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NCCR
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Engineering

Abstract Book

1-3 June 2026

Biozentrum, University of Basel, Switzerland

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Plenary Talks

Microfluidics, Automation, and Engineered Biomembranes as Enabling Technologies in Synthetic Cell Design

Yuval Elani, *Imperial College London, UK*

Synthetic cells (SynCells) are bio-inspired micromachines constructed from molecular building blocks that mimic the form and function of biological cells. Despite their promise, most SynCells remain structurally simplistic, primarily consisting of spherical liposomes, in contrast to their biological counterparts, which are highly compartmentalised. Because form and function are tightly intertwined, this lack of architectural complexity limits the emergence of more sophisticated behaviours.

In this talk, I will describe how we overcome these limitations by employing microfluidic assembly lines for SynCell production, enabling the generation of a wide repertoire of SynCell architectures. This, in turn, allows the creation of synthetic cells with emergent behaviours, including motility, bioproduction, cell–cell communication, collective actuation, and responsiveness to a variety of stimuli such as temperature, light, and magnetic fields.

In addition, we have recently expanded our toolkit to access the nanoscale by using automated approaches to generate and screen liposome libraries. Combined with rational design, this enables the construction of nano-organelles for multi-stage release of different payloads at defined time points, as well as the development of attolitre bioreactors for in situ biochemical synthesis.

Engineering Enzymes to Build Molecular Complexity: How Biocatalysis Is Reshaping Drug Discovery and Development

Ania Fryszkowska, *Novartis, CH*

The construction of molecular complexity is a central challenge in modern drug discovery and development, driven by increasingly sophisticated therapeutic modalities, rising sustainability expectations, and compressed development timelines. Enabled by advances in directed evolution, computation, automation, and analytics, biocatalysis has evolved from a niche synthetic tool into a broadly enabling and programmable technology, reshaping how molecules are designed, synthesized, and manufactured across the pharmaceutical pipeline.

This talk offers an industry perspective on the evolving landscape of biocatalysis in pharma, highlighting how integrated industrial teams are driving the adoption of enzymes as versatile, scalable, and designable catalysts across the pipeline. Through selected case studies spanning small molecules, peptides, and bioconjugates, it illustrates how engineered enzymes enable efficient C–C and C–N bond formation, access to stereochemically dense motifs, and site-selective functionalization—transformations that remain challenging for traditional chemical synthesis.

Integrative Structural Biology with Solution NMR Spectroscopy

Sebastian Hiller, *University of Basel, CH*

Structural biology is arguably at the height of its time. The integrated use of experimental and AI methods resolves problems at atomic level that have long been out of reach. Thereby, solution NMR spectroscopy is ideal to connect static structures towards their functional dynamics.

I will describe recent successes to employ solution NMR spectroscopy in such integrated setups, emphasizing the interplay between the different methods. Our journey starts with protein biogenesis in the endoplasmic reticulum (ER), where newly synthesized nascent chains are refolded by a network of molecular chaperones. We discover biomolecular condensates as the organizing principle of this chaperone network and report detailed functional and structural studies.

We then resolve the complete functional cycle of an ATP-driven molecular machine, the Hsp70 chaperone BiP, at atomic level. We create a non-equilibrium steady-state under turnover conditions inside the NMR to resolve that BiP undergoes a branched functional cycle that is regulated by two autoinhibition switches.

Finally, we leverage protein design to establish an experimental pipeline for high-throughput characterization of protein structure and dynamics by NMR. With this setup, a single operator can produce and analyze hundreds of proteins per week at minimal cost, unlocking a new regime of statistical structural biology, where sequence–structure–dynamics relationships are gained from experimental ensemble studies of suitably designed proteins.

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Reprogramming the Genetic Code to Study and Control Protein Function

Kathrin Lang, *ETH Zurich, CH*

Nature uses a limited set of twenty amino acids to synthesize proteins. In recent years, it has become possible to site-specifically incorporate designer amino acids with new chemical properties into proteins in living cells by reprogramming the genetic code. Continued advances have substantially improved the efficiency, fidelity, and scope of genetic code expansion technologies, enabling their broader application across complex biological systems. In parallel, the development of selective chemical reactions that operate within living systems has further strengthened the impact of these approaches on studying biological processes.

In this talk, I will present our lab's efforts to expand the genetic code and to endow proteins with novel chemical functionalities within their physiological environment. By engineering more efficient and versatile genetic code expansion systems,[1,2] we have enhanced the incorporation of noncanonical amino acids and enabled their application in increasingly challenging cellular contexts. Using these advances, we have developed tools to image and probe proteins,[3,4] to study protein-protein interactions and stabilize low-affinity protein complexes,[5-8] to investigate posttranslational modifications[9,10] and to re-engineer and manipulate molecular networks and biological pathways such as ubiquitylation and SUMOylation in living cells.[11-13]

We envision that ongoing improvements in efficiency and scope of genetic code expansion, together with the ability to encode complex posttranslational modifications, will enable the study of biological processes that are difficult or impossible to address by more classical methods.

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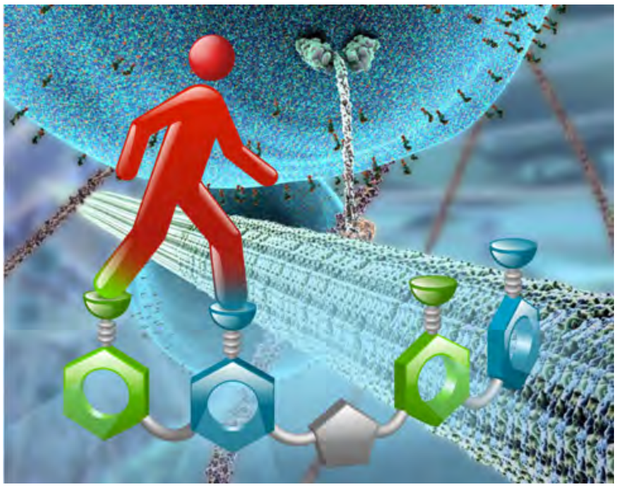
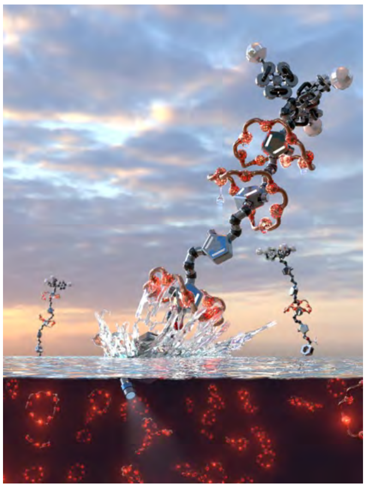
Giving Chemistry Direction

David A. Leigh, *University of Manchester, UK*

Over the last three decades examples of synthetic molecular machines and motors[1] have been developed,[2] albeit primitive by biological standards. Such molecules are best designed to work through statistical mechanisms.[3] In a manner reminiscent of Maxwell's Demon,[4] random thermal motion is rectified through ratchet mechanisms,[3–10] giving chemistry direction.

It is increasingly being recognised that similar concepts can be applied to other chemical exchange processes.[11] Ratchet mechanisms—effectively chemical engines[12] in which catalysis[5,7–9] of 'fuel' to 'waste' is used to drive another chemical process—can cause directional impetus in what are otherwise stochastic systems, including endergonic chemical reactions[13]. This is ushering in a new era of non-equilibrium chemistry, providing fundamental advances in functional molecule design and the first examples of molecular robotics,[14,15] overturning existing dogma and offering fresh insights into biology and molecular nanotechnology.





For a musical introduction, see '*Nanobot*': https://bit.ly/2M5Zwdl

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Molecular Systems Engineering in Protocell Networks

Stephen Mann, *University of Bristol, UK*

Recent progress in the chemical construction of compartmentalized semipermeable microscale objects comprising embodied cytomimetic functions is paving the way towards rudimentary forms of artificial cell-like materials (protocells/prototissues) as a step towards future proto-living technologies.

In this talk, I will demonstrate simple forms of individuated and collective molecular systems engineering in synthetic protocell networks. I will discuss recent studies on implementing programmable agency in synthetic protobiology, including: (i) enzyme-powered sensing, motility and oscillation;[1] (ii) superstructural ordering and communication[2]; (iii) information processing;[3] (iv) spatiotemporal feedback[4]; and predator-prey interactivity.[5]

These studies offer new pathways towards intelligent matter based on artificial life materials capable of autonomic behaviour and programmable agency.

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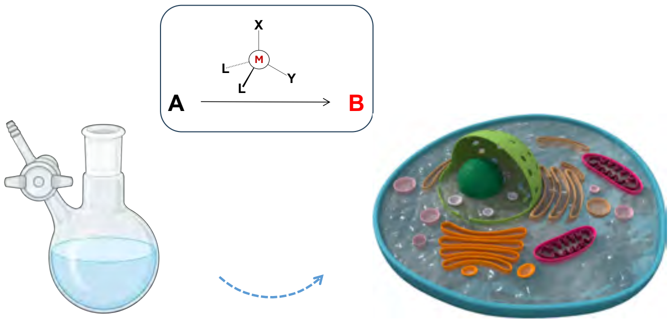
Transition Metal Catalysis: from Flasks to Live Cells

José Luis Mascareñas, *niversity of Santiago de Compostela, ES*

Transition metal complexes have proven invaluable across a broad spectrum of scientific disciplines, including catalysis, synthesis, photophysics, and supramolecular chemistry. Their diverse coordination geometries and redox properties, combined with the ability to fine-tune these characteristics through ligand modification, offer extensive opportunities for developing novel reactivities and tailored physicochemical responses.

Our research has focused on leveraging the unique features of transition metal complexes in catalysis, synthesis, and chemical biology. In recent years, we have explored the feasibility of adapting organometallic catalysis to function in biological environments, including within living mammalian cells. This endeavour poses significant challenges, particularly due to the sensitivity of many metal-catalyzed reactions to air and water, as well as the stringent demands for orthogonality and biocompatibility. Nonetheless, we have successfully developed several intracellular reactions promoted by palladium, ruthenium, and gold complexes.



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Precision Gene Editing: Rewriting the Code of Life

Krzysztof Palczewski, *University of California, Irvine, USA*

Understanding the Mysteries of the Cell: How Do Many Cell Types Arise from One Genome?

Stephen Quake, *Stanford University, USA*

In this talk I will explore to what extent the genome is a blueprint for an organism and what are some of the key open problems in interpreting the information content of the genome. Among these open problems are the fact that today it is impossible to predict the various cell types of an organism from the genome alone. This has motivated efforts to characterize the molecular composition of various cell types within humans and multiple model organisms, both by transcriptional and proteomic approaches. We used single cell transcriptomics to create a human reference atlas comprising more than one million cells from 24 different tissues and organs, many from the same donor. This atlas enabled molecular characterization of more than 400 cell types, their distribution across tissues, and tissue-specific variation in gene expression, and provides an experimental basis to understand the cell type diversity which can be generated from a single genome. We have trained large language models on this data to help understand the relationships between cell types and across evolutionary history.

Exploring Natural Products in Space and Time: from Ancient Molecules to Microbial Arms Races

Pierre Stallforth, *Hans Knöll Institute, DE*

Microbial natural products remain a critical source of therapeutic agents. These compounds are often shaped by intricate ecological interactions. Predator–prey dynamics between amoebae and bacteria represent particularly rich reservoirs of these secondary metabolites. Amoebae, as ubiquitous bacterivores, exert strong selective pressure, driving bacterial defenses against grazing and, in turn, amoebal counter-adaptations.[1,2] Within this evolutionary arms race, we investigate the biosynthesis and diversification of amoebicidal natural products, including polymicrobial modifications that expand their chemical repertoire.[3–5] Extending this perspective into the past, we exploit ancient bacterial DNA to identify and reconstruct biosynthetic genes, providing access to previously untapped natural product diversity.[6,7]

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Spatiotemporal Characterization and Modeling of Cellular Interactions Using Single-Cell and Spatial Proteomics

Savas Tay, *University of Chicago, USA*

Cells process a diverse set of signals whose type, amplitude and dynamics constantly change, and aberrant signaling leads to inflammation, infection and cancer. We have been using automated live-cell analysis, single cell methods, and computational modeling for nearly two decades to study spatial and temporal characteristics of cellular communication in the immune system. I will describe our recent results on how immune cells process combinatorial and rapidly changing pathogen and cytokine signals by modulating transcriptional dynamics. We show how single cell interactions leads to emergent spatial “patterning” of pro-inflammatory gene expression across populations and tissues. I will introduce a new single cell proteomics technology called proximity sequencing (Prox-seq), which enables simultaneous measurement of proteins, protein complexes and mRNA in thousands of individual cells. Prox-seq combines proximity ligation with single-cell sequencing to measure proteins and their dimers from all pairwise combinations, providing quadratically-scaled multiplexing. Our measurements revealed formation and dissociation of protein complexes during exposure to pathogen inputs, and reveals previously unknown protein interactions in individual cells. I will also describe the extension of proximity sequencing to intact tissues like the germinal center (spatial Prox-seq), revealing functional distributions of proteins, protein complexes, cell-cell interactions and transcripts in the same spatially resolved locations, across thousands of spots in tissue. Finally, I will describe our latest results in high throughput microfluidic systems for automated analysis of tumor cells, organoids and microbial colonies, and their use for combinatorial drug screening in personalized therapy.

Selected Talks

General Platform for Divergent Construction of Phosphorus-Modified DNA/RNA Backbones

Corentin Bon, *University of Basel, CH*

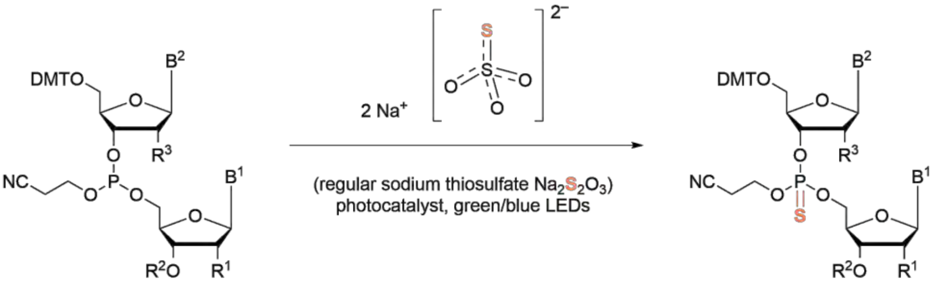
Phosphorus-containing biomolecules, including nucleotides, cyclic dinucleotides, and related cofactors, play central roles in signaling, immunity and enzyme regulation. Despite their biological importance, the highly anionic and polar nature of canonical phosphate linkages severely limits the therapeutic potential of nucleotide-based modalities by restricting membrane permeability, metabolic stability, and systemic delivery. Charge-neutral phosphorus backbones offer a promising solution, yet their development is hindered by synthetic challenges, limited structural diversity and incompatibility with oligonucleotide assembly workflows.

In this work, we present a unified platform that enables the direct modification of phosphorus centers within oligonucleotide backbones. Building on recent advances in organophosphorus radical chemistry, our strategy allows the engagement of diverse reaction partners with phosphorus centers under mild conditions, overcoming selectivity challenges typically encountered in complex nucleotide substrates. The synthetic concept opens access to previously inaccessible and unprecedented phosphorus linkages without relying on sensitive and unstable reagents or extensive protecting-group manipulations.

A Light and a Dark Method to Prepare Oligonucleotide Phosphorothioates with an Ideal Reagent

Anton Budeev, *University of Basel, CH*

With therapeutic oligonucleotides progressing from the treatment of rare genetic diseases to medications for common disorders, the synthesis of modified oligonucleotides is gaining significant momentum. To improve pharmacokinetic and pharmacodynamic properties, chemical modifications to the nucleotide backbone have thereby been particularly advantageous. Since Eckstein's pioneering discovery in 1966,[1] the phosphorothioate linkage has become the most useful oligonucleotide modification. Compared to phosphate, phosphorothioate is more resistant to cleavage by nucleases, resulting in improved elimination half-life of DNA or RNA sequence, extending from minutes to days. Moreover, the introduction of this moiety increases binding to serum proteins, amplifying the time available for uptake into target tissues.[2] This moiety can be found in 9 FDA-approved oligonucleotides.[3]



• *Benign* • *Inexpensive* • *Minimal side-oxygenation* • *Unproblematic sulfite byproduct*

However, common reagents used for the sulfurisation of phosphorothioate oligonucleotides have several disadvantages, including toxicity, cost- and atom-inefficiency, and compromised stability, underscoring the need for alternative sulfurisation protocols. In this work, we developed a more sustainable, mild and general methodology for photocatalytic sulfurisation to prepare phosphorothioates using regular thiosulfate as a benign sulfur source. Additionally, the protocol is switchable to oxygenation for the synthesis of phosphates by implementing aerobic reaction conditions. More importantly, the protocol was integrated into standard solid-phase phosphoramidite oligonucleotide synthesis using riboflavin (vitamin B2) as a photocatalyst in water. A variant of the reaction without the need of light irradiation was also developed. We believe the method represents a cost-efficient and sustainable means for the synthesis of DNA and RNA phosphorothioates and phosphates.[4-6]

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PRIME: A Modular Plasmid-Based Platform for Continuous In Vivo Protein Evolution Across Bacteria

Nicolas Huguenin-Dezot, *ETH Zurich, CH*

Directed evolution has become an essential strategy for engineering proteins with novel or enhanced functions, yet most existing approaches remain labour-intensive, low-throughput, and difficult to scale over extended evolutionary trajectories. Here, we present PRIME (Protein-primed Replication for *In vivo* Mutagenesis and Evolution), a plasmid-based, orthogonal DNA replication system that enables continuous directed evolution directly in *Escherichia coli* and *Pseudomonas putida*.

PRIME leverages the unique mechanism of protein-primed DNA replication to establish an independent replication module that operates alongside the host replication machinery. By tuning the fidelity of the dedicated DNA polymerase, mutations are selectively introduced into a target gene encoded on an orthogonal plasmid, while preserving the genomic integrity of the host cell. This design allows continuous diversification and selection to occur *in vivo* over extended time periods, facilitating the exploration of large evolutionary landscapes with minimal experimental intervention.

Importantly, the system is fully compatible with standard molecular biology workflows and does not require specialised equipment, making it readily accessible and easy to implement. The plasmid-based architecture further ensures modularity and flexibility, enabling straightforward adaptation to different targets and experimental setups.

In summary, PRIME provides a powerful and user-friendly platform for continuous *in vivo* evolution, with broad applications in protein engineering, synthetic biology, and biotechnology.

Biohybrid System to Evaluate Antibiotic Efficacy through Tissue-Like Lipid Membrane Barrier

Petra Jusková, *ETH Zurich, CH*

The increasing prevalence of drug- and multidrug-resistant pathogens poses a major challenge to the modern healthcare system, demanding the development of new tools for antibiotic treatment evaluation. Uropathogenic *Escherichia coli* (UPEC), which can invade bladder umbrella cells, is responsible for many recurrent and difficult-to-treat urinary tract infections. A subpopulation of cells residing intracellularly in host tissue is shielded by a series of membrane barriers that limit antibiotic access, making this environment difficult to reproduce and characterize *in vitro*.

In this work, we transformed the concept of artificial cells into a new platform to evaluate drug efficacy directly on living cells enclosed within tissue-like droplet-based compartments. We formed droplets containing either bacteria or antibiotics and used droplet interface bilayers (DIBs) to facilitate drug transport. We engineered an open microfluidic platform that allowed precise spatiotemporal control over droplet position and thus DIB formation. We demonstrated the possibility of exposing encapsulated bacteria to increased antibiotic concentration, drug withdrawal, and sequential antibiotic gradients. We mimicked tissue barriers using droplet networks and demonstrated that the response to antibiotic treatment depends on the number of membranes separating cells from the antibiotic source.

In summary, this platform provides a versatile *in vitro* bioassay for evaluating bacteria–antibiotic interactions under spatially controlled conditions, with potential applications in antimicrobial discovery and therapeutic development.

Engineering Protein Function through Conformational Reprogramming

Florian Leidner, *Max Planck Institute for Multidisciplinary Sciences, DE*

Proteins are soft materials that sample a large ensemble of conformations centered on a few metastable states. Enzymatic function is closely linked to the populations and interconversion of these metastable states. This suggests that the ability to manipulate conformational equilibrium would provide a powerful tool for engineering protein function. However, rational manipulation of conformational ensembles remains challenging. Structural information is usually restricted to the most populated states, and predicting the effects of amino acid substitutions on the broader ensemble is difficult. Although some computational approaches can estimate these effects, their high cost restricts exploration to a small number of candidate mutations. As a result, our ability to engineer functional landscapes by directly modulating protein dynamics remains severely limited.

To address this challenge, we developed a method to manipulate conformational ensembles through sequence design, combining physics-based simulations with deeplearning- based generative models. First, molecular dynamics simulations and Markov state models are used to characterize the conformational landscape and identify metastable states. Then, inverse folding models are used to calculate state-specific amino acid frequencies. These frequencies predict amino acid substitutions that shift the conformational equilibrium toward a target state. By explicitly considering multiple metastable states, this approach avoids a key limitation of traditional structure-based design, where mutations optimized for a single structure often introduce unintended secondary effects.

We demonstrate the feasibility of this approach using a short-chain dehydrogenase/reductase that is used in the hybrid bioorganic synthesis of taxanes. Molecular simulations showed that the catalytically competent state competes with several unproductive conformations, which limits catalytic turnover of the enzyme. Based on these results, we designed variants predicted to shift the conformational equilibrium toward the active state. Subsequent simulations revealed a significant increase in the population of the catalytically competent state. Importantly, biochemical experiments confirmed a corresponding improvement in enzymatic activity. These results demonstrate that the functional landscape of an enzyme can be altered by manipulating its conformational ensemble. More broadly, this approach provides a general strategy for designing proteins by directly modulating conformational landscapes. We currently seek to extend this framework to new systems and functions.

Short-Circuiting the SAM-Cycle in *E. coli*

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Enzyme-mediated transfer of methyl groups to specific nucleophilic functions on small metabolites, proteins, and nucleic acids is an essential activity in all known life forms. Most of these transferred methyl groups originate from the one-carbon metabolism through methyl-tetrahydrofolate-dependent methylation of homocysteine, followed by adenosylation of methionine to form the primary methyltransferase cofactor, S-adenosylmethionine (SAM). In this report, we describe a strain of Escherichia coli with a Short-Circuited SAM-Cycle (SCSC) that maintains its SAM pool exclusively by methylating S-adenosylhomocysteine (SAH) using a synthetic methyl donor. Construction of this strain was made possible by the identification of an aryl sulfonate methyl ester as a biocompatible methyl donor and methyltransferases that accept this compound as substrate for in vivo methylation of SAH. We exploited this organism for the optimization of SAH-methylating enzymes by in vivo selection and to produce isotope-labeled natural products. Looking ahead, we anticipate that strains with SCSCs will open new possibilities for methyltransferase biocatalysis, natural product discovery, and bacterial metabolomics.

Ion-Pair Breakers and Anionic Brønsted Acids for Helmholtz-Layer Catalysis with External Electric Fields in Microfluidic Capacitors

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Inspired by the intrinsic internal electric fields present in enzymes, externally applied electric fields (AEFs) have long been predicted to accelerate and direct electron movement.[1] However, the practical implementation of AEFs under standardized conditions for organic synthesis has yet to be realized. To face this challenge, we implemented catalytic Helmholtz layers in microfluidic capacitator.[2] In response to an AEF, tetrabutylammonium bisulfate in apolar solvents was found to dissociate and assemble into Helmholtz layers, generating strong effective electric fields (EEFs) that enhance the acidity of the bisulfate Brønsted acid anion, thereby catalyzing epoxide- opening polyether cascade cyclizations. To boost catalytic activity and enable unique AEF-driven reactivity, anion-binding ion-pair breakers are introduced. These amplifiers facilitate ion-pair separation and incorporate molecular recognition motifs into the catalytic Helmholtz layers. The insights gained in this study provide a conceptual mechanistic framework for the development of practical AEF catalysis for applications in organic synthesis.

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Development of Artificial Terpene Cyclases

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Terpene cyclases catalyze highly selective polycyclization cascades that transform linear polyisoprenoid precursors into complex, three-dimensional terpenoid scaffolds. Depending on their mechanism, these enzymes initiate catalysis either by diphosphate abstraction (type I) or by protonation (type II), thus triggering carbocation-driven cyclization pathways. The resulting natural products are formed with exquisite regio-, enantio-, and diastereoselectivity. However, despite their remarkable synthetic power and utility, terpene cyclases are largely restricted to substrates bearing a native substitution pattern — typically trisubstituted alkenes. Although synthetic methodologies relying on small-molecule catalysis allow a broader substrate scope, precise stereocontrol remains challenging, especially for substrates bearing electron-deficient alkenes.

Here, we present our studies toward the development of artificial terpene cyclases that utilize new-to-nature reactivity[1] to expand the biocatalytic substrate scope for polycyclization and provide a chemoenzymatic strategy for the asymmetric synthesis of bioactive abietane diterpenoids.

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A Traceless Tag for Proteins Delivery through TMU

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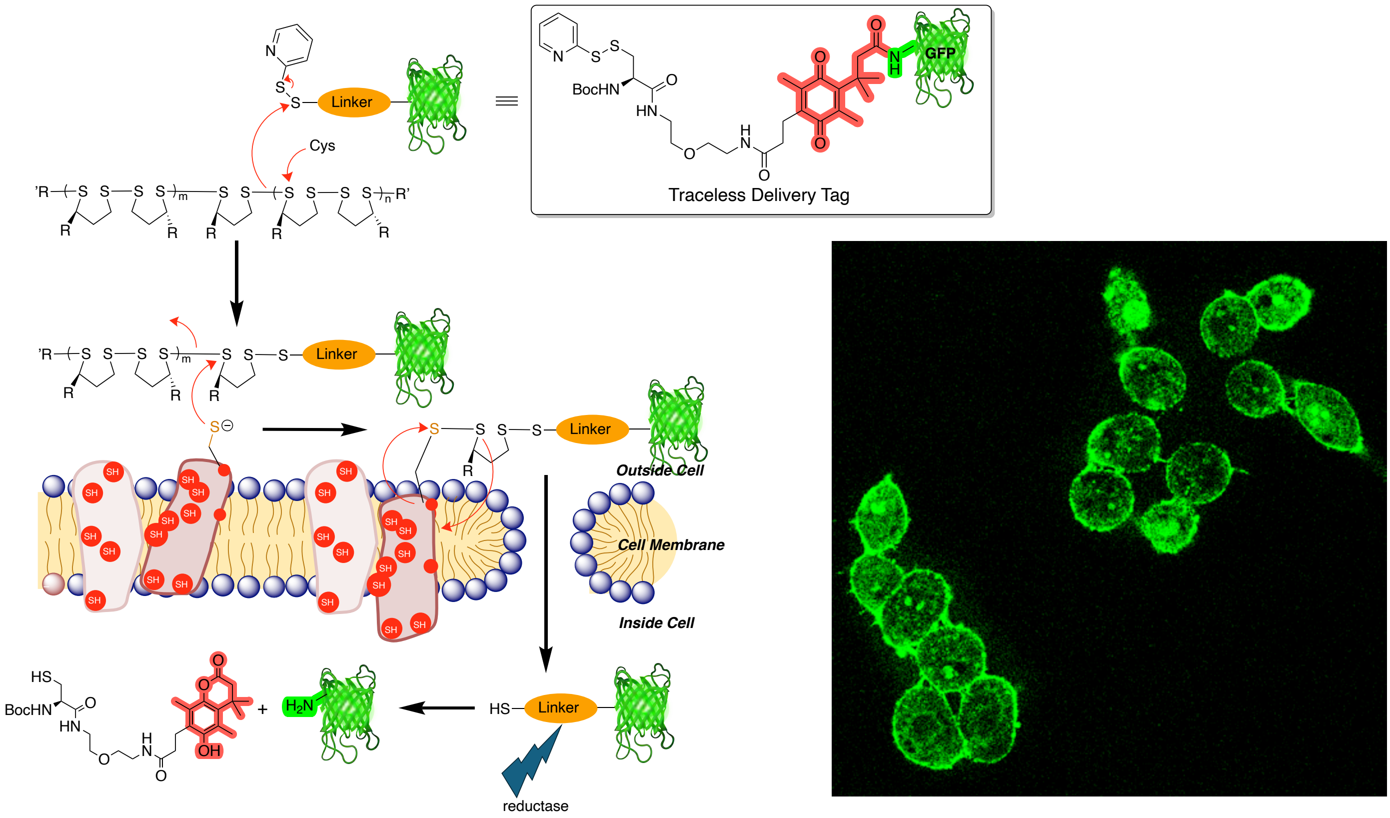
The delivery of proteins through cell membranes without modifying their structure (traceless delivery) is of vital importance but remains challenging. Among all the pathways for achieving the delivery, thiol-mediated uptake (TMU) has attracted growing attention as an unorthodox yet powerful approach,[1] involving dynamic covalent exchange networks (Figure left). Recently, the Matile group developed a new cell-penetrating polydisulfides (CPDs) method,[2] enabling the traceless delivery of cysteine-modified GFP at nanomolar concentrations via grafting-to bioconjugation.

Compared to thiols, amines are among the most abundant reactive groups on native proteins, being present on lysine side chains and at peptide N-termini. For generalising the aforementioned CPDs method to amine-based bioconjugation, as well as preserve the traceless property, we designed a delivery tag (Structure in box) in which the target proteins (GFP as an example) are linked to a trimethyl lock (red) though an amide bond which is cleavable by cellular reductase. Meanwhile, the structure incorporates an active disulfide moiety enabling rapid disulfide exchanges with the CPDs and facilitating cellular uptake via TMU.

Preliminary studies on GFP have shown promising results (Figure right), demonstrating the delivery efficiency and the tracelessness. Ongoing work is extending this approach to more complex cargos, such as antibodies and streptavidin.



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Posters

A Microfluidic Platform for Screening DNA-encoded Antimicrobial Peptides

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Antimicrobial peptides (AMPs) represent a promising class of therapeutics in the fight against the growing threat of antimicrobial resistance (AMR) in bacteria. Despite their potential, AMPs remain underexplored, partially due to technical limitations in how they are screened. Most AMPs act by selectively disrupting negatively charged bacterial membranes, a mechanism that typically requires higher peptide concentrations than drugs with high-impact targets, like intracellular enzymes. Current screening methods either suffer from low throughput (peptide synthesis and screening in well-plate formats) or cannot reach functionally relevant peptide concentrations (molecular peptide display techniques).

To address these challenges, we aim to develop a high-throughput AMP screening platform using droplet microfluidics. In this approach, members of a DNA-encoded library of AMPs are compartmentalized into individual droplets, where each sequence is amplified and expressed through in vitro transcription and translation. While maintaining compartmentalization, the resulting peptides are immobilized on hydrogel beads together with the corresponding DNA, ensuring a stable genotype–phenotype linkage. These functionalized beads are then pooled and later re-encapsulated in droplets with clinically relevant pathogens under defined conditions. By screening for antimicrobial activity via droplet or bead-based sorting, the genetic sequences of active peptides are recovered.

Our planned use of hydrogel beads enables efficient peptide capture and release at high density. This allows for screening at effective concentrations and under clinically relevant conditions – ideally bypassing the stage at which many AMP candidates fail. Additionally, we are simplifying the workflow so that it is technically accessible and compatible with widely available sorting instruments. Altogether, this project lays the groundwork for a more practical high-throughput AMP discovery platform.

Energizing Modules for Bionanotechnological Applications

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Towards the bottom-up assembly of bionanotechnological systems with diverse innovative functions, a central aim of the NCCR Molecular Systems Engineering (MSE), energizing modules are viewed as important building blocks in such systems. Liposome-based scaffolding in such systems can be conveniently accommodating for such instrumental and energizing building blocks. Attractive examples of energizing modules are the membrane proteins microbial rhodopsins which can generate a proton gradient across lipid bilayers by mere exposure to light, and ATP synthase which can utilize proton gradients for the synthesis of useful ATP that can be used in downstream functions of the system.

Microdroplet Arrays for Directed Evolution and High-Throughput Screening of Antimicrobial Peptides

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Antimicrobial resistance has become a worldwide threat, requiring novel compound development [1]. Here, antimicrobial peptides (AMPs) have been identified as promising candidates [2]. Natural AMPs must be enhanced in their bactericidal activity to become clinically viable, e.g., by directed evolution (DE) [3]. While continuous DE methods are advantageous where protein mutant performance can be coupled to host cell proliferation [4], AMP efficacy screening against pathogenic bacteria requires a compartmentalized experimental design. Droplet microfluidics is an established platform here, as it enables high-throughput and phenotype-genotype linkage [5]. However, those droplets are inaccessible, making reagent addition, long-term observation, selection, and genotype recovery extremely laborious. The goal of this project is to develop an open droplet microfluidic system for DE, that enables high-throughput mutant synthesis and identification of effective AMPs.

For this, up to 100’000 accessible aqueous nanoliter droplets are deposited on a patterned glass surface under oil to create a so-called microdroplet array, which can be observed long-term via (fluorescence) microscopy, further compounds can be added, droplets can be recovered or subjected to MALDI-MS [6]. This platform enables the distribution of (randomized) protein-encoding DNA libraries to droplets, followed by on-array protein synthesis via bacterial expression or in-vitro translation-transcription. The platform development and validation are established with fluorescent protein and enzyme libraries. Thereafter, AMP mutants will be screened against pathogenic bacteria survival. The amino acid sequence in droplets of interest is then elucidated via droplet recovery and DNA sequencing, or on-array MALDI-MS/MS for small proteins like AMPs, leading to the next DE round. Ultimately, this project aims to develop a more efficient workflow for DE of AMPs and to identify improved mutants to contribute to the efforts against globally rising antimicrobial resistance.

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Title:

From Environmental Genomes to Therapy:
Screening Ultrasensitive Channelrhodopsins for Vision Restoration

Authors: Matthew Blackburn, Morgan Chevalier, Firas Fadel, Botond Roska, Guilherme Testa-Silva

Abstract:

Channelrhodopsins are light-gated ion channels in microorganisms that facilitate ion flow in response to light, making them valuable tools for optogenetics and potential gene therapies for vision restoration. Despite recent clinical successes, current rhodopsin channels often lack sufficient single-channel conductance, sensitivity to natural light, optimal membrane expression, and kinetics, necessitating the use of light-amplifying devices to intensify retinal stimulation and requiring high expression levels, which increases the risk of cytotoxicity.

To address these challenges, we employed a metagenomic approach to investigate thousands of uncharacterized channelrhodopsins using sequence homology analysis, structural modelling, and functional comparisons. This strategy narrowed the selection to subset of candidates with promising properties, which are being further engineered to enhance their function. These optimized candidates will undergo high-throughput screening for membrane trafficking and patch-clamp assays in HEK cells, followed by functional testing in retinal organoids through whole-cell electrophysiology and imaging, and ex vivo human retinal explants using multi-electrode array (MEA) and whole-cell recordings. Our goal is to develop next-generation rhodopsin channels optimized for clinical use.

Image-guided lipid analysis of single breast cancer cells on microdroplet arrays using mass spectrometry

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Cancer cell heterogeneity can impede successful treatment of a patient due to the presence of subpopulations which can differ in drug resistance, growth rate and metastasis potential.[1] Analysis at the single-cell level provides insights in heterogeneous response to drugs and can therefore help to characterize and understand inter-cellular variations. Here, we employ a microfluidic platform, so-called microdroplet arrays,[2,3] for single-cell isolation and analysis by mass spectrometry (MS) to identify lipids in the cell plasma membrane. Thereby, we differentiate between various malignant (MDA-MB-231, MCF7 and SkBr3) and benign (MCF10A) breast cell lines.

The key component of the microarray is a glass substrate, coated with an Indium-Tin-Oxide (ITO) layer and a polysilazane layer, in which 10450 hydrophilic spots are fabricated by photolithography.[3] The array is ordered in a 190 x 55 grid with a spot diameter of 250 µm. Droplets of the cell suspensions are deposited onto the glass plate while immersed in hydrofluoroether (HFE-7500 from Puretec GmbH) to prevent evaporation. Afterwards the array is imaged by fluorescence microscopy to determine cell viability and cell positions. For single-cell matrix-assisted laser desorption/ionization (MALDI)-MS we are using an image-guided approach to target individual cells. Before measurements, a matrix compound (2,5-dihydroxybenzoic acid) is applied by sublimation as described previously.[4]
First, we validated the approach by analyzing the breast cancer cell line MDA-MB-231. Subsets of cells were stained with different concentration (0 - 15 µM) of a membrane dye (DiI) and are imaged by fluorescence microscopy as well as measured by MALDI-MS (*m/z* 833.6). The respective heatmaps together with the corresponding strip plots of the array show that the fluorescence and MS signals correlate across the concentration gradient validating the orthogonal detection of DiI in single cells. We also confirmed that the laser ablated areas after MALDI-MS acquisition correspond to the identified cells on the microarray. Next, we applied the method to perform lipid analysis of single breast cancer cells. We distributed breast cancer cell lines MDA-MB-231, MCF7 and SkBr3 and the control cell line MCF10A on a microarray. UMAP embedding and silhouette analysis revealed that MDA-MB-231 cells form an isolated cluster in contrast to overlapping clusters from MCF7, SkBr3 and MCF10A cells. The cluster can be explained by mesenchymal phenotype of triple-negative MDA-MB-231 cells in comparison to epithelial cell lines. Correlation analysis of differentially abundant m/z features across the array show that there is a strong correlation between m/z 700.35 and 728.35 while the DiI marker is independent from endogenous lipids. The m/z features are dominated by polyunsaturated phosphatidylcholine (PC) and Phosphatidylethanolamine (PE) consistent with cancer associated membrane remodeling.[5] The lipid profiles of each cell line correlate to UMAP cluster showing that cell line specific lipids drive the separation while having shared lipid characteristics.

We believe that the presented work offers a novel single cell approach to resolve lipid compositions while preserving compartmentalization using microdroplet arrays making it particularly relevant for drug testing applications where lipid remodeling is of interest.

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Drug Permeability Assessment of Functionalized Droplet Interface Bilayers

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Lipids are essential components of cell membranes, organelles and cell-originated vesicles, defining many physical and chemical properties of the membranes such as the permeability. Here, we use an artificial membrane, formed between two aqueous droplets, and mixed with lipids from breast cell-derived vesicles, to investigate how the composition effects passive drug diffusion.

Permeability (and tightness) is the key function of the cell plasma membrane that regulates molecular exchange across the membrane[1]. Permeation of molecules and drugs across the plasma membrane relies mostly on passive diffusion[2], where the lipid bilayer composition plays a pivotal role.[1] Here, we create a membrane model - a functionalized droplet interface bilayer (DIB) - by combining synthetic lipids and cell-originated vesicles to investigate the permeation rate of model compounds.

We culture different malignant and benign breast cell lines, and isolate extracellular vesicles (EVs) that are secreted from viable cells, and cell-derived vesicles (CDVs) that are formed by the cells upon incubation with a cytotoxin (cytochalasin B). We imaged EVs and CDVs (from MDA-MB-231 cells) by scanning electron microscopy. The vesicles were captured with magnetic beads that are coated by anti-CD63 antibodies which is a common membrane and EVs marker.[3] Furthermore, we used nanoparticle tracking analysis to determine the size of the vesicles, which is typically between 150 and 200 nm with CDVs slightly larger than EVs for all cell types. The lipids of the vesicles are transferred into hexadecane, and a phospholipid (DOPC) is added.

DIBs are formed within a miniaturized PMMA device, which facilitates precise droplet placement and indexing.[4] The hexadecane with DOPC and the extracted lipids from EVs and CDVs is pipetted into the container holding the PMMA chip. Aqueous droplets of 480 nL are inserted into the 80 wells, to finally create in total 40 DIB pairs.

We use the fluorescent dye rhodamine 110 (R110), filled into one droplet (“the donor droplet”) to monitor passive diffusion. Over a time period of ~1-5 hour, R110 diffused across the DIB into the acceptor droplet. The resulting changes in fluorescence intensity were monitored in real time by fluorescence microscopy. The permeation rate was calculated using Fick’s first law, relating flux across the membrane to volume, surface area, and concentration gradient.[5] When the same protocol was performed for all cell lines, we observed significant differences in the permeation rates, highlighting that different membrane composition alters the permeability. We also separated the CDV by size using an ultracentrifuge and again, significant differences were found between cell types.

The platform enables studies on functionalized membranes in a straightforward way. Future work will include permeation studies of (non-fluorescent) drugs where we analyze donor and acceptor droplets by liquid chromatography and/or mass spectrometry.

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Paracyclophenylenes as functional units and building blocks for SWCNTs

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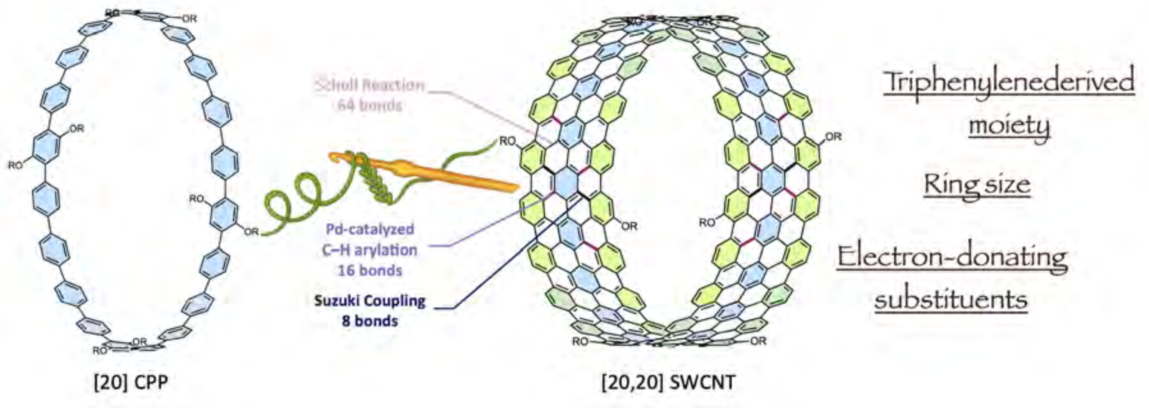
The controlled bottom-up synthesis of carbon nanotubes (CNTs) remains a major challenge in nanocarbon chemistry, as it would enable precise control over their structural and electronic properties. The π-extension of cycloparaphenylenes (CPPs) has emerged as a promising amplification strategy for CNT growth.[1-4]

The Scholl reaction provides an efficient route to extended polyaromatic hydrocarbons (PAHs), but is often accompanied by undesired rearrangements.[1, 5-7] Here, we demonstrate that two key factors are crucial for controlled π-extension: the use of sufficiently large CPP seeds ([20]CPP) to minimize strain, and the design of chlorine-containing building blocks that direct arylation toward triphenylene-based fused structures. This approach enables controlled π-extension and suppresses rearrangements, offering a pathway toward CNT-like architectures.



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Human induced pluripotent stem cell colonies sense pattern size to regulate their mechanical landscape

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Abstract: Human induced pluripotent stem cells (hiPSCs) are highly sensitive to mechanical cues, yet how their viscoelastic properties are organized within multicellular colonies remains poorly understood. Previous studies have shown that hiPSC colonies exhibit position-dependent adhesion, polarity and fate-related organization, suggesting that mechanical properties may also be spatially regulated. Here, we used atomic force microscopy (AFM)-based rheological measurements to quantify the viscoelasticity of hiPSCs at the single-cell and colony levels. Oscillatory indentation allowed us to determine the storage modulus, loss modulus and loss tangent, thereby separating elastic and viscous contributions to cellular mechanics. We first found that hiPSCs within colonies are mechanically stiffer than isolated cells, indicating that multicellular organization strengthens cellular mechanical properties. Using circular micropatterns of defined diameters, we further revealed a pattern-size-dependent mechanical landscape: larger colonies displayed pronounced radial heterogeneity in viscoelasticity, whereas smaller colonies were comparatively homogeneous. This spatial mechanical profile was associated with colony geometry and mechanics-related structures, including cytoskeletal and junctional organization. Together, our findings show that hiPSC colonies are not mechanically uniform but develop organized viscoelastic landscapes shaped by colony size and multicellular architecture. These results suggest that spatial regulation of cell mechanics may contribute to hiPSC colony organization and provide a mechanical basis for position-dependent cell behavior and fate patterning.

Keywords: hiPSCs; AFM rheology; viscoelasticity; micropatterns; mechanobiology

An Automated Experimental Synthesis Platform coupled to Artificial Intelligence Algorithms for Chemical Exploration, Synthesis Optimization and Computing

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Automated wet-chemistry platforms enable systematic screening campaigns, exploration of large chemical spaces, synthesis optimization and novel applications such as chemical computing. We present a system based on microreactors that uses scripts for varying composition and reaction conditions. The system is coupled to compound-selective analytics where individual products can be identified and quantified.

This system is used to explore chemical reaction networks (CRNs). CRNs underpin processes from prebiotic chemistry to biological function but their combinatorial complexity makes systematic exploration challenging. Although ab initio methods probe chemical compound space, open-ended searches rapidly become computationally prohibitive due to the explosion of reaction pathways. High-performance liquid chromatography and mass-spectrometry data is used to identify all compounds produced by the CRN under varying conditions. This data is directly coupled to theoretical modelling. Using SCINE[1], experiments are complemented with computed reaction pathways, activation barriers, and mechanistic predictions. Thereby, the reaction pathways of a novel CRN[2] can be explained.

Such highly branched CRNs produce a substantial amount of chemicals that are pharmaceutically attractive[2] as they are difficult to derive by conventional routes. Hence, optimizing compound yield is an attractive task but not straightforward due to cascaded pathways. As our platform provides precise control over concentrations, sequences, and boundary conditions, it defines a structured, high-dimensional input space. Bayesian optimization (BO) leverages this controllability to propose informative experiments, enabling data-efficient identification of promising reaction conditions. We present a BO framework linked to experiments and operational in closed-loop.

As another application, CRNs can act as computing systems [3] as they nonlinearly process information. By encoding composition and reaction conditions as inputs and compound quantities as outputs, a reservoir computing approach is used to solve nonlinear classifications which are benchmarked against ML algorithms.[2] Together, these applications demonstrate that automated synthesis platforms are highly versatile tools for AI-guided chemistry.

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Liquid-in-Liquid Prints: High-Density Biochemically Encoded Information Preserved in Microdroplet Arrays

Leonard Fröhlich (presenter - PhD in the Bioanalytics group of ETH Zürich), Maximilian Breitfeld, Robert Strutt, Sebastian Bargfrede, Claudius L. Dietsche, Petra S. Dittrich

Abstract:

Spatially patterning liquids is challenging because diffusion disperses local solute concentrations and thereby disrupts functional patterning. Herein, a new concept is introduced for writing and preserving information in liquid-in-liquid prints through a custom microdroplet array technology. This technology produces macro-liquid structures, composite of indexed water-in-oil droplet pixels each with a precise chemical composition, high spatial resolution and a tight inter-pixel pitch. These prints are analogous to an inkjet-printed image however each droplet pixel is maintained in the liquid state and can therefore serve as a miniature bioreactor. By applying standard and custom encoding schemes, various forms of information are written biochemically such as images, QR codes, text characters and words. As a composite material, reversible phase transitions between dissolved liquid and crystallized solid states control information encryption and decryption. Compared to current liquid printing and chemical encoding paradigms, ours introduces a fundamentally new precedent for deterministically programming information release, exchange or decay without stimuli or physical processing. These macro-liquid patterns are relevant to any application based on precise liquid handling such as materials design and biological assays.

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Macro-scale, scaffold-assisted model of the human bone marrow endosteal niche using hiPSC-vascularized osteoblastic organoids

Endosteal bone marrow (BM) niches are crucial to sustain non-steady-state hematopoiesis but are challenging to be modeled in their cellular and molecular complexity in standardized, human settings. We report a developmentally guided approach to generate a macro-scale organotypic model of BM endosteal niches (engineered vascularized osteoblastic niche [eVON]) based on human induced pluripotent stem cells and porous hydroxyapatite scaffolds. The eVON contains long-lasting vascular networks covered by pericytes and neural fibers within an osteogenic matrix. Key niche signals (CXCL12, KITLG, and vascular endothelial growth factor A [VEGFA]) are expressed in human-specific patterns. The system supports hematopoiesis in vitro and preserves hematopoietic stem and progenitor cell (HSPC) multilineage repopulation capacity in vivo. eVON perturbations at cellular (removing vasculature) and molecular (deregulating VEGF-A and CXCL12 signaling) levels enabled the investigation of the contribution of endosteal vasculature to myelopoiesis. The eVON faithfully captures phenotypic, structural, and functional features of human endosteal BM, enabling the study of pathophysiological interactions with hematopoietic cells.

Organocatalytic Microfluidic Double-Layer Capacitors

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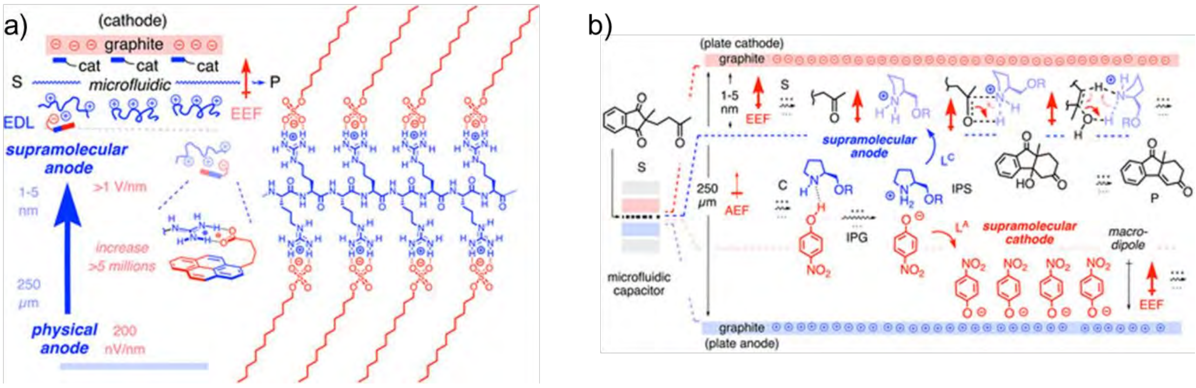
Ideas to use external electric fields to enable, accelerate and direct the movement of electrons during chemical reactions are not new. Theory and experiments under special conditions predict that electric-field catalysis (EFC) from externally applied fields could change the way we make molecules. The challenge is the incompatibility with organic synthesis under scalable bulk conditions. Access to applied electric fields (AEFs) > 1 V nm^{-1}, predicted as necessary for direct transition-state stabilization, is not possible even with electromicrofluidic systems, where the distance between the plate electrodes is minimized. Therefore, we decided to shift our attention from the applied fields to their consequences. We consider electrical double layers (EDLs) that form within a few nanometers from the plate electrodes as engineerable supramolecular electrodes, which could be generated from ion pair separation. Multiple ways have been developed to give EDLs. Applying lessons from cell-penetrating peptides (CPPs), we introduced polyarginine which forms supramolecular electrodes with effective electric fields (EEFs) that far exceeding applied fields. Protons transfer from Brønsted acid co-catalysts to secondary amines generates charges, which are separated and driven to the respective plate electrodes to form supramolecular electrodes. The charged intermediate themselves can also self-assemble into supramolecular electrodes, generating the high effective electric field required for catalysis. Ion-pair separation and the templated assembly of charged monomers into organocatalytic supramolecular 2D polymers emerge as central EFC principles that are distinct from mechanisms in stirred solution and will serve as guidelines for the discovery of organic synthesis under electric fields.



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Versatile Enzymatic Recycling Cascade for Nucleotide Cofactors

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Abstract

S-adenosyl-L-methionine (SAM) is the second most used enzyme substrate and a major methyl donor across kingdoms. Besides its crucial use in natural methylation processes, it serves as a donor for a variety of other chemical groups.[1] This study focuses on SAM as a donor for activated 2-amino propionyl groups. Those can be found in, e.g., 1-amino-cyclopropane-1-carboxylic acid (ACC), an intermediate in ethylene biosynthesis.

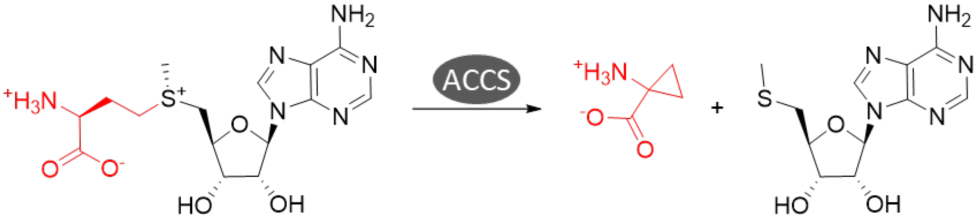

Figure 1: Reaction scheme of the production of 1-aminocyclopropane-1-carboxylic acid in plants, a precursor of ethylene in plants. 1-aminocyclopropane-1-carboxylic acid synthase (ACCS) utilizes (*S*,*S*)-SAM as a substrate to produce 1-aminocyclopropane-1-carboxylic acid (ACC) and 5-methylthioadenosine (5-MTA). In red, the 2-amino propionyl moiety of SAM and the subsequent resulting product are highlighted.

Furthermore, spermidine receives its amino-propyl group from SAM during polyamine biosynthesis. This is of particular economic interest, since spermidine is a potent geroprotector, increasing the lifespan not only of yeast and mice, but also of human cells under laboratory conditions. To exploit SAM as a 2-amino propionyl donor in preparative catalysis, we are developing a recycling cascade for nucleotide cofactors, which includes a versatile point where we propose the possibility of incorporating a variety of enzymes using SAM's 2-amino propionyl moiety to produce the product of interest. In this presentation, we discuss the progress and limitations of this project.

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A design for a photocleavable surface layer on silica nanoparticles

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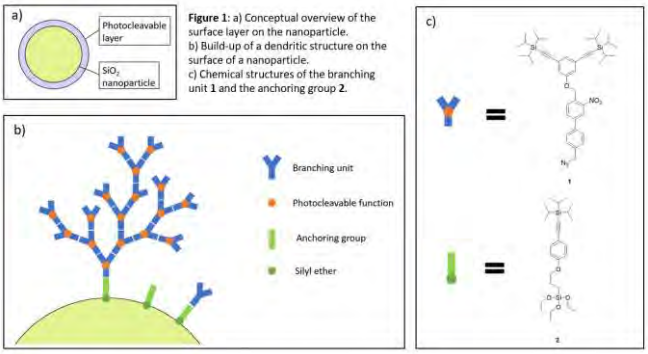

Figure 1: a) Conceptual overview of the surface layer on the nanoparticle. b) Build-up of a dendritic structure on the surface of a nanoparticle. c) Chemical structures of the branching unit **1** and the anchoring group **2**.

The wave-particle duality of matter remains an enticing topic, raising questions about whether a distinctive border between the quantum mechanical and macroscopic realm can be drawn. Quantum interferometry has proven to be a powerful tool in this regard. The future of these experiments relies on the development of new techniques that accommodate for the nature of increasingly complex molecules.[1] One promising approach involves the usage of an optical depletion mechanism, which does not rely on the species tendency to dissociate or ionize.

In this work, we propose a design for a model system, which combines photocleavable functional groups, click chemistry and the well-established surface chemistry of silica-based media. [2] We aim to functionalize silica nanoparticles with a dendritic, photosensitive surface layer (Figure **1a**). Upon irradiation, the layer is cleaved, thereby releasing particles in a controlled fashion.

The photocleavable layer is installed on the nanoparticles via a bottom-up approach involving the interplay of a branching unit and an anchoring group (Figure **1b**). Conceptually speaking, the anchoring group serves as a connection between the surface of the particle and the first generation of branching units. The branching unit contains a photocleavable midsection, while otherwise consisting of linkage sites to connect it to the anchoring group on one end and two additional branching units on the other. Repeated connections between branching units result in the build-up of a dendrimer on the surface of the nanoparticle. We designed structures **1** and **2** to fulfil the function of the branching unit and the anchoring group, respectively. The more prominent branching unit consists of a central *ortho*-nitrobenzylic ether function, which enables the photocleavage of the surface layer. The anchoring group consists of a silyl ether, which reacts readily with exposed hydroxyl groups present on silica nanoparticles. The two structures can then be connected using click chemistry.

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Synthesis of [C2]-daisy chain rotaxane structures

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Abstract

The synthesis of mechanically interlocked molecules remains challenging when highly dynamic architectures are targeted. In this work, we investigate the formation of a [c2]-daisy chain rotaxane based on a bipyridine-containing oligoethylene glycol macrocycle and a rigid axle derivative bearing bulky stopper units. The interlocked assembly is targeted through a copper-mediated Cadiot–Chodkiewicz active metal template strategy.

Preliminary analytical investigations reveal the predominant formation of the corresponding mono-threaded coupling product under the explored conditions. Ongoing studies are therefore focused on identifying the factors that could favor double-threading events and promote the formation of the targeted [c2]-daisy chain architecture.

This work aims to provide a better understanding of dynamic mechanically interlocked systems and to give insight into the delicate balance between covalent bond formation and supramolecular organization in active metal template synthesis.

From Rigid Molecular Crosses to “Molecular Chains”: Synthetic Strategy Towards Mechanically Interlocked [n]Catenane Polymers

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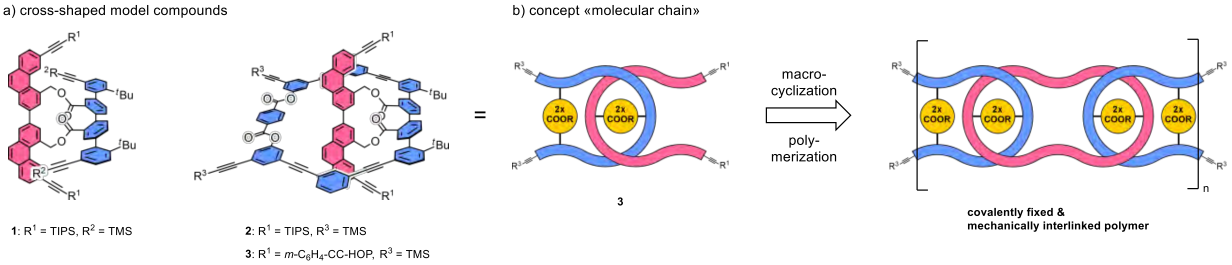
Strategies towards poly[n]catenanes are very rare and remain a significant challenge in polymer chemistry.[1] This project explores the rigid molecular cross **1**, a scaffold consisting of a rigid π-conjugated para-quarterphenyl beam and a phenanthrene strand, preorganized by cleavable ester joints. Through mild Pd-catalyzed C-C coupling, a diverse library of functionalized monomers is accessible.

We propose a bottom-up strategy to transition from this discrete cross to complex interlocked networks. The phenanthrene synthons provide precise control over the directionality of functional groups, enabling the assembly of mechanically fixed pseudo-rotaxanes **2** and **3**. The core synthetic objective is a sequence of selective acetylene liberation and oxidative coupling to yield linear, covalently fixed catenane oligomers. Subsequent cleavage of the ester joints via reduction or hydrolysis releases the mechanical constraints, resulting in poly[n]catenanes that resemble "molecular chains". This approach explores the feasibility of generating topologically interlocked polymers with potential for adaptive and stimuli-responsive behavior.



1: R^1 = TIPS, R^2 = TMS

2: R^1 = TIPS, R^3 = TMS

3: R^1 = *m*-C_6H_4-CC-HOP, R^3 = TMS

Figure 1: a) cross-shaped organic model compounds: molecular cross **1** controlling the spatial orientation of the exposed acetylenes, and covalently fixed pseudo-rotaxanes **2** and **3** with cleavable macrocyclic unit; b) concept developing a mechanically interlocked “molecular chain”.

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Grafting Cell-Penetrating Poly(disulfide)s to Substrates of Interest: a Covalent Bioconjugation for Traceless Intracellular Delivery

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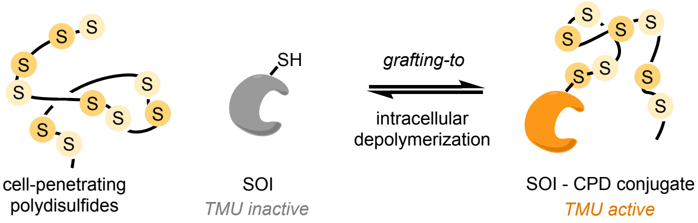
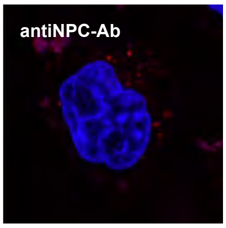
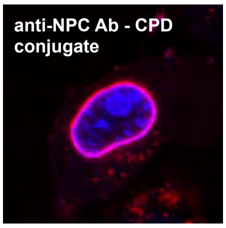
Cell-penetrating polydisulfides (CPDs) emerged in 2014 as traceless alternatives to cell penetrating peptides (CPPs) as a tool for intracellular delivery.[1] Replacement of the original peptidic scaffold with a disulfide chain allows CPDs to enter cell by thiol-mediated uptake (TMU) and be subsequently depolymerized by intracellular glutathione. CPDs have been usually grown from thiolated cargoes successfully delivered in the intracellular environment.[3] Despite many efforts, such grafting-from approach only works at high concentrations, above any biological utility. We have recently unlocked the possibility of grafting the CPDs to thiolated cargoes through a dynamic covalent process operating at concentration < 50 nM, with kinetic constant *k* = 1500 $M^{-1}s^{-1}$ in the range of the best bioorthogonal reactions.[4] Small molecular probes, bigger fluorescent proteins and functional antibodies were used to prove the effective bioconjugation and the delivery of the desired cargo with its untouched activity inside cell.







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Design of a high-throughput screening system for the evolution of an artificial metathase

Abstract: Overcoming the current limitations of directed evolution for artificial metalloenzymes is a key challenge for future biosynthetic applications. Herein we present an approach based on a high-throughput screen of an artificial metathase. This artificial metalloenzyme consists of a modified Hoveyda-Grubbs 2nd generation catalyst ([Ru1]) incorporated into a *de novo* designed Tandem Repeat Protein (dnTRP). Previous directed evolution efforts revealed the variant dnTRP R5 maintaining ring-closing metathesis activity even at neutral pH and in living *E.coli* cells. This work aims to combine this exciting finding with a fluorescent read-out of metathesis activity. The ring-closing metathesis triggered release of a fluorophore would allow the application of a fluorescence-activated cell sorting (FACS) system to screen for further improved variants in a high-throughput fashion.

Helical Porphyrin-Geländer Oligomers with Tunable Optical and Electronic Properties

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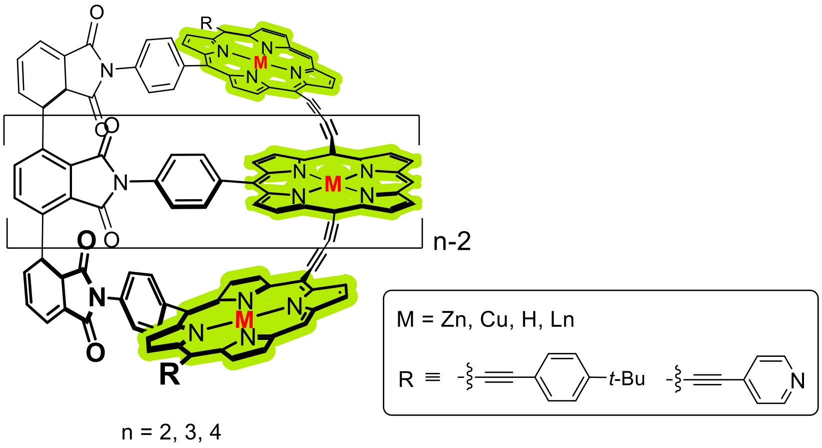
Department of Chemistry, *University of Basel*, *Switzerland*

Porphyrin-based Geländer oligomers featuring butadiyne-linked porphyrin as the banister were designed and synthesized.[1] In previous studies, analogous Geländer architectures incorporating carbazole units demonstrated length-dependent optical and chiroptical properties.[2] Building on this concept, we extended the framework to porphyrin-based oligomers, aiming to explore the impact of oligomer length and metal coordination on their physicochemical behavior. Optical and chiroptical responses (UV-vis, fluorescence, CD, CPL) varied with oligomer length, while metal substitution at the porphyrin core (e.g., Zn (II), Cu (II), Ln (III)) enables tuning of electronic properties. These systems offer a unique platform for understanding how molecular helicity, oligomer length, and metal coordination modulate chiroptical activity and circularly polarized luminescence in π-conjugated architectures, with potential relevance to single-molecule junctions and chiral-induced spin selectivity.



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PRIME: A modular plasmid-based platform for continuous *in vivo* protein evolution across bacteria

Lena Träger, ETH Zurich, CH

Abstract

Directed evolution has become an essential strategy for engineering proteins with novel or enhanced functions, yet most existing approaches remain labour-intensive, low-throughput, and difficult to scale over extended evolutionary trajectories. Here, we present PRIME (Protein-primed Replication for In vivo Mutagenesis and Evolution), a plasmid-based, orthogonal DNA replication system that enables continuous directed evolution directly in *Escherichia coli* and *Pseudomonas putida*.

PRIME leverages the unique mechanism of protein-primed DNA replication to establish an independent replication module that operates alongside the host replication machinery. By tuning the fidelity of the dedicated DNA polymerase, mutations are selectively introduced into a target gene encoded on an orthogonal plasmid, while preserving the genomic integrity of the host cell. This design allows continuous diversification and selection to occur *in vivo* over extended time periods, facilitating the exploration of large evolutionary landscapes with minimal experimental intervention.

Importantly, the system is fully compatible with standard molecular biology workflows and does not require specialised equipment, making it readily accessible and easy to implement. The plasmid-based architecture further ensures modularity and flexibility, enabling straightforward adaptation to different targets and experimental setups.

In summary, PRIME provides a powerful and user-friendly platform for continuous *in vivo* evolution, with broad applications in protein engineering, synthetic biology, and biotechnology.

Bacterial biosynthesis of azetidinium-type betaines

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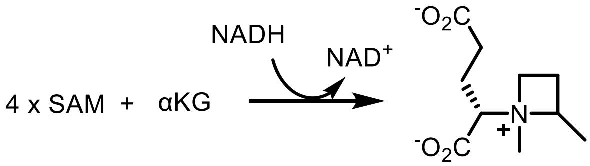
Glycine betaine is the namesake of a class of zwitterionic compounds that contain an anionic group - often a carboxylate - and a quaternary ammonium moiety. Many organisms accumulate betaines to high cellular concentrations as osmolytes, chaperones, antioxidants, or as methyl donors.

In this report, we describe a series of conserved bacterial gene cluster that codes for the production of a novel betaine-type azetidinium betaine glutamate. In this pathway, decarboxylated SAM is conjugated to an a-keto acid, followed by N-methylation and cyclization to produce an amino acid azetidinium-type betaine. *In vitro* reconstituted enzymes from the actinobacterium *Actinophytocola algeriensis* and the firmicute *Lihuaxuella thermophila* convert SAM or gamma-methylated SAM to azetidinium glutamyl betaines, and utilize pyruvate to produce the corresponding azetidinium alanyl betaines. The modularity of this pathway, the wide distribution of this gene cluster, the comparatively low sequence conservation of these enzymes, and their substrate promiscuity indicate that azetidinium-type betaines may represent a structurally diverse class of natural products.



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Synthetic Reagents for *S*-Adenosyl-L-methionine (SAM)-dependent Enzymatic Methylation

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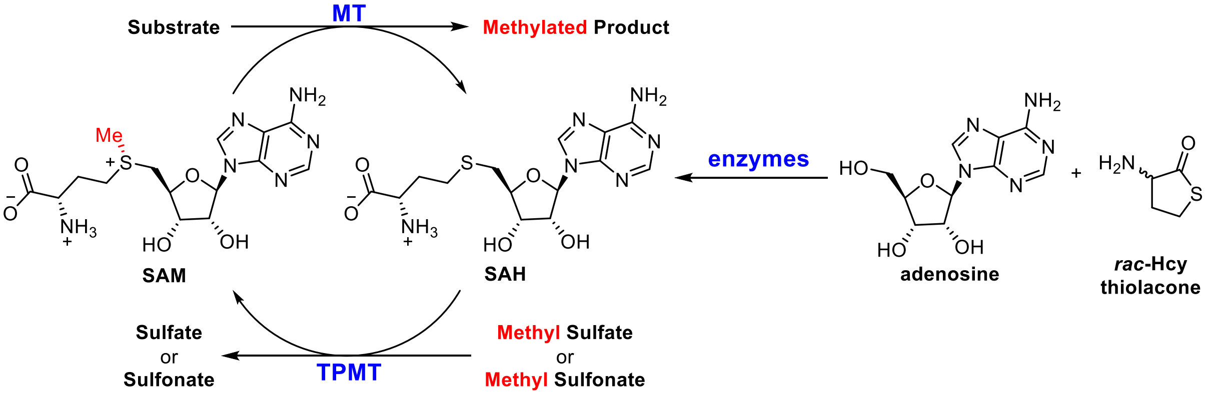
S-adenosyl-L-methionine (SAM)-dependent methyltransferases (MTs) have become important tools in the biocatalytic preparation of complex molecules because of their ability to methylate or alkylate substrates with high regio-, chemo-, and stereoselectivity.[1,2] The large-scale application of MTs suffers from their dependence on SAM as a stoichiometric and exceedingly expensive co-substrate, and product inhibition by *S*-adenosyl-L-homocysteine (SAH). As a solution to this problem, the use of methyl halides as reagents for the *in situ* regeneration of SAM has been explored.[3] However, the need to handle volatile electrophiles, such as methyl iodide (MeI), may also hamper applications at scale. As a more practical solution, we have developed a thiopurine methyltransferase (TPMT) family enzyme-catalyzed process for the regeneration of SAM from SAH with sulfate- and sulfonate-based methyl donors.[4] This development promoted SAH from an unwanted byproduct to an essential reagent. So we have also described a simple and scalable one-pot synthesis of SAH starting from racemic homocysteine thiolactone and adenosine.[5] This process has been used to supplement preparative methylation reactions with cofactor and screen *S*-nucleosyl-L-homocysteine derivatives in search of stabilized MT cofactors.



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Chemically Fuelled Control of H-H/H-T Selectivity in 9-Anthracenecarboxylic Acid Photodimerization

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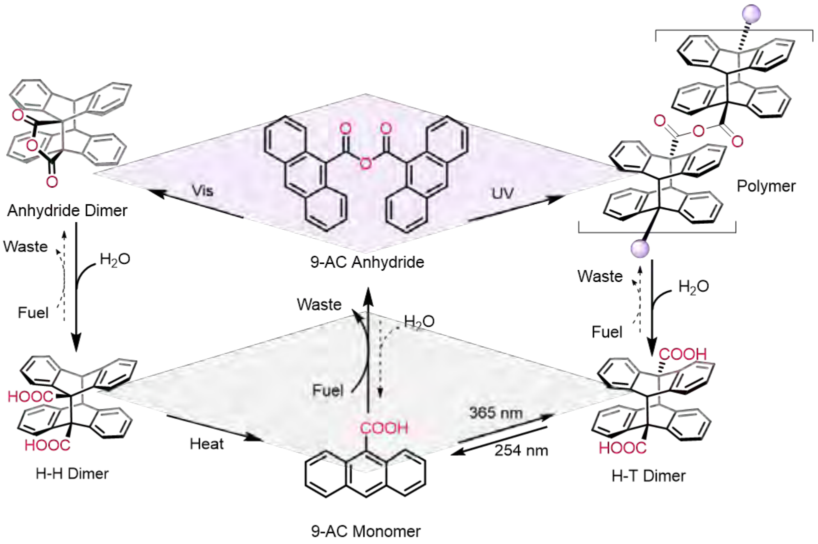
Chemical fuelling has emerged as an important strategy for driving chemical reaction networks away from equilibrium, enabling endergonic synthesis and offering new ways to control reaction outcomes beyond thermodynamic constraints.[1] Recently, Leigh and co-workers demonstrated that carbodiimide hydration can power an orthogonal endergonic Diels–Alder reaction through transient anhydride tethering, thereby biasing both product distribution and regio-/stereoselectivity under non-equilibrium conditions.[2] Inspired by this concept, we explore whether chemical fuelling can be used to control the head-to-head/head-to-tail selectivity of the photodimerization of 9-anthracenecarboxylic acid (9AC). In our design, carbodiimide-type fuel transiently activates carboxylic acid groups to generate anhydride-linked anthracene species, preorganizing two 9AC units before photoirradiation. Subsequent irradiation at 365 nm promotes [4+4] photocycloaddition, while the transient covalent tether modulates the relative orientation of the anthracene moieties and biases the formation of H-H versus H-T photodimers. By coupling fuel-driven transient bond formation with anthracene photochemistry, this work aims to establish a dissipative strategy for controlling photochemical selectivity through non-equilibrium molecular organization.



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Helical chiral conjugated macromolecule based on tetra-o-phenylenes

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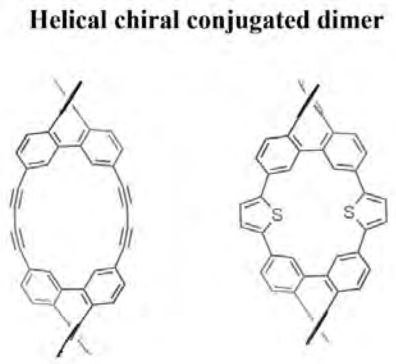

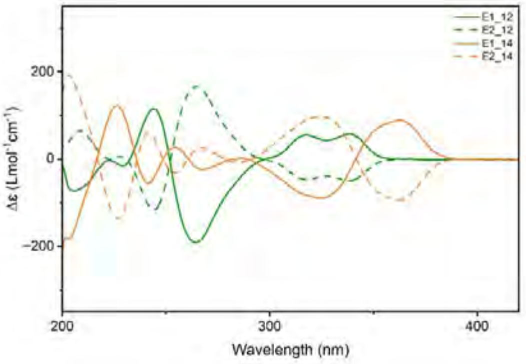

Chiral π -conjugated molecules attract continuing interest as organic materials with interesting electronic properties [1, 2]. Recognizing the tetra-*o*-phenylene as a fragment of a double helix[3], we demonstrate herein the synthesis, characterization and optical properties of conjugated double helical macromolecules that renders tetra-*o*-phenylene as a versatile building block. Dimerization was achieved via oxidative acetylene coupling, followed by conversion of the resulting butadiyne into a thiophene, affording a fully sp²-hybridized framework. Additionally, it is shown that the chiral dimers exhibited a remarkable chiroptical response.

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Studying life at the nanoscale: Pushing current boundaries in single molecule experiments

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Proteins are central to all life on earth, and their biological function is directly linked to dynamic structural changes that a given protein undergoes as it meanders across its complex energy landscape. We directly detect these dynamics to reveal and understand how proteins function at the nanoscale. We do this using time-resolved single-molecule experiments with fluorescence and FRET.

First, we directly watch CRISPR-associated-Rossman-fold (CARF) proteins, 'at work'. CARF proteins can be found in some type III CRISPR-CAS systems, where they act as a secondary line of defense in the prokaryotic immune system. They can bind to cyclic oligoadenylates (messenger molecules) in the CARF domain of the protein, which then allosterically activates non-specific RNA-cleavage in the HEPN domain. This study aims to unravel the mechanism of allosteric activation.

Single-molecule FRET (smFRET) is limited to concentrations in the low nano-molar range, which often does not represent physiological cell like conditions. In the second part we focus on the usage of nanophotonic devices, called zero-mode waveguides (ZMWs), which overcome this limitation and enable single-molecule resolution even at micro-molar concentrations. We also observe the added effect of laser induced heating of the sample. Here, we fabricate these ZMW and characterize this heating effect using the dynamics of a biomolecular model system acting as nano-thermometers. Lastly, we show that ZMWs are not only practical to reach sm-resolution at physiological concentrations but can also be used to measure at physiological temperatures like 37°C.