg/mL to 19 µg/mL directly in blood serum. Given that mAbs can induce patient-specific immune responses, anti-drug antibodies (ADAs) are frequently generated against biological medications, significantly affecting the actual bioavailability of therapeutic mAbs. Inspired by this, the nanoswitch platform was utilized to detect Infliximab in the presence of an anti-Infliximab antibody, revealing a notable decrease in the detected concentrations of Infliximab when such ADA was present. These findings demonstrated that the developed nanoswitch platform can specifically identify bioavailable antibodies, offering valuable insights for pharmacokinetic research. With its adaptable design, the current nanoswitch platform has potential as a multi-epitope nanosensor for the measurement of bioavailable mAbs and biosimilars.

Modeling Human Bone Marrow Endosteal Niches from Induced Pluripotent Stem Cells in Xeno-Free Conditions

Andres Garcia-Garcia, *University of Basel, CH*

Human induced pluripotent stem cells (hiPSCs) have emerged as a powerful tool to engineer robust and reproducible organoids to model human biology and pathology, paving the way for mechanistic and pharmacological studies in human settings. Recently, the two first bone marrow (BM) organoid models based on hiPSCs were presented, and showed to successfully recapitulate key features of human BM while modeling both healthy and malignant hematopoiesis. Although these BM organoids represent a breakthrough in modeling the functional complexity of human BM perivascular niches, they lack the bone compartment, and thus they are not suitable to model human endosteal BM niches (close to the bone surface). Moreover, these organoids remain in the micrometer scale and therefore the vascular structures are non-physiological in shape and size. Finally, they rely on the use of Matrigel as embedding material, which introduces mouse-derived proteins in the system.

Here we present a novel developmentally-guided approach combining hiPSC-derived organoids with macro-scale hydroxyapatite scaffolds to generate a standardized and physiological-like model of the human endosteal BM perivascular niche (engineered vascularized osteoblastic niche, eVON). We developed and validated a protocol to differentiate hiPSCs into vascular cells (endothelial cells and pericytes) and osteoblasts that later self-assemble to create complex and long-lasting vascular networks integrated in a dense osteogenic matrix, resembling the 3D architecture of the native endosteal BM. The eVON was first characterized through flow cytometry, high-resolution imaging and scRNAseq. Second, we demonstrated that it can persist for at least 6 weeks upon in vivo ectopic implantation and integrate within the murine tissue. Then, we assessed its potential to support human hematopoiesis and explored its customization potential by engineering different eVON using different hiPSC lines. Finally, we validated the model for pharmacological studies by targeting VEGF signaling and analyzing the effects on the vasculature. Therefore, this work provides the first standardized and physiological-like model of the human endosteal BM, offering an unprecedented possibility to dissect the contribution of BM endosteal niches to human pathophysiological hematopoiesis.

Programmable RNA Writing with Trans-Splicing

Marcos Manero Carranza, *ETH Zurich, CH*

The discovery and repurposing of CRISPR effectors has allowed for the development of a genome editing toolbox capable of performing programmable and efficient genetic interventions for a wide range of therapeutic and research purposes. However, most available tools directly modify the DNA sequence or alter gene expression level, leaving RNA modification comparatively unexplored. RNA editing, however, offers the opportunity to introduce either stable or transient modifications to nucleic acid sequences, without the risk of permanent off-target effects. This could be applied for sensing, labelling, or repairing of RNA transcripts, but installation of arbitrary edits into the transcriptome is currently infeasible. Trans-splicing-based RNA editing technologies can install diverse edits, insertions, and deletions by introducing exogenous templates via competition with the endogenous exon in the pre-spliced mRNA. However, currently they suffer from low efficiency, even after extensive screening, which hinders their applicability. Here, we describe Programmable RNA Editing & Cleavage for Insertion, Substitution, and Erasure (PRECISE), a versatile RNA editing method for writing RNA of arbitrary length and sequence into existing pre-mRNAs via 5′ or 3′ trans-splicing. We demonstrate PRECISE editing across 11 distinct endogenous transcripts of widely varying expression levels, showcasing more than 50 types of edits, including all possible transversions and transitions and a wide range of insertions and deletions. We show high efficiency replacement of MECP2 last exon, addressing most mutations that drive the Rett Syndrome; editing of SHANK3 transcripts, a gene involved in Autism; and replacement of exon 1 of HTT, removing the hallmark repeat expansions of Huntington′s disease. Furthermore, we combine payload engineering and ribozymes for protein-free, high-efficiency trans-splicing, with demonstrated efficiency in editing HTT exon 1 via AAV delivery. We show that the PRECISE achieves editing in non-dividing neurons and patient-derived Huntington’s disease fibroblasts. Our results provide the scientific community with a novel tool that markedly broadens the scope of RNA editing, is straightforward to deliver, lacks permanent off-targets, and can enable any type of edit, including those not otherwise possible with current RNA base editors. Ultimately, these findings expand the current RNA editing toolbox for therapeutic or research purposes and widen the spectrum of addressable diseases.

Synthetic Organelles to Engineer Mammalian Cells

Christopher Reinkemeier, *ETH Zurich, CH*

Engineering new functionalities into living systems has a tremendous potential for biotechnology and medicine. However, using canonical routes of evolution can be limited by the necessity to avoid interference with endogenous processes. Cells themselves do not only rely on molecular evolution to prevent undesired crosstalk, but they often utilize compartmentalization, in the form of membrane-enclosed or membraneless organelles to spatially isolate distinct molecular processes and execute complex operations that would otherwise interfere with each other.

Inspired by this, we developed synthetic organelles to equip mammalian cells with new functions. Specifically, we combined phase separating proteins such as FUS and EWSR1 with anchor proteins that tether the organelles to specific subcellular structures. With this we can generate organelles spanning vastly different shapes from kinesin-motor based micrometer sized organelles over fiber-like organelles along the microtubule cytoskeleton to film-like organelles on membrane surfaces that have a thickness of less than 100 nm.

We then applied these organelles to improve genetic code expansion (GCE) in mammalian cells. GCE is a powerful tool to control and expand protein function with single-residue precision that is widely used to perform labeling for microscopy or to photocontrol proteins. This technology relies on an orthogonal tRNA/synthetase pair that is introduced into the host, which recodes a stop codon to incorporate a noncanonical amino acid (ncAA) into the nascent chain. While this technique is codon-specific, it cannot select specific mRNAs, so naturally occurring stop codons can be suppressed, causing significant side effects. To overcome this, we integrated a GCE system into our synthetic organelles to develop orthogonally translating (OT) organelles. These OT organelles can perform mRNA specific GCE, installing ncAAs exclusively into desired proteins. We further demonstrate that it is possible to combine different OT organelles in one cell, effectively yielding cells that simultaneously execute multiple distinct genetic codes.

Our results demonstrate a simple yet effective approach to generate artificial organelles that enable customized orthogonal translation and protein engineering in semi-synthetic eukaryotic cells.

Pnictogen-Bonding Enzymes

Giacomo Renno, *University of Geneva, CH*

Pnictogens represent the last frontier of the σ-hole bonds, non-covalent interactions highly attractive for the development of powerful tools in supramolecular chemistry. In this study, artificial enzymes capitalizing on pnictogen bonding are reported as a new tool, absent in biocatalysis. To tackle this challenge, stibine catalysts were decorated with a biotin moiety and combined with streptavidin mutants. The transfer hydrogenation of fluorogenic quinolines mediated by a hydrophilic Hantzsch ester was used as benchmark reaction. σ-Hole deepening resulted into an improvement of the catalytic performances, best for mutants which position negative charges (D, E) or hydrophobic residues (I) in the active pocket. Michaelis-Menten analysis showed transition-state recognition in the low micromolar range. Lastly, an emerging stereoselectivity further corroborated the promising potentiality of pnictogen-bonding catalysts in such ordered systems.

NanoAmp: Toward Protein PCR for Rapid and Sensitive Biomolecule Detection

Edoardo Sisti, *University of Pisa, IT*

NanoAmp is an innovative PCR-based technology designed to detect antigens and antibodies through an easy and quick single-step method. Developed by UlisseBiomed S.p.A., NanoAmp leverages the target-induced increase in local concentration, mediated by interactions between nucleic acid-based elements. It combines both isothermal polymerase reactions and hot-start PCR amplification, which can be performed using a single enzyme in its most advanced form. This approach enables rapid, sensitive, and quantitative biomolecule detection in a single well within 30 minutes. Compared to traditional biomolecule detection methods such as ELISA and CLIA, which are used for high-throughput formats and involve complex, time-consuming, multi-step processes, NanoAmp offers an easier and more flexible solution. Its unique chemistry allows for testing on a few samples or even single-sample analyses without sacrificing extreme sensitivity. Additionally, compared to PCR-based protein detection methods, which are sensitive but lengthy and prone to contamination due to their multiple steps, NanoAmp addresses these challenges by providing a straightforward, single-step method equivalent to a standard PCR analysis for antigen detection. This study demonstrates NanoAmp's efficacy as a modular platform accommodating various binding moieties for diverse biological targets. The platform successfully operated with antibodies, proteins, aptamers, and peptides, showcasing its versatility and high sensitivity. Employing the DIG binding motif, the system detected anti-DIG antibodies at concentrations as low as 66 picomolar (pM) and exhibited effectiveness in crude biological samples. NanoAmp aims to revolutionize the world of immunoassays by offering a platform capable of producing highly sensitive and easy-to-use assays for one-pot detection of a plethora of antigens. Additionally, its modular nature allows for the development of customizable research use only (RUO) assays, as different analyte binding motifs can be easily paired with the core system to tailor tools for specific needs.

Pharmacological Assays in Biohybrid Artificial Cell Networks

Robert Strutt, *ETH Zurich, CH*

In vitro pharmacology is constrained by the biological relevance of the experimental system, experimental throughput and analytical method flexibility. To address these bottlenecks, fundamentally new approaches are required. Across the course of the NCCR MSE, the Bioanalytics lab has developed a series of novel techniques which blend advances from the fields of artificial cell engineering and analytical chemistry. This contribution will focus on recent developments with artificial cell networks assembled from droplets. With our experimental approach, we exert spatial-temporal control over nanoliter droplets containing chemical and biological stimuli. With this, we have unlocked pharmacokinetic / pharmacodynamic (PK/PD) modelling in single droplets and droplet networks.

In these systems, the PK component is defined by transport between droplet compartments connected by biomimetic membranes. Through assessment of an FDA approved drug library, we categorized drugs with a propensity to undergo passive diffusion. Similar physicochemical features which predict in vivo bioavailability correlate with the drug kinetics in our system. Physiologically relevant variables such as temperature, buffer, pH and membrane composition provide tools for modulating the rate of transport and improving system biomimicry. Using our approach, we can reconstitute drug concentration profiles, which capture the peak and trough flux of oral and intravenously administered drugs. In response, the PD component is measured in situ through the inclusion of living cells. By arranging the number of membrane barriers between a source of antibiotic and the target site of action, our system can mimic intracellular infections. Control over the number and arrangement of connected droplets passively distributes antibiotic throughout the system. Through modelling and simulation, we couple the experimentally measured PK and PD responses, facilitating detailed interrogation of antibiotic efficacy under subtle variations in the drug concentration profile. With this simple to use and automatable methodology, we therefore introduce a novel framework for designing and conducting pharmacological assays.

Posters

Actinorhodopsin: An Efficient & Robust Light-driven Proton Pump for Bionanotechnological Applications

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Rhodopsins are ubiquitous light-sensitive membrane proteins with diverse functions. Photosensitivity arises when the apoprotein (opsin) binds the chromogenic co-factor retinal to form the photochemically reactive retinylidene holoprotein (rhodopsin). Photon capture by the covalently bound retinal triggers its photoisomerization and leads to functionally relevant protein changes. Some microbial rhodopsins (MRs) are light-driven proton pumps, converting photoenergy into energizing electrochemical gradients useful for ATP synthesis in the host organisms.

As part of the NCCR Molecular Systems Engineering (MSE) in Switzerland, we aim to assemble bioinspired molecular factories for bionanotechnological applications. “Nanocontainers” such as liposomes and polymersomes form the scaffolding for such systems which are later functionalized with (bio)chemical modules in a bottom-up synthetic biology approach. Energizing modules (EMs) are vital in such systems. Light-driven MR proton pumps are attractive EMs as they build up proton gradients across membranes by mere stimulation with light.

We have recently identified an efficient and robust proton pumping MR from the actinorhodopsins (ActRs) family. The ActR is thus attractive for integration as an energizing building block in an artificially assembled vesicular synthetic organelle co-reconstituting F-type ATP synthase that generates ATP from the proton motive force established by ActR.

Chemical Reservoir Computation in a Self-Organizing Reaction Network

Mathieu Baltussen[1]; Thijs de Jong[1]; Quentin Duez[2]; William Robinson[1]; Wilhelm Huck[1]
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Chemical reaction networks, such as those found in metabolism and signalling pathways, enable cells to process information from their environment[1]. Current approaches to molecular information processing and computation typically pursue digital computation models and require extensive molecular-level engineering[2]. These approaches demonstrate how molecular systems may perform computation, but do not achieve the information processing capabilities of living systems. Unlocking the full potential of molecular systems requires (1) moving beyond a strict adherence to reproducing digital computation principles and (2) finding an approach that overcomes the laborious nature of bottom-up ‘molecule-by-molecule’ design patterns.

We recently reported on the discovery of emergent information processing capabilities and implementation of chemical reservoir computation in the formose reaction[3]. This prebiotic, complex, self-organizing reaction

network produces a rich diversity of possible chemical compositions that are non-linearly dependent on a small number of input chemicals. Under flow conditions, the distribution of chemical compositions can be modulated using changes in reactor input concentrations, allowing a range of complex reaction responses to be controlled with a relatively simple set of input parameters[4]. These properties, and the experimental tractability of the formose reaction, make it an excellent candidate system for exploring chemical information processing using the model of physical reservoir computation[5]. We demonstrate how the formose reaction can be used as a reservoir computer and investigate its capabilities for several computational tasks. We first show it can emulate all Boolean logic gates, and several, more advanced nonlinear classification tasks in parallel, achieving performance similar to machine learning algorithms. We next demonstrate its suitability for more complex computational tasks, by using the formose reservoir to predict the dynamics of an *E. coli* carbon-metabolism pathway in a fluctuating environment. Finally, we achieve time-series forecasting of chaotic changing environments, and evaluate the memory properties of the reservoir by quantifying the propagation of information through the formose network over time.

Our work shows how chemical reaction networks process information on the basis of self-organization, and, much like biological systems, can achieve a variety of powerful computational tasks using information from their environment, obviating the need for complex bottom-up design and creating new opportunities for scalable molecular computing. This in-chemico information processing system provides proof of principle for the emergent computational capabilities of complex chemical reaction networks, paving the way for a new class of biomimetic information processing systems.

References

1. Nurse, P. Life, logic and information. *Nature* **2008** *454*, 424–426.
2. Grozinger, L. *et al.* Pathways to cellular supremacy in biocomputing. *Nat. Commun.* **2019** *10*, 5250 (2019).
3. Baltussen, M.G., de Jong, T.J., Duez, Q. *et al.* Chemical reservoir computation in a self-organizing reaction network. *Nature* **2024**.
4. Robinson, W. E., Daines, E., Duppen, P. V., Jong, T. D. & Huck, W. T. S. Environmental conditions drive self-organisation of reaction pathways in complex prebiotic reaction networks. *Nat. Chem.* **2022** *14*, 623–631.
5. Nakajima, K. Physical reservoir computing – an introductory perspective. *Jpn. J. Appl. Phys.* **2020** *59*, 060501

High-Throughput Generation of Microdroplet Arrays for Multimodal Fluorescence- and MALDI-MS Analysis

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We introduce a method for the rapid creation of high-density open droplet arrays for the label-free determination of enzyme kinetics and concentration gradients. The droplets of sub-nanolitre volumes are analysed by fluorescence microscopy and subsequent mass spectrometry.

Testing different concentration conditions is a standard procedure in numerous applications and utilized to e.g., screen compound libraries for drug discovery, identify the optimal ratio of reagents or determine calibration curves. These tasks are usually carried out in microtiter plates by using pipetting robots. Alternatively, very small volumes for high throughput screening were realized by droplet microfluidics. However, the inaccessible channel-compartments limit the droplet analysis to optical detection and fluorescence-based methods. In recent years, we have developed a system that combines the advantages of microtiter plates (being open and accessible) with the benefits of droplet microfluidics [1,2]. Here, we

present a significantly improved platform for the rapid creation of water-in-oil droplets onto an open substrate with the possibility to form concentration gradients and perform complex assays for e.g., label-free enzyme kinetics. Our picolitre-sized droplet-arrays can be analysed by fluorescence microscopy and Matrix-Assisted Laser Desorption Ionization- Time of Flight Mass Spectrometry (MALDI-TOF MS).

We achieve droplet homogeneities of 2.9 CV% across the plate with virtually no errors (occupancy of almost 100%). We demonstrate that an array of 24 192 droplets with defined volumes from 280 to 980 pL can be formed on one plate in less than 22 minutes. Our method facilitates the creation of fine chemical gradients across the plate.

For evaluation of the droplets and concentration gradients, we formed droplets with a solution of the peptide Angiotensin II (Ang II), tagged with fluorescein (FAM). Droplet-arrays created with this fluorescently-tagged peptide were analyzed both with fluorescence microscopy and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) to confirm the performance of the droplet array as well as the analytical methods. We then show the applicability of our platform by performing a label-free kinetic reaction with the angiotensin converting enzyme 2 (ACE2) directly on our microarray. The product Angiotensin (1-7) and substrate (Ang II-FAM) were quantified by MALDI-MS at various time points to determine the kinetic constants of the enzyme-substrate reaction. The platform can be further upscaled for high throughput studies of other enzymatic reactions, enzyme inhibitor screening and other biochemical assays as required for drug discovery and protein engineering.

References

1. Haidas, D. *et al.* Microfluidic Platform for Multimodal Analysis of Enzyme Secretion in Nanoliter Droplet Arrays. *Anal. Chem.* **2019** 2066–2073.
2. Küster, S. K. *et al.* Interfacing droplet microfluidics with matrix-assisted laser desorption/ionization mass spectrometry: Label-free content analysis of single droplets. *Anal. Chem.* **2013** 1285–1289.

Vascularized Brain Organoids for Modelling Developmental Neurovascular Interactions

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Brain organoids have been shown to recapitulate the hallmarks of human brain development and have been used to study the onset of degenerative diseases. However, the lack of vasculature in these in vitro brain tissue limits its ability to model interactions between neurons and the brain vasculature, which plays a critical role in brain development, homeostasis, and neurodegeneration. Studies have begun to decipher the functional role of vasculature in the prenatal human brain, though the molecular drivers of CNS endothelial cell fate and the instructional role they play in modulating neural development is less known.

In this study, we exploit the overexpression of ETV2 to generate forebrain organoids with a vasculature-like network, which can be perfused when transplanted into an in vitro vasculature bed on microfluidic devices. Single-cell transcriptomics characterization of vascularized organoids revealed bidirectional communication between endothelial cells and neural cells in the organoid. Signaling from endothelial cells promoted neurogenesis in the organoid by upregulating genes related to neuron maturation and migration. ETV2-programmed endothelial cells also gained molecular signatures of CNS vasculature when cultured in organoids compared to 2D. Additionally, we used the pooled overexpression of a set of relevant TFs in

conjunction with ETV2 to identify those that can drive the differentiation and maturation of organ-specific endothelial cells, particularly those of the CNS vasculature. This system presents a fully in vitro model to study neurovascular interactions, and will enhance our comprehension of the organ-specific maturation of ECs and offer insights into organotypic vascular engineering.

An Evolved Artificial Radical Cyclase Enables the Construction of Bicyclic Terpenoid Scaffolds via an H-Atom Transfer Pathway

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While natural terpenoid cyclases generate complex terpenoid structures via cationic mechanisms, alternative radical cyclization pathways are underexplored. The metal-catalyzed hydrogen-atom-transfer (M-HAT) reaction offers an attractive means for hydrofunctionalizing olefins, providing access to terpenoid-like structures. Artificial metalloenzymes offer a promising strategy for introducing M-HAT reactivity into a protein scaffold. Herein, we report our efforts towards engineering an artificial radical cyclase (ARCase), resulting from anchoring a biotinylated [Co(Schiff-base)] cofactor within an engineered chimeric streptavidin. After two rounds of directed evolution, a double mutant catalyzes a radical cyclization to afford bicyclic products with a cis-5-6-fused ring structure and up to 97% enantiomeric excess. The involvement of a histidine ligation to the Co-cofactor is confirmed by crystallography. A time-course experiment reveals a cascade reaction catalyzed by the ARCase, combining a radical cyclization with a conjugate reduction. The ARCase exhibits tolerance towards variations in the dienone substrate, highlighting its potential to access terpenoid scaffolds.

Leveraging Robotics for High-Throughput Property Measurements of Mixtures

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Most chemical products consist of numerous compounds to achieve a certain combination of desirable properties. The properties of interest are typically not a simple sum of the individual properties of the constituent compounds, as they depend on complex, non-ideal interactions. This complexity complicates the prediction of properties such as solubility and surface tension, especially in mixtures. Machine learning models, however, have the potential to uncover intricate correlations and address this challenge. A prerequisite for effective machine learning models is a sufficiently large and reliable dataset, which is often scarce for pure compounds and even more so for mixtures.

This project tackles this challenge by developing high-throughput techniques for formulating mixtures and measuring their properties of interest. We employ a liquid-handling robot to not only formulate the mixtures, but also measure properties such as solubility and surface tension, using a plate reader and a pendant drop method, respectively. To select the most informative data points, a Bayesian optimization is utilized to suggest the next set of experiments. In this close-loop, automated manner, a large dataset is generated for mixtures and their property of interest, thereby enhancing the predictive capabilities of machine learning models.

Polymer-Lipid Hybrid Vesicles as Minimal Cells to Study Biological Processes

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Bottom-up synthetic biology is an approach that involves the assembly of life-like units using designed building blocks. Polymer-lipid hybrid vesicles are an alternative to liposomes and polymersomes that combine the self-assembly ability of the lipids and benefits of modern polymer chemistry. One of the applications of these life-like units is the investigation of biological processes.

While the membranes of living cells and their organelles commonly exhibit transmembrane asymmetry, this property is rarely observed in synthetic counterparts. Only limited reports show hybrid vesicles with transmembrane heterogeneity, obtained due to the assembly process. However, ideally, transmembrane asymmetry can be regulated post-assembly. We investigated the ability of lipid flippases reconstituted in hybrid vesicles, which use anionic lipids as substrates, to change the transmembrane symmetry post-assembly. ATP depletion experiments confirmed the presence of the flippase in the hybrid vesicle membrane. Furthermore, we conducted an Annexin-binding assay on both small and giant hybrid vesicles, which provided evidence of anionic phospholipid flipping, which resulted in controlling the distribution of the membrane building blocks, i.e., transmembrane asymmetry of anionic lipids in the inner leaflet. Furthermore, the transmembrane asymmetry induced changes in the spontaneous curvature of the bilayers resulting in constriction and division of the vesicles.

Moreover, we investigated the interaction between the cell membrane and biomolecular condensates. Specifically, we studied an artificial intrinsically disordered protein (IDP) that undergoes liquid-liquid phase separation and recruit a model protein, i.e., bovine serum albumin. When BSA was encapsulated into giant hybrid vesicles or giant unilamellar vesicles, and they were exposed to the IDP, the membrane show initially indentations followed by the condensate nucleation in the membrane that results in the formation of BSA-containing the IDP condensates plugged into the membrane of the vesicles without a negative impact on the overall integrity of the vesicles. This finding offers an opportunity to gain insight into the complex cell biological process of membrane repair using a minimal system.

A Multi-Epitope Protein-DNA Nanoswitch Platform for the Monitoring of Bioavailable Therapeutic Antibodies

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Monoclonal antibodies (mAbs) represent a key therapeutic option for managing chronic conditions. Therapeutic drug monitoring (TDM) presents a promising method for personalized treatment plans, especially in chronic diseases where excessive treatment can result in significant adverse effects. To advance TDM strategies, the NanoHybrid (NH) platform developed by Ulisse BioMed has been redesigned into an innovative protein-DNA nanoswitch sensor. This new configuration utilizes entire protein binding units to detect mAbs by taking advantage of multiple identifiable epitopes. Nanoswitch probes, composed of

specifically designed DNA strands conjugated with whole tumor necrosis factor α (TNFα) proteins, facilitated the one-step quantification of various mAbs such as Infliximab and Adalimumab in concentrations ranging from 2.4 µg/mL to 19 µg/mL directly in blood serum. Given that mAbs can induce patient-specific immune responses, anti-drug antibodies (ADAs) are frequently generated against biological medications, significantly affecting the actual bioavailability of therapeutic mAbs. Inspired by this, the nanoswitch platform was utilized to detect Infliximab in the presence of an anti-Infliximab antibody, revealing a notable decrease in the detected concentrations of Infliximab when such ADA was present. These findings demonstrated that the developed nanoswitch platform can specifically identify bioavailable antibodies, offering valuable insights for pharmacokinetic research. With its adaptable design, the current nanoswitch platform has potential as a multi-epitope nanosensor for the measurement of bioavailable mAbs and biosimilars.

Cell-Free Immuno-Profiling on a Chip

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Weizmann Institute of Science, IL

We developed a biosafe and quantitative cell-free synthetic biology approach to reconstitute interactions of antigens with antibodies and human receptors on a chip. We use miniaturized compartments on a silicon chip to cell-free express antigens from surface-immobilized genes. The surface is patterned by photolithography, creating a concentration gradient of fluorescently labelled antigens that interact with antibodies to generate binding curves and affinity measurements. We use the SARS-Cov2 antigens as a model to profile the specificity and affinity of monoclonal antibodies to dozens of viral epitopes expressed on one chip in a genotype-phenotype linkage. We further profile polyclonal antibodies in human sera, revealing patient-specific immuno-profiles that could not have been easily detected by conventional approaches. Co-expression of the human Ace2 receptor with the viral Receptor-Binding-Domain allowed us to demonstrate quantitative binding to different SARS-Cov2 variants. This rapid, quantitative, and on-chip genetically-programmed approach allows to study complex protein-protein interactions free of protein purification steps for human immuno-profiling.

High Throughput Screening of Transcription Factors for Subtype-Specific Neuronal Programming

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The bHLH transcription factor (TF) Neurogenin-2 (NGN2) can be used to program human induced pluripotent stem cells (hiPSCs) into populations of induced glutamatergic neuronal fate (NGN2-iNeurons). Due to the simplicity of their generation and the availability of iPSCs from different genetic/disease backgrounds, NGN2-iNs provide an interesting and unique opportunity for disease modeling and drug screening in a field otherwise limited by the availability of human primary material. However, and despite recent advances in neuronal programming, NGN2-iNs are heterogenous and cannot be assigned to defined identities within the human

brain at high confidence. To capitalize on their full potential as a model system, it is therefore necessary to further improve their programming. In this study, we establish a single-cell RNA-sequencing based screen to identify neuron subtypes that arise after overexpression of neuron subtype-specific TFs on top of the well-characterized NGN2-iN platform. We use lentiviral infection to integrate a doxycycline-inducible expression construct into iNGN2-hiPSCs, which allowed us to express both NGN2 and a target TF simultaneously. Our expression construct contains a fluorescent reporter gene (GFP), a TF coding sequence (CDS) and a feature capture sequence directly downstream of TF. The feature capture sequence is compatible with 10X genomics feature barcoding technology and enabled us to simultaneously capture transcriptomes and exogenous TF identities from single cells. Using this approach, we investigated 160 TFs in pooled format and identified 25 TFs, whose overexpression results in distinct transcriptomic changes compared to control NGN2-iNs including the upregulation of noradrenergic, dopaminergic or glycinergic markers, or the gain of regionally specific glutaminergic markers such as SLC17A7. To analyze the TF induced neuronal fate, we systematically compared our data to a primary human neuron reference atlas. Our study provides a strategy for programming distinct human neuron subtypes from iPSCs, which will greatly benefit both disease modeling as well as drug discovery. Our approach can be applied to any other cell type to identify TF combinations for subtype specific cell programming.

Nanopores Reveal the Stoichiometry of Single Oligoadenylates Produced by Type III CRISPR-Cas

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Cyclic oligoadenylates (cOAs) are small second messenger molecules produced by the type III CRISPR-Cas system as part of the prokaryotic immune response. The role of cOAs is to allosterically activate downstream effector proteins that induce dormancy or cell death, and thus abort viral spread through the population. Interestingly, different type III systems have been reported to utilize different cOA stoichiometries (with 3 to 6 adenylate monophosphates). However, so far, their characterization has only been possible in bulk and with sophisticated equipment, while a portable assay with single-molecule resolution has been lacking. Here, we demonstrate the label-free detection of single cOA molecules using a simple protein nanopore assay. It sensitively identifies the stoichiometry of individual cOA molecules and their mixtures from synthetic and enzymatic origin. To achieve this, we trained a convolutional neural network (CNN) and validated it with a series of experiments on mono- and polydisperse cOA samples. Ultimately, we determined the stoichiometric composition of cOAs produced enzymatically by the CRISPR type III-A and III-B variants of *Thermus thermophilus* and confirmed the results by liquid chromatography–mass spectroscopy (LC-MS). Interestingly, both variants produce cOAs of nearly identical composition (within experimental uncertainties), and we discuss the biological implications of this finding. The presented nanopore-CNN workflow with single cOA resolution can be adapted to many other signaling molecules (including eukaryotic ones), and it may be integrated into portable handheld devices with potential point-of-care applications.

Modeling Human Bone Marrow Endosteal Niches from Induced Pluripotent Stem Cells in Xeno-Free Conditions

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Human induced pluripotent stem cells (hiPSCs) have emerged as a powerful tool to engineer robust and reproducible organoids to model human biology and pathology, paving the way for mechanistic and pharmacological studies in human settings. Recently, the two first bone marrow (BM) organoid models based on hiPSCs were presented, and showed to successfully recapitulate key features of human BM while modeling both healthy and malignant hematopoiesis. Although these BM organoids represent a breakthrough in modeling the functional complexity of human BM perivascular niches, they lack the bone compartment, and thus they are not suitable to model human endosteal BM niches (close to the bone surface). Moreover, these organoids remain in the micrometer scale and therefore the vascular structures are non-physiological in shape and size. Finally, they rely on the use of Matrigel as embedding material, which introduces mouse-derived proteins in the system.

Here we present a novel developmentally-guided approach combining hiPSC-derived organoids with macro-scale hydroxyapatite scaffolds to generate a standardized and physiological-like model of the human endosteal BM perivascular niche (engineered vascularized osteoblastic niche, eVON). We developed and validated a protocol to differentiate hiPSCs into vascular cells (endothelial cells and pericytes) and osteoblasts that later self-assemble to create complex and long-lasting vascular networks integrated in a dense osteogenic matrix, resembling the 3D architecture of the native endosteal BM. The eVON was first characterized through flow cytometry, high-resolution imaging and scRNAseq. Second, we demonstrated that it can persist for at least 6 weeks upon in vivo ectopic implantation and integrate within the murine tissue. Then, we assessed its potential to support human hematopoiesis and explored its customization potential by engineering different eVON using different hiPSC lines. Finally, we validated the model for pharmacological studies by targeting VEGF signaling and analyzing the effects on the vasculature. Therefore, this work provides the first standardized and physiological-like model of the human endosteal BM, offering an unprecedented possibility to dissect the contribution of BM endosteal niches to human pathophysiological hematopoiesis.

Breaking the Photobleaching Limit in Single-Molecule FRET by DyeCycling

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Single-molecule observation times in single-molecule FRET (smFRET) are notoriously limited due to photobleaching of the fluorophore labels. To solve this, we developed DyeCycling, a reversible dye replenishment strategy, which achieves unprecedented single-molecule observations spanning from a hundred milliseconds up to an hour, i.e., four orders of magnitude. This dramatic gain in information per single molecule reveals slow kinetic regime changes that were previously missed by data pooling via post-hoc

ensemble averaging. By design, DyeCycling requires comparably high fluorophore concentrations potentially interfering with single-molecule resolution. Here, we demonstrate a nanophotonic solution for background reduction, and we also explore the potential of fluorogenicity for reducing background by chemical instead of physical means. Overall, we anticipate that DyeCycling will be instrumental in revealing so far inaccessible dynamic effects in numerous smFRET studies in biomolecular systems.

Oriented External Electric-Field Assisted Asymmetric Enamine Catalysis

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The vision to control the charges migrating during reactions with external electric fields is attractive because of the promise of general catalysis, emergent properties, and programmable devices. Here, we explore this idea with anion-π and cation-π catalysis, which is the stabilization of ionic transition states on aromatic surfaces. Catalyst activation by polarization of the aromatic system is most effective. This polarization is induced by electric fields. The use of electrochemical microfluidic reactors to polarize multiwalled carbon nanotubes as anion-π and cation-π catalysts emerges as essential. These reactors provide access to high fields at low enough voltage to prevent electron transfer, afford meaningful effective catalyst/substrate ratios, and avoid interference from additional electrolytes. Under these conditions, the rates and enantioselectivities of proline-catalysed enamine reactions, such as aldol reactions and Robinson annulations, are enhanced under an external electric field. While electromicrofluidics have been conceived for redox chemistry, our results indicate that their use for supramolecular organocatalysis has the potential to noncovalently electrify organic synthesis in the broadest sense.

Developing Efficient Access to Macrocycles via Glycosylation Inside the Nanoconfinement

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Medium (8-11 atoms) and large cyclic compounds (≥ 12 atoms) are fascinating compounds with significant biological activity. In contrast to the formation of five- and six-membered ring systems that are readily accessible via cyclization and cycloaddition reactions, the synthesis of medium and large rings still poses a formidable synthetic challenge. Macrocyclization is typically disfavored due to enthalpy and entropy considerations, competition with linear dimerization/oligomerization, and the need for high dilution conditions that result in high solvent usage and long reaction times, making the process both cost-intensive and environmentally impactful. Here we have developed a novel approach to facilitate macrocycles via glycosylation chemistry by catalyzing the reaction inside a closed molecular container that prevents linear oligomerization due to the finite volume of the cavity.

Macrocyclic glycosides (mostly β-isomers) are a very important class of bioactive molecules as they can interact with biological receptors, enzymes, or other biomolecules, leading to a wide range of pharmacological effects. Over the last few decades, supramolecular catalysis has started to demonstrate its potential in overcoming current limitations in synthetic chemistry. Recently our group utilized the dynamic hexameric resorcin [4]arene capsule for the highly β-selective glycosylation of diverse donors and acceptors. In this work, we observed that glycosyl donors with pendant nucleophile (-OH) undergo macrocyclization inside RS-capsule with high yield and excellent selectivity in the presence of suitable acid quencher such as basic alumina. Depending on the ring size, the formation of cyclic monomers or dimers was influenced by the choice of solvent. With this method, up to 28-membered macrocycles were synthesized with excellent yield and selectivity. Easily cleavable protecting groups i.e. benzyl and ally-protected sugars were well tolerated under the reaction condition. Ester-linked glycosyl donor delivered macrolactone with good yield and >98:2 beta/alfa selectivity, a useful precursor of a series of natural products. This approach helped to synthesize 10-membered macrocycles with excellent yield and selectivity, which is very difficult to prepare using other reported procedures.

Advancing Li-Mediated Electrochemical Ammonia Synthesis: Tackling Challenges for a Sustainable Future

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Electrochemical ammonia synthesis is emerging as a promising alternative to the traditional Haber-Bosch process, which has long been criticized for its high energy consumption and significant CO2 emissions. The electrochemical approach offers several advantages, including milder operating conditions, reduced carbon footprint, and the potential for decentralized production using renewable energy sources. Our current focus lies on better understanding nitrogen reduction mechanisms and on optimizing electrolyte systems. The goal is to enhance ammonia yield rates and Faradaic efficiency (FE) in ambient conditions, following the work of Simonov group who achieved almost 100% FE on the electrochemical nitrogen reduction reaction under 15 bar of nitrogen gas and at room temperature. Key challenges include the electrolyte solvent selection, which significantly impacts reaction selectivity and long-term operation; electrode materials, as different counter electrodes (e.g., Ni vs. Pt) affect reaction efficiency and stability; the Solid Electrolyte Interphase (SEI) layer, which plays a crucial role in reaction dynamics by acting as both a protective shield and a potential barrier; and bias application, where continuous versus pulsed bias affects lithium electrodeposition and overall reaction dynamics. Our latest study includes using DMI as an electrolyte solvent to reduce electrode surface degradation, exploring pulsed bias techniques to overcome limitations of continuous gating, and investigating the SEI layer's impact on reactant permeability and catalytic properties. While electrochemical ammonia synthesis shows great promise, scaling up to match industrial production rates remains a significant challenge. Ongoing research aims to address these issues through reactor engineering, real-time reaction monitoring, and identification of key catalytic variables. As efforts continue to overcome hurdles in electrochemical ammonia synthesis, this technology has the potential to revolutionize not only agriculture and chemical industries but also the energy sector by enabling sustainable, decentralized ammonia production using renewable energy sources.

Microfluidic-Based Platform for Automated Measurements of Concentration, Diffusion Coefficient, and Equilibrium Constant of Fluorescent Molecules

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A significant number of processes in living organisms, such as gene expression, cell signaling, transport and storage of molecules, etc., are regulated by weak noncovalent interactions. The biomolecules handling these processes transfer information by associating into complexes and dissociating into single-molecule states, e.g., DNA hybridization or hormone-receptor binding. The stability of formed complexes and, therefore, the energy of interactions are often determined by measuring the equilibrium constant (K). Various analytical techniques allow defining K in vitro, but only fluorescent ones are sensitive enough to gauge it at nanomolar concentrations and below. Among these methods, Forster Resonance Energy Transfer (FRET) is found to be the most widely used. Additionally, integrated with Fluorescence Correlation Spectroscopy (FCS), it provides information from single molecules at femtoliter volumes. Yet, the technique is limited to systems where (i) both reactants are fluorescently labeled, (ii) fluorescent moieties are in proximity after complex formation, and (iii) spectra of energy donor and acceptor overlap.

Performing control experiments in our previous studies, we noted that the molecular brightness (MB) of fluorophores alters up to 100 % while binding to other non-fluorescent molecules, depending on the fluorescent tag and environment. We took advantage of this remark, creating foundations for a new analytical method –Molecular Brightness Analysis (MBA) – that connects MB change over complex formation with FCS capability to monitor the signal of single molecules. As a result, the technique enables determining K over a series of samples, grasping the diffusion and concentration of reactants simultaneously.

Similarly to FRET, our method follows measurements at nanomolar concentration and provides comparable accuracy. Moreover, MBA functions when only one reactant is fluorescently labeled, which reduces problems with selecting dyes and tagging them at specific molecule sites. However, to measure K of a simple A + B ⇄ AB reaction, a series of different A/B concentration ratios is demanded. Considering sample preparation and the measurement itself, the procedure takes several hours. Experiments on a bigger scale, e.g., defining the impact of pH or ionic strength on a specific reaction equilibrium, are even more laborious as the total number of specimens rises by 10 to 50 folds.

Here, we solve the above-mentioned constraints of MBA by developing a device that automates the sampling and measurement procedures. We utilize our previously developed hydrodynamic traps and modified system for droplet generation with a defined periodic distribution of reactant concentrations. We present a platform for measuring the equilibrium constant of fluorescent molecules at nanomolar concentrations. Simultaneously, we take advantage of FCS and determine the diffusion coefficient and concentrations of reactants in nanoliter-volume droplets. We show the results based on DNA oligonucleotide hybridization and limit the presence of a researcher to set the experiment and calibrate a microscope.

Unveiling the Natural Progression of Initial Assembly for Diphenylalanine and its Analogs: Transition from Oligomer Equilibrium to Nanocluster Formation

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The self-assembly of peptides plays a pivotal role in various biological processes and holds significant potential for nanotechnology and biomedical applications. Despite significant advances in characterizing and understanding the assembly process at a larger scale (nano-assemblies), the mechanisms by which peptides assemble in their initial oligomeric states remain elusive. In this study, we investigate the aggregation behavior of the self-assembling peptide diphenylalanine (FF) and its analogs through a comprehensive multi-technique approach. Utilizing single nanopore analysis, we detected and characterized the initial oligomers of FF and its analogs (BocFF, FmocFF, and FmocFF5) in real-time. This method provided detailed insights into the early stages of peptide self-assembly, revealing the dynamic behavior and formation kinetics of initial oligomeric species. Furthermore, we employed mass photometry to analyze the size distribution of nano globular aggregates formed by these peptides. This technique enabled precise measurement of the mass distribution of various oligomeric states, shedding light on the intermediate phases of the aggregation process. The final assembly states of the peptides were characterized using Transmission Electron Microscopy (TEM) and Fourier Transform Infrared Spectroscopy (FTIR). TEM offered high-resolution imaging of the morphological structures, while FTIR provided information on the secondary structure and conformational changes during peptide aggregation. By integrating data from single nanopore detection, mass photometry, TEM, and FTIR, we constructed a comprehensive graph that illustrates the entire aggregation process of self-assembling peptides across different scales. This integrative approach provides a holistic understanding of peptide self-assembly, from initial oligomers to mature fibrils, enhancing our knowledge of the underlying mechanisms. The findings from this study pave the way for the rational design of peptide-based nanomaterials and therapeutic agents, offering new perspectives on their assembly pathways and structural properties.

Automated Silicon Microfluidics Platform for Controlling and Analyzing Chemical Reactions with Applications in Compartmentalized Synthesis and Chemical Computing

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Traditional chemical synthesis is performed in batch, requiring bulky glass ware and large quantities of reagents while offering only limited control over the reaction conditions, mixing or sample extraction. To vary reaction conditions and/or reagents, the entire procedure must be restarted from scratch, glass ware cleaned etc. which results in an inefficient and labor-intensive procedure. Recently, robotic systems enabled some chemical actions to be automated, ranging from dispensing of reagents to handling of vials for sample extraction, thereby offering means to automatically perform more systematic and parametric reaction screenings with the precision of a machine - not being subject to human handling variations. Additionally,

chemical reaction compartments with in- and outlet ports and internal mixing functionalities can be operated in a flow configuration. This enables a high degree of automation as the reactor can be seamlessly integrated in the chemical workflow and compounds can be fed from syringe- or pressure-based pumps supplied from large reservoirs to facilitate extensive screening campaigns. Such automated systems can further be equipped with a variety of online analytical methods, which may provide almost immediate feedback of reaction yields that can again be fed back into the design of experiments to efficiently probe multiple reaction space configurations.

In such automated or even autonomously operated synthesis platforms, the type of reactor is a key component. It defines the mixing, the mass-flow conditions, the concentration gradients and the dwell time as well as all environmental conditions of the reaction, which includes, for instance, the temperature, the electrochemical potentials, the illumination properties for photochemical processes etc. Furthermore, some reactors can further be directly functionalized with online analytics, such as optical windows for transmission experiments for UV-Vis, Raman or infrared spectroscopy, electrodes for electrical impedance spectroscopy, cyclic voltammograms etc.

We present a solid-state platform for creating such multi-purpose reactors which allows for scalable reaction volumes ranging from tens of µl down to 1 nl due to the use of enhanced semiconductor fabrication technologies. The high solvent compatibility of pristine or functionalized silicon combined with is high mechanical compliance, the ultrafast heat transfer and no diffusion of oxygen through the silicon corpus provides means for high-precision synthesis with most efficient triggers and analytics. We show how such complex microfluidic devices can be extended to the third dimension to facilitate liquid routing and how pairs of electrodes can be integrated into the channel walls yielding perfect electrostatics. Examples from droplet-based microfluidics show two to three orders of magnitude reduction in supply voltage over state-of-the-art at ultrahigh throughput rates for real-time droplet sorting and use of emulsion techniques to create functional vesicles to study biochemical reaction pathways. Another use case of a programmable synthesis system is chemical computing where complex chemical reaction networks are deployed to tackle non-linear classification tasks using a reservoir-computing approach.

Site-Selective C(sp3)–H Oxidation of Aliphatic Substrates Devoid of Functional Groups

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Although tremendous progress in the field of C(sp3)–H oxidation has been achieved over the past decades, the selective oxidation of non-activated positions on hydrocarbon skeletons is still highly challenging. It usually requires the presence of a suitable functional group in proximity to the desired oxidation site or to use it to hold and orientate the substrate. Here we present a novel approach for catalyst-directed C–H oxidation that relies on substrate binding via the solvophobic effect in fluorinated alcohols, and thus is independent of functional groups on the substrate. Enabled by the novel supramolecular catalyst Mn(mcp)-RS2, the preferential oxidation at the fifth position on aliphatic substrates was observed.

Programmable RNA Writing with Trans-Splicing

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The discovery and repurposing of CRISPR effectors has allowed for the development of a genome editing toolbox capable of performing programmable and efficient genetic interventions for a wide range of therapeutic and research purposes. However, most available tools directly modify the DNA sequence or alter gene expression level, leaving RNA modification comparatively unexplored. RNA editing, however, offers the opportunity to introduce either stable or transient modifications to nucleic acid sequences, without the risk of permanent off-target effects. This could be applied for sensing, labelling, or repairing of RNA transcripts, but installation of arbitrary edits into the transcriptome is currently infeasible. Trans-splicing-based RNA editing technologies can install diverse edits, insertions, and deletions by introducing exogenous templates via competition with the endogenous exon in the pre-spliced mRNA. However, currently they suffer from low efficiency, even after extensive screening, which hinders their applicability. Here, we describe Programmable RNA Editing & Cleavage for Insertion, Substitution, and Erasure (PRECISE), a versatile RNA editing method for writing RNA of arbitrary length and sequence into existing pre-mRNAs via 5′ or 3′ trans-splicing. We demonstrate PRECISE editing across 11 distinct endogenous transcripts of widely varying expression levels, showcasing more than 50 types of edits, including all possible transversions and transitions and a wide range of insertions and deletions. We show high efficiency replacement of MECP2 last exon, addressing most mutations that drive the Rett Syndrome; editing of SHANK3 transcripts, a gene involved in Autism; and replacement of exon 1 of HTT, removing the hallmark repeat expansions of Huntington′s disease. Furthermore, we combine payload engineering and ribozymes for protein-free, high-efficiency trans-splicing, with demonstrated efficiency in editing HTT exon 1 via AAV delivery. We show that the PRECISE achieves editing in non-dividing neurons and patient-derived Huntington’s disease fibroblasts. Our results provide the scientific community with a novel tool that markedly broadens the scope of RNA editing, is straightforward to deliver, lacks permanent off-targets, and can enable any type of edit, including those not otherwise possible with current RNA base editors. Ultimately, these findings expand the current RNA editing toolbox for therapeutic or research purposes and widen the spectrum of addressable diseases.

Engineered Advanced Hybrid Systems through Self-Assembly of Janus Nanoparticles and Polymersomes for Bio-Applications

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A significant approach in various fields such as chemistry, electronics, and technology are the self-organization of nano-objects into complex architectures. This strategy aims to generate novel systems with unique properties and functionalities[1-3]. An important step in creating interconnected artificial organelles is the DNA hybridization between synthetic assemblies, including polymersomes, nanoparticles, and micelles. These assemblies facilitate cascade reactions among different encapsulated catalytic compounds and can imitate cell signaling and interactions[4,5].

In this study, a new approach is proposed for developing a multifunctional hybrid system for specific bio-applications by investigating the self-organization of clusters between "hard" Janus nanoparticles (JNPs) and "soft" polymersomes[6]. These polymer-based JNPs provide an asymmetric platform suited for directional interaction[7] with soft polymersomes. The clusters are modularly assembled through programmed DNA hybridization. Furthermore, the vesicular architecture of the polymersomes after assembly into JNP-polymersome clusters allows the encapsulation of various kinds of catalytic compounds. The asymmetry of the JNPs has unique advantages by allowing a precise arrangement of the polymersomes and enabling, in a modular manner, various reaction configurations, including single, parallel and cascade enzymatic reactions. Additionally, these clusters, which integrate imaging and therapeutic nanocompartments, support nanotheranostic applications by enabling precise in vitro detection and simultaneously producing reactive oxygen species (ROS) to induce apoptosis.

References
1. C. Gong, S. Sun, Y. Zhang, L. Sun, Z. Su, A. Wu, G. Wei, *Nanoscale* **2019**, *11*, 4147.
2. G. Zhu, Z. Xu, Y. Yang, X. Dai, L.-T. Yan, *ACS Nano* **2018**, *12*, 9467.
3. M. R. Jones, N. C. Seeman, C. A. Mirkin, *Science* **2015**.
A. Belluati, I. Craciun, J. Liu, C. G. Palivan, *Biomacromolecules* **2018**, *19*, 4023.
4. J. Liu, V. Postupalenko, S. Lörcher, D. Wu, M. Chami, W. Meier, C. G. Palivan, *Nano Lett.* **2016**, *16*, 7128.
5. C. Kang, A. Honciuc, *ACS Nano* **2018**, *12*, 3741.
6. V. Mihali, A. Honciuc, *Adv. Mater. Interfaces* **2022**, *9*, 2101713.
7. V. Mihali, M. Skowicki, D. Messmer, C. G. Palivan, *Nano Today* **2023**, *48*, 101741.

Microscale Optical Printing for DNA-Mediated Communication of Artificial Cellular Agents

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In living systems, cells communicate with each other by transmitting diffusible signal molecules to the neighboring cells. This molecular communication facilitates collective information processing, enabling the cells to coordinate their activities and achieve complex, spatiotemporal regulation of their functions. One of the main challenges is creating artificial molecular systems mimicking such multicellular communication to provide autonomous functionality with embodied intelligence.

To date, several strategies have been explored to address this challenge, particularly focusing on the use of liposomes or proteinosomes as protocells. These protocells can encapsulate various biomolecules, including DNA, which can serve as a signal molecule to mediate intercellular communication. However, current technologies lack stable methods of patterning multiple types of protocells with precise control over their populations and spatial arrangements, limiting systematic understanding of how each of these factors determines the overall spatiotemporal behavior of the system.

In this study, we develop a general microfluidic platform that addresses these challenges by enabling communication between artificial cellular agents that are precisely patterned within a channel. Our approach utilizes a microscale continuous optical printing (μCOP) strategy, which allows for the fabrication of micropatterned arrays of hydrogel posts that serve as the cellular agents. The sizes, arrangements, and populations of the hydrogel posts can be freely designed by modifying the UV illumination patterns, providing a high degree of flexibility and control over the system’s architecture.

The posts in our platform are functionalized with DNA strands that enable them to transmit and receive DNA to neighboring posts through diffusion. Through a combination of experimental and simulational approaches, we demonstrate that the micropatterned posts can activate or inhibit neighboring posts by exchanging specific DNA strands, which is observable by fluorescence microscopy. Moreover, we observe that the local addition of triggering DNA in the channel induces spatially biased chemical communication of the posts. We believe our study helps the experimental validation of artificial cellular communication and becomes a first step toward developing future microfluidic systems with life-like intelligence.

Synthetic Organelles to Engineer Mammalian Cells

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Engineering new functionalities into living systems has a tremendous potential for biotechnology and medicine. However, using canonical routes of evolution can be limited by the necessity to avoid interference with endogenous processes. Cells themselves do not only rely on molecular evolution to prevent undesired crosstalk, but they often utilize compartmentalization, in the form of membrane-enclosed or membraneless organelles to spatially isolate distinct molecular processes and execute complex operations that would otherwise interfere with each other.

Inspired by this, we developed synthetic organelles to equip mammalian cells with new functions. Specifically, we combined phase separating proteins such as FUS and EWSR1 with anchor proteins that tether the organelles to specific subcellular structures. With this we can generate organelles spanning vastly different shapes from kinesin-motor based micrometer sized organelles over fiber-like organelles along the microtubule cytoskeleton to film-like organelles on membrane surfaces that have a thickness of less than 100 nm.

We then applied these organelles to improve genetic code expansion (GCE) in mammalian cells. GCE is a powerful tool to control and expand protein function with single-residue precision that is widely used to perform labeling for microscopy or to photocontrol proteins. This technology relies on an orthogonal tRNA/synthetase pair that is introduced into the host, which recodes a stop codon to incorporate a noncanonical amino acid (ncAA) into the nascent chain. While this technique is codon-specific, it cannot select specific mRNAs, so naturally occurring stop codons can be suppressed, causing significant side effects. To overcome this, we integrated a GCE system into our synthetic organelles to develop orthogonally translating (OT) organelles. These OT organelles can perform mRNA specific GCE, installing ncAAs exclusively into desired proteins. We further demonstrate that it is possible to combine different OT organelles in one cell, effectively yielding cells that simultaneously execute multiple distinct genetic codes.

Our results demonstrate a simple yet effective approach to generate artificial organelles that enable customized orthogonal translation and protein engineering in semi-synthetic eukaryotic cells.

Pnictogen-Bonding Enzymes

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Pnictogens represent the last frontier of the σ-hole bonds, non-covalent interactions highly attractive for the development of powerful tools in supramolecular chemistry. In this study, artificial enzymes capitalizing on pnictogen bonding are reported as a new tool, absent in biocatalysis. To tackle this challenge, stibine catalysts were decorated with a biotin moiety and combined with streptavidin mutants. The transfer hydrogenation of fluorogenic quinolines mediated by a hydrophilic Hantzsch ester was used as benchmark reaction. σ-Hole deepening resulted into an improvement of the catalytic performances, best for mutants which position negative charges (D, E) or hydrophobic residues (I) in the active pocket. Michaelis-Menten analysis showed transition-state recognition in the low micromolar range. Lastly, an emerging stereoselectivity further corroborated the promising potentiality of pnictogen-bonding catalysts in such ordered systems.

NanoAmp: Toward Protein PCR for Rapid and Sensitive Biomolecule Detection

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NanoAmp is an innovative PCR-based technology designed to detect antigens and antibodies through an easy and quick single-step method. Developed by UlisseBiomed S.p.A., NanoAmp leverages the target-induced increase in local concentration, mediated by interactions between nucleic acid-based elements. It combines both isothermal polymerase reactions and hot-start PCR amplification, which can be performed using a single enzyme in its most advanced form. This approach enables rapid, sensitive, and quantitative biomolecule detection in a single well within 30 minutes. Compared to traditional biomolecule detection methods such as ELISA and CLIA, which are used for high-throughput formats and involve complex, time-consuming, multi-step processes, NanoAmp offers an easier and more flexible solution. Its unique chemistry allows for testing on a few samples or even single-sample analyses without sacrificing extreme sensitivity. Additionally, compared to PCR-based protein detection methods, which are sensitive but lengthy and prone to contamination due to their multiple steps, NanoAmp addresses these challenges by providing a straightforward, single-step method equivalent to a standard PCR analysis for antigen detection. This study demonstrates NanoAmp's efficacy as a modular platform accommodating various binding moieties for diverse biological targets. The platform successfully operated with antibodies, proteins, aptamers, and peptides, showcasing its versatility and high sensitivity. Employing the DIG binding motif, the system detected anti-DIG antibodies at concentrations as low as 66 picomolar (pM) and exhibited effectiveness in crude biological samples. NanoAmp aims to revolutionize the world of immunoassays by offering a platform capable of producing highly sensitive and easy-to-use assays for one-pot detection of a plethora of antigens. Additionally, its modular nature allows for the development of customizable research use only (RUO) assays, as different analyte binding motifs can be easily paired with the core system to tailor tools for specific needs.

Exploring the Phenotype Landscape of Signaling Domain Engineered CAR T Cells Using Machine Learning on Transcriptomics Data

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Chimeric antigen receptors (CARs) are synthetic receptors designed to transduce extracellular antigen recognition to an intracellular signaling cascade leading to T cell activation. T cell activation is the result of a complex interplay of multiple signaling pathways which determine the behavior and properties of a given T cell population – the T cell phenotype. Second-generation CAR designs, including clinically approved cancer therapies, contain a 41BB costimulatory signaling domain alongside the CD3z domain of T cell receptors and elicit a predominately central memory phenotype, which is correlated with improved clinical efficacy. Here, we propose mutational profiling on the 41BB signaling domain in the CAR T cell context (anti-CD19 41BB CD3z)1. We will perform deep mutational learning (DML)2, a machine learning-guided protein engineering method to discover variants of the 41BB CAR able to induce a broad spectrum of T cell phenotypes, which will be characterized via single-cell RNA sequencing (scRNAseq)3. DML applied to scRNAseq data will enable us to explore and study the relationship between phenotypes and the 41BB mutational landscape. These insights may allow us to understand the signaling domain design rules that make 41BB-based CARs outperform others, apply it to different cancer settings, and predict new 41BB variants that induce novel T cell phenotypes.

Pharmacological Assays in Biohybrid Artificial Cell Networks

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In vitro pharmacology is constrained by the biological relevance of the experimental system, experimental throughput and analytical method flexibility. To address these bottlenecks, fundamentally new approaches are required. Across the course of the NCCR MSE, the Bioanalytics lab has developed a series of novel techniques which blend advances from the fields of artificial cell engineering and analytical chemistry. This contribution will focus on recent developments with artificial cell networks assembled from droplets. With our experimental approach, we exert spatial-temporal control over nanoliter droplets containing chemical and biological stimuli. With this, we have unlocked pharmacokinetic / pharmacodynamic (PK/PD) modelling in single droplets and droplet networks.

In these systems, the PK component is defined by transport between droplet compartments connected by biomimetic membranes. Through assessment of an FDA approved drug library, we categorized drugs with a propensity to undergo passive diffusion. Similar physicochemical features which predict in vivo bioavailability correlate with the drug kinetics in our system. Physiologically relevant variables such as temperature, buffer, pH and membrane composition provide tools for modulating the rate of transport and improving system biomimicry. Using our approach, we can reconstitute drug concentration profiles, which capture the peak and trough flux of oral and intravenously administered drugs. In response, the PD component is measured in situ

through the inclusion of living cells. By arranging the number of membrane barriers between a source of antibiotic and the target site of action, our system can mimic intracellular infections. Control over the number and arrangement of connected droplets passively distributes antibiotic throughout the system. Through modelling and simulation, we couple the experimentally measured PK and PD responses, facilitating detailed interrogation of antibiotic efficacy under subtle variations in the drug concentration profile. With this simple to use and automatable methodology, we therefore introduce a novel framework for designing and conducting pharmacological assays.

High-Resolution Nanopore Studies of Single Proteins Using Novel Glass Chips with Low Capacitance

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Nanopores are label-free single-molecule detectors for biomolecules, e.g. DNAs, proteins, small molecules[1]. Recently we introduced the nanopore electro-osmotic trap (NEOtrap)[2,3] to trap and sense single proteins and their conformations using a solid-state nanopore and DNA-origami. Here, we present new nanopore glass chips with significantly lower capacitance than conventional silicon chips, thus offering nanopore reads with much lower current noise. In particular, we found a solution to achieve the required high etching selectivity for the glass substrate over the SiN forming the free-standing membrane. This advance offers great opportunities for electrical and electro-optical nanopore experiments.

References
1. Fuentenebro-Navas, D.; Steens, J.; De Lannoy, C.; Noordijk, B.; Pfeffer, M.; De Ridder, D.; H.J. Staals, R.; Schmid, S. *ACS Nano* **2024**, accepted.
2. Schmid, S.; Stommer, P.; Dietz, H.; Dekker, C. *Nat Nanotechnol* **2021**, *16*, 1244-1250.
3. Wen, C.; Bertosin, E.; Shi, X.; Dekker, C.; Schmid, S. *Nano Lett* **2023**, *23*, 788-794.

Iron(III) Carbene Complexes with LMCT Luminescence Lifetimes up to 100 Nanoseconds

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The long-standing interest in photoactive 3d6 compounds with long-lived metal-to-ligand charge transfer (MLCT) excited states led to the discovery of an Fe(II) complex with an MLCT lifetime of 0.5 nanoseconds a few years ago. An isostructural Fe(III) compound subsequently turned out to be luminescent from a ligand-to-metal charge transfer (LMCT) excited state featuring a lifetime of 100 picoseconds. This sparked broader interest in photoactive 3d5 Fe(III) complexes, for example in the context of photocatalysis.

Now we report new Fe(III) complexes with energy-tunable LMCT excited states and lifetimes in the range of 10 picoseconds to 100 nanoseconds. One of these two Fe(III) complexes undergoes photoinduced electron transfer with various donors and acceptors. Photocatalytic reactions not previously reported for this

compound class, in particular the C-H arylation of diazonium salts and the aerobic hydroxylation of boronic acids, were achieved with low-energy red light excitation. Doublet–triplet energy transfer (DTET) from the luminescent 2LMCT state to an anthracene annihilator permitted the first proof-of-principle for triplet–triplet annihilation upconversion based on a molecular iron photosensitizer. This advance enabled the development of new types of Fe(III) complexes, employing an anthracene chromophore as a triplet reservoir to decelerate the 2LMCT excited-state decay via reversible intramolecular DTET. This modification elongated the lifetime by approximately 350 times compared to the parent complex, reaching a 100 nanosecond LMCT lifetime. These findings are relevant for the development of iron complexes featuring photophysical and photochemical properties competitive with noble metal-based compounds.

Characterizing the Inhibition Mechanisms of Gasdermin D Pore Formation by the Drug Disulfiram

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Gasdermin D (GSDMD) is a pore-forming protein identified as the executioner of pyroptosis (a programmed cell death) and serves as conduit to release inflammatory mediators, including inflammatory cytokines (interleukins IL-1β, IL-18, and IL-6) and alarmins. The key role of GSDMD in infection and inflammation has prompted the investigation of therapeutics targeting GSDMD. Previous work showed that disulfiram (DSF), an FDA-approved drug for treating chronic alcoholism, inhibits human GSDMD (hGSDMD) pore formation by covalently modifying Cys 191. We investigated by force-distance curve–based (FD-based) atomic force microscopy (AFM) and limited proteolysis coupled to mass spectrometry (LiP-MS) the effect of DSF on hGSDMD pore formation. We find that DSF inhibits most of the GSDMD-processing proteases that activate the auto-inhibited full-length hGSDMD to the lytic hGSDMD N-terminus, thus reducing the proteolytic activation of hGSDMD. Our data also show that DSF inhibits both wild-type and C191A mutant oligomerization and pore-formation, therefore, suggesting that DSF may target a different cysteine other than Cys 191 or act via additional alternative mechanisms. Indeed, we observe that DSF causes both hGSDMD wild-type and mutant C191A aggregation thereby reducing the protein available for pore formation. Besides, we find that DSF increases the mechanical stiffness of the supported lipid membrane, which appears to hinder hGSDMD membrane insertion. In summary, our data show that DSF inhibits hGSDMD pore formation through different mechanisms, namely - by inhibiting hGSDMD proteolytical activation, by covalently modifying hGSDMD cysteines, by causing hGSDMD aggregation, and by stiffening the membrane.

Repurposing Myoglobin into an Abiological Asymmetric Ketoreductase

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Thanks to recent advances in enzyme repurposing, hemoproteins have gained significant attention as versatile biocatalysts that catalyze a variety of transformations, ranging from oxidation to redoxneutral reactions. To complement these achievements, we report herein on our efforts to repurpose myoglobin (Mb)

into an asymmetric ketoreductase, using PhSiH3 as reductant. Two rounds ofmutagenesis afforded a doublemutant capable of reducingwith high enantioselectivity a broad range of prochiral aliphatic and aromatic ketones in the presence of whole cells. Additional rounds of directed evolution afforded a quintuple mutant with opposite enantioselectivity. Mechanistic investigations suggest that a fleeting Fe–H species undergoes heterolytic hydride transfer to afford enantiopure alcohols from the corresponding ketones. The excellent saturation kinetic profile, combined with the practicality of whole-cell biocatalysis under aerobic conditions, highlights the potential of repurposed Mb as an asymmetric ketoreductase with a broad substrate scope, thus expanding the reaction repertoire catalyzed by hemoproteins.