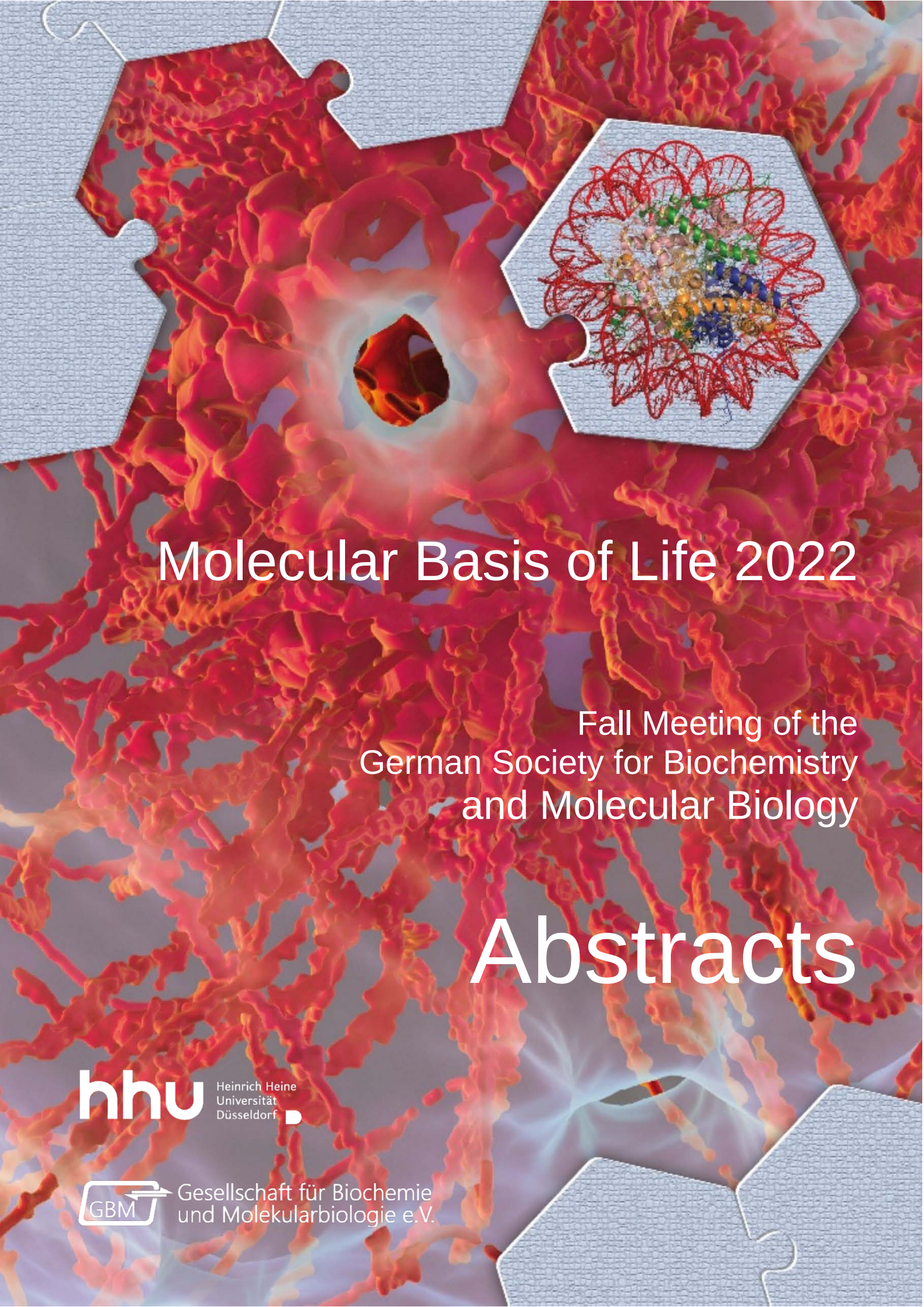

Molecular Basis of Life 2022

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Abstracts

hhu Heinrich Heine Universität Düsseldorf

GBM Gesellschaft für Biochemie und Molekularbiologie e.V.

Content

A 001 [10]
Functional Analysis of Intercellular Transfer of Glycosylphosphatidylinositol-Anchored Proteins: Use of Transwell-Coculturing and Chip-Based Sensing

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Glycosylphosphatidylinositol-anchored proteins (GPI-APs) are anchored at the outer leaflet of plasma membranes (PM) only by a carboxy-terminal GPI glycolipid. GPI-APs with the complete GPI anchor attached were recovered from rodent and human serum, dependent on age and metabolic state. To test for a physiological role of intercellular transfer of GPI-APs in control of metabolism, mutant erythroleukemia (EL) cells, mannosamine-treated human adipocytes or methyl-ß-cyclodextrin-treated rat adipocytes were used as potential acceptor cells for GPI-APs, based on defective PM expression of GPI-APs. Incubation of these cells with total full-length GPI-APs, prepared from rat adipocytes and embedded in micelle-like complexes, or with EL cells and human adipocytes with normal expression of GPI-APs as putative donor cells in transwell co-cultures increased the amount of GPI-APs at PM of the acceptor cells, as monitored by chip-based sensing, in parallel to stimulation of glycogen and lipid synthesis. Both transfer and syntheses were diminished by serum, serum GPI-specific phospholipase D and albumin. This inhibition was abrogated by synthetic phosphoinositolglycans, which closely resemble the GPI glycan core and dissociate GPI-APs from serum proteins. The data demonstrate that full-length GPI-APs can be transferred between adipocytes and blood cells which results in stimulation of lipid and glycogen synthesis and is controlled by proteinaceous (downregulation) and small-molecule (upregulation) serum factors. This argues for the (patho)physiological relevance of the intercellular transfer of GPI-APs, in general, and for a role in paracrine *vs*. endocrine (dys)regulation of glucose and lipid metabolism, in particular.

A 002 [17]
Mes-Mer-ising; Insights into Meiotic Recombination via the Mer2 and Mer3 complexes

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Meiosis is a specialised form of cell division that results in the generation of haploid gametes. During meiosis I, it is essential that homologous chromosomes be linked in order that they be properly segregated. Linkages are generated through crossovers, arising from recombination mediated repair of double-stranded DNA breaks (DSBs). Meiotic DSBs are non-random, instead they arise through the control of the conserved topoisomerase Spo11 and its chromosome associated cofactors.

In the budding yeast *S. cerevisiae*, Mer2 is a Spo11-associated factor. Using recombinant proteins and synthetic nucleosomes, combined with hybrid structural biology and biochemistry we extensively characterise Mer2. We discover several novel and unexpected interactions that provide exciting insights into the regulation of Spo11, and the formation of meiotic DSBs.

Once DSBs are formed they are resected to ssDNA and enter into the homologous recombination (HR) pathway. In meiosis the homologous chromosome is used as a template. A number of factors bias the formation of crossovers from nascent HR repair intermediates. We discover that the helicase Mer3, protects "D-loop" repair intermediates by antagonising the anti-crossover factor Sgs1.

Taken together, our *in vitro* efforts have provided much needed clarity on the initiation of meiotic recombination, its regulation, and on how the normally deleterious formation of crossovers is facilitated.

A 003 [29]
The mechanism of ISW1-mediated nucleosome spacing

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Regular nucleosome arrays are found throughout all eukaryotic kingdoms, ordering their chromatin. These nucleosome arrays are generated by ATP-dependent chromatin remodellers, which are able to alter DNA-histone contacts to set a species-specific nucleosome pattern. To achieve this, eukaryotes have evolved a multitude of different, highly conserved chromatin remodelleling enzymes, underscoring the importance of regulated nucleosome positioning. However, the mechanism of their function and the role of regular nucleosome arrays are still poorly understood. Further research on ATP-dependent chromatin remodellers is especially important since they are widely involved in cancers, human congenital anomalies, as well as neurodevelopmental diseases, and only a proper understanding of them can lead to developing promising therapeutics.
To understand how ATP-dependent chromatin remodellers can set regular nucleosome arrays we developed a sophisticated genetic model using *Saccharomyces cerevisiae*. These *S.cerevisiae* strains lack all known nucleosome spacing remodellers as well as RNA Polymerase II, which results in a highly disrupted nucleosome organization. By supplementing these strains with mutant alleles of the *S.cerevisiae* chromatin remodeller ISW1, we can dissect its mechanism of nucleosome spacing in vivo. I will discuss preliminary insights that we obtained using this strategy. We anticipate that similar strategies will become useful to dissect other chromatin remodelling enzymes in the future.

A 004 [33] Altered tRNA dynamics during translocation on slippery mRNA visualized by 3D-smFRET as determinant of spontaneous ribosome frameshifting

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When reading consecutive mRNA codons, ribosomes move by exactly one triplet at a time to synthesize a correct protein. Some mRNA tracks, called slippery sequences, are prone to ribosomal frameshifting, because the same tRNA can read both 0- and –1-frame codon. The result is the production of aberrant frameshifted peptides, a major translation error compromising the fitness of cells. Here, we determine the molecular mechanism that leads to spontaneous frameshifting.
We developed a three-color (3D) smFRET system to follow tRNA translocation pathways by TIRF microscopy in the absence of frameshifting and on a slippery mRNA where a significant fraction of ribosomes changes from 0 to –1 frame. Our smFRET approach allows us to identify potential heterogeneity within the ribosome population, to visualize local tRNA fluctuations, and to monitor how internal movements of the ribosome control frameshifting.
We show that during EF-G-catalyzed translocation on slippery sequences a fraction of ribosomes spontaneously switches from rapid, accurate translation to a slow, frameshifting-prone translocation mode where the movements of peptidyl- and deacylated tRNA become uncoupled. While deacylated tRNA translocates rapidly, pept-tRNA continues to fluctuate between chimeric and posttranslocation states, which slows down the re-locking of the small ribosomal subunit head domain. After rapid release of deacylated tRNA, pept-tRNA gains unconstrained access to the –1-frame triplet, resulting in slippage followed by recruitment of the –1-frame aa-tRNA into the A site.
Our data reveal how altered choreography of tRNA and ribosome movements reduces the translation fidelity of ribosomes translocating in a slow mode.

A 005 [34]
The disulfide bridge of AK2 is critical for its import and stability

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Adenylate kinase 2 (AK2) is an enzyme of the mitochondrial intermembrane space catalysing the exchange of a phosphate group between adenine nucleotides. It is imported into mitochondria via the MIA40 disulfide relay system, a rather slow import mechanism. During import a disulfide bridge is introduced including an intramolecular isomerization. In this study we investigated further possible roles of AK2's disulfide bridge besides its function in import. This way we could show it to have a stabilizing function on AK2 *in vitro* and *in vivo*. However, it is not required for the activity of AK2.

A 006 [40]
Connecting dynamic microtubules to mitotic chromosomes: insights from biochemical reconstitution

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Error-free cell division requires proper connections between mitotic chromosomes and microtubules. These connections are established by the Ndc80 complex, a 60-nm protein complex that binds microtubules at one end and is embedded in kinetochores on the other. At kinetochores, dozens of Ndc80 complexes cooperate in microtubule binding.

To unravel how Ndc80 binds dynamic microtubules, it is therefore essential to reconstitute the multivalency of the system. Towards this end, we covalently conjugated precisely three Ndc80 complexes to customized streptavidin-derived scaffolds. These trimeric modules span distances well over 100 nm, mimic the natural organization of Ndc80 on phosphorylated scaffold proteins, bind to microtubules, and are stable at picomolar concentrations.

Single-molecule biophysics experiments demonstrated that multivalent Ndc80 modules hold on to the ends of shortening microtubules and harness the energy generated when microtubules depolymerize. This energy moves cargo, mimicking what happens when chromosomes segregate in a dividing cell. We used our reconstituted system to untangle how the Ndc80 tail, the mitotic kinase Aurora B, the microtubule binding Ska complex, and Ndc80-Ndc80 interactions contribute to chromosome segregation.

A 007 [45]
Phosphorylation of PLPPR3/PRG2 in the regulation of filopodia formation and axonal branching

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In developing neurons, PI3K signaling positively regulates axon growth and branching via the accumulation of PIP_3 and F-actin patches. This pathway is antagonized by the ubiquitous and constitutively active phosphatase PTEN. PLPPR3 is an axonal transmembrane protein that binds and locally inhibits PTEN, thus facilitating filopodia and branch formation. Super-resolution imaging shows that PLPPR3 forms nanoclusters at the plasma membrane and live-imaging experiments characterize that filopodia form preferentially near the PLPPR3 clusters. Our data further indicate that PLPPRs form homo- and heteromultimers. We hypothesize that the assembly of PLPPR3-based clusters involved in regulating actin dynamics and filopodia formation depends on phosphorylation of the PLPPR3 intracellular domain (ICD). Indeed, the ICD is highly phosphorylated. Using mass spectrometry we identify 30 high-confidence phosphorylation sites. Furthermore, we find that PLPPR3 is a substrate for PKA. Using site-directed mutagenesis we show that S351 is a PKA phosphorylation site. Intriguingly, we identified BASP1/CAP23 as interaction partner of the PLPPR3 ICD, and show that this interaction is regulated by

phosphorylation of S351. BASP1 has functions in axonal outgrowth and actin dynamics, and the proposed mechanism involves sequestration of PIP_2 at the plasma membrane. Future work will explore whether PLPPR3-BASP1 have cooperative functions in axonal morphogenesis.

A008 [49]
Insights into the biogenesis of the human mitochondrial ribosome

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The human mitochondrial ribosome (mitoribosome) is of central importance as it synthesizes the 13 mitochondrial DNA-encoded subunits of the oxidative phosphorylation (OXPHOS) system, which is the major source of cellular energy. Defects in mitoribosome biogenesis and function leads to OXPHOS deficiency and subsequently to severe human diseases mostly affecting high energy demanding tissues such as heart and brain. Thus, it is crucial to understand this complex process of mitoribosome assembly and the molecular function of involved factors. Although the mitochondrial and the bacterial ribosome derived from a common ancestor, recent structural analyses reveal substantial differences in structure and composition of these two particles. The 55S human mitoribosome has reduced its RNA content while it gained more protein mass leading to a reverse protein:RNA ratio as found in bacteria. During the last decade it became apparent that also the biogenesis of the mitoribosome differs from its bacterial counterpart with the requirement of additional assembly factors. Here, we present mechanistic insights into the late maturation steps of the large mitoribosomal subunit by combining genetic-engineered perturbation with biochemical and structural approaches. This enabled us to determine the function of several mitoribosome biogenesis factors and to reveal the sequential folding of the peptidyl transferase center, one of the last quality control steps during large subunit maturation.

A 009 [51]
A new fold in TANGO1 evolved from SH3 domains for the export of bulky cargos

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Bulky cargos like procollagens, apolipoproteins, and mucins exceed the size of conventional COPII vesicles. During evolution a process emerged in metazoans, predominantly governed by the TANGO1 protein family, that organizes cargo at the exit sites of the endoplasmic reticulum and facilitates export by the formation of tunnel-like connections between the ER and Golgi. Cargo recognition appears to be mediated by an SH3-like domain, however, just how the vastly different cargos are recognized remains elusive. Based on structural and dynamic data as well as interaction studies from NMR spectroscopy presented here, we show that the luminal cargo-recognition domain of TANGO1 adopts a new functional fold for which we suggest the term MOTH (MIA, Otoraplin, TALI/TANGO1 homology) domain. Also, differences in the structural properties within the domain family suggest fundamentally different mechanisms of cargo-recognition in vertebrates and invertebrates. Similarly, in vertebrates, it is proposed that cargo-specificity is mediated by structural differences within the vertebrate TANGO1 and TALI MOTH domains themselves.

A 010 [52]
Dysferlin – essential for the T-tubule system and for muscle contraction

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Dysferlin is a multi-C2-domain tail-anchored protein with functions in myocytes, including T-tubule biogenesis and maintenance as well as calcium handling and membrane repair in skeletal muscle. Absence or

dysfunction of dysferlin leads to muscular dystrophies called dysferlinopathies. We discovered that dysferlin enables membrane tubulation in non-muscle cells. We propose that dysferlin plays an essential role in shaping T-tubule by contributing to membrane curvature. Moreover, we found that the interaction of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) with dysferlin is required for T-tubule biogenesis. In cardiomyocytes, we showed that dysferlin-deficiency alters the structure of the transversal-axial-tubule system (TATS). Furthermore, dysferlin is important for calcium handling in cardiomyocytes under β-adrenergic stress. We propose a model describing that loss of dysferlin leads to disturbed TATS structures resulting in a diminished interaction of L-type calcium channels and ryanodine receptors and impaired excitation-contraction coupling. Our current research focusses on structure and function of dysferlin in the secretory pathway and in membrane repair.

A 011 [53]
ADP-ribose is a novel RNA cap which prevents RNA degradation and translation

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Posttranscriptional modifications are essential for RNA function. Recently, it was shown that RNA can be modified with ADP-ribose by ADP-ribosyltransferases of the PARP family *in vitro*, potentially representing a new RNA modification. Traditionally, ADP-ribosylation by PARP enzymes has been studied as a protein posttranslational modification and to date, it is not clear whether ADP-ribosylated RNA exists in cells.

To determine whether RNA ADP-ribosylation occurs in cells, we extracted different RNA pools from cells, which we blotted and analysed using an ADP-ribose antibody. These experiments reveal that ADP-ribose is present on diverse RNA species. The ADP-ribosylation signal increases when we overexpress diverse transferases, or when we knockdown hydrolases TARG1, PARG and ARH3. We also observed that diverse stressors can modulate the amount of ADP-ribosylated RNA in cells, implying that the modification is added as part of a dynamic stress response. We next tested potential consequences of modification for mRNA and found that ADPr-mRNA is not degraded by XRN1 and is also not translated. T4 RNA ligase 1 can ligate ADPr-RNA in absence of ATP, resulting in a non-canonical ligation product with incorporated abasic site. Further work is required to identify the RNAs that are modified in cells and to follow their fate in cells.

We thus provide the first evidence that ADP-ribosylated RNA exists in mammalian cells and postulate potential functions of this novel RNA modification.

A 012 [**64**]
Nanodisc reconstitution and NMR analysis of the vesicular SNARE Protein Synaptobrevin-2

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Membrane proteins play a central role in most biochemical and biophysical processes leading to the interaction between cells, transport of metabolites, and exchange of information. As an example of information exchange, the neuronal SNARE complex induces membrane fusion between neurotransmitter-filled vesicles and the cell membrane of synapses. Synaptobrevin-2 is a small SNARE protein that is anchored in the vesicle membrane. This protein is intrinsically disordered in its monomeric pre-fusion form but forms an α-helix during SNARE complex formation.

By optimizing the expression of synaptobrevin-2, large amounts of protein can be produced, which are necessary for structural studies. The protein is isotopically labeled for NMR analysis with ^{13}C and ^{15}N. It is reconstituted into lipid nanodiscs to study synaptobrevin-2 in a near-physiological lipid environment. Such nanodiscs are a powerful and well-tunable tool for studying membrane proteins in a lipid bilayer environment.

Using the method of solution NMR, we assess the reconstitution of synaptobrevin-2 into lipid nanodisc. This will be compared to ^{1}H detection solid-state NMR spectra recorded on the same sample. Those solid-state

NMR spectra are not limited by the weight-limitation that solution NMR faces.

We aim at currently unavailable experimental structural insights on the C-terminal transmembrane region of Synaptobrevin-2 in the lipid bilayer membrane. Further, we want to obtain detailed structural and dynamic insights into the Synaptobrevin-2 SNARE motif and linker domain region interacting with the lipid bilayer membrane.

A 013 [66]
An easy and reliable way to perform single molecule FRET measurements

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Single Molecule studies and – more specifically – single molecule FRET methodologies have become a standard tool for studying dynamic structural changes in proteins and nucleic acids. These types of measurements can reveal dynamic events on time scales covering several orders of magnitude from ~ns to several seconds. This allows studying e.g., chain dynamics, binding, folding, allosteric events, oligomerization and aggregation. The power of these methodologies is highlighted by the study of Intrinsically Disordered Proteins (IDPs) whose biological relevance has been increasingly studied over the recent years.
In this poster we show how easily these measurements can be performed with Luminosa single photon-counting confocal microscope and how all necessary correction parameters are automatically determined requiring no interaction from the user by employing methodologies benchmarked by the scientific community. We will also show how the variable PSF feature can be used in such measurements to fine-tune the observation window of freely diffusing biomolecules.

KEY WORDS: Luminosa , single molecule Fluorescence, Foerster Resonance Energy Transfer (FRET), confocal fluorescence microscopy, dynamic structural biology, single photon counting confocal

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A 014 [68]
Structural basis for targeted p97 remodeling by ASPL as prerequisite for p97 trimethylation by METTL21D

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p97 is an essential AAA+ ATPase that extracts and unfolds substrate proteins from membranes and protein complexes. Through its mode of action, p97 contributes to various cellular processes, such as membrane fusion, ER-associated protein degradation, DNA repair, and many others. p97 function and protein interaction is regulated by a large number of adaptor proteins. Alveolar soft part sarcoma locus (ASPL) is a unique adaptor protein that regulates p97 by disassembling functional p97 hexamers to smaller entities. Another way to regulate the activity and interactions of p97 is by post-translational modifications (PTMs). Although more than 140 PTMs have been identified in p97, only a handful of those have been described in detail. Here we explain how the p97-remodeling adaptor protein ASPL enables the metastasis promoting methyltransferase METTL21D to bind and trimethylate p97 at a previously inaccessible lysine side chain. The crystal structure of the trimethylated p97:ASPL:METTL21D heterotrimeric complex in presence of cofactors ATP and S-adenosyl homocysteine uncovers a novel role of the second region of homology (SRH) present in the first ATPase domain of p97 in binding of a modifying enzyme and shows the importance of p97 remodeling by ASPL to enable modification of inaccessible residues and to create new interaction interfaces. Investigation of this interaction in human, fish and plant reveals fine details on the mechanism and significance of p97 trimethylation by METTL21D across different organisms.

A 015 [69]
Interactions guiding the folding and maturation processes of the lipase A of Pseudomonas aeruginosa PAO1

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The bacterium *Pseudomonas aeruginosa* acts as an opportunistic human pathogen and possesses a large repertoire of membrane-embedded systems for efficient secretion of multiple virulence factors. The major extracellular lipase of *P. aeruginosa* is the lipase A (LipA), which represents a putative virulence factor. LipA follows the type 2 secretion system (T2SS) pathway. The precursor form of LipA is predicted to be translocated via the Sec system to the periplasm. Upon transport and secretion processes, LipA undergoes periplasmic interactions with the lipase-specific foldase LipH which facilitate its folding and maturation. Besides, the main interactions with LipH, further periplasmic interactions with prominent periplasmic chaperones Skp, FkpA, SurA, YfgM and PpiD are possible and were investigated within this study. We were aiming to elucidate factors that determine the targeting and maturation process of LipA. Our findings demonstrate that, among all tested periplasmic chaperones, only the trimeric chaperone Skp prevents the lipase from the assembly of fibrillar aggregates. Skp greatly stabilizes the soluble form of the lipase and ensures the functionally important hand-over to LipH. The effect of Skp is particularly pronounced at elevated ionic strength that triggers the aggregation of the lipase and inhibits the charge-driven assembly of LipA:LipH, while LipA:Skp interactions are apolar. Structural analysis of the Skp highlight the importance of hydrophobic contacts for the periplasmic chaperone:client interactions. The aim of the present study is to gain knowledge on important transport and maturation mechanisms of putative virulence factors from potential bacterial pathogens, that are biomedically relevant.

A 016 [71]
Structural dynamics of the Sars-Cov-2 non-structural protein three (nsp3) ubiquitin-like domain studied by NMR spectroscopy

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This project aims to understand better the molecular mechanisms involved in forming the Sars-Cov-2 replication organelle (RO), which drives the coronavirus replication in the host cell. Double-membrane vesicles (DMVs) are critical for establishing the viral RO in the infected host cell. If the formation of these DMVs can be blocked, replication of the coronavirus is inhibited. The viral non-structural protein 3 (nsp3) plays a central role in forming DMVs. The N-terminal Ubiquitin-like domain 1 (Ubl1) is exposed and presumably interacts with several viral l factors, e.g., the viral N-protein or viral RNA. We are investigating the internal and conformational dynamics of the nsp3 Ubl1 domain as well as its dynamic binding interactions by Nuclear Magnetic Resonance (NMR) spectroscopy. NMR provides insights into the protein's structural dynamics and dynamic binding interactions with atomic resolution.
For this purpose, we have successfully expressed and purified the ^{15}N and ^{13}C isotopically labeled Ubl1 domain to characterize its internal dynamics. NMR spectroscopy will probe dynamic binding interactions of the Ubl1 domain.

A 017 [74]
Ferredoxin-dependent bilin reductases (FDBRs)

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Ferredoxin-dependent bilin reductases (FDBRs) are a class of enzymes which are able to convert biliverdin

into bilins wchich are chromophores found in cyanobacteria and chloroplasts of red algae and higher plants. Bilins are involved in light harvesting and light perception of these organisms. In this project we focus on the reactions catalyzed by a specific FDBR, from *Klebsormidium nitens* (KflaHY2). The distinguishing feature of KflaHY2 is its surprising catalytic activity. While it shows highest sequence similarity to the phytochromobilin synthase HY2 from land plants, it converts BV instead to phycocyanobilin (PCB) like the FDBR PcyA found in cyanobacteria and green algae. Therefore, KflaHY2 might represent an intermediate in the evolutionary step in the development of HY2 variants in terrestrial plants.The streptophyte KflaHY2 is currently not well characterized, and a 3D-structure is missing. To overcome this, we apply an extensive orthologue screening based on the analysis genetic information of the candidates. In addition, to probe the mechanistic abilities of KflaHY2, we perform a characterization of the substrate/product profiles of several of these representatives. Here we present the preliminary results for expression, purification and screening of the candidates. Additional HPLC analys will be included in order to provide quantitative results, and isolate the individual post reaction compounds.

A 018 [76]
Architecture and functional dynamics of a large, virulence-associated DNA helicase from enterohaemorrhagic Escherichia coli

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Production of flagella in enterohaemorrhagic *Escherichia coli* (EHEC) supports EHEC virulence and requires the EHEC-specific nucleic acid-dependent NTPase, Z5898. Z5898 has been suggested to represent a separate clade of DExH-box RNA helicases in EHEC and related bacteria. Here, we find that Z5898 actually exhibits efficient 3′-to-5′ directional DNA helicase activity, but lacks RNA helicase activity. Cryogenic electron microscopy-based structural analyses of Z5898 in complex with DNA and an ATP analog revealed core helicase domains as also found in the antibiotics resistance DNA helicase, MrfA. Although MfrA-like domains in Z5898 are interspersed with and expanded by a dimerization domain, a duplex binding domain, three zinc-binding domains and two phospholipase D-like domains, they assemble a MrfA-like core structure in 3D. The dimerization motif can flexibly connect two Z5898 molecules, and the oligomeric state depends on the chemical environment and the DNA-binding mode. Comparison of Z5898 in various structural states revealed a dynamic interplay between several domains, structural motifs and bound DNA. Structure-guided mutagenesis in conjunction with functional assays pinpointed a DNA-binding rope, an ATPase activation clamp and a DNA gate as key molecular tools of the helicase. Our results reveal that Z5898-like nucleic acid-dependent NTPases have to be re-classified as DNA helicases and hint at possible strategies to interfere with the virulence-related activities of these enzymes.

A 019 [79]
Inhibition of a bacterial global gene regulator by a phage protein via forced hyper-oligomerization

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Most bacteria with sequenced genomes encode ρ, a hexameric RNA-dependent ATPase. In many of these species, ρ is essential. ρ is a paradigmatic transcription termination factor that defines the ends of 20-30 % of transcription units in *Escherichia coli*. ρ activity involves conformational transitions between an inactive open-spiral and an active closed-ring state, modulated, among others, by nucleotide and RNA binding. On RNA polymerase, ρ-dependent termination can be counteracted by various transcription factors, but only two known protein inhibitors target isolated ρ, including the polarity suppression protein, Psu, of pirate bacteriophage P4. Presently, the molecular basis of Psu-mediated ρ inhibition is unknown. We elucidated

cryogenic electron microscopy structures of ρ-Psu complexes, in which multiple Psu dimers laterally clamp two ρ spirals, stabilizing them in an open conformation. Remarkably, Psu increases the helical pitch of the spirals, fostering their expansion by further ρ subunits up to the nonameric state. Consistent with Psu trapping ATP analogs at their binding sites on ρ, ATP stabilizes the ρ-Psu interaction, and Psu reduces ATP binding kinetics, thereby inhibiting the ρ ATPase. Also fully consistent with the structures, Psu counteracts RNA binding at the center of the ρ spirals, which requires ring closure. Structure-guided exchange of ρ-Psu contact residues reduced complex formation, decreased Psu-mediated ρ ATPase inhibition and undermined Psu-mediated inhibition of ρ-dependent termination *in vivo*. Our findings reveal that Psu implements a unique mechanism – forced hyper-oligomerization – to inhibit ρ, which may inform the development of novel, ρ-targeting anti-microbials.

A 020 [**80**]
Studying dynamic conformational changes in multi-domain nonribosomal peptide synthetases (NRPSs) using FRET

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Nonribosomal peptide synthetases (NRPSs) are large modularly organized enzymes that synthesize a plethora of therapeutically important peptides from over 500 different monomers in an assembly-line fashion [1]. A module consists of discrete domains that have to interact specifically with each other on the intramolecular level to facilitate a sequence of coordinated reactions in a directional manner. In this process, the domain arrangement adopts multiple conformational states. Although several crystal structures of NRPS multidomain constructs were reported, we are far from a comprehensive picture about the structural and temporal dynamics of these inter- and intramodular interactions.
In order to overcome these shortcomings, we applied Förster Resonance Energy Transfer (FRET) spectroscopy to NRPS by introducing donor and acceptor fluorophores into catalytically active constructs [2, 3]. Although FRET is a widely established technique to study protein dynamics [4], its application to NRPS is not trivial due to their size and complexity. Our investigations will advance the knowledge of dynamic NRPSs and help to overcome the obstacles of their bioengineering. New results along these lines will be presented.

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A 021 [86]
Towards understanding the substrate translocation of a type 1 secretion system

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The selective and controlled transport of molecules across the membrane is crucial for all living cells, be it for nutrient uptake, export of signalling molecules or secretion of virulence factors in case of pathogens. The latter transmembrane transport is facilitated by designated membrane proteins, one of the largest protein families for this being the ABC superfamily. For Gram-negative bacteria it is especially challenging, since secretion means to traverse two membranes, and it is often attained by type 1 secretion systems (T1SS). Our research focuses on the hemolysin A (HlyA) T1SS from *Escherichia coli*. The translocation machinery is composed of the outer membrane protein TolC and the so-called inner membrane complex. This inner membrane complex is formed by the ABC transporter hemolysin B (HlyB) and the membrane fusion protein hemolysin D (HlyD). Together, they secrete the 1024 aa large pore forming toxin hemolysin A (HlyA) in one step across both membranes.
In our efforts to understand the substrate recognition and translocation we chose an approach based on homologous T1SS. We identified various systems *in silico* and cloned, expressed and purified several of the ABC transporters. One homolog from *Avibacterium paragallinarum* showed exceptional stability in detergent solution, being a promising candidate for

crystallization. Furthermore we created chimeric transporters using the *Kingella kingae* transporter RtxB. By exchanging the C39 peptidase-like domain (CLD), the transmembrane domain (TMD) or nucleotide binding domain (NBD) of HlyB we identified not only the CLD to be essential for secretion, but also the NBD to play a more important role than merely to energize the system.

A 022 [93]
Regulation of Tom70 association with the mitochondrial outer membrane TOM complex

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In the yeast *Saccharomyces cerevisiae*, 99% of the ~1000 mitochondrial proteins are encoded by the nuclear DNA and synthesized in the cytosol. Many of the newly synthesized mitochondrial proteins are initially targeted to Tom70 at the mitochondrial surface for subsequent transfer to Tom40, the central pore-forming component of the translocase of the outer mitochondrial membrane (TOM) [1,2].

However, Tom70 is only transiently associated with the TOM complex and the mechanisms that determine the transfer of proteins from Tom70 to the TOM complex are unclear. We investigated the abundance of Tom70/TOM interactions by comparing authentic Tom70 with a phosphomimetic variant of Tom70. We found that docking to the TOM complex was blocked for the phosphomimetic variant of Tom70, but substantially facilitated using non-phosphorylated Tom70, suggesting a metabolic regulation by phosphorylation of Tom70.

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A 023 [103]
Disulphide formation in the matrix protein NDUFAF8 modulates complex I assembly

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The vast majority of mitochondrial proteins are synthesized in the cytosol and follow different routes to reach different subcompartments of the mitochondria. Import pathways usually drive proteins exclusively to dedicated mitochondrial subcompartments. However, there is an increasing number of proteins which do not bear a specific targeting feature. We identified a subgroup of small proteins that follow an unconventional import route into the mitochondrial matrix that modulates their stability by oxidative folding in the IMS. For further investigations on the relevance of this import pathway, we then focused on NDUFAF8, an assembly factor of Complex I. Interestingly, NDUFAF8 harbours a TwinCx9C motif as well as a MTS. The interaction of NDUFAF8 with the mitochondrial matrix protein NDUFAF5 indicates a mitochondrial matrix localization. The import pathway of NDUFAF8, however, remained elusive. We obtained a detailed understanding of the unusual import pathway and found out that the disulphide formation in the IMS modulates complex I assembly. We also investigated the redox regulated proteolytic events *en route* to the mitochondrial matrix. Structural analysis of NDUFAF8 could show that beside the oxidative folding also the uncleavable MTS is essential for the interaction with NDUFAF5. Together, these findings reveal a novel import pathway including a new subgroup of proteins relying on multiple targeting features to end up in the mitochondrial matrix.

A 024 [111]
Ribosomal protein S1 is required for translation of IGR IRESs in Escherichia coli.

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Internal ribosome entry sites (IRESs) are RNA elements capable of recruiting ribosomes and initiating translation on an internal portion of an mRNA. The intergenic region (IGR) IRES of the Dicistroviridae virus family folds into a triple pseudoknot (PK) tertiary structure, allowing it to recruit the ribosome and initiate translation in a structure-dependent manner. This IRES has also been reported to drive structure-dependent translation in E. coli and to date is the only described translation initiation signal that functions across domains of life. However, here we show that unlike in the eukaryotic context the tertiary structure of the IGR IRES is not responsible for prokaryotic ribosome recruitment. Additionally, IRES translation efficiency is heavily dependent on ribosomal protein S1 and an A-rich Shine- Dalgarno-like element supporting a model where the translational activity of IGR IRES is due to canonical prokaryotic translation and not tertiary RNA structure.

A 025 [113]
Learning from reconstitution: metamorphosis of HORMA domain proteins ATG13 and ATG101 at the basis of human autophagy initiation and lipid transfer

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Macro-autophagy is a process of regulated degradation, which eliminates damaged or unnecessary cellular components. A timely initiation of autophagy is key to maintain survival of cells under stress and starvation. After autophagy induction, bulk cytosol is taken up within minutes by autophagosomes and transported to the lysosome for degradation and recycled into small-molecule biosynthetic precursors. The double membrane enclosed autophagosome is generated *de novo* at ER contact sites, where lipids flow into the growing autophagosome.

A crucial unresolved question is how the initial contact sites are assembled on-demand, and thereby build the autophagy initiation machinery at the right time and place. Autophagy is initiated by the recruitment of three autophagy initiation complexes to the ER contact site, followed by lipid transfer and membrane tethering complexes. However, the architecture of the contact site remains largely uncharacterized. Do the autophagy initiation complexes and lipid transfer machinery coalescence in a stable contact site super-complex? What triggers the assembly of these complexes at the right place and time? We report the biochemical reconstitution of novel early autophagy initiation and lipid transfer complexes entirely from recombinant full-length proteins. Building on our previous biochemical reconstitutions of HORMA-domain based signaling networks, we hypothesized that the metamorphosis of HORMA-domain proteins ATG13-101 create rate-limiting and obligatory interactions that dictate the assembly of a larger effector complex. Here, we report the main conclusions from exploring this hypothetical assembly mechanism and the consequence of coincidence of functional subcomplexes.

A 026 [116]
Structure and sequence requirements of regulatory mRNA regions for specific recognition by RNA-binding proteins

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mRNA fate is regulated via 5'- and 3'-untranslated regions (UTRs). UTR-contained cis-regulatory elements represent binding sites for RNA-binding proteins (RBPs), which account for mRNA transport, translation, packaging and degradation. Cis-elements can be short sequences or folded RNA motifs, the latter highly abundant within viral UTRs. They occur in variable numbers, sequential or functional redundancy and thus challenge specific recognition by RBPs. Evidence of RNA structure in UTRs has established an additional layer of mRNA regulation, translating individual cis-trans pairs

into 3D-space and spotlighting roles e.g. in mRNA regulation. mRNA 3'-UTRs cluster cis-elements in regulatory hubs that integrate binding by numerous RBPs. Additionally, the gross of RBPs exploit multiple RNA-binding domains for multivalency. However, the extend of cis-trans interactions and their spatial arrangement within an mRNA is still incompletely understood. A technical challenge is the systematic understanding and structural depiction of entire regulatory hubs, if not complete mRNA UTRs. This appears particularly critical considering the inherent dynamics of RNA and its influence, e.g. on the availability of folded cis-elements. This talk provides selected insight into combinatory approaches centered around solution NMR spectroscopy to determine and evaluate cis-elements, their structures and binding by regulatory RBPs. In doing so, the integration of high-resolution information from single cis-trans pairs into full UTR secondary and tertiary structures has been successfully accomplished. Examples are given for highly conserved viral UTRs and multiple elements within target mRNAs for an immunoregulatory multidomain RBP.

A 027 [128]
Structural studies of modification enzymes of class I lantibiotic from Clostridium maddingley

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Ribosomally synthesized and post-translationally modified peptides (RiPPs) such as lantibiotics have a rising interest due to their high structural diversity and consequently, their wide variety of bioactivities. Understanding the mechanistics of their modification enzymes will open up new avenues for the development of new molecules against multi-drug resistant microorganisms. One of the most important aspects regarding selectivity and activity of the modification enzymes is encoded in the leader peptide of the lantibiotics itself. Mutational studies and the corresponding structures will provide a closer insight into the mode of binding and regioselectivity of the modification reactions, which play a crucial role for future endeavours.

Here, we focused on a class I lantibiotic called maddinglicin. Although maddinglicin possesses some characteristic features of the well-studied class I lantibiotic nisin, it also has some distinguishing features. Based on the structures and functional analysis, we could determine the influence of these differences on the modification machinery. Furthermore, a detailed comparison of the structure of these post-translationally modifying enzymes to the nisin homologues was also performed, revealing interesting differences and insights into their mode of action.

A 028 [129]
In vitro characterization of the ABC transporter Pdr5 reconstituted into liposomes

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Pdr5 is the most abundant ABC transporter in *Saccharomyces cerevisiae* and plays a major role in the pleiotropic drug resistance (PDR) network against a large number of structurally unrelated compounds. Due to an asymmetry in one of its nucleotide binding sites (NBS), Pdr5 serves as a perfect model system for asymmetric ABC transporter such as its medical relevant homologue Cdr1 from *Candida albicans*.

In the past 30 years this ABC transporter was intensively studied *in vivo* and in plasma membrane vesicles. Nevertheless, these studies were limited since it was not possible to isolate and reconstitute Pdr5 in a membrane system while maintaining its activity.

Here, we describe the functional reconstitution of Pdr5 in a native-like environment and first activity assays. We could demonstrate that reconstituted Pdr5 is capable of translocating short chain fluorescent NBD lipids from the outer to the inner leaflet of the proteoliposomes. Moreover, this transporter revealed its ability to utilize other nucleotides to accomplish the transport of substrates in a reconstituted system. Besides, we were able to estimate the NTPase activity of reconstituted Pdr5 and determine the kinetic parameters for ATP, GTP, CTP and UTP.

Based on the functional reconstitution of Pdr5, we now possess the capability to study Pdr5 in an isolated and defined system *in vitro* and on a molecular level.

A 029 [130]
Functional analysis of the linker domain of the ABC transporter Pdr5 from S. cerevisiae

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The ABC transporter Pdr5 from *S. cerevisiae* was discovered more than 30 years ago and studied extensively ever since as its host presents a model organism to study fungal PDR proteins. The PDR proteins are part of the fungal pleiotropic drug resistance network and the overexpression of these proteins leads to a multidrug resistance phenotype. The arising number of human infections with multidrug resistant, pathogenic fungi is alarming as the number of antifungal agents is very limited.
Although studied extensively for more than three decades, Pdr5 evaded the resolution of its protein structure. Recent advances in the purification of the protein as well as the progress in single-particle electron cryo-microscopy (cryo-EM) of membrane proteins enabled the elucidation of the structure of the ABC transporter Pdr5.
The analysis of the structure of Pdr5 revealed the presence of a linker domain located in between the two parts of the protein. This linker domain possesses a conserved motif MQKGEIL whose amino acid residues are in very close proximity to the nucleotide ATP bound in the degenerated nucleotide binding site (NBS) 1. This motif was analyzed through a mutational study investigating the influence of the resulting Pdr5 mutants on the resistance against several cytotoxic compounds as well as their ATPase activity. The results show that the degenerated NBS1 not only senses the binding of a nucleotide but also communicates with the catalytically active NBS2. The Pdr5 mutants are able to confer resistance against the tested cytotoxic compounds even at reduced ATPase activity indicating that Pdr5 operates as an uncoupled transporter.

A 030 [135]
Structure and condensate formation of the axonal transmembrane protein PLPPR3

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Phospholipid-phosphatase-related (PLPPR) proteins are a class of five brain enriched transmembrane proteins involved in neuronal growth. Although closely related to the Lipid Phosphatases (PLPPs), PLPPRs lack common phosphatase activity. Here, we characterize structure-function relationship of PLPPR3, one of two members with an over 400 amino acid long intracellular domain (ICD) in neurons. PLPPR3 is present in neuronal axons, where it binds the lipid phosphatase PTEN and antagonizes its activity at the plasma membrane. We could show that this local inhibition of PTEN resulted in a stabilization of $PI(3,4,5)P_3$ and thereby induced axonal branching. In accordance, $PLPPR3^{-/-}$ show less axon branches compared to wildtype control neurons. At the structural level, we identify that PLPPR3 can homo/hetero-multimerise with other members of the PLPPR family to form higher-order oligomers. However, any detailed structural information of PLPPR3 is currently missing. Here, we present our work that aimed at characterizing the topology of PLPPR3 using Cryo electron microscopy. We also discussed the possibility that the intracellular domain supports the formation of liquid-liquid phase separated (LLPS) condensates that may act as biochemical reactors for actin polymerization to facilitate nascent branch formation.

A 031 [136]
Towards the in vitro reconstitution of the hemolysin A type 1 secretion system

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Protein secretion is important for bacterial virulence. Among Gram-negative bacteria this is often attained by type 1 secretion systems (T1SS). T1SS secretion occurs in a single step from the cytosol to the extracellular space without periplasmic intermediates. Our research

focuses on the hemolysin A (HlyA) T1SS from *Escherichia coli*. The translocation machinery of the HlyA T1SS is composed of the outer membrane protein TolC and the so called inner membrane complex (IMC). This inner membrane complex is formed by the ABC transporter hemolysin B (HlyB) and the membrane fusion protein hemolysin D (HlyD). The systems substrate is the toxin hemolysin A (HlyA).
Aim of this project is the *in vitro* reconstitution of the HlyA T1SS to gain further insights into the systems structure, stoichiometry and function. The *in vitro* system is build up by a giant unilamellar vesicle (GUV) mimicking the outer membrane and a lipoprotein nanoparticle mimicking the inner membrane. Purification, labeling and reconstitution of the dye labelled outer membrane protein TolC into liposomes was successful. Those proteoliposomes were grown to GUVs using an osmotic shock method.
For the inner membrane complex the focus of this work lies on the ABC-transporter HlyB. Expression, purification and reconstitution of HlyB was achieved using Saposin A and lysolipids. Furthermore expression of the inner membrane complex was tested in different *Escherichia coli* strains but issues with the stability were observed during purification. Therefore the components of the inner membrane complex were purified separately and combined for co-reconstitution afterwards. A co-reconstitution with Saposin A and lysolipids delivered first promising results.

A 032 [142]
The role of NDUFAF1 in respiratory complex I assembly

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Complex I is the largest complex of the respiratory chain and its structure has been described in great detail. The intricate biogenesis of human complex I requires more than 15 assembly factors [1]. The molecular function of individual assembly factors is largely unknown. Recent structural studies showed how NDUFAF2 mediates attachment of the N module and shed light on the role of plant specific assembly factor GLDH in joining two bulding blocks of the membrane arm [2,3]. We have engineered a deletion of the canonical assembly factor NDUFAF1 in our yeast model organism *Yarrowia lipolytica*. The deletion strain assembles exclusively the distal proton pumping module but all other complex I building blocks are absent. After complementation with tagged NDUFAF1 we purified NDUFAF1 associated assembly intermediates and determined their structures by cryo-EM. Unexepectedly, we found a link between complex I assembly and cardiolipin remodelling.

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A 033 [159]
Binding preferences of the SARS-CoV-2 nucleocapsid for genomic RNA elements

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The virus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) possesses a large RNA

genome of almost 30kb, flanked by highly-conserved regulatory untranslated regions (UTRs). Genome packaging into virions is crucial for viral progression. Key player of this process is the versatile nucleocapsid protein (N) - one of four coronaviral structural proteins - that is further involved in RNA transcription. N harbors five functionally distinct parts, including two folded domains, the RNA-binding NTD and the dimerization-mediating CTD, flanked by intrinsically disordered regions.
Despite numerous publications on RNA-binding by SARS-CoV-2 N, high-resolution structural data of complexes with RNA is missing, and no systematic examination of its specificity for genomic RNA is available. However, a structural basis for specific interactions of N with viral RNAs would pave a way to new medication. We here give a detailed insight into the interaction of N's NTD with 5'-*cis* regulatory elements SL1-SL5, using an NMR-centered approach. We specify preferences of the NTD for sequences within the 5'UTR occurring in distinctive structural contexts. In fingerprint spectra, we further define signature residues located in the RNA-interacting regions of the NTD that are likely sensors for the distinction between specific vs. non-specific target sequences. Our results will serve as a basis for further studies that aim at understanding precise determinants of nucleocapsid RNA specificity.

A 034 [162]
The hemolysin A type I secretion system of Escherichia coli

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The hemolysin A (HlyA) type I secretion system (T1SS) is one of best-known T1SSs in gram-negative bacteria. The system is named after its transported substrate, the pore forming toxin HlyA. The transport machinery is a tripartite system, which consists of two inner membrane proteins, the ABC transporter hemolysin B (HlyB) and the membrane fusion protein (MFP) hemolysin D (HlyD), and of the outer membrane protein TolC. Despite extensive research, little is known about the complete structure, stoichiometry and precise mechanism of the machinery. This work focuses especially on the underrated MFP HlyD. HlyD is a highly sophisticated protein which is involved in substrate recognition, TolC recruitment and TolC opening. Additionally, HlyD harbors structural features unknown in other MFPs, such as an N-terminal amphipathic helix with unknown function. Thus, investigation of HlyD could further reveal unknown mechanisms. In this work, the purification of a detergent-solubilized dimeric subcomplex was successfully established for the first time. Furthermore, the dimer was successfully reconstituted into nanodiscs. The structure of the detergent-solubilized as well as of the reconstituted subcomplex will be probed via cryogenic electron microscopy, which will reveal insights into the illusive MFP HlyD and will lead to a better understanding of the whole T1SS. In addition, first trials in co-reconstituting HlyD with the ABC Transporter HlyB into lipid nanoparticles were performed. Complex formation was biochemically confirmed and functional and structural studies will be performed. This *in vitro* co-reconstitution of different integral membrane proteins will further make a huge step in understanding membrane protein complexes.

A 035 [179]
CaMKII activity spreads by interholoenzyme phosphorylation, not subunit exchange

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Ca^{2+}-calmodulin dependent protein kinase II (CaMKII) is indispensable for long-term potentiation (LTP) of synaptic responses, a cellular mechanism implicated in learning and memory formation. CaMKII is unique in the kinase world because it comprises a dodecameric holoenzyme. In unstimulated neurons CaMKII is autoinhibited and quiescent. Activation is triggered by a brief Ca^{2+} signal, for example, from NMDA-type glutamate receptors which releases the autoinhibition of the kinase. CaMKII remains active through autophosphorylation of its inhibitory domain, which is proposed to spread and outlive the initial calcium signal. Because of this ability to remain active long after the initial trigger, CaMKII has been proposed to serve as a kind of a memory molecule, which would propagate the

information received by Ca^{2+} to downstream signaling events. The prevailing model for the spread of CaMKII activity is via the exchange of CaMKII subunits between activated and unactivated holoenzymes, and subsequent trans-autophosphorylation. We challenged this model by imprisoning subunits within their parent holoenzymes with genetically-encoded crosslinkers, and followed the consequences of activation with a panel of biochemical assays, single molecule TIRF microscopy, and mass photometry. Crosslinking coupled to mass spectrometry allowed us to build a structural picture of how CaMKII holoenzymes propagate activity. We show that CaMKII subunit exchange, if it occurs at all, is a rare event, and rather CaMKII maintains and spreads its activity though phosphorylation between different holoenzymes- namely inter-holoenzyme phosphorylation.

A 036 [180]
Evolutionary conserved assembly of a physiological transport complex in cilia

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Specific recognition of cellular cargo and efficient transport to its correct intracellular destination is an infrastructural challenge faced by most eukaryotic cells. This remarkable deed is accomplished by processive motor proteins that are subject to robust regulatory mechanisms. The first level of regulation entails the ability of the motor to suppress its own activity. This autoinhibition is eventually relieved by specific cargo binding. To better understand the role of the cargo during motor activation, we dissected the activation mechanism of the ciliary homodimeric kinesin-2 from *C. elegans* by its physiological cargo. In functional reconstitution assays, we identified two cargo adaptor proteins that together are necessary and sufficient to allosterically activate the autoinhibited motor. Surprisingly, the orthologous adaptor proteins from the unicellular green algae *C. reinhardtii* also fully activated the kinesin-2 from worm, even though *C. reinhardtii* itself lacks a homodimeric kinesin-2 motor. The latter suggested that a motor activation mechanism similar to the *C. elegans* model existed already well before metazoans evolved, and prompted us to scrutinize predicted homodimeric kinesin-2 orthologs in other evolutionarily distant eukaryotes. We show that the ciliate *Tetrahymena thermophila* not only possesses a homodimeric kinesin-2 but that it also shares the same allosteric activation mechanism that we delineated in the *C. elegans* model. Our results point to a much more fundamental role of homodimeric kinesin-2 in IFT than previously thought and warrant further scrutiny of distantly related organisms towards a comprehensive picture of the IFT process and its evolution.

A 037 [181]
Structural and dynamic analysis of the third conformational state of T4 Lysozyme by photoinduced electron transfer

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In this work, a novel structure elucidation approach based on dynamic fluorescence quenching bye photoinduced electron transfer (PET) is used to find a structural solution for the recently discovered third conformational state (C3) of T4 Lysozyme (T4L) [1]. Furthermore, the associated dynamics are resolved by fluorescence correlation spectroscopy (FCS).
Twelve possible structures for the C3 state were generated considering the structural information from the previous work [1]. In order to find the most suitable structural model, a new approach for structure elucidation is applied. This approach based on PET between the fluorescence quencher tryptophan and the fluorophore Alexa488 bound to T4L. To find the best structural model, the dynamic quantum yields of selected PET pairs were simulated [2] and compared to the respective experimentally obtained dynamic quantum yields.
The enzyme T4L undergoes a dynamical exchange between two states, an open (C1) and a closed (C2) state, which arises in the course of substrate binding and conversion. The newly defined compact C3 state is thought to play a major role in formation of the enzyme-product complex and the subsequent product release [1]. Based on the single molecule MFD experiments [1], it was shown that the transitions between the C1, C2 and the C3 states obey a reversible sequential three-states

kinetic, in which the exchange between C2 and C3 follows a slow dynamic in the range of hundreds of microseconds [1]. In the second part of this work, the dynamic nature of this slow transition is detected by PET-FCS.

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A 038 [184]
Characterisation of the Vancomycin resistance protein VanW

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Antibiotic resistance is a long-known threat in the treatment of bacterial infections. Vancomycin, an antibiotic already discovered in the 1950s, was long thought to be a secure antibiotic with respect to the development of resistance due to its unique involvement of disrupting peptidoglycan (PG) synthesis. Vancomycin is binding to the PG precursor lipid II, thus preventing PG synthesis and cell growth. Vancomycin resistance is mainly divided into two classes, defined by the gene clusters VanA and VanB. The latter alters lipid II synthesis to contain a pentapeptide with a C-terminal Ala-Lac as opposed to the natural di-d-Ala C-terminus. VanW is located within the VanB resistance cluster, but close homologs can be found isolated or in related Vancomycin resistances. The function and structure of VanW are still unknown. When *Clostridioides difficile* is treated with an antimicrobial peptide specifically the VanW gene is upregulated. Our goal is the identification of substrates and functional characterization of VanW. We choose to take a heterologous approach by expressing the protein in *E. coli*. The purified protein was used for intrinsic tryptophane fluorescence measurements using compounds of the peptidoglycan layer: N-acetylmuramic acid (MurNAc), N-acetyl-D-glucosamine (GlcNAc) and the pentapeptide which is part of lipid II (L-Ala-D-Glu-L-Lys-D-Ala-D-Ala) as well as Vancomycin. We were able to determine K_D-values with the result, that MurNAc and pentapeptide have a four times higher affinity than GlcNAc towards VanW. Vancomycin was shown to also influence VanW.
We succeeded in expressing and purifying the protein VanW and initiated the biophysical characterization of the protein and its possible ligands.

A 039 [188]
Biophysical characterization of the N-terminal domain of TDP-43

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TDP-43 is an RNA binding protein that presents four domains compromising an N-terminal region, two RNA recognition motifs, and a C-terminal region[1]. This protein has shown to aggregate in patients with amyotrophic lateral sclerosis and fronto-temporal dementia, where it is highly phosphorylated, polyubiquitinated and truncated [2,3]. The N-terminal domain has a relevant role in the oligomerization and the *splicing* activity of TDP-43. In this work we have expressed, purified, and biophysically characterized the region that includes residues 1 to102, and contains the nuclear localization signal. We have evaluated the equilibrium of oligomerization of this protein fragment observing a dependence on the protein concentration and the presence of reductive agents. Also, we have determined changes in the tertiary structure and its stability at a broad range of pH values, by means of different spectroscopic methods. Additionally, we used bioinformatics and molecular dynamics simulations to study the fragment comprising the first 77 residues, which is known to be well folded. We evaluated the motion of this domain in its monomeric and dimeric form to get insights into the conformational flexibility of this TDP-43 module. Our results suggest that, whereas this domain is folded, it exhibits considerable conformational plasticity, concerning tertiary and quaternary structure. This fact may help to understand some features of TDP-43 oligomerization and stability.

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A 040 [189]
Single-molecule FRET measurements uncover an unexpected conformation of hAgo2-RNA complexes during target-directed miRNA degradation

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Human Argonaute 2 (hAgo2) is a key player of RNA interference. Target messenger RNAs are recognized by complementarity to a hAgo2-bound miRNA-derived guide RNA. Downregulation of targets occurs by translational inhibition or hAgo2-mediated cleavage depending on the degree of complementarity between guide and target RNA. Tight control of the amounts of cellular miRNAs prevents dysregulation. Target-directed miRNA degradation (TDMD) was described as a cellular pathway for the regulation of miRNA levels. In the context of TDMD, miRNA guides as well as the hAgo2 protein itself is subject to degradation.
The varying fates of hAgo2 and hAgo2-bound nucleic acids are probably determined by structural rearrangements enabling e. g. interactions with other proteins. We site-specifically engineered fluorophores into native hAgo2 and the bound nucleic acids to uncover dynamics and hidden conformational states using single-molecule FRET. The analysis of conformations along the catalytic cycle enabled insights in an unexpected dynamic behavior of hAgo2 even in complex with a guide and a target RNA with full base pairing potential. Furthermore, we found that complexes composed of hAgo2-guide RNA and a TDMD target lead to a so far unobserved conformation in which the guide RNA moves significantly out of its binding channel within the protein. In summary, our study underscores the importance of conformational flexibility of hAgo2 and hAgo2-nucleic acid complexes as a pre-requisite to function.

A 041 [190]
The influence of oligomeric structures on α-synuclein aggregation

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On the field of protein aggregation, often it is not the insoluble amyloid aggregates found in patients' brains that are deemed most cytotoxic but the smaller oligomeric species. Due to both forms consisting of the same building blocks, the processes which form them cannot truly be separated and should be analyzed in conjunction with each other. Similarly, different proteins might interact to detrimental effect in neurodegenerative diseases such as Parkinson's Disease, Alzheimers Disease and Dementia with Lewy Bodies.
In this *in vitro* study, we observe the effects of oligomeric species of multiple proteins on the aggregation of α-synuclein, a well-known part of the pathogenesis of Parkinson's Disease, by trying to disentangle the different processes.

A 042 [196]
Maturation strategy influences expression levels and cofactor occupancy in Fe-S proteins

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Iron-sulfur (Fe-S) clusters are ubiquitous cofactors that are required for fundamental life processes. Structural and spectroscopic analysis of iron-sulfur [Fe-S] cluster-containing proteins is often limited by the occupancy and yield of recombinantly produced proteins. In the model organism *Escherichia coli* there are two different machineries that are responsible for the assembly of Fe-

S clusters and their incorporation into apoproteins: the iron-sulfur cluster (ISC) machinery is used for maturating housekeeping proteins under standard growth conditions whereas the sulfur formation (SUF) machinery is used under stress conditions. Since recombinant genes are expressed to an excessively high level for protein production but the level of the Fe-S assembly proteins remains unchanged, these machineries are not capable of keeping up with the production of the cofactor leading to incomplete maturation of the target Fe-S proteins in regular *E. coli* BL21 (DE3) cells. However, the Fe-S cluster content in partially occupied proteins can be enhanced by different methods, including *in vitro* reconstitution or complete *in vivo* maturation in cell strains that are specialized to produce high amounts of Fe-S clusters. Here, we report a systematic investigation of different maturation strategies for three well-established target [4Fe-4S] proteins: aconitase B, 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (IspH), and quinolinate synthase (NadA) and the less well-studied Radical SAM protein thurincin B (ThnB) and shed light on the differences of *in vitro* and *in vivo* Fe-S cluster maturation.

A 043 [201]
The role of DNA nanostructures in the catalytic properties of an allosterically regulated protease

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DNA nanotechnology is an ideal platform for the precise binding and arrangement of guest molecules with nanometre-scale accuracy. Interestingly, many studies on enzyme cascades demonstrate that the close proximity of multiple enzymes immobilized onto DNA nanostructures often results in increased catalytic activities. Comparably fewer studies on single enzyme-DNA systems report the same phenomenon, thus raising the question whether multiple factors may contribute to the intriguing observations. To tackle this question, we used thrombin, an allosterically regulated serine protease, specifically bound *via* aptamers to DNA origami structures with a variable degree of confinement. We compared the hydrolysis of three substrates that differ only in their net-charge due to a C-terminal amino acid residue that is likely involved in the allosteric regulation of the enzyme. Our data show that, for all substrates, the reaction speed increases with the level of DNA tethering but the extent of this effect is strongly dependent on DNA/substrate interactions. For substrates of opposite charge, enzyme confinement within a DNA origami cavity leads to completely different kinetic behaviours, turning the worst substrate into the best performing one. Applying the transition state theory and the kinetic linkage scheme for thrombin, we formulate a model wherein the negatively charged DNA environment nearby the enzyme is likely to affect local electrostatic features on the protein surface, thereby interfering with charge-dependent mechanisms of substrate recognition. This ultimately influences the catalytic performance of the system and may offer an alternative tool to regulate allosteric processes through spatial confinement.

A 044 [203]
Dynamics of γD-crystallin undergoing liquid-liquid phase separation

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γD-crystallin belongs to a family of highly homologous mammalian eye lens crystallin proteins. This globular protein undergoes liquid-liquid phase separation (LLPS) at high concentrations and low temperature, marked by a cloudy appearance of the protein solution which is like what is observed in cataract formation. LLPS is the driving force behind the formation of biomolecular condensates in cells which can have either a detrimental effect like cataract or a functional role like membraneless organelles. The LLPS onset in γD-crystallin was found to be modulated by cosolutes, temperature and pressure [1,2]. The phase diagram of this protein is well characterized, making γD-crystallin an ideal model protein for studying LLPS[1].

To probe the site-specific side chain dynamics of γD-crystallin while undergoing LLPS, we labeled the protein with a nitroxide label, and investigated by continuous wave EPR the dynamics of the spin-labeled side chain at varying temperatures to identify the spectral fingerprint of the condensate. We additionally studied the effect of macromolecular crowding agents like PEG or Ficoll and of cosolutes like trimethylamine-*N*-oxide (TMAO) and NaCl on the phase separation behavior of γD-crystallin. The temperature and cosolute dependent appearance of a distinct immobile spectral feature in the presence of LLPS is correlated with turbidity measurements on the same samples. The changes in side chain dynamics upon formation of the protein condensate solutions are discussed.

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A 045 [229]
Cluster formation of MHC class I proteins on the plasma membrane

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At the plasma membrane of mammalian cells, major histocompatibility complex class I molecules (MHC-I) present antigenic peptides to cytotoxic T cells. Following the loss of the peptide and the light chain beta-2 microglobulin, the resulting free heavy chains (FHCs) can associate into homotypic complexes (clusters) in the plasma membrane. We have investigated the stoichiometry and dynamics of these MHC-I FHC clusters with an antibody micropatterning two-hybrid assay including fluorescence recovery after photobleaching (FRAP), and with single- molecule co-tracking. We identify non-covalent MHC-I FHC dimers, with dimerization mediated by the alpha-3 domain, as the prevalent species at the plasma membrane. MHC-I FHC dimers show increased tendency to cluster into higher order oligomers, as concluded from an increased immobile fraction with higher single-molecule colocalization. In vitro studies with isolated proteins in conjunction with molecular docking and dynamics simulations suggest a structure for the FHC clusters. We will show current results on their interaction and on possible biological roles.

A 046 [237]
Rep15 interacts with several Rab GTPases and has a unique fold for a Rab effector.

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In their GTP-bound (active) form, Rab proteins interact with effector proteins that control downstream signaling. One such Rab15 effector is Rep15, which is known to have a role in receptor recycling from the endocytic recycling compartment but otherwise remains poorly characterized. Here, we report the characterization of the Rep15:Rab15 interaction and further in a systematic yeast-two hybrid screen against a human Rab library, we have identified Rab3 paralogs and Rab34 as novel Rep15 interacting partners. Rep15 co-localizes with the Rab3 paralogs at exocytic vesicles. Biochemical validation of the interactions is presented and crystal structures of the Rep15:Rab3B and Rep15:Rab3C complexes provide additional mechanistic insight. We find that Rep15 adopts a globular structure that is distinct from other reported Rab15, Rab3 and Rab34 effectors. Structure-based mutagenesis experiments explain the Rep15:Rab interaction specificity. Rep15 depletion in U138MG glioblastoma cells impairs cell proliferation, cell

migration and receptor recycling, underscoring the need for further clarification of the role of Rep15 in cancer.

A 047 [238]
Actin as the target and activator of bacterial toxins: a structural perspective

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Actin is a highly conserved protein involved in essential cellular processes. As such, it is the target of numerous bacterial protein toxins.
ExoY is a virulence factor of the human pathogen *Pseudomonas aeruginosa*. Inside bacteria, the toxin is inactive, but once it enters target cells, it interacts with filamentous actin (F-actin) and becomes a potent nucleotidyl cyclase (Belyy et al., 2016). Using single-particle cryo-EM, we show that in comparison to the apo-state, two flexible regions of ExoY become ordered and interact with F-actin. Our MD simulations and biochemical assays demonstrate that the specific stabilization of these regions leads to allosteric stabilization of the nucleotide-binding pocket and thus activation of the enzyme (Belyy et al., 2021). Our findings pave the road for developing of novel antidotes against toxins of microbial origin.
TccC3 is an effector from the insect pathogen *Photorhabdus luminescens*. Once TccC3 is translocated into the target cell, the enzyme ADP-ribosylates F-actin, resulting in clustering of the cytoskeleton and ultimately cell death (Lang et al., 2010). By combining NMR spectroscopy and cryo-EM we show in atomic detail how TccC3 modifies actin. Binding of TccC3 to its substrate occurs via an induced-fit mechanism that facilitates access of NAD^+ to the nucleotide-binding pocket. The following nucleophilic substitution reaction results in the transfer of ADP-ribose to F-actin. This site-specific modification of F-actin prevents its interaction with depolymerization factors, which impairs actin network turnover and leads to steady actin polymerization (Belyy et al., accepted). Our findings reveal a new mechanism of action of a bacterial toxin through specific modification of F-actin.

A 048 [239]
Structural basis of actin filament assembly and aging

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The dynamic turnover of actin filaments (F-actin) controls cellular motility in eukaryotes and is coupled to changes in the F-actin nucleotide state. It remains unclear how F-actin hydrolyzes ATP and subsequently undergoes subtle conformational rearrangements that ultimately lead to filament depolymerization by actin-binding proteins. Here, we present cryo-EM structures of F-actin in all nucleotide states, polymerized in the presence of Mg^{2+} or Ca^{2+}, at resolutions (~2.2 Å) that allow for the visualization of hundreds of water molecules. The structures reveal that the monomeric to F-actin transition induces the relocation of water molecules in the nucleotide binding pocket, activating one of them for the nucleophilic attack of ATP. Unexpectedly, the back door for the subsequent release of inorganic phosphate (P_i) is closed in all structures, indicating that the F-actin conformation that allows for P_i release occurs transiently. The small changes in the nucleotide-binding pocket after ATP hydrolysis and P_i release are sensed by a key amino acid, amplified and transmitted to the filament periphery. Furthermore, differences in the positions of waters in the nucleotide binding pocket explain why Ca^{2+}-actin exhibits slower polymerization rates than Mg^{2+}-actin. Our work elucidates the solvent-driven rearrangements that govern actin filament assembly and aging and lays the foundation for the rational design of drugs and small molecules for imaging and therapeutic applications.

A 049 [242]
Biosynthesis of aryl polyenes: An uncommon type II PKS system

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The aryl polyenes (APEs), a widespread class of bacterial polyketides, are produced by uncommon type II polyketide synthase (PKS) systems. The associated biosynthetic gene clusters are part of the most common clusters among gram-negative bacteria.[1] Here, we present the 3D-structures of all oligomeric protein complexes involved in the APE biosynthesis, such as the hetero- and homodimeric ketosynthases (ApeO:C, $ApeR_2$), the tetrameric ketoreductase ($ApeQ_4$), the heterodimeric dehydratases (ApeI:P), the tetrameric thioesterase-like protein ($ApeK_4$) and $ApeK_4$ bound to one of the two acyl-carrier proteins forming a so far unknown heterooctameric complex ($ApeE_4$:K_4, $ApeF_4$:K_4).[2]
Furthermore, we provide underlying mechanisms and report combined elements of fatty acid and polyketide biosynthesis involved in the APE biosynthesis.

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A 050 [260]
Unveiling the impact of farnesylation on hGBP1 dynamics, structure and function using smFRET.

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The human Guanylate binding Protein 1 (hGBP1) is a human immune protein, which acts against viral and microbial attacks within the cell. This protein can be posttranslationally modified with a farnesyl moiety, which allows the protein to perform its most observed activities: polymerisation and membrane binding. Over the course of these activities, major conformational changes could be observed in literature [1]. To better understand the impact of the farnesyl moiety on hGBP1 dynamics, both non-farnesylated and farnesylated specifically labelled hGBP1 samples were synthesized and measured with single molecule FRET (smFRET). Through this approach, it was possible to refine and expand previously observed dynamics [2] for non-farnesylated hGBP1 and further explore the activity pathway of the farnesylated hGBP1 with its impact on structure and dynamics. It could be shown that dynamics observed for non-farnesylated hGBP1 play a vital role in the pathway of farnesylated hGBP1, while also being being able to show that the structure of hGBP1 is completely extended within the polymer. All in all, it was possible to observe hGBP1 in its monomeric, dimeric and oligomeric form to further the knowledge of this protein's activity pathway.

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A 051 [**274**]
Utilizing an azobenzene photoswitch to investigate the structure formation of amyloid β (1-40) fibrils

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Amyloid β (Aβ) is an intrinsically disordered peptide that is known for its potency to form amyloid fibrils, which are a hallmark of Alzheimer's Disease. Despite their high medical relevance their structure is still not completely solved and several structural models are under discussion.
We investigate the 40 amino acid variant of Aβ for which two major classes of structures are proposed, one comprising U-shaped Aβ peptides and one comprising Aβ with an extended conformation.
To discriminate these conformations, we replaced three amino acids of the potential turn region with an azobenzene photoswitch. This compound can be switch between a cis- or trans-conformation upon irradiation with light of the corresponding wavelength and therefore force the Aβ peptide in a U-shaped or extended conformation, respectively.
We show that the photoswitch can be successfully incorporated in the Aβ peptide and the conformation of the hybrid-construct can be manipulated by irradiation. Furthermore, the fibrilization behavior of the constructs depends on the predetermined conformation of the Aβ peptide.
Our investigations suggest, that only Aβ with an extended conformation is able to form fibrils and that this extended structure is retained in mature fibrils. These findings are confirmed by NMR experiments looking at characteristic amino acid contacts of modified Aβ.

A 052 [277]
Structural analysis of tRNA modifying KEOPS complex

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Modifications of tRNA bases near the anticodon are suggested to ensure the reliability of translation. One of these modifications is the cyclic N^6-threonylcarbamoyladenosine (ct^6A) at position 37 of ANN decoding tRNAs. Ct^6A is built through the transfer of a threonylcarbamoyl moiety onto the tRNA. The transfer is catalysed by the KEOPS (kinase, endopeptidase and other small molecules) complex, a heterooligomeric protein complex consisting of five proteins in *S. cerevisiae*. To better understand the transfer mechanism of the threonylcarbamoyl moiety, we investigate the structure of the KEOPS complex. Additionally, the function of each subunit is examined through *in vitro* complex assembly as well as activity assays.

A 053 [281]
High-resolution structural analysis of the Dam1 complex and associated proteins

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Accurate segregation of chromosomes during mitosis is one of the basic processes of life. During mitosis, paired sister chromatids are separated and partitioned into the two forming daughter cells. This is mediated via kinetochores, large multi-subunit protein assemblies that connect chromosomes to dynamic microtubules (MTs) of the mitotic spindle. In the model organism *Saccharomyces cerevisiae,* one of the key players in this process is the Dam1 complex (Dam1c). Dam1c oligomerizes as a ring around the plus-end of MTs and links them to chromosomes via a connection to the kinetochore. Bim1 and Bik1, Dam1c-affiliated members of the plus end-associated protein family, are also important for MT structure, MT dynamics regulation and interaction with the kinetochore. Previous studies have shown that the interactions between these proteins occur in a specific order and are vital to cell cycle progression. However, there is currently a lack of detailed structural information on these interactions and the underlying molecular

mechanisms. Here we present preliminary results of our efforts to thoroughly characterize the Dam1 complex and its key interactions using cryo-electron tomography and single particle analysis.

A 054 [282]
Structural Analysis of Black Widow Spider Neurotoxins

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Black widow spiders, the members of the genus Latrodectus are widely known because of their potent neurotoxic venom called, Latrotoxins (LaTXs). LaTXs are presynaptic pore-forming neurotoxins. The effects of the toxins are similar but are caused by phylum-specific toxins for different phyla. One of them targeting vertebrates (α-latrotoxin (α-LTX)), five specialized in insects (α, β, γ, δ, ε- latroinsectotoxins (LITs), and one on crustaceans (α-latrocrustatoxin (α-LCT)). LaTXs bind to specific receptors on the surface of neuronal cells or insert themselves into the membrane to form ion-permeable pores, inducing the massive release of neurotransmitters from the nerve terminals. A high-resolution structure and precise mechanism of action of LaTX have yet to be determined despite extensive studies over the past decades. We report the structures of the α-LCT monomer and the δ-LIT dimer. The structures reveal a four-domain organization of the LaTXs. A C-terminal domain of ankyrin-like repeats shields a central membrane insertion domain of six parallel α-helices. Both domains are flexibly linked via an N-terminal α-helical domain and a small β-sheet domain. Based on this study, we propose a cyclic mechanism of oligomerization, which takes place prior to membrane insertion. In artificial membranes, both recombinant α-LCT and δ-LIT form channels that are stabilized by Ca2+ ions and allow calcium flux at negative membrane potentials. Comparing α-LCT and δ-LIT provides the first crucial insight into the molecular mechanism of the LaTX family.

A 055 [283]
Reconstitution of the BiP-GRP94 chaperone complex in vitro

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BiP and GRP94 are protein chaperones in the secretory pathway of mammalian cells. They localize to the endoplasmic reticulum (ER) but are also found in post-ER compartments, such as the Golgi apparatus or the extracellular space. Through specific induced unfolding of the Halotag2 (HT2) domain (Hydrophobic tagging) in the Golgi, a relocalization and retention of unfolded HT2 to the ER was observed[1]. Moreover, unfolded HT2 was associated with the chaperones BiP and GRP94[1], which are members of the Hsp70 and Hsp90 protein family, respectively, and known interactors[2]. We are using HT2 as an *in vitro* model substrate to characterize the BiP-GRP94 interaction and to investigate Golgi protein quality control mechanisms. We could show a direct interaction between BiP and the unfolded HT2. Additionally, BiP and GRP94 form a stable complex. The ternary complex of BiP-GRP94-HT2 was enriched and characterized by dynamic light scattering. Taken together, we were able to reconstitute the BiP-GRP94 interaction with a model substrate *in vitro* and we are currently exploring the structure of the complex by electron microscopy and cross-link mass spectrometry. For a physiological approach, amyloidogenic antibody light chains, a destabilized secretory cargo, are studied as substrate proteins of the BiP-GRP94 complex.

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Poster Abstracts: (B) - Biophysical chemistry and cell biology of interfaces and functional phase separation

B 001 [13]
Mapping water thermodynamics in α-Elastin liquid-liquid phase separation

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Liquid-liquid phase separation (LLPS) is a fundamental process for membraneless compartmentalization in cells. Realizing this motivated efforts to understand the biophysical principles of LLPS leading to biological condensates.[1] While many studies focus on the interaction of the biomolecules itself, we have directly measured the interaction of water within the biological condensates with terahertz (THz) spectroscopy with attenuated total reflection geometry (ATR).[2] This allowed us to identify spectroscopic fingerprints of water molecules during LLPS. By adopting a recently introduced approach to directly link spectroscopic to thermodynamic quantities [3], we proposed that water, as a solvent, plays an active role in driving LLPS.[2] In particular, using α-Elastin as a model for concentration and temperature dependent LLPS, we were able to tune LLPS and adjust the relative contributions from two distinct populations of water revealed by spectroscopic fingerprints.[4] These are water molecules hydrating hydrophobic and hydrophilic patches on the proteins surface.[2] Based on the THz experiments and accompanying simulations, we propose that these populations act in opposite directions for driving LLPS.

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B 002 [**35**]
Genetically-encoded sensors for measuring macromolecular crowding at the lipid membrane interface

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Biochemical processes within the living cell occur in a highly crowded environment. The phenomenon of macromolecular crowding is not an exclusive feature of cytoplasm and can be observed in the densely protein-packed, non-homogeneous cellular membranes and membrane interfaces. The impact of the membrane crowding on function and behavior of single proteins and complex protein machineries is usually not considered *in vitro* experiments, although various effects like hampered diffusion rates, oligomerization, clustering, folding of proteins as well as transport processes and membrane remodeling were shown to be affected [1]. Simulation and study of crowding effects in artificial systems require systematic reconstruction and characterization of crowding-induced confinement in the physiologically relevant range.
Here we describe membrane-adapted genetically encoded sensors that target the membrane interface and are suitable for measuring the interfacial crowding both *in vivo* and *in vitro*. The sensor consists of two fluorescent proteins forming a FRET pair [2], which are connected via a flexible linker and a hydrophobic transmembrane domain. The sensor is sterically compressed by soluble and membrane-tethered crowders, so the associated changes in FRET signal report on the lateral confinement at the membrane interface. We demonstrate that the crowding induced by either proteins or polymers of varying sizes may be probed using the sensor, and the measurements may be carried out also in native cellular membranes, thus offering a robust approach for crowding analysis in complex environments.

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Poster Abstracts: (B) - Biophysical chemistry and cell biology of interfaces and functional phase separation

B 003 [48]
Unsaturated fatty acids augment protein transport via the SecA:SecYEG translocon

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Protein transport through the bacterial cytoplasmic membrane is mediated by the Sec machinery, which is composed of the membrane embedded channel, SecYEG, and the ATPase SecA. The amphipathic N terminal helix of SecA interacts with the anionic lipids, phosphatidylglycerol, and cardiolipin, which are essential for protein transport. However, the effect of acyl chain composition on protein transport has never been investigated.

Here, we reveal that the unsaturated fatty acids (UFAs) of the membrane phospholipids, including the UFA rich tetraoleoyl-cardiolipin, stimulate SecA:SecYEG mediated protein translocation (Kamel et al., 2022). MD simulations and laurdan fluorescence spectroscopy show that increasing UFAs content induces loose lipid packing at the interface, where the N terminal amphipathic helix of SecA docks. While UFAs do not affect SecYEG folding, they promote SecA binding to the membrane, which is then converted to the enhanced transport. Ongoing studies aim to elucidate the exact binding interface of SecA to the membrane. Additionally, we show that the net charge on the N terminal helix of SecA and its hydrophobicity play a crucial role in SecA membrane interactions. This might explain how SecA homologs interact with different membrane compositions across different bacterial species, highlighting the role of SecA N terminal helix in Sec mediated protein transport.

B 004 [122]
Functional Mechanism of MbxA Protein

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Repeats in **T**o**X**ins (RTX) protein family is a group of exoproteins secreted by Type 1 secretion systems (T1SS) of gram-negative bacteria. HlyA is the prototype among RTX toxins, secreted by *E. coli,* that causes urinary tract infections. However, the activity of HlyA and other RTX toxins beyond pore formation remains elusive as it is known to manipulate host cell functions in various ways. This project aims at the functional characterization of a poorly charecterised RTX protein, MbxA, from *Moraxella bovis*. We established the heterologous expression and secretion of MbxA by the HlyA T1SS and found that, MbxA, which was earlier thought to infect only bovine cells has also the potential to induce cytotoxicity to human epithelial cells and T-cells. The LC50 of epithelial and T-cells was within the low nanomolar range. Furthermore, it was also demonstrated that these cells lysed within minutes with the appearance of membrane blebs. Elevation and modulation of free cytosolic calcium ion concentrations are basic strategies of host cell manipulation by pathogens. Like other RTX toxins, a significant amount of calcium ion influx into HEp-2 cells was observed with MbxA, which further validates our finding of MbxA induced cell death of HEp-2 cells. Moreover, nuclear shrinkage and PS externalization of HEp-2 cells on treatment with MbxA was observed which give new insights into MbxA induced cell death of the HEp-2 cells. Additionally, to visualize the localization of MbxA on HEp-2 cells, MbxA was purified and labeled using Atto-488. In the direction of identifying the cell surface receptor of the MbxA and mechanism of pore formation, we performed liposome leakage experiments with MbxA to understand the lipid dependency of MbxA binding and pore formation.

B 005 [204]
Characterizing the dynamic structural ensembles of the phase separating protein FUS

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The formation of biomolecular condensates through liquid-liquid phase separation (LLPS) of the intrinsically disordered protein Fused in Sarcoma (FUS) and their direct association with neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) have recently gained significant attention. However, due to the lack of a stable tertiary protein structure charge combined with a strong sensitivity to environmental conditions the morphology and chain dynamics of singular FUS monomers are not yet completely understood. Using *in vitro* biophysical studies based on multiparameter fluorescence detection (MFD), we are mapping out the intrinsic interaction motives of FUS dependent on the environmental ionic strength. Overall, we find that FUS can be best described as a dynamic ensemble which is highly prone to conformational switching. Single-molecule FRET (smFRET) measurements show a continuum of conformations which shifts to larger end-to-end distances upon increasing KCl concentration while filtered FCS (fFCS) resolves rapid chain dynamics with relaxation times from 100 ns to 10 µs. Effective hydrodynamic radii of rotational and translational diffusion determined by polarization resolved FCS (pFCS) indicate a threefold FUS compaction evoked by the salting-in effect. The lower degree of hydration results in an average shape change into a denser, more oblong FUS. Corroborating with the smFRET studies, ensemble Tyrosine-Tryptophan FRET measurements display a stepwise decrease in FRET efficiency between Tyr and Trp at the saturation concentration suggesting that upon cooperation into condensates the natively compact FUS chain expands and thus enters a favorable state for LLPS associated protein self-interaction.

B 006 [249]
Family Tradition: Liquid-Liquid Phase Separation of the Bacterial ParB Protein Family

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Liquid-liquid phase separation has emerged as a widespread molecular process to govern protein and nucleic acid compartmentalization in eukaryotes. A variety of long known cellular structures such as the nucleolus or stress granules have been shown to form through this biophysical principle. While more and more eukaryotic proteins are being associated with liquid-like compartments, little is known about similar compartmentalization strategies in prokaryotes. By *in vitro* reconstitution, we investigate the phase behavior of the important bacterial ParB protein family. We find novel mechanisms of regulation of the formed condensates and establish evolutionary conserved phase properties. Lastly, we use a ParB paralogue to understand how protein condensates can interact with lipid membranes. Taken together, our findings establish the ParB protein family as a potential master regulator of bacterial protein phase separation with precise and conserved regulative mechanisms.

B 007 [280]
Interface resistance can govern molecular transport across condensate interfaces.

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Cells can achieve compartmentalization of biochemical processes via organelles by the selective admission of biomolecules. Organelles are enclosed by a membrane or, in the case of biomolecular condensates, by the condensate-bulk interface. While transport across membranes has been studied for decades, how biomolecular condensates regulate transport across their interface is less clear. Using a combination of in vitro live-imaging and theory, we show that the flux of molecules across the condensate-bulk interface exhibits transients that

cannot be explained by local equilibrium between the coexisting phases, a phenomenon also referred to as interface resistance. It is unclear whether this interface resistance stems from molecules adsorbing at the interfacial domain or from a kinetic barrier reflecting molecules at the interface. Using single-particle fluorescence microscopy of PGL-3 droplets, we observe no accumulation of molecules at the interface. This observation suggests that molecules are not adsorbed, but rather reflected at the interface. We quantify the strength of interface resistance within a model that can account for molecule dynamics outside, inside, and at the droplet interface. We thus provide a framework to accurately characterize molecular fluxes across condensate-bulk interfaces and, for the first time, quantify the magnitude of interface resistance.

C 001 [4]
DNA Nanocomposites as functional materials in cells and in cell-free systems

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DNA hydrogels are a new class of biomaterials with high potential for a wide range of biotechnological applications and have recently been further developed into complex DNA nanocomposites originating from DNA-functionalized silica nanoparticles (SiNPs) and carbon nanotubes (CNTs). These materials are characterized by the unique properties of nucleic acids, such as molecular recognition, programmability and excellent biocompatibility, and can be tuned regarding properties such as stiffness by adjusting the ratio of the composites' starting materials. These properties enable applications in cell culture as well as use as a new material for cell-free protein synthesis (CFPS).
The presented work investigates the scope of a recently developed composite material, which is synthesized using nanoparticles as the starting point of the polymerization of long DNA single strands, which are produced by rolling circle amplification (RCA). The integration of plasmids coding for fluorescent marker proteins provides SiNP/CNT-DNA composites with genetic transcribable information. Flow cytometry and confocal microscopy showed that the materials are very efficiently taken up by different eukaryotic cell lines, which can continue to divide while the ingested material is evenly distributed among the daughter cells. Although the encoded protein was not expressed in the living cells, the composites proved to be efficient templates for CFPS with eukaryotic cell lysate. This work contributes to the understanding of the molecular interactions between DNA composites and the functional cellular machinery.

C 002 [50]
Optimized metrics for combinatorial CRISPR screens identify directional genetic dependencies.

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To map combinatorial gene dependencies, choosing an efficient CRISPR system is of utmost importance. Cas9 and Cas12 systems are widely used, however, a side-by-side comparison and recommendation for when to use which system remains unavailable. Moreover, two combinatorial screening approaches are currently established, i) the "anchor" approach in which an anchor-edit is made and subsequently screened for dependencies, and ii) the dual-editing approach in which two edits are introduced simultaneously. These approaches are, however, fundamentally different and it remains unclear which identifies genetic interactions (GIs) most robustly. Here, we systematically compared combinatorial Cas9, Cas12a and CHyMErA systems and identified Cas12a's RNA-processing activity to confound combinatorial hit-calling. Applying optimized screening parameters, we compared anchor- and dual-editing in their ability to identify GIs, and demonstrate that cell-engineering and smart library designs enable the identification of directional gene relationships in which the phenotypes of A→B and B→A differ. Investigating >230.000 gene combinations among cancer druggable genes, we identified hitherto unexplored directional relationships between clinically-approved cancer drug targets. In sum, we present optimized metrics for functional CRISPR genomics as well as directional gene relationships among cancer druggables. We believe our approach has the potential to reveal synergistically iterative drug regimens.

C 003 [92]
Systems integration reveals molecular mechanisms driving the stress responses to mitochondrial protein misfolding

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Maintaining mitochondrial function and proteostasis is key for cellular survival. Perturbations, such as mitochondrial protein misfolding, modulate a complex network of stress responses to avoid cellular damage. However, the precise outcomes, both on a molecular and systems biology level, remain unclear and limit our understanding of the mechanisms driving the required responses. Carrying out a genome-wide CRISPR/Cas9, we identified that reducing mitochondrial protein import was sufficient to induce mitophagy, without the

need of a loss of mitochondrial membrane potential. Integrating multi-level proteomics of the insoluble protein fraction, interactomes and the mitochondrial proteome revealed that mitochondrial protein misfolding sequesters the PAM complex, which is required for protein import, from the mitochondrial translocation machinery to induce mitophagy. By developing a novel method to quantitative monitor protein import kinetics of the mitochondrial proteome, we further revealed integration between import rates, mitochondrial and cytosolic translation and control of cytosolic stress regulation. Together, our findings reveal how mitochondrial protein misfolding induces mitophagy and provide first insight into the complex protein import rate changes during mitochondrial stress.

C 004 [278]
A modular cloning (MoClo) toolkit to study mitochondrial biology in yeast

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Modular Cloning (MoClo) allows the combinatorial assembly of plasmids from standardized genetic parts. It is a very powerful strategy which enables highly flexible expression patterns without the need of repetitive cloning procedures. In this study, we describe an advanced MoClo toolkit that is designed for the baker's yeast *Saccharomyces cerevisiae* and optimized for the targeting of genes of interest to specific cellular compartments. Comparing different targeting sequences, we developed signals to direct proteins with high specificity to the different mitochondrial subcompartments, such as the matrix and the intermembrane space (IMS). Furthermore, we optimized the CEN/ARS regions of single copy plasmids to minimize the loss of plasmids. The plasmids generated by this MoClo strategy have a comparable stability than other plasmid series commonly used in the yeast community, but their generation is much more versatile and faster.

C 005 [296]
Production of bimetallic nanoparticles using cell-free extract from engineered E. coli cells

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Yearly, over 3000 patients in the Netherlands are diagnosed with head and neck cancer. The treatment of these cancers is highly dependent upon the type and stage, and can come with toxic side-effects or (partial) loss of speech. A novel, highly precise treatment for head and neck cancers is photothermal therapy. It relies on using absorbents that convert near infrared light to thermal energy for cancer tissue ablation. Metallic nanoparticles are effective absorbents, a property which can be enhanced by modifying the structure of the nanoparticles to a jagged sphere consisting of a silver core and gold spikes. This composition and structure not only shifts the absorption spectrum to a more suitable wavelength for effective photothermal therapy, but also provides improved optical properties compared to monometallic nanoparticles. In this project, we produce jagged spherical bimetallic nanoparticles composed of silver and gold by using engineered *E. coli* cells. These engineered strains contain three genes to enhance the release of reducing agents from the bacterial cells that can convert the metallic ions into nanoparticles. To account for biosafety, cell-free extract is used to produce these nanoparticles. We show that a microbial-based cell-free system can produce bimetallic nanoparticles with a jagged spherical structure. We have established a minimally-toxic, environmentally-friendly, and cost-effective platform for the production of jagged bimetallic nanoparticles with the potential to be upscaled.

D 001 [67]
Prediction of enzyme kinetic parameters and substrate scopes using artificial intelligence

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The Michaelis constant K_M and the enzyme turnover number k_{cat} are crucial parameters when studying enzyme kinetics and cellular physiology. The function of all enzyme-encoding genes as well as kinetic parameters for enzymatic reactions are required for genome scale metabolic models that account for cellular resource allocation. We developed general prediction models for K_M, k_{cat}, and for enzyme-substrate pairs, which can be applied to any enzyme with known protein sequence. The machine learning models for K_M and k_{cat} achieve coefficients of determination of R^2=0.53 and R^2=0.42, respectively, on independent test sets. Our binary prediction model that predicts whether a metabolite is a substrate for a given enzyme achieves an accuracy over 90% on an independent test set. In part, this accuracy was achieved by representing enzymes through a modified transformer model with a trained, task-specific token, and by representing small molecules with graph neural networks. Our methods outperform previous approaches developed for the tasks of predicting the substrate scope of enzymes and of predicting Michaelis constants K_M, which are moreover limited to small groups of enzymes and require dense training data sets. Our prediction of the turnover number k_{cat} leads to a similar accuracy as in a previous study, which was however limited to enzymatic reactions from *Escherichia coli* and which requires much more detailed input data, such as metabolic flux estimates and active site information, which are not available for the vast majority of enzymes. To allow an easy use of our trained models, we implemented python functions and webservers.

D 002 [138]
FRET-assisted integrative modeling of biomolecules

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Förster Resonance Energy Transfer (FRET) experiments provide information on the dynamics and the tertiary and super-tertiary structure of dynamic biomolecules. Combining FRET experiments with other experimental and computational methods in integrative approaches allows to generate structural models of biomolecules with high accuracy and precision. We used single-molecule FRET measurements together with FRET-restrained structural modeling, molecular simulations and SAXS measurements in a hybrid approach to obtain structural models for an RNA four-way junction. Such integrative structural models can be deposited in PDB-Dev, which is a prototype archiving system for deposition of integrative and hybrid structural models obtained from various experimental sources. The recently developed FLR dictionary allows for the deposition of integrative structural models using data from FRET measurements. The dictionary includes the description of samples, fluorescent probes, analyses, structural modeling procedures and the resulting structural models.
In order to facilitate FRET-based structural modeling of proteins and nucleic acids, we present a methodology that (i) helps planning and designing of FRET experiments for structural studies by providing suggestions for potential labeling positions and FRET pairs, thereby reducing the number of necessary measurements, and (ii) includes screening and scoring of an ensemble of conformations against experimental FRET data, which allows to identify conformers from the ensemble which best agree with experimental data.

D 003 [194]
FRET-assisted structural modeling of dynamic protein ensembles

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The biological function of proteins and its interactions with other biomolecules are highly dependent on its structural and conformational dynamics. Dynamic protein ensembles consisting of structural heterogeneities of interconverting conformers can provide valuable information about its function. Integrative approaches, the combination of Förster Resonance Energy Transfer (FRET) experiments with computational methods like molecular simulations,

have the ability to generate dynamic ensemble representations and to resolve static and dynamic heterogeneities based on the distributions of FRET observables.
We use this hybrid approach for the analysis of the conformational transitions of syntaxin 1a, a part of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein complex, which is essential in the membrane fusion process of neuronal exocytosis. Syntaxin 1a switches between a closed state preferred in presence of the regulator protein Munc18-1 and an open conformation needed for the assembly into the SNARE complex.
The FRET observables efficiency, inter-dye distance and fluorescence weighted lifetime of the donor in presence of FRET are utilized jointly to screen a pool of simulated conformations against these experimental FRET observables, to identify conformers with the best agreement to the experimental data and to generate an accurate and precise dynamic ensemble which can describe the open-to-closed conformational transitions of syntaxin 1a.

D 004 [195]
On the robustness of Maximum Entropy Method-derived posterior conformational ensembles of flexible systems using FRET measurements

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Maximum Entropy Methods (MEMs) are statistical approaches based on Bayes' theorem, used to determine posterior distributions by combining experimental data with prior information. We apply MEM to recover the conformational ensemble of the steric chaperone Lif in the apo state, highly flexible protein with potential local disordered regions, which exceeds the complexity of systems studied so far. As prior information, we used five distinct sets of conformational models with corresponding population fractions, obtained from multiple, microsecond-long MD simulations with different force fields. We refined ensembles using time-resolved ensemble-averaged FRET data, acquired for a network of FRET pairs. Use of ensemble-averaged FRET data, in combination with high conformational flexibility of apo Lif, defines the challenge of this study. Therefore, we first computed synthetic FRET data, and the structural models underlying ground truth are known. Using the synthetic data and varying priors, we assessed the accuracy of reconstructed posterior ensembles across different levels of model representation. With 50 optimally chosen FRET pairs, MEM performs reliably for ensemble-integrated representations, such as inter-residue distograms or electron density maps, yielding resolutions of 5-8 Å. However, at atomistic resolution, MEM yields ambiguous solutions heavily dependent on the prior. Going to our experimental data acquired for a network of six FRET pairs, and the five varying priors, we then obtained only three classes of posterior electron density maps, which provide first insights into the ensemble properties of apo Lif.

D 005 [233]
Bioinformatic analysis of different 2D and 3D culture conditions of an established melanoma cell model

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More than 2000 established cell lines can be used to investigate cellular responses, drug response or general biological processes of cells. Currently, 2D cultures of adherent or non-adherent cells are mostly used in biomedical research.

It is well known that the cellular environment has direct impact onto cell behavior and cellular response. Thus, the discrepancies between the frequent 2D cultures and the 3D *in vivo* situations can be rather big. Due to that we investigated the gene expression of an established melanoma cell line in different 3D culture conditions. For this purpose, cells were cultured for 2 days in 2 different 3D hydrogels either promoting (Matrigel) or non-promoting (Cellink Bioink) cell cycle progression. Afterwards we performed RNA-sequencing followed by differential expression analysis comparing the two 3D culture conditions. Functional enrichment analysis concerning the identified differential expressed genes (DEGs) confirmed the differences in cell cycle activity and cellular processes, especially in regards of adhesion, locomotion and migration as well as RNA and DNA metabolic and chromosome organization processes.

Further gene set enrichment analysis using the top scored DEGs revealed a subset of transcription factors which seem to have an impact on the detected differences between the cell culture conditions. Comparison of these results with the results of transcriptomic analysis of 2D cultures shows the significant changes associated with the inclusion of the cellular environment.

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E 001 [115]
Integration of photosynthetic complex I into liposomes for ferredoxin-dependent electron transfer towards plastoquinone

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The two light-driven electron pathways, linear and cyclic electron flow, operate in all organisms that perform oxygenic photosynthesis. The linear electron flow (LEF) is driven by the two photochemical reaction centers photosystem I (PSI) and photosystem II (PSII) for the synthesis of ATP and NADPH. Photosynthetic complex I (NDH-1), which is structurally and functionally related to respiratory complex 1 from mitochondria and bacteria, enables cyclic electron flow (CEF) around PSI, exclusively leading to the formation of ATP. Using liposomes as an artificial membrane system, we were able to observe NDH-1 *in vitro* activity by monitoring NADPH fluorescence decay in the presence of ferredoxin (Fd), ferredoxin-NADPH-reductase (FNR) and NADPH. The components of the established assay and the properties of the proteoliposomes after integrating NDH-1 and its terminal electron acceptor decyl-plastoquinone (DPQ) were analyzed by various biochemical and biophysical methods. NdhV, an extrinsic subunit of NDH-1 which is only transiently binding and usually lost during purification of the complex, is proposed to play a significant role in the binding of Fd. A reconstitution of the complex with the recombinantly expressed subunit was successfully performed and verified by biochemical and biophysical analysis to further elucidate its function. Our preliminary results, however, do not show an elevated electron transfer via the complex in the presence of NdhV, thus indicating another subunit being the major driver for the binding of Fd. Our assay will provide further insight into the electron transfer of the complex and creates further possibilities for optimization and modification, e.g., to monitor the proton-pumping activity of the complex.

E 002 [121]
Isolation of three different Photosystem II complexes from Thermosynechococcus elongatus via Strep-tagged PsbO

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The multi-subunit membrane protein complex Photosystem II (PSII) catalyzes the light-driven oxidation of water and with this the initial step of photosynthetic electron transport in plants, algae, and cyanobacteria. Chromatographic isolation of PSII complexes from the thermophilic cyanobacterium *Thermosynechococcus elongatus* yields stable protein complexes of high purity, which are well suitable for structural and functional analysis. In the present study, we purified three different PSII complexes from a mutant line in which the extrinsic subunit PsbO, characteristic of active PSII, was fused with an N-terminal Twin-Strep-tag. Three different PSII fractions, which differ in oligomeric state and subunit composition, were separated by ion-exchange chromatography after the initial affinity purification. The activity observed for the highly abundant, active dimeric PSII is around 6.000 µmol O_2·(mg Chl·h)$^{-1}$, which is due to the absence of inactive complexes within the sample, as seen by the chlorophyll a fluorescence properties. Because of their high homogeneity, we propose that these complexes are suitable for all kinds of investigations on PSII, in particular the oxygen-evolving complex (OEC). Moreover, one of the isolated PSII fractions revealed differences in subunit composition, activity, manganese content, and chlorophyll a fluorescence properties. This suggests a disturbed OEC, which indicates a complex related to PSII biogenesis or repair.

E 003 [198]
Utilizing photosystem I for light driven in vitro hydroxylation of mid-chain alkenes by a CYP153 oxidoreductase

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Nowadays, the chemical industry has a great interest in the sustainable production of chemicals such as ω-hydroxylated hydrocarbons, which are applicable as chemical feedstocks. As an alternative to the chemical synthesis, the enzymatic approach is considered promising. CYP153 oxidoreductases, a family of the P450 monooxygenase superfamily, are known to catalyze the hydroxylation of alkanes and fatty acids. In situ, CYP153 enzymes are part of a NAD(P)H dependent three component system consisting of a reductase and a ferredoxin (Fd) as an electron carrier. In this work, a CYP153 candidate was functionally coupled to the photosynthetic electron transfer to overcome the NAD(P)H dependency. As a proof of concept, an in vitro based photosystem I (PSI) driven cascade was established. For this, $P700^+$ reduction kinetics were used to screen for a suitable Fd that interacts with both PSI and the reductase. This method allows, in principle, to test various electron mediator candidates for their ability to accept electrons from PSI. The established cascade consists of an electron donor system, PSI from *Thermosynechococcus vestitus* BP-1, a Fd from *Acinetobacter* sp. OC 4 and CYP153A16 from *Polaromonas* sp. JS666. Finally, the strictly light dependent substrate conversion of mid-chain alkanes to the corresponding ω-alkanols or alkanediols by the cascade was demonstrated.

E 004 [200]
Structure and function of cyanobacterial membrane protein complexes

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Membrane protein complexes play a central role in cyanobacterial bioenergetics. We have recently solved several structures of cyanobacterial membrane protein complexes by cryo-electron microscopy and complemented the work by spectroscopic analysis or molecular dynamics simulation. Photosynthetic complex I (aka NDH-1) is a redox-driven proton-pump involved in respiration and cyclic electron flow (CEF). Structural analysis of the complex revealed adaptations, which facilitate binding of the photosynthetic electron mediator ferredoxin, thus enabling cyclic electron flow via photosystem I. Alternative versions of photosynthetic complex I are also involved in so-called carbon concentrating mechanisms (CCMs) and structural analysis in combination with MD-simulation of one alternative complex I (NDH-1S) provides detailed insights into the molecular mechanism of CO_2 conversion by a novel type of carbonic anhydrase. Recently, we also solved the structure of a partially functional Photosystem II (PSII) assembly intermediate. It contains three assembly factors (Psb27, Psb28, Psb34) and provides novel insights into their molecular function. Binding of Psb28 induces large conformational changes at the PSII acceptor side, which distort the binding pocket of the mobile quinone (Q_B) and replace the bicarbonate ligand of non-heme iron with glutamate, a structural motif found in reaction centers of non-oxygenic photosynthetic bacteria. These results reveal novel mechanisms that protect PSII from damage during biogenesis until water splitting is activated. Our structure further demonstrates how the PSII active site is prepared for the incorporation of the Mn4CaO5 cluster, which performs the unique water splitting reaction.

F 001 [38]
Fast analysis with minimal user interaction in Fluorescence Lifetime Imaging

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Fluorescence Lifetime Imaging (FLIM) has become more attractive in recent years as it offers increased specificity in many assays as well as the possibility of multiplexing the read out of many markers with a small number of detectors.

Here we present how FLIM modalities are implemented in Luminosa, the new single-photon counting confocal microscope by PicoQuant. Thanks to a dynamic bining format and GPU-based algorithms FLIM images of 1024x1024 can be anaylsised in a few seconds. The FLIM analysis workflow suggests the best fitting model based on statistical arguments and requires minimal user interaction making these modalities become accessible to new users who can then confidently start working with FLIM and incorporate it into their research toolbox combining the strengths of phasor plots with decay fitting.

KEY WORDS: Luminosa , rapidFLIM, Fluorescence Lifetime Imaging (FLIM), Foerster Resonance Energy Transfer (FRET), confocal fluorescence microscopy, Multiplexing, single photon counting confocal

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F 002 [89]
About Dents and Buldges: Super-Resolved Imaging of Oligodendrocyte Progenitor Cell Process Tips with Correlated Scanning Ion Conductance and Expansion Microscopy

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Oligodendrocytes myelinate neuronal axons in the nervous system. Their progenitors, oligodendrocyte progenitor cells (OPCs), are highly motile cells as they have to migrate across the nervous system to reach their target cells. Upon reaching their target, they can form myelin sheets to insulate axons. A thorough understanding of the molecular machinery driving the migration and target finding of OPCs could increase our understanding of demyelination disorders such as multiple sclerosis or periventricular leukomalcia.

The process tips of OPCs appear to be similar to neural growth cones. However, OPC tips have not been investigated as extensively as growth cones. Thus, we here investigated the correlation between the cytoskeleton and membrane topography of fixed OPC process tips using a novel combination of 4x expansion microscopy [1] and scanning ion conductance microscopy (SICM) [2]. This combination of super-resolution fluorescence microscopy and scanning probe microscopy enabled us to relate the cytoskeletal components to dents and buldges in the membrane.

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[2] Gesper A, Hagemann P, Happel P, 2017. A low-cost, large field-of-view scanning ion conductance microscope for studying nanoparticle–cell membrane interactions. Nanoscale, 9(37).

F 003 [123]
FRET-nanoscopy: a correlative approach with Ångström resolution.

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Introduction Biomolecules organize in 3D assemblies to drive the fundamental processes of life. To access this scale, we present a correlative FRET-nanoscopy approach where we make the transition from 2D to 3D at a molecular scale. Using localization on two-channel STED images (colocalization, cSTED), we resolve xy-projections with high precision (<4 nm, seamless

resolution), which is increased to 4 Å precision using particle alignment and averaging.By applying appropriate corrections and using sub-ensemble averaging, we obtain accurate FRET-based distances under STED conditions with an uncertainty of less than 5 Å, independent of the molecular orientation. Uniquely, FRET measures distances independent of molecular orientation (hypotenuse), whereas localization measures the projected xy-distance (adjacent), using Pythagoras' theorem we make the transition into 3D molecular resolution on short (<12 nm) scales. **Results** To calibrate our method, we perform FRET-nanoscopy on a rectangular origami platform labeled with two FRET pairs separated by 75 nm, showing that FRET and cSTED data yield distances with 4 Å precision compared to predictions. **Application** We apply our approach in vitro to the protein hGBP1, which has been suggested to undergo a conformational change to an extended state and self-assemble into a complex polymer structure upon activation. Inaccessible to either STED or FRET alone, we provide direct evidence for the extended state by resolving a donor and acceptor distance of 28 nm.

1 Budde, J., van der Voort, N.T.M. et al. arXiv:2108.00024v2

F 004 [197]
Timelapse Imaging of Migrating Oligodendrocyte Progenitor Cells

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Oligodendrocyte progenitor cells (OPCs) eventually give rise to myelinating oligodendrocytes. In the OPC stage, the cells migrate in the direction of their targets. So far, the molecular mechanisms driving OPC migration have not fully been resolved. One hypothesis proposes a fine-tuned combination of coordinated cytoskeleton rearrangements and ion and waterfluxes as a possible driving force. Prior investigations suggest, that OPC soma movements require water and ion fluxes through various channels and transporters [1][2]. Additionally, the direction of the soma movement appears to be determined by the position of the processes of the progenitor cell. The processes, their tips and their relation to soma dislocation have so far not been studied extensively. Thus, we here investigated changes in the actin skeleton and mitochondrial network of OPC somata and processes using fluorescence timelapse imaging.

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[2] Schwab A, Nechyporuk-Zloy V, Fabian A, Stock C. (2006). Cells move when ions and water flow. *Pflügers Archiv - European Journal of Physiology*, *453*(4).

F 005 [255]
How to stop transcription at the start.

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Transcription is a key step in gene expression that regulates crucial processes such as cell differentiation and organismal development. To ensure the fastest possible response to the changing environment the transcribing RNA polymerase II (Pol II) is paused shortly after transcription starts and released when the gene output in needed.
The paused Pol II under certain conditions is terminated by the metazoan specific Integrator complex. Integrator is a 15-subunits complex that cleaves nascent RNA using its endonuclease activity to terminate Pol II. Integrator also recruits protein phosphatase 2A (PP2A) that counteracts paused Pol II activation.
To gain insight into Integrator mediated transcription termination, we firstly reconstituted a 34-subunit complex consisting of paused Pol II complex and the Integrator-PP2A complex *in vitro*. Using our *in vitro* system, we showed Integrator cleaves RNA only in the context of Pol II elongation complexes suggesting endonuclease activation upon Pol II binding. To explore the molecular basis of Integrator activity, we determined a cryo-electron microscopy structure of our reconstituted complex. Our structure explains how Integrator recognizes the paused Pol II complex. The endonuclease is positioned optimally near the nascent RNA and adopts an open conformation that is vital for RNA binding and cleavage. Our structure also shed

light on how Integrator and PP2A complex oppose Pol II activation into productive elongation and exposes how Integrator interacts with the C-terminal domain (CTD) of Pol II.
Our structure provides molecular insights into how integrator terminated Pol II at pause sites and serves as a starting point to investigate integrator function in genome-wide regulation of Pol II

G 001 [81]
Ubiquitylation and deubiquitylation at the TOM complex

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Mitochondria are double membrane-bound organelles well known for their crucial role in cellular respiration, metabolism, and signaling. The majority of mitochondrial proteins are synthesized as precursors on cytosolic ribosomes. The translocase of the outer membrane (TOM complex) serves as the main entry site for mitochondrial precursor proteins. Defects during the import of precursor proteins lead to cellular stress and proteotoxicity. To overcome import impairment the cell employs quality control mechanisms. We discovered the mitochondrial protein translocation-associated degradation (mitoTAD) pathway which is actively involved in the removal of non-imported precursor proteins at the TOM complex. Ubx2 interacts with the TOM complex and mediates release of ubiquitylated stalled substrates by recruiting the cytosolic AAA-ATPase Cdc48. These ubiquitylated substrates are targeted for degradation by the proteasome and hence mitoTAD mediates the clearance of the clogged TOM complex. How the precursor proteins are ubiquitylated remained unclear. Here we were able to identify new molecular components responsible for ubiquitylation and deubiquitylation of accumulated precursor proteins at the TOM complex. These findings revealed important quality control steps employed by the cell at the TOM complex.

G 002 [82]
The MIM complex functions as main translocase for α-helical outer membrane proteins

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The mitochondrial outer membrane contains a few dozen integral membrane proteins with a diverse range of functions. Outer membrane proteins can be divided into two types. While five have a transmembrane β-barrel in yeast mitochondria, the majority contains a single or multiple α-helical transmembrane domain. The translocase of the outer membrane (TOM) complex and the sorting and assembly machinery (SAM) mediate the translocation across and insertion of β-barrel proteins into the outer membrane. The mitochondrial import (MIM) complex, formed by Mim1 and Mim2, was shown to insert multi-spanning proteins into the outer membrane. The import of single spanning α-helical proteins is less understood, despite being the largest group of outer membrane proteins.
We investigate the role of the MIM complex in the biogenesis of α-helical proteins. The MIM complex insert precursors of single-spanning TOM subunits into the outer membrane.
Here, we report that this protein translocase plays a major role in the import of single-spanning signal- and tail-anchored precursors into the outer membrane by identifying new substrates of the MIM pathway. Additionally, we show that the MIM complex dynamically cooperates with both TOM or SAM complexes in protein import. The TOM-MIM interaction allows the transfer of precursor proteins from the receptor Tom70 to the MIM complex for insertion. Whereas the SAM-MIM complex recruits small Tom proteins to promote early steps of the TOM complex assembly. Our findings shed light on the versatility of the MIM complex as the main protein translocase for α-helical proteins.

G 003 [217]
State-dependent protein interaction networks of a GTPase regulating plant growth and metabolism

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Small GTPase are molecular switch proteins. They cycle between a membrane-associated active form binding GTP, and a cytosolic inactive form binding GDP. In plants, they regulate cytoskeletal dynamics, membrane trafficking, cell wall synthesis, cellular signalling pathways, nitrogen metabolism, and sugar homeostasis. Discovering interactors of such central

regulators is a way to better understand how all those mechanisms are co-regulated.

Interactome studies in plants recently benefit from the advances made with biotin ligase-based technologies. Using it, we identified in *Arabidopsis thaliana* the interactome of Rho GTPase Of Plant 2 (ROP2) in its active and inactive forms. Beyond discovery of individual interactors, the sets of proteins identified in the proximity of ROP2 in its different activity states enables us to determine which biological processes and signaling pathways are preferentially associated with each GTP/GDP binding forms. In particular, previously unknown links between GTPase signaling and hormonal and calcium signaling pathways have been identified. Such network-level analyses will enable us to uncover the dynamic molecular landscape that coordinates cellular responses and allow plants to thrive under changing environmental conditions.

Key words: ROP2, protein interaction network, *Arabidopsis thaliana*

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G 004 [257]
The Na,K-ATPase facilitates the unconventional secretion of Fibroblast Growth Factor 2

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FGF2 is a tumor cell survival factor that is exported from cells by an ER/Golgi-independent secretory pathway. This unconventional mechanism of protein secretion is based on direct translocation of FGF2 across the plasma membrane. The Na,K-ATPase has been recently revealed as a recruitment factor for FGF2, including a direct physical interaction between FGF2 and the α1 subunit of the Na,K-ATPase. However, the underlying biological role in the secretory pathway remains unclear. Hence we study the function of the Na,K-ATPase in the context of FGF2 secretion. We are interested in determining the oligomeric state of FGF2 at the α1 interface *in cellullo*. We inspect the activity of the Na,K-ATPase and its impact on FGF2 secretion using microscopy techniques. We also attend to identify the effect of FGF2 on the plasmatic membrane potential and membrane integrity. Based on the combined approaches we consider the Na,K-ATPase to be the initial recruitment contact in the inner membrane leaflet for FGF2. The interaction serves as a double functional step. The Na,K-ATPase quality check for biological active FGF2 molecules to be dimerize and continue further down the secretory pathway while modulates the activity to secure the membrane potential and preserve membrane integrity during FGF2 translocation. Based on these approaches, we propose the Na,K-ATPase as the initial facilitator of FGF2 direct membrane translocation. Our findings will define the initial recruitment steps at the inner plasma membrane leaflet required for efficient membrane translocation of FGF2 across membranes.

G 005 [268]
Atg9 interactions via its transmembrane domains are required for phagophore expansion during autophagy-

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During autophagy, a group of conserved autophagy-related proteins (ATGs) catalyzes the *de novo* formation of cup-shaped structures known as phagophores. These precursors sequester cytoplasmic material and/or organelles while they grow, and close resulting in double-membrane transport vesicles called autophagosomes. The autophagosomes fuse with lysosomes/vacuoles to allow the degradation and recycling of their cargoes. The sequential binding of yeast Atg2 and Atg18 to Atg9, the only conserved transmembrane protein in autophagy, mediates the

establishment of membrane contact sites between the phagophore and the endoplasmic reticulum. As the Atg2-Atg18 complex transfers lipids between adjacent membranes *in vitro*, it has been postulated that this activity and the scramblase activity of the trimers formed by Atg9, are required for the expansion of the phagophore.
Here, we present evidence that Atg9 indeed promotes Atg2-Atg18 complex-mediated lipid transfer *in vitro*, although this is not the only requirement for its function *in vivo*. In particular, we show that the mutation of F627, a highly conserved residue located in the interface between the transmembrane domains of the Atg9 monomers, dramatically compromises the function of this protein. The F627A mutation blocks the phagophore expansion and thus autophagy progression. Importantly, $Atg9^{F627A}$ has identical scramblase activity *in vitro* to Atg9, and enhances the Atg2-Atg18-mediated lipid transfer to a similar extent as the wild-type protein. Collectively, our data reveal that the interactions of Atg9 trimers via their transmembrane segments play an essential role in phagophore expansion beyond the role of Atg9 as a lipid scramblase.

G 006 [275]
In vitro reconstitution and analysis of Pseudomonas aeruginosa Sec-mediated protein transport

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The pathogenicity of *Pseudomonas aeruginosa* is associated with multiple secreted virulence factors. The general secretory pathway (Sec) mediates the translocation of many virulence factors. The protein precursors, known as preproteins, are transported via the universally conserved protein-conducting channel SecYEG with help of the ATPase SecA. Even though investigations on SecA:SecYEG are established for *Escherichia coli*, the heterologous reconstitution of the Sec system of the opportunistic human pathogen *P.* aeruginosa is still to be elucidated.
The key components, incl. SecA, SecYEG and the chaperone SecB of *P. aeruginosa* were heterologously expressed, purified and employed to reconstitute transport of preproteins *in vitro*. A range of preproteins, incl. extracellular, periplasmic and outer membrane proteins were employed in their precursor form. In contrast to a model preprotein proOmpA of *E. coli*, transport of native proteins from *P. aeruginosa* appeared to be challenging *in vitro*. To investigate the role of preprotein-specific targeting, a set of cytoplasmic chaperones, such as SecB, CsaA, Trigger factor, and heat shock proteins HtpG, DnaJ and DnaK was examined. Among the investigated preproteins, the primary subunit of the Fap amyloids, FapC showed the highest transport efficiency. Further analysis will focus on the targeting process *in vivo*, and the roles played by lipids in activation of SecA:SecYEG machinery of *P. aeruginosa*.

G 007 [276]
Investigating the inner membrane complex of the Pel exopolysaccharide secretion system of Pseudomonas aeruginosa.

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Pseudomonas aeruginosa is a gram-negative human pathogen and the major morbidity cause of cystic fibrosis patients. It persists in long and aggressive antimicrobial therapy, due to the formation of antibiotic-resistant biofilms. Biofilms are built of extracellular DNA, secreted proteins and exopolysaccharides (EPS). The EPS serves as a structural scaffold and a protective barrier. *P. aeruginosa* contains three major EPS: alginate, Psl and Pel. Each EPS harbors its own gene cluster, encoding for proteins involved in synthesis, modifications, and secretion. The secretion machinery is barley studied and differs for each EPS.
Our research focusses on the inner membrane proteins involved in Pel secretion. While the outer membrane components PelB and PelC have been previously described, the organization and the functional mechanism of the inner membrane synthase/secretion complex remain obscure. The complex consists of the membrane proteins PelD, PelE and PelG, which interact with the cytosolic glycosyltransferase PelF. With its c-di-GMP-binding domain, PelD is the putative regulatory subunit of the complex. We observe dimeric and trimeric species of the isolated PelD, which are

further used for liposome/nanodisc reconstitution towards the structural and functional analysis. Biophysical tools are currently established to probe PelF interactions with the individual membrane components, and the assembled complex will be employed for studying secretion of Pel EPS *in vitro*.

G 008 [295]
Non-canonical ATPase activity modulates SynPspA membrane constriction

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To protect the inner membrane, many bacteria activate the bacterial phage shock system (psp) with the main effector phage shock protein A (PspA). A distinctive feature of PspA is its ability to form ring- or rod-shaped homooligomers of MDa size. Recently, PspA was identified as a bacterial member of the ESCRT-III superfamily based on its ESCRT-III fold and phylogenetic analyses revealing common ancestors with eukaryotic/archaeal ESCRT-III proteins. An important factor in the assembly/disassembly of previously known ESCRT-III complexes is the AAA+-ATPase Vps4 that is thought to remove or add subunits in individual ESCRT-III polymer structures by ATP hydrolysis, thus contributing to the remodeling of lipid membranes. Although some psp systems possess an AAA+-ATPase (PspF), it was not found to be essential for the membrane remodeling properties of PspA, as not all organisms that contain PspA also contain PspF (e.g. cyanobacteria).

Here we show that PspA from the cyanobacterium *Synechocystis* sp. PCC 6803 (*Syn*PspA) binds and hydrolyzes ATP with a non-canonical NTP-binding motif by solving a total of 46 *Syn*PspA assembly structures in presence of ATP, ADP and /or lipids in various diameters. We observed that ATP binding/hydrolysis affects the diameter of rod-shaped *Syn*PspA assemblies via three hinge regions in the monomer. Therefore, our structures suggest that a non-canonical ATPase activity modulates *Syn*PspA membrane constriction thereby leading to vesicle fission and/or membrane defect excision by *Syn*PspA. Due to the close structural and functional relationship between PspA and ESCRT-III proteins, the observed constriction mechanism of PspA may also be found in the eukaryotic ESCRT-III superfamily.

H 001 [3]
Development of PROTAC Molecule Targeting Splicing Machinery

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New strategies of drug development are urgently required to target currently undruggable proteome. The Proteolysis-targeting chimeras (PROTACs) are heterobifunctional small molecules to specifically degrade protein of interest (POI) through chemically induced proximity. PROTAC consists of a chemical warhead to catch a POI and a recruiter of E3 ubiquitin ligase to form ternary complex between POI and E3 ligase for ubiquitination. Because of lack of chemical binders splicing machinery so far is not PROTACable.
Previously, we performed HTS and identified a small molecule (O4I2) able to support the generation of human pluripotent stem cells. To understand the molecular mechanism involved, we performed proteomics analysis on the biotinylated O4I2 and identified Splicing Factor 3B1 (SF3B1) as the top interacting protein. Introducing thalidomide, a ligand of CRBN E3 ligase into the thiazole moiety of O4I2 we successfully generated a novel degrader, called PROTAC-O4I2. We demonstrated that PROTAC-O4I2 efficiently induced SF3B1 degradation in cells. Interestingly, this degradation is independent whether SF3B1 is mutated. As a result, we found nearly equal anti-proliferation effects of PROTAC-O4I2 in cells expressing $SF3B1^{WT}$ or $SF3B1^{K700E}$, a frequent mutation in myelodysplastic syndromes. However, this degradation is dependent on ubiquitin-proteasome pathway in cells expressing functional CRBN. Thanks to the conversed role of SF3B1 in the development, PROTAC-O4I2 also significantly increased survival in a Drosophila intestinal tumor model, implicating that noninhibitory chemicals can be used as POI binders and Drosophila could be an alternative to mouse model in the development of PROTACs.

H 002 [14]
Exploring applications of cell permeable anti-Survivin VHHs in cancer

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Despite its small size (16.5 kDa), Survivin (Svv) fulfills two well characterized functional roles in cancer cells. On the one hand Svv takes part in the inhibition of apoptotic cell death and on the other hand as part of the chromosomal passenger complex, Svv is crucially involved in mitotic regulation, promoting cell proliferation. Overexpression of Svv in cancer cells is associated with decreased patient survival, frequent recurrences and with a resistance against chemo- and radiotherapy. Svv does not exhibit any kind of enzymatic activity, all effects are mediated by direct protein-protein interactions (PPI). Therefore, it is commonly referred to as being undruggable by small molecule inhibitors.

Common strategies to achieve cancer selectivity use either antibodies or selective tags to modulate drug-uptake which has an obvious downside: the drug remains universally toxic. In this study we now aim to perturbate the functions of Svv simply by binding of VHHs (15 kDa antibodies) to functionally relevant PPI domains and thus harm cancer cells selectively. VHH-bound epitopes and binding characteristics were analyzed by ITC and Bio SAXS. Compared to common strategies, selectivity and effect are mediated by the VHH itself while uptake might occur unselectively. Achieving cell permeability was thus the first hurdle to overcome. We coupled VHHs to small peptides and used this strategy to bind multiple VHHs on ultra-small gold nanoparticles via copper-click chemistry. Analogously, we bound VHHs to cell-permeable peptides. Both concepts indeed mediate fast VHH uptake at high rates of bioavailability. Moreover, first *in situ* results hint towards a promising strategy to allow selective targeting of Svv in cancer cells.

H 003 [182]

PACMAN - Proteolytic Antigen Cleavage Mediated AmplificatioN: Introducing a novel class of proteolytic immunotherapeutics to tackle amyloid diseases

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In amyloid diseases, a pathological build up of aggregated proteins or peptides leads to toxicity and triggers a plethora of mislead processes ultimately leading to the diseases' symptomatic expressions. In Alzheimer's disease, the amlyoid-β (Aβ) peptide is one of the disease's amyloid culprits. Conventional immunotherapeutics, with monoclonal antibodies, aim to bind the amyloid antigen and remove it via endogenous degradation pathways. Unfortunately, these approaches have consistently proven insufficient in clinical trials.
We propose proteolytically active antibodies as a novel therapeutic approach to tackle amyloid diseases which might supersede and fundamentally outcompete conventional immunotherapeutics. Proteolytic antibodies offer a great advantage – they cleave their antigen and do not merely bind to their antigen. Furthermore, proteolytic antibodies do not form stable immune complexes with their amyloid antigen, which have been linked to severe negative side effects seen in recent clinical trials against Aβ using conventional antibodies. Here we present the PACMAN method as means to develop this novel class of proteolytic immunotherapeutics to utilize their potential against amyloid diseases.

H 004 [187]

Fluorescence Spectroscopic Methods to Investigate RNA Constructs

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Fluorescence spectroscopic methods provide a versatile toolbox to non-invasively investigate biological samples under physiological conditions with Ångström precision. Fluorescent dye labels such as Cyanine 5 (Cy5) are sensitive to their local environment [1] and therefore provide an excellent means of probing the sequence and structure of biomolecules.
We combine ensemble techniques and single-molecule spectroscopy to quantify not only sequence-dependent effects, but also dynamical changes of the structure of Cy5-labeled RNA duplexes (ds-RNA) and RNA three-way junctions (3WJs) by monitoring the fluorescence lifetime and anisotropy [1]. We further use Förster Resonance Energy Transfer (FRET) [2] to gauge the amplitude of helical motions in 3WJs.
Ensemble measurements have the benefit of an excellent signal-to-noise ratio, while single-molecule methods can resolve heterogeneities in samples.
Using the corresponding sequence of ds-RNA as a reference system for the 3WJ will reveal motions of the global structure and resulting dye interactions with other helices, causing a prolonged fluorescence lifetime and increased anisotropy. For ds-RNA, we correlate dye properties with structural motives and are thereby able to assign deviating dye properties for 3WJs to dye interactions with helices of the construct.

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H 005 [191]
ENGINEERING ENZYMES TO STUDY THE ROLE OF LYSINE ACYLATION IN CHROMATIN STRUCTURE AND DYNAMICS

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Lysine acetylation has a major impact on chromatin structure and gene expression. Using protein engineering strategies, we develop new tools to investigate the molecular principles of these processes. In particular, we employ the incorporation of non-standard amino acids in proteins to monitor the interaction of lysine deacylases with other proteins, create new enzymatic assays and design engineered variants.
Various different types of lysine acylation besides acetylation have been reported. Deacylases display a broad spectrum of activities towards these modifications, hindering the deconvolution of their contribution to cellular processes. We have developed a luciferase-based KDAC assay and a bacterial selection system for the directed evolution of KDACs towards particular acyl substrates and allele-specific inhibitors. Using this selection system, we have produced variants of *E. coli* CobB and human SirT1 that are no longer able to remove lysine crotonylation but still show high activity for other lysine acylations. Using these enzymes, we aim to disentangle the physiological roles of this diverse family of protein modifications.
Using the same directed evolution strategy, we have created a variant of a human lysine deacylase that now catalyzes a unique bioorthogonal reaction. This enzyme will be used to develop an antibody-directed enzyme prodrug therapy (ADEPT) to locally activate drug precursors in tumor tissue. Combined with a suitable precursor that will penetrate cells after activation by this enzyme, this could lead to a breakthrough in ADEPT-based cancer therapy.

H 006 [199]
Do immunoglobulin M (IgM) antibodies represent a natural immune mechanism against amyloid diseases?

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Amyloid diseases represent a range of severe, yet incurable diseases. In patients suffering from those diseases, such as Alzheimer's disease, Parkinson's disease and *Diabetes mellitus* Type 2, amyloid aggregates are found as small soluble, but highly toxic oligomers as well as insoluble, structurally highly ordered fibrils. These amyloid aggregates are characterised by β-sheet-rich structures and arise from the aggregation of natively harmless intrinsically disordered peptides or proteins. For yet unknown reasons, these aggregates accumulate in amyloid diseases, possibly due to an altered balance of the protein homeostasis or due to increased production of the respective amyloid proteins.

We explore a novel approach to eliminate these amyloid structures by utilizing proteolytically active antibodies. Proteolytic antibodies combine the function of proteases and the superior specificity of antibodies. Proteolytic antibodies are a natural phenomenon and have been found in humans. In immunoglobulin M (IgM) class antibodies a particularly high proteolytic activity has been observed.

In first studies of our research group, the amyloid peptides amyloid-β (Alzheimer's disease associated), α-synuclein (Parkinson's disease associated) and islet amyloid polypeptide (*Diabetes mellitus* Type 2 associated) were incubated with polyclonal IgM antibodies from serum and analysed by SDS-PAGE and RP-HPLC. It was found that the purified IgM antibodies were proteolytically active against all those peptides, but not against globular proteins such as ovalbumin. This observation raises the question whether proteolytically active antibodies represent a natural immune mechanism against amyloidogenic peptides.

H 007 [254]
Directional dependencies in chemo-resistant non-small cell lung cancer

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Cancer is a complex and multifaceted disease that depends on the concerted actions of genes. Similarly, cells adapting to drugs gain multiple mutations to account for the toxic stress. Here, we hypothesize that the occurring order of mutations is crucial and contains unexplored genetic vulnerabilities. While understanding of such phenotypes would greatly improve our mechanistic understanding of sequential dependencies for therapeutic exploitation, major technical limitations remain towards their unbiased identification. Therefore, we propose to mimic the directional occurrence of "loss-of-function" mutations among cancer druggable genes by a newly developed CRISPRi-dCas9 system. To distinguish genetic interactions from directional dependencies, we will perform parallel (simultaneous edits) and directional (timely-separated edits) high-throughput screens. Providing conceptual feasibility, our proof-of-concept study already identified three directional dependencies among tumor-suppressor and core-essential genes (TP53→C3orf17, ARNT→XRN2, NF2→DIMT1), suggesting an uncontrolled proliferation to generate a dependency on essential genes. Our overreaching goal is to investigate the molecular mechanisms underlying directional phenotypes to understand the biology of, and enable the therapeutic exploitation of chemo-resistant NSCLC.

H 008 [270]
Quantifying cardiovascular disease biomarkers for molecular diagnostics

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The identification and quantification of disease markers is still a major challenge in clinical application of proteomics in cardiovascular diseases. Nevertheless, the detection of molecular signatures in tissue samples or body fluid has the potential to provide new clues for the elucidation of disease mechanisms. In order to discover new biomarkers for cardiovascular disease, a data independent acquisition approach has been established. This label-free approach is highly adaptable, even after the start of the study. To get more comprehensive results we use sample specific spectral libraries which are built from data dependent acquisition runs on the sample type of interest including retention time calibration. Afterwards, data is acquired in data independent acquisition mode using LC-coupled high-resolution mass spectrometry. Using an optimized sample preparation procedure about 2.1 mg solubilized proteins were extracted from approx. 8 mg heart tissue. A library for mouse heart from 50 µg starting material containing more than 7,000 proteins was constructed. Finally, following DIA experiments led to over 2,000 reliably quantified proteins within the library. The second emphasis lies in building MS-based Protein Assays. Targeted proteomics detects a self-chosen panel of proteins of interest with high sensitivity, quantitative accuracy and reproducibility. In example, we quantify a panel of 13 apolipoproteins in plasma. Therefore, a selected reaction monitoring assay was used to determine the endogenous amounts of the corresponding proteins in plasma.

I 001 [27]
Laboratory reconstruction of the recent evolution of a highly efficient xenobiotic-degrading enzyme

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Background: We wish to understand how new enzyme functions emerge in evolution. Enzymes acting on anthropogenic substances (herbicides, industrial waste, etc.) are suitable model systems to study this problem. The goal of this work was to reconstruct the evolution of AtzB, a hydroxyatrazine ethylaminohydrolase (HAE), which is part of the Atz pathway, acting on the herbicide atrazine.

Results: AtzB showed low promiscuous guanine deaminase (GD) activity and its closest homologues, $AtzB_{Hom1}$ and $AtzB_{Hom2}$, were demonstrated to be natural GDs (k_{cat}/K_M = 1.8 x 10^3 $M^{-1}s^{-1}$). Using these homologues as templates, the introduction of four amino acid exchanges yielded an AtzB variant with a decent activity for guanine (k_{cat}/K_M = 4.1 x 10^3 $M^{-1}s^{-1}$), corresponding to a 740-fold improvement over wild-type AtzB.
Introducing the inverse residue exchanges into $AtzB_{Hom1}$ and $AtzB_{Hom2}$ yielded variants with HAE activities of 3.1 x 10^5 $M^{-1}s^{-1}$ and 4.4 x 10^2 $M^{-1}s^{-1}$. For the $AtzB_{Hom1}$ variant, this represents an increase by 4-5 orders of magnitude, surpassing wild-type AtzB.

Significance: The recent emergence of novel metabolic functions in response to man-made chemicals showcases the remarkable adaptability of enzymatic systems. Our findings provide insights into mechanisms, constraints, and epistatic effects of this process by establishing novel substrate specificities through only a few mutations. These results are also relevant for efforts to mitigate the ecological effects of anthropogenic substances.

I 002 [77]
Modified Cell SELEX: Identification of clickmers binding prostate cancer cells

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In terms of therapeutics and diagnostics, aptamers possess a great deal of potential. However, limited chemical diversity of natural nucleobases constrains the potential interactions with a given target. By the addition of functional groups to the nucleobases it is possible to overcome this obstacle. The split–combine click-SELEX procedure offers a new approach for screening multiple modifications to the target of interest in one selection. In this study, we applied this technology to prostate cancer cells *in vitro*. We identified a new class of clickmers, modified with different clicked moieties, that are capable to recognize prostate cancer cells. Each clicked moiety affected the binding to prostate cancer cells. In addition, dependent on the clicked moiety these clickmers exhibited altered binding properties and specificities. For instance, the clickmer dubbed S1 aptamer recognized PC3 and MCF-7 cells, respectively depending on its clicked moiety.

I 003 [101]
Investigating the role of newly identified AAA+ ATPases in Glycosomal Biogenesis in Trypanosoma brucei parasites

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Trypanosomes parasites are kinetoplastid protozoans that contain unique peroxisome-related organelles known as glycosomes. Glycosomal proteins are post-translationally imported into the organelle by a group of proteins called peroxins (PEX). Glycosomes are essential for the survival of parasites and glycosome biogenesis has been validated as an attractive drug target. AAA+ ATPases represent a class of over 30000 proteins associated with diverse cellular functions, which are widely distributed among various organisms. We have identified TbPEX1 and TbATAD1 as the homologs of Pex1 and Msp1, the known AAA-ATPases associated with peroxisomes in both yeast and humans. TbATAD1 has been previously characterized in our group for its subcellular localization to both glycosomes and mitochondria. We established a conditional knockout model of TbATAD1 and we show that ATAD1 is not essential for the survival of the bloodstream form parasites. We endogenously tagged PEX1 and using super resolution microscopy demonstrate its subcellular localization to the

glycosomes. Using RNA interference approach, we show that TbPEX1 is essential for the parasite survival. We also show that PEX1 plays a crucial role in the recycling of cargo receptors, as PEX1 RNAi resulted in degradation of the import receptors TbPEX5 and TbPEX7, in a proteasome dependent manner. Further, we provide evidence that PEX1 RNAi leads to partial mislocalization of glycosomal enzymes to the cytosol due to impaired glycosomal biogenesis resulting in significant depletion of cellular ATP levels which kills the parasites. Our results validate newly identified TbPEX1 or its interaction with the known TbPEX6 as promising drug targets against Trypanosomiasis.

I 004 [104]
Indole-functionalized clickmer interacts with and exhibits neutralizing potential against CoV-2 Spike variants

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Cell entry of SARS-CoV-2 is primarily mediated by Spike glycoprotein interaction with the human ACE2 receptor. The inhibition of this initial contact point of SARS-CoV-2 viral infection is a potential treatment strategy. Despite the speedy development pace of neutralizing antibodies, the appearance of SARS-CoV-2 immune escape variants shows that additional classes of affinity ligands with different binding modes and no antibody-dependent enhancement are needed. Aptamers are a class of affinity molecules that can be developed via *in vitro* process, SELEX. The chemical repertoire of unmodified oligonucleotides is limited and addition of various functional groups extends the chemical space by which an aptamer can interact with its target, in turn increasing the chance of successful SELEX. Here we implemented split-combine click-SELEX to introduce several functional groups using Copper-catalyzed azide-alkyne cycloaddition (CuAAC) click chemistry to a DNA library and screen for chemical moieties mediating best binding. We identified modified aptamer N2.5, clickmer, that interacts with CoV-2 Spike variants such as Mu, Delta and Omicron with high affinity (< 20 nM) upon indole functionalization solely. Cell culture experiments using SARS-CoV-2 spike-pseudotyped virus and live SARS-CoV-2 reveal neutralizing potential of the N2.5 against viral variants. Our findings provide proof of principle for modified aptamers and the methodology applied against a medically relevant viral target. Currently, we pursue generating homo-dimers of the truncates and seek to establish hetero-dimers with other characterized CoV-2 aptamers to improve neutralizing potency with a novel design.

I 005 [106]
Identification of the inhibitors of glycosomal membrane protein import machinery for anti-trypanosomatid drug development

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Insect-transmitted infections of trypanosomatid parasites lead to trypanosomiasis and leishmaniasis, which pose a major burden for public health. Glycosomes are peroxisome-related organelles found only in trypanosomatid parasites. Glycosomes compartmentalize the enzymes of glycolytic and other essential metabolic pathways. Previously, we demonstrated that the small molecule inhibitors of the glycosomal matrix protein import show a therapeutic effect in a mouse model of Human African Trypanosomiasis. PEX19 is the cytosolic receptor for the newly synthesized peroxisomal membrane proteins (PMPs). The PMPs contain membrane peroxisome targeting signals (mPTS) that are recognized by PEX19. Disrupting the PEX19-PMP protein protein interaction (PPI) would result in mislocalization of both matrix as well as membrane proteins, which is lethal for parasites. Therefore, this machinery provides an excellent target for developing new drugs against African sleeping sickness, Chagas disease, and leishmaniasis caused by the Trypansomatid parasites. In this project, we target parasite PEX19-PMP interaction with small molecule inhibitors to block the glycosome membrane biogenesis. To this end, we have identified and characterized the mPTS in different PMPs. An AlphaScreen® based assay has been established and optimized using recombinant PEX19 and synthetic His_6-tagged mPTS peptides. We have performed pilot screens and identified 3 compounds from the FDA approved drug repurposing library which inhibit

PEX19-mPTS interaction *in vitro*. Further, we are investigating the biological activities of these compounds against cultured *Trypanosoma* parasites for anti-parasitic activity and against mammalian cells for cytotoxicity.

I 006 [108]
Functional analysis of the TatA and TatB subunits of the Tat machinery in chloroplasts

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The Twin-arginine translocation (Tat) mechanism, one of the mechanism catalyzing protein transport across cellular membranes, is featured by its ability to translocate proteins in a fully folded conformation. It is found at the cytoplasmic membranes of bacteria and archaea, as well as at the thylakoid membrane of chloroplasts. Tat machinery consists of three subunits: TatA, TatB and TatC. TatB and TatC constitute the hetero-oligomeric TatBC-complex within the thylakoid membrane which provides the receptor function for the Tat substrates. In contrast, TatA is assumed to be responsible for the actual membrane translocation step in a yet unknown manner. Remarkably, TatA has a dual localization within chloroplasts, integral in the thylakoid membrane as well as soluble in the stroma, despite the fact that it shows considerable structure and sequence homology with TatB, which is found exclusively within the membrane. In order to characterize the roles of TatA and TatB during Tat translocation in more detail, we have developed an *in organello* reconstitution assay in which the intrinsic TatA or TatB activity is eliminated by specific antibodies followed by supplementing the assays with soluble TatA or TatB obtained from *in vitro* translation or heterologous overexpression. With this approach, we aim to quantify TatA/TatB demand during Tat transport and to unravel the potential functional relation between TatA and TatB.

I 007 [126]
The role of late endosomal chloride/proton exchangers in the autophagic-lysosomal pathway

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The intracellular CLCs, ClC-3 through -7, function as chloride/proton (Cl^-/H^+) exchangers in the degradative pathway, with distinct localisation on endosomal compartments. Their dysfunction leads to severe diseases. Loss of the renal ClC-5 impairs endocytosis resulting in kidney stones. Mutations in all other vesicular CLCs lead to a variety of neuronal and further disorders.
Gain-of-function variants of ClC-6 were recently identified in patients with an early-onset neurodegeneration. In addition, we identified a mutation that uncouples chloride from proton transport by ClC-6 in a patient with West syndrome, an epileptic disorder. We found that this mutation impairs the clearance of autophagosomes by blocking autophagosome-lysosome fusion.
ClC-7 is ubiquitously expressed on lysosomes and additionally on the ruffled border of bone-resorbing osteoclasts. Its dysfunction leads to osteopetrosis in mice and humans, often accompanied by a neurodegenerative lysosomal storage disease. We have analysed the impact of various mutations that differentially affect ion transport by ClC-7. One gain-of-function mutation, underlying a severe neurodegeneration, results in a tremendous enlargement of autophagosomal/lysosomal compartments. For another mutation, which accelerates the gating of ClC-7, we generated a knock-in mouse. Interestingly, the mouse develops severe osteopetrosis but no extraskeletal phenotypes.
These mutations in late endosomal Cl^-/H^+ exchangers, while differentially impinging on ion transport and underlying variable alterations in lysosomal morphology and function in various disorders, demonstrate the importance of Cl^-/H^+ exchange-mediated ion homeostasis in the autophagic/lysosomal pathway.

I 008 [150]
Aptamers as therapeutics – CCL17/22 targeting aptamers inhibit immune cell chemotaxis

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The chemokines CCL17 and CCL22 are associated with the pathogenesis of several autoimmune and inflammatory disorders. As ligands of the CCR4 receptor, CCL17 is mainly involved in the recruitment of T cells towards inflamed tissue, whereas CCL22 is also known to recruit regulatory T cells. Despite their different functions, previous studied mouse models for allergic contact dermatitis (ACD) showed that CCL17 and CCL22 deficiency reduces skin inflammation. The targeted inhibition of CCL17 and CCL22 in the dermis may therefore represent a new treatment approach for ACD.

Aptamers are a perfect tool for the development of inhibitory therapeutic agents. As synthetic single-stranded oligo(deoxy)nucleotides they can be selected to specifically bind to a target protein with high affinity and can impair the biological function of the protein upon binding. Previously, we reported the generation of RNA aptamers that bind to murine CCL17 and inhibit migration of CCR4+ cells *in vitro* and *in vivo* in an ACD mouse model. The aptamer application significantly alleviates ACD symptoms as evidenced in reduced ear swelling and less infiltration of immune cells into the ear skin.

We now identified two DNA aptamers, AJ1 and AJ2, specifically recognizing murine CCL22 to study their potential as therapeutic agent. AJ1 and AJ2 effectively inhibit the migration of CCR4+ T cells in an *in vitro* transwell-migration assay and are currently being tested in an ACD mouse model and as therapeutic agent for skin application. The results of this study will help us understand the function of CCL22 in the context of contact allergies and will reveal the potential of inhibitory aptamers for fundamental research and treatment.

I 009 [153]
ADP-ribosylation is a novel RNA modification with a potential role in RNA-ligation

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The function of RNA relies on a chemically diverse set of modifications, including ADP-ribose (ADPr) that was recently added. However, all data about ADP-ribosylated RNA were generated *in vitro* and evidence about the actual existence in mammalian cells or about potential functions was lacking so far.
Recently, we could demonstrate ADP-ribosylated RNA to be a highly dynamic modification in mammalian cells, by probing cellular RNA fractions with an ADPr-specific antibody. We identified candidates that catalyse and reverse RNA ADP-ribosylation in cells and characterized tRNA 2'-phosphotransferase 1 (TRPT1) as *bona fide* RNA-specific ADP-ribosyltransferase. The function of mammalian TRPT1 is largely unexplored. Tpt1, the fungal homolog of TRPT1, is an essential component of the fungal tRNA splicing and ligation machinery where it processes 2'-PO^4 splice-junctions to generate mature 2'-OH RNA. TRPT1/Tpt1 homologs are evolutionary highly conserved in bacteria, archaea and metazoa although their tRNA processing differs from that of fungi and does not result in 2'-PO^4 junctions.
We found that human TRPT1 ADP-ribosylates monophosphorylated RNA with 5'-terminal base specificity. ADPr protects the RNA against XRN-1 mediated 5'→3' nuclease activity. Furthermore, ADPr-RNA can be ligated by T4 RNA ligase 1 in the absence of ATP, resulting in an unconventional ligation product with an abasic site. In cells, we have identified small RNAs as a main substrate of TRPT1 catalysed ADP-ribosylation. This pool contains a mix of RNA species including 5S rRNA and tRNA. Through prospective research we aim to identify RNA substrates of TRPT1 mediated ADP-ribosylation and to understand the potential role of mammalian TRPT1 in (t)RNA ligation and repair.

I 010 [160]
Molecular Crowding: Effects on DNAzyme Activity

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Desoxyribozymes (DNAzymes) are artificially obtained, single stranded DNA molecules that are able to catalyse a broad spectrum of chemical reactions. One of the most prominent RNA-cleaving variants is the 10-23 DNAzyme. To this point, however, DNAzymes show high activity *in vitro* but poor activity in complex cellular systems, limiting their application *in vivo*. In this work, we demonstrate structural and functional analyses under simulated molecular crowding conditions *in vitro* using natural and synthetic macromolecules. Our results show that the crowder's concentration dependent influence on physical and chemical solution properties has a greater effect on DNAzyme activity rather than crowder type and size. In our structural analyses we could not observe any conformational changes in the size of the DNAzym:RNA complex. We further show that charged small molecules limit DNAzyme activity more rather than non-charged polymers. This study provides first insights into the limitations of molecular crowding effects on DNAzyme activity and targets towards modification strategies.

I 011 [173]
Pharmacological Readthrough as a therapeutic strategy for the treatment of Rett syndrome

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Translational readthrough (TR) describes the decoding of stop codons as sense codons through binding of near-cognate tRNAs to stop codons. As a result, the translational elongation continues in the 3'-UTR until the ribosome reaches the next in-frame stop codon which ultimately leads to C-terminally extended proteins. We co-discovered the first functional human 'readthrough' proteins, the extended isoforms of lactate dehydrogenase B (LDHB) and malate dehydrogenase 1 (MDH1) that naturally get extended by translational readthrough and carry a functional peroxisomal targeting signal in the readthrough extension. When we characterized endogenous (functional) TR, we realized that the stop codon context, i.e. the nucleotides in the vicinity of the stop codon, significantly influenced readthrough. Pharmacological TR induction can be used as a potential therapeutic approach to treat diseases caused by nonsense mutations, which account for 11% of all human genetic disease cases. Several different nonsense mutations in the gene *methyl-CpG-binding protein 2 (MECP2)* lead to the neurodevelopmental disorder Rett syndrome (RTT). We investigate the potential of readthrough therapy in a variety of RTT-causing *MECP2* nonsense mutations, considering the corresponding stop codon and the stop codon context.

I 012 [174]
Inhibition of viral RNA synthesis of SARS-CoV-2 by ASOs and siRNA targeting the 5'-UTR

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a pandemic virus of the genus Betacoronavirus. So far, it has infected 420 million people worldwide and caused approximately 5,9 million deaths as of June 2022. Despite its unique proofreading mechanism carried out by a 3'–5' exonuclease, several mutated variants emerged since the start of the pandemic.

Promising therapeutic strategies against viruses include nucleic acid-based drugs. Ideally, the nucleotide sequences of local target regions are conserved and structurally accessible. Therefore, we decided to targeted the 5'-UTR of SARS-Cov-2 by means of antisense oligonucleotides (ASO) and siRNAs.

The 5'-UTR sequence contains a highly conserved leader sequence (position 14-69). It consists of five conserved stem loop structures and an upstream open

reading frame of which the function is not determined yet.

Based on the 5'-UTR sequence we designed ten ASO targeting the 5'-UTR sequence. To study the inhibitory effect, we constructed a reporter gene containing the 5'-UTR sequence of SARS-Cov-2 upstream of the NanoLuc luciferase gene and analyzed each ASO effect by dual-luciferase reporter assay. Inhibition studies were performed by transient transfection in human HEK293T cells. The ASOs were compared at different concentrations and for the three most potent inhibitors a dose-response relationship was measured. Three ASOs reduced the expression by 80 % - 90 %. From the most potent ASOs we derived siRNAs and measured their inhibitory effects in the same cell system. We observed a maximal inhibition of 95 % and an IC_{50} value in the order of 1 nM. Ongoing studies focus on the two most potent siRNAs.

Based on these data, we plan to finally use cell systems that allow viral replication.

I 013 [185]
Real time detection of back-splicing and circular RNA by a stainable aptamer in cis.

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Circular RNAs (circRNA) are in the focus of RNA biology and cancer research. A majority of publications address human circular RNAs as disease marker and regulators involved in malignant cell proliferation. CircRNAs are thought to contain microRNA binding sites thereby being involved in regulatory processes. The ´sponge model´ considers circRNA as a regulator for microRNA. While the majority of knowledge about circRNA has been derived from theoretical studies, up-to-date, no conclusive experimental evidence exists for the biological function and role.

Based on our previous *in vitro* and cellular studies we attempt to describe the role of circRNA in human cells using transiently expressed circRNA containing microRNA binding sites. We used plasmids containing an expression cassette for circRNA which consists of long inverted repeats upstream and downstream of the segment containing the RNA of interest. These repeats allow back-splicing of the initial linear transcript and give rise to circRNA.

Here we describe a concept for the real-time detection of nascent circular RNAs in human cells using RNA aptamer technology. For this the aptamer sequence "RhoBAST" was split into two fragments and only back-splicing reconstitutes a circRNA containing a functional fluorescence light up aptamer (FLAP) element. We make use of the RNA-aptamer's specific quencher-dye-conjugate TMR-DN (Tetramethylrhodamine-dinitroaniline). The aim is to detect circRNAs in prolonged live-cell imaging for spatial and time-resolved studies and also enabling quantitative and qualitative analyses directly at the RNA-level.

I 014 [227]
INA (in-vitro detection of drug allergy) - Proteomics

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Medication can sometimes result in health deterioration by causing a harmful allergic response known as 'drug allergy'. Such drug allergic response is very relevant form of adverse drug reactions, as it is hard to diagnose and its symptoms can range from simple rash to life threatening condition.
The overall aim of the INA project is to investigate - by means of innovative, state-of-the art genomics and proteomics methods - basic principles which could facilitate the development of an in-vitro diagnostic test suitable for routine diagnosis of drug allergy. In particular, our proteomics research focuses upon identifying signature proteins from peripheral mononuclear cells (PMBCs) of drug-allergic patients, after in-vitro stimulation with the suspected drug as compared to those of non-allergic test persons.
Using bottom-up proteomics workflow, we have analyzed detectable proteins in in-vitro cultured PBMCs using liquid chromatography-mass spectrometry (LC-MS). In the biomarker discovery phase, using the MS proteomics data from 14 non-allergic (healthy) and allergic (patient) person's sample treatments (drug-

treatment versus no-treatment), we have identified around 80 proteins of interest. In future, we will analyse an independent set of samples using an untargeted/targeted LC-MS method to confirm these differential protein signatures upon drug allergic reaction.

I 015 [247]
In vitro assembly, positioning and contraction of a division ring in minimal cells

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Abstract
Constructing a minimal machinery for autonomous self-division of synthetic cells is a major goal of bottom-up synthetic biology. One paradigm has been the *E. coli* divisome, with the MinCDE protein system guiding assembly and positioning of a presumably contractile ring based on FtsZ and its membrane adaptor FtsA. Here, we demonstrate the full *in vitro* reconstitution of this machinery consisting of five proteins within lipid vesicles, allowing to observe the following sequence of events in real time: 1) Assembly of an isotropic filamentous FtsZ network, 2) its condensation into a ring-like structure, along with pole-to-pole mode selection of Min oscillations resulting in equatorial positioning, and 3) onset of ring constriction, deforming the vesicles from spherical shape. Besides demonstrating these essential features, we highlight the importance of decisive experimental factors, such as macromolecular crowding. Our results provide an exceptional showcase of the emergence of cell division in a minimal system, and may represent a major breakthrough towards developing a synthetic cell.

I 016 [263]
New insights into the resistance mechanism for the BceAB-type transporter SaNsrFP.

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A major challenge of our time is the treatment of bacterial infections due to the evolved resistance mechanisms of pathogens against antibiotics. To counteract this problem, it is necessary to understand the mode of action of the drug and the mechanism of resistance of the pathogen. The biosynthesis of the peptidoglycan (PGN), which is a critical feature of bacteria is one of the most potent antibiotic targets. Antimicrobial peptides (AMPs), such as nisin and colistin, targeting PGN synthesis are considered promising weapons against multidrug-resistant bacteria. However, human pathogenic bacteria conferring resistance to these compounds evolved by the expression of an ATP-binding cassette transporter of the Bacitracin efflux (BceAB) type that is localized in the membrane. In *Streptococcus agalactiae*, the BceAB transporter *Sa*NsrFP is known to confer resistance to the antimicrobial peptide nisin. The exact mechanism of action for *Sa*NsrFP is poorly understood. For a detailed characterization of the resistance mechanism, we heterologously expressed *Sa*NsrFP in Lactococcus lactis. By treating *Sa*NsrFP expressing *L. lactis* cells with different antibiotics, analyzing the peptidoglycan and peptidoglycan precursor accumulation, we demonstrated that *Sa*NsrFP conferred resistance to a structurally diverse group of antimicrobial peptidoglycan (PGN)-targeting compounds.
Our data elucidates a target protection mechanism, implying a direct involvement of *Sa*NsrFP in resistance by shielding the membrane-localized target of these antimicrobial peptides, resulting in modification of the cell wall.

I 017 [279]
Mistargeting of the mitochondrial processing peptidase to the cytosol leads to severe proteotoxic stress

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Mitochondria consist of many hundreds of different proteins that are synthesized on cytosolic ribosomes. Mitochondrial protein import mechanisms have been extensively studied in the past. Aminoterminal presequences ensure the reliable targeting of these

proteins into mitochondria. Subsequently to the import of these proteins, these presequences are proteolytically removed in the mitochondrial matrix by the mitochondrial processing peptidase, MPP. Whereas the import of proteins across the outer and inner membrane was well characterized, the processes occurring right before the translocation of a polypeptide remain unclear. In order to better understand the timing of the synthesis and import of precursor proteins, we engineered a yeast strain which expresses MPP in the cytosol. Expression of this cytosolic MPP (cytoMPP) is highly toxic as MPP cleavage in the cytosol obviously competes with mitochondrial import of precursor proteins. We plan to use this tool to address the following questions: How are different precursors sequestered to the mitochondrial surface? What determines post- or co-translational protein import? Which proteins are particularly sensitive to cleavage by cytoMPP? Which factors determine the import efficiency into mitochondria? On my poster, I will show the first exciting data obtained with this powerful cytoMPP tool.

I 018 [301] Possible Applications of Combining Optical Tweezer Measurements with Confocal Fluorescence Imaging

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Optical tweezers are a common tool to investigate the unfolding kinetics of single proteins. Combining this method with confocal microscopy allows studying in detail interactions with different fluorescently labelled small molecules or ligands. In future studies, we will track the binding of the fluorescently labelled HSP70/HSP40 system or dexamethasone to the ligand binding domain of the Glucocorticoid Receptor in real time. To minimize background noise caused by the fluorophores, switching the measuring conditions in < 1 s is required. To achieve this, a microfluidic setup is utilized.

Fluorescently-labelled proteins can also act as force sensors. Especially forces in the low piconewton regime are difficult to resolve. FRET shows a high dependency on distance changes in the nanometer range. One possible candidate for a force sensor is the Aslov2-Zdk2 complex. This complex shows a significant FRET-gradient around 3 pN. The peptide unbound state is used to calibrate the maximum fluorescence intensity, which is required for calculating the FRET-efficiency.

I 019 [298]
Exploration of the Kinetics of Toehold-Mediated Strand Displacement Using Single-Molecule Force Spectroscopy

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DNA/RNA toehold-mediated strand displacement (TMSD) is one of the fundamental reactions to achieve programmable molecular dynamic behavior in DNA nanotechnology and DNA/RNA circuits. Previous studies usually measured the kinetics of TMSD using fluorescent reporters, which alters the properties of the nucleic acids and could only display slow kinetics behavior. In this work, we firstly addressed an exploration of the dynamic branch migration process of the TMSD at a single-molecule level using single-molecule force spectroscopy (SMFS). DNA/RNA hairpins with the same toehold length were investigated by adding different invader strands. We observed the binding of the invader strand to the toehold and consecutive invading in two separate events. We also introduced different mismatches on the invader strand to fine-tune the branch migration kinetics. In constant distance measurements, we were able to study repetitive invading and reinvading at equilibrium conditions. Also, our study revealed the difference in the kinetics of strand displacement among DNA/DNA, RNA/DNA, and RNA/RNA hairpin-invader duplex combinations. Finally, we further estimated the TMSD rates via extrapolating to zero force conditions. These experiments provide a new perspective on the branch migration process of TMSD at a single-molecule level, exhibiting a more rapid and detailed equilibrium transition process.

I 020 [211]
APEX-based proximity labeling for time-resolved, quantitative cilia proteomics reveals proteome dynamics during active signaling

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The primary cilium is a central signaling hub and provides a special environment that concentrates signaling proteins to generate adequate responses to external stimuli. Effective cilia signaling depends on the dynamic transport of signaling components such as receptors and effectors into and out of cilia. Yet, apart from select factors, the extent of the proteomic remodeling of primary cilia during active signaling remains largely unknown.

We employ proximity labeling methods using cilia-localized ascorbate peroxidase (cilia-APEX) in combination with tandem-mass-tag-based quantitative mass spectrometry approaches to reveal the comprehensive proteomic alteration of primary cilia in response to signal. By profiling the cilia proteome in a time-resolved manner after inducing the hallmark primary cilium signaling pathway by Sonic Hedgehog stimulation, we could reconcile described changes in the localization of known Hedgehog signaling components. Importantly, we revealed a rapid removal of the cAMP-dependent protein kinase (PKA) holoenzyme, including the orphan GPCR GPR161, which functions as the A-kinase anchoring protein (AKAP) in primary cilia. Hierarchical clustering identified the putative phosphatase PALD1 that accumulates in cilia in response to active Hedgehog signaling to dampen signaling in a cell type-specific manner. Surprisingly, we find PALD1 enriched in cilia also in response to other stimuli, suggesting a more general function in ciliary signal transduction.

Our unbiased analyses demonstrate that proximity labeling in combination with quantitative proteomics allows time-resolved proteomics of primary cilia and provide novel insights into how primary cilia orchestrate signaling processes.

J 001 [36]

Dissecting the molecular functions of the RNA-binding, LCD-containing protein Rbfox1 in ovaries

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Drosophila Rbfox1 is a human RBFOX protein family homolog, deregulation of which is associated with multiple seemingly unrelated human diseases, such as autism, diabetes, obesity, epilepsy and spinocerebellar ataxia. We have previously shown that Rbfox1 is promiscuously included in different types of stress-dependent RNA granules and that its expression is regulated by a stress-related miR-980. Rbfox1 is an RNA-binding alternative splicing factor that contains a highly conserved RNA recognition motif (RRM). In addition, our data indicate that Rbfox1 has multiple low complexity domains (LCDs), which are domains crucial for liquid-liquid phase separation of cell proteins into liquid RNP granules. To understand the role of these Rbfox1 domains, we have generated mutant flies that lack either the RRM or some of the LCDs using CRISPR/Cas9-mediated mutagenesis. We found that these mutants have different phenotypes depending on a cell type and whether stress was applied, which means that different Rbfox1 domains have different functions in vivo. Since Rbfox1 deregulation leads to an abnormal stress response, a further analysis of CRISPR/Cas9 mutants will help to dissect the functional role of Rbfox1 domains. This knowledge should advance our understanding of how Rbfox1 deregulation contributes to deleterious symptoms, which comprise a vast spectrum of human disorders.

J 002 [58]

A mutation in the Ca2+ regulatory protein phospholamban (PLN R9C) disturbs cardiac excitation/contraction coupling with consequences for mitochondria and ER

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Defective Ca^{2+} handling impairs cardiac function. To assess the consequences of impaired Ca^{2+} cycling on excitation/contraction coupling, mitochondrial function and endoplasmic reticulum (ER) stress response, we used mice with a missense mutation in the Ca^{2+} regulator protein phospholamban (PLN^{R9C}-tg).
To monitor Ca^{2+} cycling and excitation/contraction coupling Fura-2 or edge detection was applied, respectively, and cardiac function was assessed by echocardiography. PLN^{R9C}-tg revealed an impaired diastolic Ca^{2+} re-uptake and speed of relaxation, and fractional shortening was significantly reduced. Interestingly, the respiration of isolated mitochondria of PLN^{R9C}-tg mouse hearts was disturbed, production of reactive oxygen species was enhanced, and ATP-production was reduced compared to wildtype (Wt). In line with these data, tetramethylrhodamine staining revealed a collapse of the mitochondrial membrane potential (mtMP) in cardiomyocytes isolated from PLN^{R9C}-tg mice. Also, expression levels of proteins involved in ER stress response were upregulated in PLN^{R9C}-tg compared to Wt mice as detected in an untargeted mass spectrometry approach and subsequent Western Blots. Early correction of Ca^{2+} defects by simultaneous overexpression of RKIP, an endogenous protein known to moderately stimulate β-adrenergic receptor signalling in cardiomyocytes, restored Ca^{2+} cycling in PLN^{R9C}-tg mice. Consequently, this also normalized cardiac contractility, adjusted proteostasis, restored mitochondrial respiration and ROS production.
This study validates the benefit of early Ca^{2+} correction and highlights its central role for the functional rescue of mitochondria and ER in the pathomechanism of heart failure.

J 003 [72]
Establishing a multi-lineage liver organoid from non-transformed cell lines

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Liver cancer, liver metastases and other liver diseases are a global health burden. Studies of cellular communication have to take interactions such as direct cell-to-cell contacts between different cell types, extracellular matrix and mechanical properties of tissue-like structures into account. Here, in vitro models such as organoids may be helpful. We utilized non-transformed cell lines derived from human hepatocytes (THLE-5b), umbilical vein endothelial cells (Ci-HUVEC), and mesenchymal stroma cells (Ci-MSC) as a model for certain aspects of liver tissue. Cells were co-cultured on matrigel according to an organoid protocol adapted from Takebe et al. (2014). Ci-HUVECs formed a layer or, when seeded on top of co-cultures containing THLE-5b cells and Ci-MSCs, Ci-HUVECs were recruited into the middle of the structures. However, Ci-HUVECs died within a few days. Endothelial cells need adhesion to collagen I for survival that is absent from matrigel. Indeed, Ci-HUVECs alone proliferated for 14 days on a mixture of matrigel and collagen I. However, in combination with THLE-5b cells and Ci-MSCs, Ci-HUVECs were lost again. Moreover, Ci-MSCs did not survive in 3D co-cultures in DMEM with 10% FCS that we apply for regular 2D-culture. However, they survived in a 3D co-culture with a medium with serum replacement. In 3D co-culture systems, conditions additional to growth factors in the medium and matrix composition have to be taken into account for Ci-HUVECs and/or medium requirements change compared to 2D-culture.

J 004 [84]
MS-based analysis of the PARP10 and CHIKV-nsP3 interactome reveals potential host factors regulated by MARylation

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Mono-ADP-ribosylation (MARylation) is a post-translational modification involved in host pathogen conflicts. Several PARP enzymes are induced by type I interferons or pathogen-associated molecular patterns, linking MARylation to innate immunity. This is supported by the fact that several positive single-stranded RNA viruses, such as Chikungunya Virus (CHIKV) or SARS-CoV2 virus, encode macrodomains (MDs). These MDs have been identified as hydrolases capable of reversing MARylation. However, how MARylation controls viral infections remains unknown.

To understand the role of MARylation in host-virus conflicts, we aimed at finding host factors regulated by MARylation. Through complementary mass spectrometry analysis of PARP10 and CHIKV-nsP3, harboring the viral macrodomain, we identified common interactors. Using co-immunoprecipitation experiments, we confirmed several of these interactions. Next, we used *in vitro* MARylation and hydrolase assays to analyze these proteins as substrates for MARylation/de-MARylation. One of the proteins identified as a common interactor and substrate *in vitro* and in cells is the essential stress granule (SG) protein G3BP1. G3BP1 is essential to CHIKV replication. Also, some of the IFN-induced PARPs are recruited to SGs or even trigger SG assembly upon overexpression.
We aim to understand functional consequences of G3BP1 MARylation and how CHIKV benefits from reversing this modification.

J 005 [90]
Taspase1 facilitates Topoisomerase IIβ-mediated DNA double-strand breaks driving estrogen-induced transcription

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Human protease Taspase1 plays a role in developmental processes and cancerous diseases by processing critical regulators, such as the leukemia proto-oncoprotein MLL. Despite almost two decades of intense research, Taspase1's biology is still poorly

understood and so far its cellular function was not assigned to one superordinated signalling pathway or a specific protein interacting network.
Our data functionally link Taspase1 and hormone induced, Topoisomerase IIβ-mediated transient DNA double-strand breaks, leading to active transcription. We identified this development- and cancer-relevant protease as an interactor of Topoisomerase II. This interaction enhances Topoisomerase IIβ-induced DNA double-strand breaks that are a precondition for stimulus-driven gene transcription. Moreover, the protease alters the epigenetic chromatin signature upon estrogen stimulus by cleavage of chromatin modifying enzyme MLL. As Estrogen-driven transcription and MLL-derived epigenetic labelling are reduced upon *TASP1* siRNA transfection, we finally characterize Taspase1 as a co-activator of estrogen-stimulated transcription.

J 006 [95]
A network biology-based approach for the evaluation of novel SMN-independent treatment targets for Spinal Muscular Atrophy (SMA)

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Spinal Muscular Atrophy (SMA) is caused by mutations of the survival of motoneuron 1 (*SMN1*) gene. Thus, lack of SMN protein leads primarily to the degeneration of α-motoneurons. Therapies increasing SMN protein levels improve symptoms and survival of patients, but several patients do not respond to therapy. In addition, previously non-visible multi-organ symptoms emerge in treated, severely affected patients. In this project, we aim to characterize alterations of molecular networks in the central nervous system both common and different to peripheral organs. For a better understanding of the central signaling pathways in SMA, we performed (phospho-) proteomic analyses of lumbar spinal cord in an SMA mouse model at symptom onset. We detected altered expression of 307 out of 3904 proteins and, 664 out of 9786 phospho-sites. 10 out of 40 most dysregulated proteins were known from literature, highlighting the reliability of data. Ingenuity Pathway Analysis displayed altered synaptogenesis and cholesterol biosynthesis. Since SMA is a multi-organ disease, we combined these neuronal data with proteomic analyses of peripheral organs from SMA mice. We identified cell cycle dysregulation present in lung, kidney, and spinal cord samples. These data highlight altered pathways as potential targets to find combinatorial treatment options for SMA.

J 007 [96]
Pex19 is required for peptide secretion in Drosophila neurons

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Peroxisomes are highly dynamic metabolic organelles that closely interact with other organelles such as mitochondria, endoplasmic reticulum, lysosomes, and lipid droplets. In *D. melanogaster*, loss of the peroxisomal biogenesis factor Peroxin 19 (Pex19) induces mitochondrial damage, delayed development, early lethality, and lipotoxicity evoked by upregulation of Lipase 3. Lipase 3 is a putative phospholipase that triggers free fatty acid accumulation. Peroxisome function declines in Diabetes patients; studies in mice show that a Peroxin 5 deletion causes impaired glucose homeostasis, increased apoptosis, elevated levels of very-long chain fatty acids (VLCFAs), and mitochondrial deterioration in pancreatic β-cells. We found that in *D. melanogaster*, peroxisome loss impairs insulin signaling and thereby the ability of the animal to react to nutrient restriction. Furthermore, loss of peroxisomes in a mutant for Pex19 impairs the secretion of Drosophila insulin-like peptides from the insulin-producing cells (IPCs), a set of neuroendocrine cells in the brain of the animal. This leads to reduced levels of insulin-like peptide in the hemolymph, and impairs insulin signaling in peripheral tissues. Lastly, we linked impaired secretion to Lipase 3 hyperactivity. Here we aim at unraveling how peroxisomes or Pex19 contribute to insulin-containing dense core vesicle trafficking and neuropeptide secretion.

J 008 [98]
Peroxisomes and Pex19 in growth control depending on nutritional context

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Peroxisomes are integral metabolic organelles assembled by peroxins. Mutations in the peroxisome biogenesis factor Peroxin19 (Pex19) lead to peroxisome loss and result in lethality in adult Drosophila melanogaster and humans. Pex19 mutants are sensitive to nutrient restriction, while the addition of medium-chain fatty acids rescues their lethality, as shown by Sellin et al. (2018).
Here, we monitor the larval growth in Pex19 mutants under various diets, while looking for differences in signaling pathways such as TORC1 and the respective microbiome. We could demonstrate that loss of Pex19 leads to the inability of using dietary protein for growth efficiently. As for lipids, Pex19 mutants elicit cholesterol toxicity under carbohydrate restriction as pupation is not achieved. Contrarily, adding cholesterol to a balanced diet impedes normal growth in wildtype in comparison to normal nutrient supply. Moreover, the absence of a carbohydrate source can be connected to a shift in the microbiome of Pex19 mutants: wildtype larvae react to carbohydrate restriction with an enrichment of *Lactococcus species*, which is absent in Pex19 mutants. Instead, various other bacteria species are enriched. We hypothesize that peroxisomes are required for appropriate nutrient signaling, which extends to microbiome composition. Our results suggest that peroxisomes are required for protein-driven organismal growth, which might be linked to a specific microbiome composition.

J 009 [99] ·
The ER protein Creld regulates ER-mitochondria contact dynamics and respiratory complex 1 activity

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Dynamic contacts are formed between endoplasmic reticulum (ER) and mitochondria that enable the exchange of calcium and phospholipids. Disturbed contacts between ER and mitochondria impair mitochondrial dynamics and are a molecular hallmark of Parkinson's disease, which is also characterized by impaired complex I activity and dopaminergic neurodegeneration. Here we analyzed the role of Cysteine-rich with EGF-like domain (Creld), a poorly characterized risk gene for Parkinson's disease, in the regulation of mitochondrial dynamics and function. We found that loss of Creld leads to mitochondrial hyperfusion and reduced ROS signaling in Drosophila melanogaster, Xenopus tropicalis, and human cells. Creld fly mutants show enhanced, but less functional ER-mitochondria contacts: phospholipid transfer at mitochondria-associated membranes (MAM) is reduced. This impairs respiratory complex I activity. The resulting low hydrogen peroxide levels are linked to disturbed neuronal activity and lead to impaired locomotion, but not neurodegeneration, in Creld mutants. We conclude that Creld regulates ER-mitochondria communication and thereby hydrogen peroxide formation, which is required for normal neuron function.

J 010 [100]
Nutrient-dependent trafficking of organelles in neurons of the CNS of Drosophila melanogaster

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Peroxisomes are vital and highly dynamic organelles that compartmentalize metabolic reaction in eukaryotic cells. They are involved in complex metabolic reactions such as the β-oxidation of very-long-chain fatty acids (VLCFAs), the formation of ester phospholipids (like plasmalogen), the catabolism of branched-chain fatty acids, the production of bile acid, polyamine oxidation and amino acid catabolism. Mutations in peroxin (*pex*) genes, responsible for peroxisome assembly and function, result in the loss of functional peroxisomes and lead to the development of peroxisome biogenesis disorders (PBDs) in humans. Peroxins are highly conserved in *Drosophila melanogaster*, making them a convenient model organism to gain insight into human disease. From previous studies of the Drosophila PBD model Pex19 mutant, we found that they are developmentally delayed and have disturbed insulin signaling. To investigate insulin-like peptide secretion, Drosophila insulin-like peptides (DILPs) were observed from the insulin-producing cells (IPCs) in the brain under controlled nutrition condition. Molecular relation between DILPs and pex3, pex19 and peroxisome were specifically studied, and how they contribute to vesicle

trafficking in IPCs. We found that the kinesin unc-104 contributes to Pex19-dependent DILP release.

J 011 [114]
Oxidative stress dependent regulation of Ataxia Telengiectasia Mutated (ATM) kinase at the central nervous system

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The brain is composed of billions of neurons, which connect to each other using electrical or chemical signals. To sustain this complex system, the brain consumes an intensive amount of oxygen and glucose to fulfil the needs in ATP. As a result, neurons produce a robust amount of reactive oxygen species (ROS). Uncontrolled production and insufficient elimination of ROS from the antioxidant system can be detrimental and damage essential macromolecules such as enzymes and structural proteins leading to brain dysfunction. The ataxia-telangiectasia mutated (ATM) kinase is mutated in the disease ataxia-telangiectasia, a rare autosomal disorder characterized by neuronal degeneration, cancer, immune deficiency, increased sensitivity to ionizing radiation, growth retardation and premature aging. Aside from the classical nuclear function of ATM in sensing DNA double strand breaks, ATM also exploits cytosolic signaling pathways by responding for example to oxidative stress. In fact, several mechanisms point towards a protective effect of ATM against ROS-induced damage of the brain. For example, the ATM regulation of metabolic pathways as well as of autophagy may safeguard against neuronal dysfunction. Recently we showed that ATM can phosphorylate the actin binding protein Drebrin to improve the stress resilience in dendritic spines, the post-synaptic site of glutamatergic synapses suggesting that ATM control of the actin cytoskeleton may also protect against ROS induced synapse dysfunction. Further understanding how dysregulation of ATM may lead to anomalies in the physiological properties of the synapse will open new avenues on therapeutic strategies for patients with ataxia-telengiectasia or other neurodegenerative diseases

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J 012 [183]
Dissecting the molecular mechanisms of neurodegeneration in a Drosophila SWS/NTE neuropathy model

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Mutations in human Neuropathy Target Esterase (NTE) gene cause motor neuron disease called spastic paraplegia, in which long spinal axons degenerate leading to limb weakness and paralysis. *Drosophila* Swiss cheese (SWS) is a highly conserved orthologue of vertebrate NTE, phospholipase that can degrade endoplasmic reticulum-associated phosphatidylcholine. Similar to humans, mutations of *sws* in *Drosophila* cause progressive neurodegeneration, shortened lifespan, and interestingly, activated innate immunity response. NTE/SWS is required for the glial wrapping of neurons; however, the role of SWS in glia is not clear. NTE/SWS is expressed in the glia, forming the blood-brain barrier (BBB) and its loss or downregulation affects BBB integrity. Importantly, glia-specific expression of *Drosophila* SWS or human NTE in *sws* mutant background fully rescues the organization of the surface glia and could partially rescue BBB permeability, suggesting a conserved function of NTE/SWS in glia. Moreover, the BBB phenotype can also be alleviated by anti-inflammatory agents. We hypothesize that abnormal phosphatidylcholine metabolism caused by the absence of NTE/SWS phospholipase results in abnormal amounts of unsaturated fatty acids. Understanding the molecular mechanisms of inflammation in neurodegenerative diseases may help to promote the use of anti-inflammatory therapy and dietary supplements for age-dependent neurodegeneration.

J 013 [216]
NAADP redox cycle during T cell activation

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NAADP is a potent Ca^{2+} releasing second messenger important in T cell activation. After stimulation, NAADP is rapidly formed, contributing to the first Ca^{2+} signals (Wolf, Diercks et al. 2015. Science Signaling 8(398): 1-13). These signals later influence T cell effector functions, making the NAADP signaling pathway an attractive target for pharmacologic interventions. The therapeutic potential of modulating NAADP signaling was demonstrated using the NAADP-antagonist BZ194 in a mouse model of multiple sclerosis (Cordiglieri et al. 2010. Brain 7(133): 1930-1943, 2010), inviting further research. To identify the NAADP forming enzyme during T cell activation, we established a membrane-based assay for NAADP synthesis. Using a high-performance liquid chromatography (HPLC) system, we discovered that NOX-family NADPH oxidases are able to synthesize NAADP from its reduced precursor NAADPH under physiological conditions. Additionally, we identified glucose-6-phosphate-dehydrogenase as the only cellular dehydrogenase able to reduce NAADP to its inactive precursor. Knockout of dual NADPH oxidases (DUOX1/2) reduces Ca^{2+} microdomains in CD4+ T cells during CD3/CD28 stimulation. This effect is independent of H_2O_2 generation and not further reduced by BZ194, pointing towards a NAADP redox cycle controlling initial Ca^{2+} signals during T cell activation (Gu, Krüger, Roggenkamp et al. 2021. Science Signaling 14(709): eabe3800). We are currently using a modified version of the HPLC assay and high resolution Ca^{2+} imaging with different knockout models and inhibitors to investigate the regulation of the NAADP redox cycle and will share our first insights on its integration into existing networks controlling T cell activation.

J 014 [220]
Interaction of the NAADP binding protein HN1L/JPT2 with its target receptors in T cells

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During an immune response, T cell activation significantly depends on changes in the free cytosolic calcium concentration ($[Ca^{2+}]_i$). In the early phase of T cell activation nicotinic acid adenine dinucleotide phosphate (NAADP) is formed within seconds and mobilizes Ca^{2+} from internal stores[1]. Still, the target organelles and channels involved remain controversial. We hypothesize that in T cells, NAADP acts on the ryanodine receptor 1 (RYR1) in the endoplasmic reticulum (ER) via a small cytosolic NAADP binding protein, which we and others independently identified as HN1L/JPT2[2,3].
Here, we want to understand the exact mechanisms connecting HN1L/JPT2 and RYR1 to NAADP signaling. A novel fluorescence-based photoaffinity labeling assay of HN1L/JPT2 will be presented; this assay is a prerequisite to characterize NAADP's binding properties to HN1L/JPT2.

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K 001 [56]
Exchange of the structural determinant defines different functions of monothiol and dithiol Glutaredoxins

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Glutaredoxins are divided into two major subfamilies: monothiol and dithiol Glutaredoxins. Members of both subfamilies are able to coordinate FeS cofactors. Whereas most of the monothiol Glutaredoxins lack the activity as oxidoreductase but shows functions in Fe metabolism, dithiol Glutaredoxins are oxidoreductases, but are not functioning in Fe homeostasis. We identified the structural difference determining the functions and thereby the two subfamilies of Glutaredoxins. Engineered mutants revealed that we are able to turn a dithiol Glutaredoxin into a monothiol Glutaredoxin and vice versa. Different spectroscopic methods demonstrated that on a molecular level, the structural changes determine orientation of the two monomers in the holo dimer and thereby FeS cluster stability and GSH binding. On a functional level, the structural changes regulate processes such as heme biosynthesis in zebrafish.

K 002 [63]
A permanent AIFM1-MIA40/CHCHD4 complex drives complex I assembly through efficient import of NDUFS5

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AIFM1 mediates mitochondrial import of MIA40/CHCHD4, the import receptor of the disulfide relay. Here, we demonstrate that AIFM1 and MIA40/CHCHD4 cooperate beyond MIA40/CHCHD4 import. We show that AIFM1 and MIA40/CHCHD4 form a stable long-lived complex not only in vitro but also in different cell lines and tissues. Remarkably, in AIFM1 knockout HEK293 cells, levels, localization and redox state of MIA40/CHCHD4 are unchanged. In absence of AIFM1, MIA40/CHCHD4 fails to efficiently interact with specific substrates and thus their import is impaired. This is especially severe for NDUFS5, a subunit of complex I of the respiratory chain. As a consequence, NDUFS5 accumulates in the cytosol and undergoes rapid proteasomal degradation. Lack of mitochondrial NDUFS5 results in stalling of complex I assembly at a specific intermediate. Collectively, we demonstrate that AIFM1 serves two overlapping functions: importing MIA40/CHCHD4 and constituting an integral part of the disulfide relay that ensures efficient interaction of MIA40/CHCHD4 with specific substrates.

K 003 [73]
Castles made of… oxygen? Challenging the detection of sulfenic acids in biological samples

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The formation of cysteinyl sulfenic acids is often thought to be the initial oxidative protein modification in redox signaling and under so-called oxidative stress. Dimedone and its derivates are used as selective probes for the nucleophilic detection of sulfenic acids in biological samples. However, qualitative analyses suggested that dimedone also reacts with cyclic sulfenamides. Furthermore, under physiological conditions, dimedone must compete with the highly concentrated nucleophile glutathione. Here we show that glutathione kinetically outcompetes dimedone by several orders of magnitude and that the origin of dimedone-labeled peptides from biological samples cannot be unambiguously assigned to cysteinyl sulfenic acids. Comparative *in vitro* and intracellular dimedone labeling experiments point towards unidentified reaction pathways or unknown redox species. We would therefore like to recommend (i) to include glutathione competition assays for the future development and application of nucleophilic probes against sulfenamides, sulfenic acids and other

electrophilic cysteinyl modifications, and (ii) to interpret proteomic data from dimedone labeling studies with care.

K 004 [253]
A Non-Canonical PDZ Domain Involved in Ciliogenesis and Planar Cell Polarity

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The Rab family of small GTPases plays a pivotal role in the organization of intracellular trafficking and maintenance of organelle identity. The spatiotemporal control of GTPase activity requires guanine nucleotide exchange factors (GEFs), which act as the activators of small GTPases. Recently, the Tri-Longin-Domain-Rab (TLDR) GEFs has been identified as a sub-family of GEFs based on bioinformatic analysis[1]. The TLDR GEF family comprises the heterodimeric complexes Mon1-Ccz1, BLOC-3 and Inturned-Fuzzy in metazoans, and the Mon1-Ccz1 complex is conserved all the way to yeast. We previously determined the mechanism of nucleotide exchange by Mon1-Ccz1 from the structural and biochemical characterization of a catalytic core complex[2] and derived a model for membrane recruitment of the complex based on the structure of the full complex[3].
Continuing along these lines, we are currently establishing the similarities and differences between Mon1-Ccz1 and the related BLOC-3 and Inturned-Fuzzy complexes on a structural and functional level.
A unique feature within the TLDR GEF family is the N-terminal PDZ domain of Inturned. PDZ domains are a common protein-protein interaction motif and facilitate target binding with affinities in a micromolar range. By structural, bioinformatic and biochemical approaches we have determined that the Inturned-PDZ domain represents a novel non-canonical PDZ-like fold. We are currently working towards identifying its binding targets and functional roles.

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K 005 [299]
Functional characterization of Arabidopsis thaliana CC-type glutaredoxin ROXY9

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Glutaredoxins (GRXs) are small proteins with a conserved thioredoxin fold. Class I GRXs with a CPYC consensus active site are mainly active as oxidoreductases using glutathione (GSH) as an electron donor. In contrast, class II GRXs with a CGFS active site bind Fe-S clusters as a dimer along with two GSH molecules. Plants possess a third class of GRXs with the unusual CC(M/L)(C/S) active site motif. The *Arabidopsis thaliana* class III GRX ROXY9 exhibits a similar fold as class I AtGRXC2. In contrast to AtGRXC2, ROXY9 shows no or only weak oxidoreductase activity on glutathionylated substrates. When subjected to a range of GSH/GSSG ratios, ROXY9 forms a disulphide bridge at a midpoint redox potential of around -240 mV. In contrast, AtGRXC2 was either glutathionylated at the first active site cysteine or formed a disulphide over a wide range of redox potentials. Since GSH-dependent redox reactions require the glutathionylated intermediate, the altered GSH binding mode of ROXY9 most likely explains its poor oxidoreductase activity. The feature is not due to the peculiar active site, as replacing the motif by CPYC does not enable stronger activity, but most likely due to other amino acids in the GSH binding groove.

L 001 [15]
Rational targeting of Taspase1 nuclear import and biological function by supramolecular ligands

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Taspase1 is a threonine-aspartase that plays an important role in cancer. While it is generally overexpressed, but not mutated in most tumor cell lines, many of its substrates are associated with tumor development, the most prominent being Mixed Lineage Leukemia (MLL). Taspase1 is therefore classified as a non-oncogene-addiction-protease.

Taspase1 is synthesized as an inactive pro-enzyme and has to enter the nucleus first to activate itself by autoproteolysis. To enter the nucleus, Taspase1 has to interact with Importinα by its Nuclear Localization Sequence (NLS). Mutations in this sequence render Taspase1 inactive and similar effects should be possible by blocking the NLS with ligands.

Our aim is therefore the development of rationally designed supramolecular ligands that interfere with Taspase1 function by blocking either the active site or the NLS of Taspase1. After the successful development of the first known supramolecular disruptor of the Taspase1/Importinα interaction, we have now even developed supramolecular inhibitors with a dual-inhibition mechanism that can disrupt the interaction with Importinα, thereby disrupting the activation process, as well as inhibit the enzymatic activity of activated Taspase1 directly.

L 002 [16]
Targeting Taspase1 with divalent molecular tweezers

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Threonine aspartase 1 (Taspase1; TASP1) is an evolutionarily conserved threonine protease and a member of the type 2 asparaginase family. To date, 27 potential target proteins of TASP1 are known, including the oncologically relevant proteins MLL, TFIIA and USF2. While TASP1 is normally expressed during embryonic development and is scarcely present in differentiated adult tissues, the protease is overexpressed in many tumor cell lines. Although TASP1 alone is unable to transform benign cells into tumor cells, the protein plays a crucial role in the development and maintenance of cancer, making it an interesting target for future cancer therapies. We identified a flexible loop with a two-part basic nuclear localization signal (NLS) as a target structure. This loop is essential for the protein activation process, as TASP1 is synthesized as an inactive proenzyme and must be imported into the nucleus through the interaction of the NLS with Importin α. There, TASP1 is autocatalytically cleaved and rearranged to an active heterotetramer. In this study, we want to address the NLS with lysine/arginine binders. We investigated the effects of three different divalent tweezers combined with calixarenes or AIE units. First, the binding of the tweezers to TASP1 was confirmed by NMR and anisotropy experiments. Next, we analyzed the inhibitory effect of the divalent tweezers on TASP1 activity using cleavage assays. The calixarene tweezer significantly reduced the proteolytic activity of TASP1, in contrast to the other divalent tweezers showing a moderate inhibitory effect. Using pull-down experiments, we demonstrated that the AIE tweezer disrupts the TASP1/Importin α-interaction, whereas the calixarene tweezer seems to act rather enhancing.

L 003 [31]
NMR to study Supramolecular Chemistry on Protein Surfaces

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Nuclear Magnetic Resonance (NMR) spectroscopy is ideally suited to monitor ligand binding to proteins with single-residue resolution. However, binding of large supramolecular ligands to proteins and protein-protein complexes poses additional challenges due to the size and complexity of the systems.

Here, we present two examples how NMR methodology has been extended to study supramolecular ligand binding to proteins.

Side chain specific experiment for lysine and arginine residues directly observe the atoms encapsulated by ligands that recognize these cationic side chains, e.g. supramolecular tweezers or calixarenes. Monitoring the side chain NMR signals allows a precise ranking of multiple ligand binding sites on a given protein.

Aggregation-induced emission (AIE) fluorophores show an increase in fluorescence once their intramolecular motion is restricted. The combination with one or multiple protein recognition motifs results in a fluorescence turn-on signal upon protein binding for a convenient and direct read-out.
We solved the NMR solution structures of an AIE fluorophore carrying one peptide, both alone and in complex with the hPin1-WW domain. These structures give insight how contacts between the aromatic AIE core and the protein restrict intramolecular motion and thus induce the increase in fluorescence.

L 004 [44]
The Alternative Complement Pathway is Active in the Urine of Nephrotic Mice and Humans

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Introduction:
Complement activation is often involved in glomerulopathies. In nephrotic syndrome (NS), high proteinuria and aberrant filtration of complement factors are common. However, the activation of the alternative complement pathway (AP) in nephrotic urine from mice and humans is ill-defined.

Methods:
Activation of the AP (C3, D & B) was studied using urine samples from nephrotic mice (n=12) and humans (n=14). Human samples were obtained from patients with acute NS. In wild-type or C3, CFD & CFB deficient mice, NS was induced with doxorubicin. Western blot was analyzed, under reducing (R) and non-reducing (NR) conditions, with primary antibodies against CFD, CFB and C3.

Results:
The C3, CFB and CFD antibodies were specific after validation against mice knock-out samples. Under NR conditions, full-length C3 (≈ 186 kDa), CFB (≈ 90 kDa), and CFD (≈ 40 kDa in mice and ≈ 38 kDa in humans) were excreted in the urine of nephrotic mice and humans. Under R conditions, the clear degradation products in mice samples were C3 α′-chains ((≈ 100 kDa and 115 kDa), C3 α′-chain fragments (≈ 41 and 38 kDa), Bb fragments (≈ 60 kDa), Bb degradation products (≈ 38, 30 and 25 kDa). In complement-deficient mice, the full length and degradation products were absent. About 70% of the human samples show activation of the AP with C3, FB and FD fragments or degradation products.

Conclusion:
NS leads to increased excretion of complement factors of the AP into the urine which undergo activation and degradation. C3 can be active in the absence of CFB, but it requires CFD for full activation, while CFB requires CFD for activation. Urinary complement activation might play a role in kidney disease.

L 005 [47]
Exploring the Inhibition of Spinocerebellar Ataxin-3 Aggregation by Molecular Tweezers

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Spinocerebellar ataxia type 3, also known as Machado-Joseph disease, is a neurodegenerative disorder caused by polyglutamine tract expansion and further fibrillogenesis of the Ataxin3 (ATXN3) protein. The

molecular tweezer CLR01 is a synthetic small molecule that selectively binds lysine (K) and arginine (R) residues and could potentially inhibit toxic protein aggregation. Here, Gaussian accelerated Molecular Dynamics (GaMD) simulations were performed on selected models of the Josephin domain (JD) of ATXN3 protein to understand the effect of CLR01. Our results showed that the half-open form of JD is stabilized when complexed with the CLR01 molecules while the flexibility of the domain decreases. We identified four binding sites that could explain the chemical shift, observed in NMR studies, for residues in the vicinity of the K/R encapsulated by the tweezers. In addition, we estimated the free energy changes for the binding of CLR01 to the JD and revealed those positions in which the interaction of the molecular tweezer to exposed K/R is favored. Thus, we propose the encapsulation of those identified residues by CLR01 as key mechanistic insights for the modulation of JD-JD binding. Our work provides a better understanding of the role of molecular tweezers in the inhibition of the first steps of the ATXN3 aggregation pathway.

L 006 [88]
NMR-based interaction studies between functionalised Au-Nanocarriers and E. coli Peptide Deformylase (ecPDF)

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The rising number of drug-resistant pathogens poses a global health threat. One strategy to overcome the cell wall barrier from drug resistant gram-negative bacteria is to combine Gold-Nanoparticles (AuNP) as drug delivery systems with antibiotic ligands. AuNP can be coated with antibiotics and thereby alternate their physicochemical properties, while they have been reported to exhibit antimicrobial effects on their own depending on their size.

Here, we demonstrate the proof-of-principle of this dual-mode approach in terms of functionalisation of AuNP, their binding capability to their target protein, and the possibility of releasing their antimicrobial payload. To this extent, we coated AuNP (50 nm) with the well-known antibiotic actinonin, a high-affinity ligand for bacterial Peptide Deformylase (PDF). PDF is involved in bacterial protein maturation processing and therefor a potential drug candidate.

Interactions between ^{15}N-enriched His-ecPDF 1-147 and actinonin-coated AuNP were investigated *via* 2D ^{1}H-^{15}N-HSQC NMR spectra. Here, we report the successful preparation of actinonin-covered AuNP (50 nm) and a direct binding of AuNP to the PDF protein. Subsequently, the ligand could be successfully released from the AuNP by adding reducing agent, and it was indeed still able to bind ecPDF. This was also true for ultra-small PMBA-AuNP (2 nm), indicating that carboxylic acid groups are able to coordinate the protein metal ion as well.

L 007 [141]
Small Oligomers of Molecular Tweezers disrupt the essential NDC80 Interaction with Microtubules

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Cell division is a critical step of the cell cycle in which the kinetochore ensures faithful segregation of chromosomes in eukaryotes. Chemical compounds that modulate kinetochore-microtubule interactions thus are of interest for chromosome segregation studies and for designing anti-mitotic compounds. Here we report on the development and characterization of ligands that target a conserved interface between the Ndc80 complex and the microtubule lattice. Surface-exposed lysines of the Ndc80 calponin-homology domain are addressed with Lys/Arg-directed molecular tweezers. Characterization of tweezer conjugates with varying numbers of binders shows that the trimeric compound ABS198 and the small dimeric compound ABS265 bind Ndc80-CH with low micromolar affinity. Analysis of tweezer-CH domain interactions by NMR reveals that these tweezers target biologically relevant lysine residues on Ndc80 which is supported by MD simulations. Direct observation of microtubule binding via TIRF microscopy showed that the tweezers inhibited the interaction between the full Ndc80 complex and microtubules in a dose-dependent manner while

having minimal impact on microtubule dynamics. Our study provides a proof-of-principle for the application of molecular tweezers in the kinetochore-microtubule system and identifies ABS198 and ABS265 as scaffolds for future compounds with increased selectivity for Ndc80.

iGEM Team Düsseldorf:
CosMIC - sustainable biomanufacturing on earth and beyond

Our project "CosMIC" is tackling the problem of limited lifetime of supplies and the lack of adequate manufacturing options on remote expeditions, focusing especially on the conditions in space.

We are aiming to establish an independent and self-sustaining alternative manufacturing system, containing a 3D-printable bioink produced by soil bacterium *Azotobacter vinelandii*. By overexpression of genes associated with alginate biosynthesis and introduction of a cellulose expression-system, our bacteria form a hydrogel meeting the required mechanical properties to be used as 3D-printable bioink. By linking the cellulose synthase to an optogenetically inducible system, various characteristics and stability can be achieved upon desire. In doing so, we aim to provide a solution for self-sustained and ecologically friendly manufacturing in the future - on earth and beyond.

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iGEM Team Bonn:
Cyan Energy

Cyanobacteria has the capability to produce hydrocarbon such as alkane.
Since the biosynthetic hydrocarbon is very similar chemically and structurally than the ones found in fossil fuels, it has the potential to be a replacement.
The enzymes aldehyde deformylating oxygenase (ADO) and acyl-ACP reductase (AAR) have been confirmed by researchers to be responsible for catalyzing the production of bio-hydrocarbon in cyanobacteria.
In order to optimize the expression of the two enzymes, expressions of other less important enzymes are inhibited. We do this by knocking out genes.
Once the modified bacteria produce enough biomass, we can harvest the alkane by centrifuging it. This will let the alkane sink to the bottom and create a pellet. The supernatant can be discarded and pellet can be resuspended in organic solvent.

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iGEM Team Wien:
Project Pichitecture

Creating an environmentally friendly building material with the help of Synthetic Biology The 2019 IEA Global Status Report for Buildings and Construction [1] revealed that 11% of global CO2 emissions are caused by the production of building materials. Inspired by the work of Heveral et al. (2020), we set out to create a brick material from two microorganisms: the cyanobacterium synechocystis PCC 6803, due to their ability to biomineralize, and the yeast pichia pastoris, the strain of which used here also has a carbon fixation ability that aids their growth.
In this work, we create plasmids vie Golden Gate Assembly for the following biopolymers: spider silk (MaSp1 & PySp), gelatine and PHB. The plasmids are codon optimized for both expression on our yeast and in the cyanobacteria strain.
Meanwhile, we culture cyanobacteria in conditions optimized for biomineralization.
After mixing the produced components with desert sand and lignin, we measure the brick properties at room temperature and 4°C drying conditions.

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iGEM Team Aachen:
Supporting the basis of life – MetaPhos

Phosphate is an important but finite resource and is used on a large scale, for example in agriculture, as it is an essential growth factor for plants. However, reserves are dwindling and massive use is causing accumulations of the substance in groundwater. This is where MetaPhos comes in - modified enzymes targeting phosphate recycling. We address both of these problems by recovering valuable phosphate from groundwater and processing it for industrial reuse. This is achieved by using modified phosphate binding proteins which can be activated and deactivated by blue light irradiation.

Interplay of inositol pyrophosphate pathway and iron-sulfur cluster biogenesis

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The bifunctional kinase/pyrophosphatase Asp1 of Schizosaccharomyces pombe is classified as a member of the highly conserved PPIP5K/Vip1 family. The structural composition of Asp1 enables a dual function, where the N-terminal kinase domain catalyzes phosphorylation reactions on inositol pyrophosphate molecules (IPPs), thus generating highly-energetic pyrophosphate functional groups. The second half of the dual function is assumed by the pyrophosphatase domain, which catalyzes the hydrolytic cleavage of phosphate groups from the pyrophosphate functionality. Thus, Asp1 is autonomously able to generate a specific class of inositol pyrophosphates (IPPs) that act as signaling molecules. Furthermore, extensive research has revealed that Asp1 is involved in many essential biological processes, including cell morphogenesis, chromosome segregation and microtubule stability. Recently, it was discovered that the pyrophosphatase domain of S. pombe Asp1 binds an Fe-S cluster. However, the structure and role of this cofactor remain elusive. Here we present our results focusing on the nature of the iron-sulfur cluster, its coordination environment, and its biological function. Our results contribute to a better understanding of this fascinating bifunctional protein of the PPIP5K/Vip1 family.

All-optical Super-resolution Imaging of Molecules in Their Nanoscale Cellular Context

Jörg Bewersdorf

Super-resolution optical microscopy has become a powerful tool to study the nanoscale spatial distribution of proteins of interest in cells over the last years. Imaging these distributions in the context of other proteins or the general cellular context is, however, still challenging. I will present recent developments of our lab which facilitate access to super-resolution microscopy for a broader community: A new fluorogenic DNA-PAINT probe enables fast, high-quality, 3D whole-cell imaging without the need for optical sectioning, adding a versatile and easily accessible tool to the toolbox of single-molecule super-resolution probes [1]. Labeling proteins and other cellular components in bulk in our recently developed pan-Expansion Microscopy method provides ultrastructural context to the nanoscale organization of proteins, replacing complex correlative light/electron microscopy by an all-optical approach to imaging cells and brain tissue sections [2, 3].

Financial Interest Disclosure: J.B. has financial interest in Bruker Corp. and Hamamatsu Photonics and is co-founder of a startup company related to Expansion Microscopy.

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Mitochondrial complex I: evolution, assembly and mechanism

Ulrich Brandt

Abstract not submitted

Direct Interaction of Smoothened and PKA Opens Up a New Window into Hedgehog Signaling

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Hedgehog signaling is important for the development of a complex organism and tissue homeostasis in adults. Dysregulation leads to severe developmental defects or cancer. The extracellular morphogen hedgehog (Hh) activates Hh gene transcription via a network of spatiotemporally regulated components. A central, yet poorly understood step of the Hh cascade is the inhibition of cAMP-dependent protein kinase (PKA) by the G-protein coupled receptor (GPCR) Smoothened (SMO). Active SMO inhibits the phosphorylation of the transcriptional regulator GLI by the catalytic subunit of PKA (PKA-C). Canonically, PKA is inhibited at low cAMP levels and GPCRs regulate intracellular cAMP levels by the regulation of adenylyl cyclases. Although SMO couples inhibitory G-proteins, those are not responsible for Hh mediated PKA-C inhibition by SMO. The recently demonstrated membrane recruitment of PKA-C by the SMO C-tail suggests a direct interaction. We employed Surface Plasmon Resonance (SPR) to characterize wildtype and mutant PKA-C binding to the recombinant SMO C-tail. An activity assay for PKA was then used to verify the inhibition of PKA-C by the SMO C-tail in vitro. It could be shown that PKA-C binds to a sequence in the SMO C-tail which resembles the consensus recognition sequence of PKA-C found in other PKA pseudosubstrate inhibitors. The SMO C-tail also inhibits phosphorylation of peptide substrates by PKA. These results suggest a direct inhibition of PKA-C by the SMO C-tail which acts as a pseudosubstrate and physically separates PKA-C from its substrate GLI. Our results point to a novel mechanism of PKA regulation by GPCRs, allowing SMO to transduce Hh signals. This provides a new perspective on the spatiotemporal regulation of PKA.

Visualizing and Manipulating Cell Surface Proteins

Johannes Broichhagen

Selective targeting of biomolecules for labeling, visualization and functional manipulation is at the forefront of Chemical Biology. We employ approaches from photopharmacology and fluorophore design to enable biomolecule labeling and control, while bringing dye properties to the next level. As such, azobenzene photoswitches enable reversible ON and OFF remote control of metabotropic glutamate receptor 2 (mGluR2), a class C G Protein Coupled Receptor (GPCR) involved in neurotransmission with light. This optical regulation of membrane excitability has led to the latest generation of photoswitches to probe working memory with light in vivo. More generally, the precise labeling of GPCRs in defined cellular compartments is important to differentiate functional receptor pools. For this reason, we extend labeling techniques with new properties to separate and interrogate different cellular protein pools, such as the membrane bound and the intracellular populations. We successfully highlight this by advanced SNAP-tag substrates to determine different GPCR stoichiometries in various cellular compartments. Expanding on this, we recently targeted endogenous GPCRs with custom- tailored fluorescent antagonists. In order to pave the way for better image quality, we think in and pursue unconventional ways in the design and synthesis of fluorophores for super- resolution imaging in live cells. In our latest efforts, we endow fluorophores with deuterium to yield dyes with increased fluorescent lifetimes, higher photostability and augmented brightness, thereby opening new applications in nanoscopy and single molecule tracking.

Organelle communication in neuron function and physiology

Margret Bülow

Organelles form dynamic contacts with each other to exchange material and signaling cues. An important type of organelle communication exists between mitochondria and peroxisomes. Peroxisomes assist mitochondria in fatty acid oxidation (FAO) and use the same machinery for fission. We found that Drosophila mutants for the peroxisome biogenesis factor Pex19 deregulate mitochondrial FAO, which results in depletion of medium-chain fatty acids. This shortage induces a lipolytic program that leads to the depletion of storage fat and accumulation of free fatty acids (FFA). FFA exert lipotoxic effects on mitochondria and disturb the secretion of neuropeptides such as insulin-like peptides. This alters neuron physiology and systemic metabolism of Pex19 mutants.

Contacts between the endoplasmic reticulum (ER), mitochondria and peroxisomes regulate the redox status of a cell. Pex19 interacts with the ER protein Creld, and loss of Creld function impairs peroxisome biogenesis in neurons. We found that Creld is required for the formation of ER-mitochondria contacts in dopaminergic neurons. In response to low respiratory complex I activity, ER-mitochondria contacts promote phospholipid flux, which supports complex I function.

In Creld mutants, constant complex I inactivity reduces H2O2 formation in dopaminergic neurons and impairs their activity. Our studies show the impact of organelle communication on neuron function.

Sequential genetic dependencies identified by time-resolved orthogonal gene editing

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CRISPR-based gene editing is a powerful tool for functional genomics that enables unbiased investigations of single and combinatorial genotype-to-phenotype associations. In the light of efforts to map all combinatorial gene dependencies in cancer, choosing a robust CRISPR system is a very important consideration. Cas9 and enCas12a have been used for combinatorial screening, however, a side-by-side comparison of these two enzymes in combinatorial CRISPR screens remains elusive. Moreover, genetic dependency screens have so far focused on the simultaneous perturbation of two genes of interest. This is, however, in contrast to the development of cancer or therapy resistance, in which mutations occur sequentially, with their order directing clonal evolution. In this work, we performed a side-by-side comparison of parallel and orthogonal combinatorial gene editing approaches and present optimized metrics for combinatorial genotype-phenotype associations. Our analyses identified Cas9 to yield higher phenotypic resolutions in single and combinatorial conditions when compared to enCas12a. This is likely explained by the overall slow establishment of enCas12a phenotypes, which we demonstrate to depend on the pre- crRNA processing activity of enCas12a. Interestingly, we reveal the orthogonal gene editing system CHyMErA to also suffer from Cas12a pre-crRNA processing activity and present an arrayed gRNA-expression system (CHyMErA.v3) that circumvents gRNA processing, enabling highly efficient orthogonal gene editing screens. Lastly, we applied our learned parameters and developed a time-resolved orthogonal gene editing system which we used to uncover the hitherto unexplored concept of sequential genetic dependencies.

Cell-cell metabolite exchange creates a pro-survival metabolic environment that extends lifespan

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Metabolism is deeply intertwined with ageing. Effects of metabolic interventions on ageing have, thus far, been explained with intracellular metabolism, growth control and signalling. Studying chronological ageing in yeast, we reveal a so far overlooked metabolic property that influences ageing via the cooperative exchange of metabolites. We observed that metabolites exported by young cells are re-imported by chronologically ageing cells, resulting in cross-generational metabolic interactions. Then, we used synthetic cell communities as a tool to boost cell-cell metabolic interactions, and achieve significant lifespan

extensions. Combining proteomics, metabolomics and modelling, we reveal a specific role of long-lived methionine consumer cells. These were enriched with ageing, but also increased the export of protective metabolites that, in turn, extended the lifespan of cells that supplied them with methionine. Our results establish cooperative metabolite exchange interactions as a determinant of cellular ageing, and show that microbial communities shape their metabolic environment to extend lifespan.

Living therapeutic devices: on-demand drug production and delivery in your body

Aránzazu del Campo

Living Therapeutic Materials are implants that contain drug producing cells encapsulated in a matrix that supports and controls proliferation and on-site drug production, while confining them at the therapeutic site and preventing escape and direct interaction with the cells of the host. When properly designed to allow diffusion of nutrients and metabolites, this simplified "biofactories" allow long-term production and delivery of drugs in physiological environments. Encoding sensing and regulatory photoswitches in the drug-producing bacteria allows for controlled and eventually personalized production and delivery. Recent developments in this topic will be presented.

The dynamic exchange of core components of the type III secretion system shapes its assembly and function

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To interact with and influence their environment, bacteria have evolved a variety of secretion systems. These systems enable the specific transport of proteins across bacterial membrane(s) and into target cells. Secretion systems are important weapons for pathogens, but also shape bacterial communities by mediating competition or symbiosis. Key secretion systems have been characterized in structural detail and appear as defined, apparently stable protein complexes. However, this impression can be deceiving. Like many biological processes, protein secretion is inherently dynamic and adaptive at multiple levels, and subunits exchange during the assembly and function of the secretion systems. In the type III secretion system (T3SS), we found that essential cytosolic subunits are not only exchanged with a pool of freely diffusing molecules, but that this exchange is linked to the function of the T3SS, protein secretion. We verified and exploited this finding by implementing an optogenetic interaction switch with one of the dynamic components to control T3SS activity with high spatial and temporal precision. Bacteria benefit from these protein dynamics: the exchange allows them to adapt T3SS activity to external conditions much more rapidly than other mechanisms. Surprisingly, we now discovered that protein exchange is not limited to the cytosolic subunits of the T3SS, but also occurs in key structural components of the system. This dynamic behavior supports the assembly and strongly influences the function and regulation of the system. Our data provide a new view of the molecular working mechanism of the T3SS and highlight the physiological importance of protein dynamics for bacterial secretion systems and other molecular machines.

Control of aberrant phase transitions in neurodegenerative diseases

Dorothee Dormann

Abstract not submitted

Altered cytoskeleton signaling in cardiac disease determines defective cellular cargo internalization

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Disturbed signal transduction from the cytoskeleton to the plasma membrane (PM) can cause severe disease, and its role in cardiac disease is incompletely understood. To study these signaling defects at the organelle but also nanoscale levels, we employed a human model of induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). Human iPSC-CMs carried inherited cardiac disease-specific mutations (MUT) in proteins of the sarcomere, a key functional component of the cytoskeleton in cardiomyocytes. We detected in MUT iPSC-CM patho-phenotypic signaling defects such as abnormal calcium handling and impaired contractile force transduction. Using this system in combination with isogenic CRISPR/Cas9 engineered controls, biochemical methods, and high-resolution STED imaging, we found disrupted interactions of sarcomeres with other cytoskeleton filaments and the PM in MUT iPSC-CMs. As a result, cargo internalization and endosome functions were found to be altered in MUT iPSC- CMs compared to WT controls. Of note, these defects were reversed in isogenic, mutation- corrected iPSC-CMs. Our studies showed these molecular signaling defects to be connected to disrupted sarcomere function in presence of cardiac disease-specific mutations. Chemical or genetic manipulation of these dysfunctions could, to different extend, reverse the observed defects. Moreover, we found that rescue of cargo internalization and altered endosome functions in MUT iPSC-CMs also recovered cardiomyopathy endpoint phenotypes such as sarcomere protein organization. Together, a better understanding of signaling defects in human cardiac disease models may reveal potential druggable targets for future therapeutic directions.

A redox-dependent thiol-switch and a Ca2+ binding site within the hinge region hierarchically depend on each other in α7β1 integrin regulation

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Integrin-mediated cell contacts with the extracellular matrix (ECM) are essential for cellular adhesion, force transmission, and migration. Several effectors, such as divalent cations and redox-active compounds, regulate ligand binding activities of integrins and influence their cellular functions. To study the role of the Ca2+ binding site within the hinge region of the integrin α7 subunit, we genetically abrogated it in the α7hiΔCa mutant. This mutant folded correctly, associated with the β1 subunit and was exposed on the cell surface, but showed reduced ligand binding and weaker cell adhesion to its ligand, laminin-111. Thus, it resembles the α7hiΔSS mutant, in which the redox-regulated pair of cysteines, closeby to the Ca2+ binding site within the hinge, was abrogated. By comparing both mutants in adhesion strength and cell migration, we show that both Ca2+ complexation and redox-regulation within the hinge interdepend on each other. Moreover, protein-chemical analyses of soluble integrin ectodomains containing the same α7 hinge mutations suggest that integrin activation via the subunit α hinge is primed by the formation of the cysteine pair–based crosslinkage. Then, this allows Ca2+ complexation within the hinge, which is another essential step for integrin activation and ligand binding. Thus, the α hinge is an allosteric integrin regulation site, in which both effectors, Ca2+ and redox-active compounds, synergistically and hierarchically induce far-ranging conformational changes, such as the extension of the integrin ectodomain, resulting in integrin activation of ECM ligand binding and altered integrin-mediated cell functions.

A fluorescent nanosensor paint detects dopamine release at axonal varicosities with high spatiotemporal resolution

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The neurotransmitter dopamine (DA) controls vital brain functions and is involved in several prevalent brain diseases. Despite this importance, our understanding of the molecular mechanisms that control DA release has been limited by the low spatial resolution of DA detection methods. Dopaminergic secretion sites (varicosities) are functionally and structurally highly diverse, and a detection method with high spatial resolution is required to dissect the molecular basis underlying this diversity. We developed and present a technique that uses small (0.7 nm x 200 nm) near-infrared fluorescent DA nanosensors for the optical detection of DA secretion from cultured murine dopaminergic neurons: AndromeDA (adsorbed nanosensors detecting release of dopamine). Through imaging of millions of nanosensors in parallel, AndromeDA detects local DA secretion events (hotspots) from up to 100 varicosities simultaneously. Using AndromeDA, we describe discrete hotspots, and find that they occur at only ~17% of all varicosities, indicating that many varicosities are functionally silent. In addition, using a mouse KO model, we find that Munc13 priming proteins are required for DA release. Imaging with AndromeDA is versatile and readily applicable to other in vitro systems, with an unprecedented ability to spatially correlate DA secretion events to subcellular structures. This method will enable a detailed dissection of the molecular mechanisms that give rise to the heterogeneity of the DA system

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Blocking bacterial pathogenicity by novel quorum sensing inhibitors for the treatment of chronic Pseudomonas aeruginosa infections

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The antimicrobial resistance (AMR) crisis is considered to be a major healthcare challenge, which is steadily exacerbating and projected to outpace cancer as a major cause of death by 2050.[1] This “silent pandemic” urgently requires the discovery and development of novel anti- infective agents with new modes-of-action (MoA).

One critical pathogen is Pseudomonas aeruginosa (PA) that causes severe chronic lung infections in patients suffering from cystic fibrosis or bronchiectasis. This opportunistic, ubiquitous gram-negative bacterium is able to switch to the biofilm mode of life, which serves as a physical barrier to survive antibiotic treatment and host immune defense. PA develops high resistance towards antibiotics resulting in maintenance of chronic infections and high mortality of infected patients. The Pseudomonas Quinolone Signal (PQS) quorum sensing (QS) system is essential for bacterial virulence and biofilm formation rendering it a suitable drug target to block PA’s pathogenicity.[2]

By systematically probing potential target proteins within the PQS QS system, we identified innate transcriptional regulator PqsR (MvfR) as the most promising point-of-intervention.[3] Following a fragment-based approach, we discovered novel structure-divergent PqsR-targeting QS inhibitors.[4] An extensive lead generation and optimization campaign yielded candidate molecules with potent virulence-attenuating properties combined with suitable pharmacokinetics and safety profiles. Notably, our QSI synergize with aminoglycoside antibiotics in biofilm eradication in vitro as well as in reducing bacterial burden in vivo. Early (non-GLP) preclinical studies are the next steps aiming at eventually translating this unprecedented MoA into the clinics.

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Exploring the Dynamic World of Membrane Systems and Biocatalysis

Manuel Etzkorn

To understand life on a molecular level, it is of fundamental importance to study structure, interactions, and dynamics of life's key players – proteins, nucleic acids, and lipids – in their native environment. NMR spectroscopy has a unique potential to provide high spatial and temporal resolution of the target molecules in complex environments. However, sample preparation, low sensitivity, and ensemble averaging pose clear limitations in increasingly complex systems.
The presentation will summarize our recent contributions to overcome central restrictions via the development and application of novel methods. This will include our optimized isotope-labelling1, sample-preparation2, and polarization-usage3 strategies as well as selective hyperpolarization via functionalized ligands4.
Furthermore, it will be discussed how the application of the developed methods can provide new insights into: (i) membrane-induced aggregation of the Parkinson's associated protein α--synuclein5, (ii) signalling of central membrane receptors6,7, and (iii) DNA-mediated biocatalysis8.

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FRET measurements resolving structures, dynamics and transitions of various biomolecules

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Fluorescence spectroscopy and imaging are important biophysical techniques to study biomolecules in vitro. The use of more than one fluorophore per molecule opens additional opportunities arising from photon densities, coincidences and dipolar coupling by Förster Resonance Energy Transfer (FRET) to study the stoichiometry, structure, dynamics and transitions of biomolecular systems. This phenomenon we applied to a wide range of biomolecules with a varying size from 100 kDa to 1400 kDa showing different dynamics and kinetics on timescales from µs to hours. To study this we combine established methods like Multiparameter Fluorescence Detection (MFD) of freely diffusing molecules and Total Internal Reflection (TIRF) microscopy using immobilized molecules, together with new approaches pushing the collection efficiency to detection signals to hundreds of kHz. The outcome are observables describing (a) different structural states and dynamics of the human Guanalyte Binding Protein (hGBP1) and its behavior in the farnesylated state, (b) the live exchange of different molecular states of a freely diffusing 4-way junction on a µs timescale and (c) the structural behavior of a Tc toxin on a ms-timescale giving insights into fast/straight forward and complex/distributed transitions crucial for its biological functionality.

Rational design of biophotoelectrodes for in vitro biocatalysis

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The use of photosynthetic protein complexes for the fabrication of solar energy conversion devices is a promising strategy due to their natural abundancy and high quantum efficiency. Particularly one of the main photosynthesis-driving enzymes, photosystem I (PSI), is a stable protein complex able to convert visible light into high-energy electrons – making it an attractive candidate for the fabrication of biohybrid devices. One of the challenges in such devices is to overcome short-circuiting processes between the light-generated electrons and the electrode. One approach is oriented immobilization of PSI complexes in so-called Langmuir monolayers by taking advantage of their amphiphilic character, thus enabling anisotropic electron flow. Residual charge recombination occurring at the gaps between PSI trimers could be successfully closed by additionally employing smaller PSI monomers, resulting in increased surface coverage and overall performance. To promote efficient wiring between PSI complexes and the electrode, rationally designed redox-active polymers can be used as an effective tool. They enhance electron transfer and film stability and furthermore enable deposition of additional enzymes such as oxidoreductases to the photoelectrode to make use of the high energy electrons generated by PSI, as could be shown for a hydrogenase, resulting in light-driven H2 production. Further optimization of PSI-based biohybrid devices can be achieved via the use of transparent, 3D-structured electrodes that enable a significantly increased protein loading density and via decorating PSI complexes with additional light-harvesting antennae, closing the "green gap" and thus increasing the system's overall quantum efficiency.

Molecular mechanisms of macromolecular assembly during cellular stress responses

Titus Franzmann

Abstract not submitted

Fundamental nature of the droplet state of proteins

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Dense, liquid-like state of proteins, termed as the droplet state plays key roles in structural and functional organisation of the cellular matter. We propose that at high concentrations in the cell, the droplet state should be considered as a fundamental state of proteins together with the native and amyloid states. Using condensed matter physics approaches we show that most proteins are close to their critical concentrations in the cell and can sample the droplet state. Formation of the droplet state is driven by multivalent, non-native interaction motifs. We relate interaction fuzziness of multivalent motifs to folding frustration and propose a general model for phase separation. In particular, we demonstrate that the droplet state exploits interactions, which are also present in stoichiometric protein complexes. Based on this framework, we demonstrate that most proteins, regardless of their structural order, possess sequence motifs, which enable their partitioning into condensates. Finally, we apply this generic model to predict perturbations in the condensed states, which can lead to a variety of dysfunctions and protein condensation diseases.

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Good things come to those who bait: docking and recycling at the peroxisomal membrane

Christos Gatsogiannis

Abstract not submitted

Multi-TaG: Next generation bioorthogonal probes for multi-method targeted analysis of glycoproteins

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Glycosylation is the most prevalent posttranslational modification (PTM) that substantially affects the structure and functions of proteins. Heterogeneity and dynamic changes, make the analysis of glycoproteins very challenging, which demands for new tools and strategies. One established method is the use of metabolic labelling with non-natural functionalized sugars and subsequent covalent tagging with analytical probes by means of bioorthogonal chemistry. However, established bioorthogonal probes are often limited regarding their experimental read-out options and their applicable bioorthogonal attachment chemistry. Here, we present a strategy for the generation of bioorthogonal probes that allow the combination of multiple experiments by using only one tag that is attached to the sample of interest. Our approach uses solid phase peptide synthesis to combine multiple functional units (fluorophores, cross linkers, cleavable linkers, affinity units and isotopic labels etc.) in a building block fashion on a solid support. Using a special linker, our synthesis strategy further allows probe release and C-terminal functionalization in one step. This approach provides high flexibility regarding the bioorthogonal handle that is introduced in the final step of the synthesis. We show a library of multi-functional probes that allow smart workflows and concerted analysis of glycoproteins with different methods, such as imaging, isolation and targeted or quantitative glycoprotiomics.

On the Road to Finding the Right Key: Structural Characterisation of Three Active States of the Human Neuropeptide Y Type 4 Receptor (hY4R) at the Molecular Level

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Human neuropeptide Y receptors (hYxR) are rhodopsin-like, peptide-binding G-protein coupled receptors (GPCRs), which modulate a plethora of biological functions (e.g. energy homeostasis, vasoconstriction, circadian rhythm), and also play a central role in the insurgence of several wide-spread pathologies such as cancer progression, obesity/anorexia and epilepsy.[1] They are often referred to as membrane trafficking proteins, whose functions and dysfunctions are tightly dependent on the specific extra- and intracellular coupling partners, as well as on the type of lipid membrane in which they are embedded. Understanding this complex interplay of receptor structures/bound ligands/physical environment has received much attention in the biochemical and biophysical communities, especially over the last 20 years,[2] often motivated by the urgent need to develop selective therapeutics, based on receptor-specific key–lock mechanisms. Here, we present our latest results on the structural dynamics of the human Y receptor sub-type 4 (hY4R), which is the only member of the hYxR family to show remarkable selectivity for one specific endogenous ligand, namely pancreatic polypeptide (PP), and which, surprisingly, has received very little attention compared to the rest of the hYxR family. Through cell-free expression strategies, we introduce site-specific isotopic labels at the receptor,[3] which allow us to follow structural changes at the molecular level using solid-state NMR spectroscopy.

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Arctic TREx – Combining Cryofixation with Ten-fold Robust Expansion Microscopy to Image Neural Cells

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When preparing biological samples for microscopy, chemical fixation with aldehydes is one of the most common approaches. However, aldehyde treatment can drastically change cytoskeletal morphology, damage membrane integrity, or hinder epitope accessibility. Thus, it is advisable to switch from chemical fixation to cryofixation whenever possible as cryofixation has been shown to better preserve the native sample structure[1]. This year, Laporte et al have published a method to combine cryofixation and 4x expansion microscopy (Cryo-ExM)[2] thereby supplying an easy to follow protocol implementing cryofixation into super-resolution fluorescence imaging. Unlike other super-resolution techniques, expansion microscopy (ExM)[3] works with a common widefield fluorescence microscope to achieve resolutions below the diffraction limit. Instead of relying on optical advances or statistical calculations, the sample itself is physically expanded by anchoring it to an expandable hydrogel. Here, we further extended the protocol of Laporte et al by combining it with Ten-fold Robust Expansion Microscopy (TREx)[4]. Using this new technique, which we call arctic TREx, we imaged the subcellular structures of primary neural cells from postnatal rats achieving resolutions below 100 nm.

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Mechanism of SUMO-mediated phase separation at PML nuclear bodies

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The mammalian cell nucleus contains a variety of subnuclear structures, such as the well-described nucleolus. The assembly of many (if not all) of these macromolecular assemblies may be mediated by liquid-liquid phase separation (LLPS). Promyelocytic Leukemia nuclear bodies (PML NBs) have been implicated in DNA repair and recombination but their biochemical function in these processes are not known yet. We are interested in the assembly mechanism of PML NBs to gain more insight into their functional properties. The PML protein is polySUMOylated and a polySUMO/SIM (SIM: SUMO-interation motif) interaction network seems to contribute to NB assembly. Here we describe coarse-grained in silico modeling of the assembly of a complete PML NB employing molecular dynamics simulation. In an iterative process between computer predictions and wet-lab assays we have identified and functionally characterized a novel SIM in SUMO. Strikingly, short polySUMO peptides assemble to dynamic biomolecular condensates independently of PML NBs in live-cell nuclei. Observations of their biophysical properties (FRAP, FCS, RICS, N&B) are consistent with a liquid droplet behaviour of SUMO chains in the nucleus. These findings suggest a novel purely polySUMO chain-driven mechanism of liquid-liquid phase separation at PML nuclear bodies. Furthermore, in silico 'virtual microscopy' allowed us to assess the spatial relationship within the polySUMO/SIM scaffold at PML NBs at nanometer resolution.

Cutting RNA to power life: Molecular basis of RNA processing in human mitochondria

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Mitochondria contain an organellar genome, which is expressed by dedicated molecular machineries that differ from those found in other cellular compartments or in bacteria. We have previously elucidated the structural basis of transcription in human mitochondria (Hillen et al., Cell 2017a, 2017b). However, the mechanisms of RNA processing and maturation in human mitochondria remain poorly understood. The mitochondrial RNA polymerase produces polycistronic transcripts that contain mRNAs and rRNAs flanked by tRNAs, which need to be processed to liberate the individual RNA species. The first step of RNA processing is carried out by mitochondrial RNase P (mtRNase P), which cleaves the transcripts at the 5′ end of tRNAs. In contrast to most other RNase P enzymes, human mtRNase P is not a ribozyme and is instead comprised of three protein subunits that carry out both pre-tRNA cleavage and methylation. We have recently determined the cryo-EM structure of human mtRNase P bound to pre-tRNA, which reveals the mechanism of this dual-function RNA processing machine (Bhatta et al., NSMB 2021). The subunits MRPP1 and MRPP2 form a subcomplex that interacts with conserved elements in mitochondrial tRNAs, including the anticodon loop, by a unique mechanism and positions the pre-tRNA for methylation. The endonuclease MRPP3 is recruited and activated through interactions with its PPR and nuclease domains, which ensures precise cleavage of the pre-tRNA. Mapping of disease mutations to the structure additionally provides insights into the molecular basis of human mitochondrial disorders. These results reveal the molecular mechanism of the first step of mitochondrial RNA processing, and provide a framework for studying mitochondrial RNA metabolism.

Molecular basis of heme-driven pathway activation in hemolytic disorders

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Hemolysis results in an accumulation of labile heme, which leads to proinflammatory and prothrombotic complications. As a regulatory molecule, heme can affect the function and/or stability of proteins through binding to short, surface-exposed amino acid stretches. As such, the stimulation of the complement and coagulation system through direct heme binding to participating proteins (e.g., C3, fibrinogen, and APC) was described. On the cellular level, heme-triggered proinflammatory and procoagulant effects under hemolytic conditions were attributed to the activation of the TLR4- signaling pathway, but direct binding of heme to the receptor has not been analyzed so far. Herein, the functional influence of heme binding to a selection of proinflammatory and procoagulant proteins is demonstrated, characterized by the combination of biochemical approaches. The heme-binding properties of these proteins were analyzed by using surface plasmon resonance spectroscopy. In order to characterize the respective heme-binding sites, the proteins were screened for potential heme-binding motifs by using the webserver HeMoQuest. Subsequently, these motifs were synthesized as nonapeptides and analyzed for heme binding via UV/vis spectroscopy. Promising sites were further evaluated by molecular docking simulations of the heme- protein complexes. Taken together, these results extend the understanding of hemolysis-derived heme as a regulator within the triad of inflammation, the complement, and the blood coagulation system on the molecular level, which will support an improved understanding of the progression of thrombosis and inflammation under hemolytic conditions.

Metastable amyloid-β oligomers – Kinetics, mechanisms, interactions

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Metastable oligomers of amyloid-β peptide (Aβ) are more effective than Aβ amyloid fibrils at triggering Alzheimer's disease-related processes such as synaptic dysfunction and Tau pathology. Here we elucidate the mechanism of Aβ oligomer (AβO) formation and identify physiological conditions that promote AβO generation. AβO formation was determined utilizing Aβ42 as well as the dimeric Aβ construct dimAβ that facilitates analysis of the oligomerization kinetics. AβO formation, clustering, and release were imaged by atomic force microscopy. The structure of the smallest AβOs was investigated by cryo-EM. The capacity of AβOs to bind to dendritic spines, to induce Tau missorting, and to impair neuronal function were studied in primary mouse neuronal cell cultures. The rate of AβO assembly is accelerated 8,000-fold upon pH reduction from extracellular to endo-lysosomal pH, at the expense of amyloid fibril formation. The pH-induced promotion of AβO formation and the high endo-lysosomal Aβ concentration together enable extensive AβO formation of Aβ42 under physiological conditions. Exploiting the enhanced AβO formation of dimAβ we furthermore demonstrate targeting of AβOs to dendritic spines, potent induction of Tau missorting, a key factor in tauopathies, and impaired neuronal activity. The results suggest that the endosomal/lysosomal system is a major site for the assembly of pathomechanistically relevant AβOs.

Rhomboid-Catalyzed Intramembrane Proteolysis Requires Hydrophobic Matching with the Surrounding Lipid Bilayer

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Membrane thinning of the rhomboid GlpG has been proposed to reduce the hydrophobic mismatch between the enzyme and its surrounding lipid environment. Here, we directly show that the membrane environment of the rhomboid influences the velocity of substrate cleavage. We first measure the impact of GlpG on the hydrophobic thickness in phosphatidyl-choline membranes of varying thickness, where the rhomboid only marginally alters the surrounding membrane. However, in an E. coli relevant lipid mix of phosphatidyl-ethanolamine and phosphatidylglycerol, a decrease in hydrophobic thickness of -1.1 Å per leaflet is observed. The cleavage velocity of GlpG is highest in DMPC followed by POPC, POPE/POPG and DLPC, while in the thickest membranes (DPPC/cholesterol) enzyme function is abolished. This suggests that an optimal window of membrane thickness (between ~24 – 26 Å) exists while headgroup specificity does not seem to be decisive for protein function. We infer from these results that the lipid environment can fine-tune GlpG function. By adjusting membrane thickness, for instance through dynamic domain formation, the cell can regulate membrane protein function.

Coiled-coil modules for designed molecular and cellular logic circuits

Roman Jerala

Coiled-coil (CC) dimers are ubiquitous building modules in natural proteins. Rules that define interactions in CC dimers are relatively understood, which allows us to design new CC dimers with selected stability and selectivity. Although CC dimers occur frequently in natural proteins, designed CC dimers can be used for cell regulation and construction of new building modules to regulate cellular processes. Designed CC dimer-forming peptides can be fused to other proteins, enabling regulation of biological processes, including localization within mammalian cells, augmented transcriptional response based on chemical regulators (CCCtag) and faster kinetics based on combination of split proteases with coiled-coil modules (SPOC logic)(1,2). Those building blocks have also been used to construct genetically encoded orthogonal secretion systems for fast secretion of proteins based on the ER-retained proteins (3). Furthermore, formation of CC dimers has been used to introduce allosteric regulation of function of diverse proteins, comprising different folds and biological role, from enzymes, DNA

binding proteins, signaling mediators, antibodies, which can be generate switches and logic functions with response time within minutes in mammalian cells (4) and introduce new type of control for research or biotechnological or therapeutic applications.

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Catch Me if You Can - Entrapment of Methylated Arginine by Supramolecular Hosts

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The World Health Organization estimated that 1.28 billion adults aged 30-79 years worldwide suffer from hypertension (2021). Patients with elevated blood pressure develop more cardiovascular complications. Therefore, cardiovascular diseases are major cause of premature death. Nitric oxide (NO), produced by the endothelial isoform of NO synthase (eNOS) in the vascular endothelium, plays a key role in the regulation of blood pressure. Patients with hypertension, hypercholesterolemia, diabetes, and chronic kidney failure show an elevated level of asymmetric dimethylarginine (ADMA), which acts as an endogenous competitive inhibitor of eNOS. The aim of our study is to introduce a supramolecular host to entrap ADMA and thus prevent the inhibition of eNOS.

First, we tested three different classes of supramolecular hosts to identify the best ADMA binder [1]. We are currently establishing an in vitro and two in vivo assays to quantify host binding to ADMA and their effect on the eNOS activity. We already expressed and purified recombinant eNOS for the in vitro inhibition assay. In addition, two in vivo models are investigated: (a) human umbilical vein endothelial cells (HUVEC), which are able to express eNOS and thus produce NO themselves, and (b) we will be establishing an inducible expression HEK cell line system for external control and regulation of eNOS expression.

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Cryo-EM structure of the actinobacterial respiratory supercomplex: an efficient generator and potential drug target

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Respiratory chain cytochrome (cyt) bc complexes (complex III) and cyt c oxidases (complex IV) are major generators of the proton motive force that fuels ATP synthesis. They are proposed to assemble into supercomplexes to optimise the energy conversion efficiency, but less is known at the level of atomic detail. Here, we report the 2.8-Å resolution cryo-EM structure of the obligate complex III2-IV2 (cyt bcc-aa3) supercomplex of the actinobacterium Corynebacterium glutamicum [1]. The resolved catalytic position of menaquinol as well as proton channels provide insights in the basis for concerted release of electrons and protons limiting wasteful and deleterious bypass reactions in the cyt bcc complex. A previously unknown menaquinone binding Qc site and a tightly bound lycopene indicate the presence of a built-in free radical handling system. The conformational states of four conserved key protonable groups provide the basis for controlled proton uptake, loading and release and thus for effective proton pumping in cyt c oxidases. Our results show how safe and efficient energy conversion is achieved in a respiratory supercomplex. The well-resolved conformations of inhibitor as well as native substrates in the cryo-EM structure of the supercomplex may aid the rational design of drugs against actinobacteria that cause diphtheria and tuberculosis [2].

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Expanding the repertoire of de novo protein assemblies

Alena Khmelinskaia

Abstract not submitted

Bispecific NK Cell Engagers Activating NK Cells via NKp30 to Improve Tumor Cell Lysis and Pro-Inflammatory Cytokine Release

Katja Klausz

Activating Natural Killer (NK) cell receptors represent promising target structures to elicit potent anti-tumor immune responses. Novel Fc comprising NK cell engagers (NKCEs) were generated that bridge the activating NK cell receptor NKp30 on NK cells with epidermal growth factor receptor (EGFR) on tumor cells in a bispecific IgG-like format. The Fab arm was derived from Cetuximab and either yeast surface display-derived affinity-optimized N-terminal IgV domains of the natural ligand B7-H6 (ΔB7-H6) or NKp30 binding camelid VHH single domain antibodies were fused to activate NK cells via NKp30. Biochemical and functional characterization of ΔB7-H6-derived NKCEs revealed an up to 45-fold enhanced affinity for NKp30 and up to 87-fold improved NK cell-mediated, EGFR-dependent killing of tumor cells compared to the NKCEs based on the wild-type ΔB7-H6 domain. Using NKp30-specific VHHs against different epitopes on NKp30 revealed that those NKCEs built with VHHs that compete with B7-H6's natural ligand binding site were significantly more potent in eliciting tumor cell lysis of EGFR-positive tumor cells than NKCEs harboring VHHs that target different epitopes on NKp30. Nevertheless, these non-competing NKCEs were capable to leave physiological processes between NKp30 and membrane-bound B7-H6 unaffected and still elicited tumor cell killing at low picomolar concentrations. Furthermore, release of interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α) was vastly increased by all NKCEs – irrespective of using ΔB7- H6 or NKp30 VHH. Importantly, all NKCEs could be further improved by concomitant engagement of Fcγ receptor IIIa (FcγRIIIa) and NKp30 on NK cells. These NKCEs carrying an active Fc were even more potent than the clinically approved antibody Cetuximab in terms of tumor cell killing and pro-inflammatory cytokine release, both features which might be beneficial for anti-tumor therapy. Thus, NKCEs activating NK cells via NKp30 show unique modes of action and might represent an effective strategy for cancer immunotherapy.

Single cell transcription during yeast cell cycle

Edda Klipp

We use the yeast Saccharomyces cerevisiae as the model organism for eukaryotic cells allowing to comprehensively analyzing regulatory networks and their integration with cellular physiology. We focus on processes during the lifetime of a single cell along one period of the cell division cycle and study the changes of metabolism, gene expression, or ion and nutrient transport during the growth of that cell.

We use a modular and iterative approach that allows for a systematic integration of cellular functions into a comprehensive model allowing to connect processes that are strongly interlinked in cellular life, but measured separately. The modular concept also permits to zoom in and out if different aspects of regulation or dynamics come into focus.

During cell cycle, transcription of most genes changes in a roughly systematic manner giving rise to the concept of cell cycle oscillations. Here, we investigate the noise or uncertainty of the genes involved in cell cycle regulation, which often exhibit very low levels of expression, yet still must ensure tight regulation.

Exploiting supramolecular chemistry to modulate protein function

Shirley Knauer

Due to their cancer relevance, a detailed biological understanding of the apoptosis inhibitor and mitosis regulator Survivin and the protease Taspase1 is of broad interest and key for innovative interference strategies. Both protein functions are regulated by

their dynamic cellular localization, dimerization, and protein interactions. We thus aimed at the development of supramolecular interference strategies for intracellular protein transport signals; as proof-of-concept, we successfully targeted Survivin's nuclear export signal (NES, aa 89VKKQFEELTL98) and the import signal (NLS, aa 197KRNKRK-x14-KKRR220) of Taspase1. An addition of peptidesderived from Survivin's dimer interface (partially overlapping the NES) to a molecular tweezer increased binding, regioselectivity, and signal specificity. Moreover, multivalent ligands, e.g., from the coupling of tweezers to ultrasmall gold nanoparticles let to more potent binders. Moreover, multivalent glutamate/aspartate binders with guanidiniocarbonylpyrrole (GCP) motifs allowed a selective targeting and dissection of the biological Survivin/Histone H3 interaction. Additional structural modifications allowed to access distinct Survivin regions: Duplication of the GCP-units combined with changes in their steric orientation resulted in ligands that either perturbed the Histone H3- or the NES/CRM1 interactions, even in cells. For Taspase1, PEGylated GCP-equipped oligomers inhibited the interaction of its NLS with the import receptor Importin α, presumably by sterically shielding the flexible loop. Moreover, multi-armed GCP-ligands covered an extended surface area and affected Importin α binding, proteolytic activity, and cancer cell viability.

The allosteric landscape of a fundamental molecular switch

Tanja Kortemme

Molecular switch proteins whose cycling between states is controlled by opposing regulators are central to biological signal transduction. As switch proteins function within highly connected interaction networks, the fundamental question arises of how functional specificity is achieved when different processes share common regulators. We have focused on a paradigm molecular switch, the small GTPase Ran/Gsp1, which regulates diverse processes including nucleocytoplasmic transport, cell cycle progression, and RNA processing. Using systematic mutational perturbations of the switch, quantitative genetic interaction mapping, analysis of rewiring of physical interaction networks, in vitro biochemistry, and NMR, I will propose a model of how different biological processes are sensitive to different quantitative regimes of switch function. Our results highlight a considerable role of allostery in regulating the switch.

Engineering DNA-templated nonribosomal peptide synthetases

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Nonribosomal peptide synthetases (NRPSs) protect microorganisms against environmental threats by producing siderophores or antibiotics, for instance, and are predisposed for biosynthetic engineering because of their modular molecular structure. We have explored several strategies for the redesign of NRPS specificity. Notable examples are the incorporation of a clickable amino acid through targeted binding pocket mutagenesis [1] or specificity transfer through swapping of small protein fragments [2, 3]. Incorporation of clickable amino acids has further enabled a strategy for high-throughput sorting of mutagenized NRPSs displayed on yeast [4]. Here, we demonstrate the engineering of DNA-templated supramolecular complexes to facilitate NRPS reprogramming [5]. We have split the NRPS for the cyclic decapeptide gramicidin S into modules. Up to four modules were later reassembled on a DNA template using DNA binding domains with high specificity and affinity, and loosely binding intermodular docking domains. The complex nonribosomal machinery showed astonishing tolerance for structural variations when the DNA spacers between modules were altered in length. In the future, DNA programmable NRPSs might allow to write the sequences of natural product-like peptides into short DNA templates to speed up NRPS design.

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Structural dynamics of the intrinsically disordered SNARE proteins at the membrane interface: Recent insights by a combined solution and solid-state NMR approach

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Neurotransmitter release at the neuronal synapse is a fundamental process for signal transduction between neurons. The SNARE proteins play a crucial role here by eliciting the fusion of the synaptic vesicle membrane with the presynaptic plasma membrane. A fusion pore will open, and the neurotransmitters are released into the synaptic cleft. In their pre-fusion state, the SNARE proteins are intrinsically disordered. They do not exhibit a well-defined structure and show high internal flexibility. The SNARE proteins are membrane-anchored. However, the mode of interaction between the SNARE proteins and the lipid membrane is not well understood. Here NMR spectroscopy can provide novel structural and dynamic insights at an atomic resolution, as NMR is favorably suited for studying highly dynamic systems, such as intrinsically disordered proteins. We use the SNARE proteins as a model system for developing novel NMR methods to characterize the inner and conformational dynamics of intrinsically disordered proteins that interact with lipid membranes or a membrane-anchored. We address a large range of timescales, from pico- to milliseconds. We employ both solution NMR and proton- detected solid-state NMR methods at very fast MAS (magic angle spinning) frequencies. The aim is to better describe the conformational space of intrinsically disordered proteins at the lipid membrane interface. At the conference, we will present recent (unpublished) insights into the structural dynamics of the SNARE proteins synaptobrevin-2 and SNAP25 at the lipid membrane interface.

Isolation of three different Photosystem II complexes from Thermosynechococcus elongatus via Strep-tagged PsbO

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The multi-subunit membrane protein complex Photosystem II (PSII) catalyzes the light-driven oxidation of water and with this the initial step of photosynthetic electron transport in plants, algae, and cyanobacteria. Chromatographic isolation of PSII complexes from the thermophilic cyanobacterium Thermosynechococcus elongatus yields stable protein complexes of high purity, which are well suitable for structural and functional analysis. In the present study, we purified three different PSII complexes from a mutant line in which the extrinsic subunit PsbO, characteristic of active PSII, was fused with an N-terminal Twin-Strep-tag. Three different PSII fractions, which differ in oligomeric state and subunit composition, were separated by ion-exchange chromatography after the initial affinity purification. The activity observed for the highly abundant, active dimeric PSII is around 6.000 µmol O2·(mg Chl-·h)-1, which is due to the absence of inactive complexes within the sample, as seen by the chlorophyll a fluorescence properties. Because of their high homogeneity, we propose that these complexes are suitable for all kinds of investigations on PSII, in particular the oxygen-evolving complex (OEC). Moreover, one of the isolated PSII fractions revealed differences in subunit composition, activity, manganese content, and chlorophyll a fluorescence properties. This suggests a disturbed OEC, which indicates a complex related to PSII biogenesis or repair.

Post-translational lysine acetylation regulates the transcriptional regulator RutR as shown by using a structural biological and synthetic biological approach

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Bacteria live in rapidly changing environments with consequences on nutrient availability. They precisely sense the cellular metabolic state and translate this in altered protein functionalities. The TetR-family member RutR (pyrimidine utilization repressor) is a transcriptional repressor for the rutA-G operon involved in pyrimidine breakdown and it activates transcription of the carAB operon involved in pyrimidine biosynthesis. RutR is structurally composed of an N-terminal DNA-binding domain (DBD) and of a C-terminal ligand-binding domain. It is lysine acetylated at distinct sites in the DBD and LBD. How lysine acetylation affects RutR function is not known. Applying the genetic code expansion concept (GCEC) we produced site-specifically lysine- acetylated RutR proteins suitable for biophysical studies including X-ray crystallography. We solved a crystal structure of K52-acetylated RutR showing how K52-acetylation switches-off RutR DNA-binding exerting a steric and electrostatic mechanism. The interactions of acetylated RutR-variants with carAB and rutAB dsDNA operator sequences were analysed thermodynamically ITC and by EMSAs. We applied the GCEC in E. coli in vivo showing that RutR acetylation modulates its transcriptional regulator activity. RutR acetylation is catalysed enzymatically by E. coli lysine acetyltransferases and non-enzymatically by acetyl-phosphate. The sirtuin deacetylase CobB can revert RutR acetylation at all functionally important sites. We present a model explaining how prokaryotes apply lysine acetylation of transcriptional regulators as sensors of the cellular metabolic state directly adjusting gene expression allowing to adjust to changing environmental conditions.

A possible role of the ubiquitin kinase PINK1 in protein quality control

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Degradation of dysfunctional, damaged, or misfolded proteins is a crucial component of the protein quality control network to maintain cellular proteostasis. Dysfunction in proteostasis regulation due to imbalances in protein synthesis, folding, and degradation challenges the integrity of the cellular proteome and favors the accumulation of aggregated proteins that can damage cells by a loss of their functions and/or a gain of adverse functions. Ubiquitination is an essential player in proteostasis regulation but also in orchestrating signaling pathways in response to various stress conditions. Both cellular degradation systems, the proteasome and autophagy, employ ubiquitin for selection and targeting of substrates to the degradative machineries. We recently found that linear ubiquitin chains generated by LUBAC are implicated in the clearance of protein aggregates associated with neurodegenerative diseases, such as Huntington's disease or Parkinson's disease. In this study, we are analyzing a possible role of PINK1 in protein quality control, based on our observation that PINK1 is co-localizing with pathogenic protein aggregates.

Cholesterol promotes head group visibility and clustering of PI(4,5)P2 driving unconventional secretion of Fibroblast Growth Factor 2

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Fibroblast Growth Factor 2 (FGF2) is a cellular survival factor involved in tumor-induced angiogenesis. It is

one of the most prominent examples of extracellular proteins that lack signal peptides and are secreted by ER/Golgi-independent secretory pathways. Biochemical reconstitution experiments and imaging in living cells have shown that FGF2 is secreted by direct translocation across the plasma membrane. This process is initiated by PI(4,5)P2- dependent FGF2 recruitment at the inner plasma membrane leaflet. This in turn results in the formation of membrane-spanning FGF2 oligomers within toroidal membrane pores. Here, using both biochemical reconstitution experiments and live-cell imaging, we demonstrate that PI(4,5)P2-dependent FGF2 recruitment at the inner plasma membrane leaflet is positively modulated by cholesterol in both . Based on extensive molecular dynamics simulations and free energy calculations, we propose cholesterol to increase the negative charge density of the membrane surface and to induce clustering of PI(4,5)P2 molecules stabilizing FGF2 binding through increased avidity. Our findings have general implications for phosphoinositide-dependent protein targeting membranes and explain the highly selective targeting of FGF2 towards the plasma membrane.

Picturing biological water oxidation by snapshot crystallography

Johannes Messinger

Oxygenic photosynthesis is the primary means of fuelling life on Earth. This process, carried out by cyanobacteria, algae and plants, converts solar energy into chemical energy and provides us with the oxygen we breadth. The major 'invention' has been the ability to utilize the abundant and stable water as electron and proton source for carbon dioxide reduction to carbohydrates. The water oxidation reaction is carried out by Photosystem II, which thereby is the starting point of the photosynthetic electron transport chain of photosynthesis.

Oxidizing water to molecular oxygen by the energy of visible photons requires the coordination of four ultrafast (picoseconds) light-driven one-electron charge separations with the slow (milliseconds) four-electron and four-proton water oxidation chemistry. For interfacing these different time scales and stoichiometries Photosystem II utilizes a tetra-manganese calcium oxygen (Mn_4CaO_5) cluster that cycles to a set of five oxidation states.

In my presentation I will present recent progress in visualizing and deriving the mechanism of biological water oxidation. Special focus will be on the utilization of snapshot crystallography performed at x-ray free electron lasers (XFELs), which allowed deriving high-resolution structures of most key intermediates of this reaction cycle. In addition, experiments allowing the identification of one of the substrate waters and a tentative mechanism will be presented.

Elucidating Receptor Oligomerization States in CD95 Signaling with Super-Resolution and Multiparametric Image Spectroscopy

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CD95 oligomerization state can decide a cell's fate. CD95 (Cluster of Differentiation 95), also known as FAS or Apo-1, is a membrane death receptor initiating the signaling cascade for controlled cell death (apoptosis) after ligand-induced activation. Some types of cancer show a deregulation of this CD95 apoptosis mechanism leading to proliferation rather than cell death1. As the switch from signaling for death to life is hypothesized to occur via different CD95 activity states, we here investigate mechanism of molecular oligomerization of CD95 on the cell membrane.

Multiparametric imaging approach. Measuring the structural state of proteins at the single-molecule level remains challenging, in particular when molecular oligomerization states are to be distinguished2. For this reason, we use a multiparametric spectroscopic approach to provide quantitative insights. Time-resolved FRET live-cell experiments are used to measure the supramolecular state of CD95. As an orthogonal measure, super-resolved STED images and FCS measurements reveal the supramolecular size and diffusion characteristics, respectively. Recently, we are moving to the single molecule level and develop EGFP-bleaching step analyses using a confocal setup.

We report on the CD95 oligomerization states as well as on novel experimental/analytical methodologies.

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Manipulating and Labeling of Proteins by Selectively Addressable Thiol Moieties

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Introducing fluorophores, biophysical probes and other defined chemical modifications into proteins is an enabling technology for their biophysical, biochemical and cellular investigations. Cysteine is arguably the most versatile proteinogenic amino acid in terms of both the importance for protein structure and catalysis and the chemical reactivity of the thiol side chain. Classical thiol bioconjugation with reagents like maleimides provides unique opportunities for the chemo- and regioselective introduction of various labels into proteins. However, its scope is severely restricted for proteins containing an essential or multiple cysteines. Bioorthogonal reactions can circumvent these limitations, but they require the incorporation of non-natural reactive groups, which often amounts to elaborate and expensive procedures and requires specialized chemical synthesis skills. Therefore, we have sought to expand the scope of the established thiol bioconjugation chemistry with its plethora of commercially available reagents at reasonable costs. Split inteins reconstitute proteins from two segments by protein trans-splicing. We have explored short sequence tags with a pre-modified single cysteine that can be appended by protein trans-splicing to a purified protein or to a protein in a living cell. Furthermore, we have developed non-natural amino acids with caged thiol side chains that can be selectively deprotected and hence allow for a defined labeling of two thiol groups in one protein, for example. Homocysteine with its altered reactivity of the thiol moiety further serves to manipulate and probe structural and catalytic properties of proteins.

Chromatin remodeling in condensed and phase separated chromatin

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Chromatin compacts and regulates the genomes of eukaryotes. Recently, it was discovered that chromatin can undergo phase separation. Phase separation provides profound challenges for cellular factors. (How) can chromatin factors enter these dense condensates, can they work in such a crowded environment, and how can they diffuse efficiently through the condensate? In fact, highly condensed chromatin has previously often been considered to be largely inaccessible to cellular factors.

We challenge this view at the example of the chromatin remodeling enzyme ISWI. Drawing energy from ATP hydrolysis, it slides nucleosomes along DNA. We show that neither chromatin folding nor condensation impedes nucleosome sliding in vitro. To this end, we developed an imaging-based nucleosome sliding assay, which allowed us to compare remodeling rates in- and outside of chromatin condensates. Using optical tweezers, we documented that ISWI hardens chromatin condensates whenever ATP hydrolysis is not permitted. Active hydrolysis is also required for ISWI's mobility inside condensates. ATP hydrolysis has therefore a dual use: it is not only needed for nucleosome sliding but also for propelling ISWI through dense condensates.

We propose a 'monkey-bar' model, in which ISWI can grab a neighboring nucleosome, or withdraw from it, in an ATP hydrolysis dependent manner. Molecular dynamics simulations of the model broadly agree with our data. We speculate that such 'monkey-bar' mechanisms could be shared more broadly by other chromatin factors. Our findings further suggest that pathologies induced by corrupted chromatin remodelers might be caused in part by changes in chromatin dynamics, and not exclusively by disruption of canonical remodeler functions.

The kinetochore: an intrinsically divisive molecular machine

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Chromosome bi-orientation is the pre-condition for successful cell division, but how it is achieved on the molecular level in settings as diverse as mitosis and meiosis remains poorly understood. Kinetochores play a decisive role in promoting chromosome bi-orientation and in imparting fidelity to the chromosome segregation process. In addition to binding microtubules, they recognize and correct improper microtubule attachments, and act as control centers to make the timing of cell division contingent on completion of bi-orientation through the spindle assembly checkpoint. How are these different activities regulated and integrated within the kinetochore's structure? To answer this question, our laboratory took up the long-term goal of reconstituting kinetochores and their functions in vitro, focusing on human kinetochores as model system. The reconstitution is challenging, because kinetochores consist collectively of ~35 core subunits [1], and several additional regulatory subunits, for a total of ~100 different polypeptides. The challenge is compounded by the embedding of kinetochores in the complex and incompletely understood environment of the centromere, a specialized chromatin domain whose organization promotes epigenetic propagation of the kinetochore assembly site through cell generations. As a summary of our work so far, I will present three large reconstitutions, comprising two major stable kinetochore sub-complexes (each with molecular mass ³ 1 MDa), and the signaling ensemble of the spindle assembly checkpoint [1-3]. I will illustrate what organizational principles have emerged from this work, often through parallel structural work. All three reconstitutions reflect stable interactions at thermodynamic equilibrium, and therefore cannot be considered "alive". The ultimate challenge for future in vitro work on the kinetochore, and a more general challenge for any in vitro reconstitution, is to ignite the energy-dissipating reactions that promote functional regulation and informational processing. We would like to build particles that, like their cellular counterparts, sense bi-orientation (or lack thereof) and turn the checkpoint on or off depending on context. This will require the addition of enzymes, most notably mitotic kinases and phosphatases, whose opposing regulation determines, at any given time, appropriate context-dependent signaling outcomes.

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OptoRiboGenetics: Light-dependent Control of RNA function through PAL-RNA platform

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Sensory photoreceptors nurture light-dependent adaptations in nature and enable the optogenetic control of organismal behaviour and physiology. One such photoreceptor is PAL. PAL was identified in the a gram-positive actinobacterium Nakamurella multipartite. The acronym PAL is derived from its unique architecture, comprising of Per-ARNT-Sim (PAS)[4], AmiR and NasR transcription antitermination regulators (ANTAR) and light-oxygen-voltage (LOV) domains. A crystal structure reconciles the unusual receptor architecture of PAL with C-terminal LOV photosensor and N-terminal effector units. This protein sequence-specifically binds to artificial RNA hairpin structures when exposed to blue light. This light-activated PAL-RNA complex is exploited for the spatio-temporal regulation of cellular processes such as transcription, translation, and mRNA processing in bacteria and mammalian cells. Furthermore, the PAL:RNA system is being established in vivo to control the gene expression in Drosophila Melanogaster using CRISPR activation.

Spatial metabolomics technology for life science and medicine

Prasad Phapale

Metabolomics technologies have proved their potential in early disease diagnosis and monitoring health conditions. This has also opened a new window into understanding the biochemistry of health and diseases such as cancers, cardiovascular disease, and other metabolic disorders. With recent advances in mass spectrometry imaging and computational methods, metabolites and lipids can be mapped at single-cell resolution from any tissue sections, organoids, and cell culture models. This emerging field of spatial metabolomics is complementing our spatial molecular understanding with a new dimension. The ability to label-free profiling 100s of metabolites from a biological system can provide unprecedented molecular insights into pathophysiological mechanisms with new therapeutic options for previously unmet medical conditions. In my talk, I will review the recent advances in metabolomics technologies and how they are shaping medical diagnosis as well as contributing to our molecular and spatial understanding of new biochemical pathways. The talk will also provide an overview of the potential of our latest MALDI imaging technology with post-ionization for comprehensive metabolite imaging.

Engineering Bioactive Dimeric Transcription Factor Analogs via Flow Synthesis and Palladium Rebound Mediated Protein Cross-Coupling

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Dysregulation of the transcription factor MYC is involved in the majority of human cancers. The dimeric transcription factor complexes MYC/MAX and MAX/MAX bind to the same enhancer box (E-Box) DNA. MYC/MAX activates, and MAX/MAX inhibits gene transcription. Inspired by the MAX/MAX activity, we engineered covalently linked, synthetic homo- and heterodimeric analogs of MYC, MAX, and Omomyc, to inhibit MYC-dependent gene transcription. We prepared the dimers (167-231 residues) in a single shot via chemical flow-synthesis or via palladium mediated protein cross- coupling. All variants displayed correct folding, DNA binding activity, and enhanced structural stability compared to their non-covalent counterparts. The dimers are intrinsically cell-penetrating and inhibit cancer cell proliferation in low micromolar concentrations. Via RNA sequencing and RT-qPCR we showed that the synthetic dimers specifically downregulate MYC-dependent transcription. This work shows the intriguing potential synthetic protein based modalities for artificial gene regulation.

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How cells defend their cytosol against bacteria: LPS ubiquitylation and other tricks

Felix Randow

Abstract not submitted

Regulating mitochondrial protein biogenesis

Peter Rehling

Abstract not submitted

Recent uncovered protective strategies in neurons against lipotoxicity in brain, an organ with very high fat content, rapid ATP turnover and large oxygen consumption

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Human brain has 60% fat / dry weight. It is thus an organ with one of the highest body fat content. This high amount is explained by the large degree of membranal lipids required for brain's functional integrity. But, due to inherent properties, neurons have very high ATP demand, and consequently a high oxygen consumption. However, free fatty acids (FFA) exert deleterious activities on mitochondrial

conversion of redox energy into ATP (see refs. in [Schönfeld P, Reiser G, 2013, Why does brain metabolism not favour burning of fatty acids to provide energy? . J. Cerebral Blood Flows & Metabolism 33, 1493-1499]). In addition, FFA stimulate mitochondria to production of reactive oxygen species (ROS), either as hydrogen delivering substrates or by partial electron transport block within the respiratory chain. Due to their poor antioxidative equipment, neurons respond particularly sensitive to FA-linked ROS generation. Several processes enhance cerebral FFA levels, such as uptake of FFA from circulation into brain tissue or increased hydrolytic degradation of membrane phospholipids in traumatic or hypoxic brain injuries. We propose the following main strategies to protect neurons against FFA-linked lipotoxicity: 1) spurning β-oxidation in mitochondria of neurons. 2) the supply from astrocytes of relevant metabolites to neurons for antioxidants synthesis. 3) neural autophagy of ROS-emitting mitochondria combined with transfer of degradation-committed FFA for disposal in astrocytes. This is an eminently potent protective strategy against ROS and harmful FFA activities. 4) estrogens and, generally, neurosteroids as protective triggers via ERK- and PKB-mediated signaling to initiate expression of neuronal survival genes via CREB.

TomoTwin: Generalized 3D localization of macromolecules in cryo-electron tomograms

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Cryoelectron tomography enables the visualization of cellular environments in extreme detail through the lens of a benign observer; what remains lacking however are tools to analyze the full amount of information contained within these densely packed volumes. Detailed analysis of macromolecules through subtomogram averaging requires particles to first be localized within the tomogram volume, a task complicated by several factors including a low signal to noise ratio and crowding of the cellular space. Available methods for this task suffer either from being error prone or requiring manual annotation of training data. To assist in this crucial particle picking step, we present TomoTwin: a robust, first in class general picking model for cryo-electron tomograms based on deep metric learning. By embedding tomograms in an information-rich, high-dimensional space which separates macromolecules according to their 3-dimensional structure, TomoTwin allows users to identify proteins in tomograms de novo without manually creating training data or retraining the network each time a new protein is to be located. TomoTwin is open source and available at https://github.com/MPI-Dortmund/tomotwin-cryoet

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Highly efficient stem cell genome editing to study the genetic basis of modern humans and disease-relevant mutations

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CRISPR-Cas9 genome editing has revolutionized molecular biology as it allows targeted modification of a gene of interest. This helps to investigate the molecular basis of life, the genetic basis of modern humans, as well as disease-relevant mutations. The first step in CRISPR-Cas9-mediated genome editing is the cleavage of target DNA sequences that are complementary to spacer sequences in the respective CRISPR gRNA. Cellular repair of CRISPR induced DNA double-strand breaks is dominated by error prone end joining pathways that outcompete inefficient homology-directed repair (HDR) that would allow precise introduction of a mutation present in a supplied synthetic DNA donor. I will present methods to increase CRISPR-Cas9 mediated cleavage of DNA (up to 1000-fold) by an engineered gRNA and to increase HDR-dependent precise genome editing (up to 90%) by modulation of DNA repair pathways.

Shedding light on the regulation and execution of necroptosis

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Necroptosis is an inflammatory form of regulated cell death implicated in neurodegeneration, cancer and immunity against infection. Execution of necroptosis depends on Mixed Lineage Kinase domain-Like (MLKL), a pseudokinase whose activity remains poorly understood. We combine membrane biophysics with

biochemistry, and structural and cell biology to understand MLKL function and regulation. We identified that pore formation is a core mechanism in necroptosis, which is linked to the activation of calcium fluxes through the plasma membrane and the endoplasmic reticulum. To get insight into the structure-function relation of MLKL, we investigate the activity and biological function of different MLKL isoforms. We uncovered a fundamental mechanism of how MLKL molecules work to trigger necroptosis. Specifically, we found a new site in MLKL that is so central to its function that interfering with it completely abrogates the necroptosis activity of naturally occurring isoforms. MLKL isoforms counterbalance each other to modulate necroptosis sensitivity, which may play a role in fine-tuning the level of inflammation in response to gram-negative bacterial infection. We exploited this structure-function relation knowledge and devised a new strategy for specifically targeting MLKL with a new class of allosteric inhibitors. These inhibitors emerged as a new tool to study the consequences of MLKL chemical inhibition in mouse models, opening new possibilities for the treatment of necroptosis-related diseases in humans.

A scale-spanning integrative modeling strategy to study structure, dynamics, and function of complex molecular machines

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Life is motion driven through cellular processes carried out by molecular machines. In order to understand and manipulate such cellular processes, scale-spanning knowledge of structure, dynamics, and function of molecular machines is needed. This knowledge cannot be obtained by one method alone. Therefore, I developed a strategy that combines ab initio structure prediction, molecular dynamics (MD) simulation, and quantum chemical calculations with data from structure resolving experiments to investigate the assembly and functional cycle of molecular machines from the electron up to the molecular level.

First, experimental data is converted into static structural models of different mechanistic states using bioinformatics methods. Second, active sites are refined at sub-Å resolution and intermediate states inaccessible by experiments are identified employing QM/MM simulations. Third, the obtained refined snapshots are merged to map the dynamics of the processes in their native environment using MD simulations. The strategy provides insights into the interplay between local processes like chemical reactions and global conformational changes driving cellular function. These insights lead to mechanistic hypotheses and key functional residues that motivate targeted experiments, e.g. mutagenesis studies of the suggested residues, to validate the structural dynamic models.

This generally applicable strategy was applied e.g. to obtain insights into ATP driven protein recycling by the proteasome (Hung et al, Nature Communications 2022) or the assembly of proteins involved in photosynthesis like photosystem II (Zabret et al, Nature Plants 2021) or vesicle inducing protein in plastids Vipp1 (Gupta et al, Cell 2021).

Dynamic framework for large-scale modeling of biomembranes and flexible peripheral proteins

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Membrane-associated peripheral proteins are, among other tasks, responsible for shaping and remodeling biomembranes. The necessary cooperative action of a multitude of curvature-inducing proteins is facilitated by indirect membrane-mediated interactions. Quantitative analysis of the dynamics of a collection of peripheral proteins in a consistent model that incorporates membrane kinetics as well protein flexibility and lateral dynamics is essential in fully describing protein aggregation and membrane remodeling processes. Here, we present our dynamic membrane modeling framework, in simulation setups including varying concentrations of flexible membrane-bound proteins. We present quantitative results on membrane-mediated interactions and investigate the aggregation kinetics, stationary distributions, and free energy landscapes governing the formation and break-up of protein clusters on the surface of the membrane. We demonstrate how protein flexibility plays a significant role in highly selective macroscopic aggregation behavior. Relying on transition-based reweighting analysis method (TRAM), we obtain accurate estimates of membrane entropy, and discuss how the balance between

entropic and enthalpic forces in protein cluster formation shifts with surface concentration.

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Relation between loop motion and catalytic activity by the example of the (βα)8-barrel enzyme HisF

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Motions of active site loops are essential for catalytic activity. Enzymes with the prevalent (βα)8- barrel fold are highly suitable for studying the relationship between loop dynamics and catalysis. We analysed the β1α1-loop of the cyclase subunit HisF of imidazole glycerol phosphate synthase which catalyses the formation of the imidazole ring in histidine biosynthesis. During the catalytic cycle this loop undergoes a transformation from a flexible, entropically rich form into a stiff ligand-bound conformation. The aim of this work was to define the functional significance of β1α1-loop mobility and its structural transitions for the catalytic activity of HisF. Steady-state kinetics showed that certain amino acid substitutions within the β1α1-loop reduce the turnover velocity of the enzymatic reaction, whereas substrate affinity is hardly affected. In- depth analyses of HisF mutants with altered loop dynamics did not evince a direct correlation between loop flexibility and turnover velocity. Instead, NMR spectroscopy revealed that catalytically active HisF mutants bind substrate via an induced-fit mechanism involving the β1α1-loop. Stopped-flow analysis under single- and multiple-turnover conditions suggested that turnover velocity of HisF is not limited by product release. In addition, substrate binding and product release steps are fast in comparison to the turnover number kcat, rendering the chemical conversion reaction as the rate-determining step in the catalytic cycle. Hence, although the structural changes of the β1α1-loop associated with the transition from the inactive to active conformation are not rate-limiting for catalysis, the induced-fit motion is a prerequisite for effective enzymatic function.

Life as a matter of function

Petra Schwille

Compared to physics and chemistry, biology has always been lacking something like a simplified model system such as the hydrogen atom that would allow to formulate and scrutinize first principles and laws required for a fundamental understanding of the phenomenon of life. The reason is that biology's study object is a moving target, as life ever since its origin on earth several billions of years ago has been complexifying through evolution, and although there is the conceptual agreement that the cell should be considered the basic unit of life, nothing is "basic" about this unit, the smallest representations of which still are incomprehensively complicated chemical reaction systems with more than thousands of genes alone. Our hypothesis is that if one ever wants to have in hands and under the microscope a truly minimal living system, one will have to build it from scratch. In contrast to origin-of-life research, however, we do not focus too strongly on the actual molecules nor aim to reproduce the plausible series of events that presumably led to the life we find on earth today. Instead, we understand life as a organizational form of matter that is primarily distinguished by a set of key functions, which can however be abstracted from their specific representatives in various organisms. In the past years, it has been our ambition to identify such a set of key functions for one of life's most central features, self-division. Our experimental work focuses on the reconstitution of a dramatically reduced number of elements of the bacterial cell division system, which however appear to emerge basic features of division in protocell compartments. From our work so far that I will present in my talk, we feel encouraged to believe that the complex cellular division machineries may indeed be deduced to a very limited set of general functional elements, and that some of these rudimentary functions may even still be partly conserved in "modern", highly specialized, proteins.

Cross-talk between cAMP and c-di-AMP signaling via carbon sensor protein SbtB: Linking CO2 homeostasis with diurnal metabolic switch

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PII superfamily consists of widespread signal transduction proteins found in all domains of life. In addition to canonical PII proteins involved in C/N sensing, structurally similar PII-like proteins evolved to fulfill diverse, yet poorly understood cellular functions. For efficient CO2 fixation at low ambient concentrations, cyanobacteria evolved highly specialized carbon concentrating mechanism, to augment intracellular inorganic carbon (Ci) levels. Recently, we identified the PII- like protein SbtB as Ci sensing module via sensing various adenine nucleotides including the second messenger nucleotides cAMP, and c-di-AMP, involved in global cellular homeostasis. We showed that cAMP acts as carbon signal, whereas adenyl-nucleotide binding links SbtB signalling to the energy state of the cells. The c-di-AMP signaling through SbtB turned out pivotal for day-night acclimation of cyanobacteria via regulation of glycogen metabolism. To our knowledge, this is the first signaling protein known integrating both cAMP and c-di-AMP signaling. Moreover, SbtB possess a C-terminal extension with a disulfide bridge, which we call R-loop. We revealed an unusual ATP/ADP apyrase activity of SbtB that is controlled by the R- loop. We followed the sequence of the hydrolysis reactions from ATP to AMP in crystallographic snapshots and revealed the structural mechanism by which changes of the R-loop redox state modulate apyrase activity. This highlights SbtB as a central switch-point in cyanobacterial cell physiology, integrating not only signals from the energy state (adenyl-nucleotide binding) and the carbon supply via cAMP binding, but also from the diurnal status reported by the R-loop redox-switch and c-di-AMP binding.

Structure of the lysosomal membrane fusion machinery

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Lysosomes are essential for cellular recycling, nutrient signaling, autophagy and the invasion of pathogenic bacteria and viruses. Lysosomal fusion is fundamental to cell survival and requires HOPS, a conserved heterohexameric tethering complex. On the membranes to be fused, HOPS binds small membrane-associated GTPases and assembles SNAREs for fusion, but how the complex fulfils its function remained speculative. Here, we used cryo-electron microscopy to reveal the structure of HOPS. Unlike previously reported, HOPS is surprisingly rigid and extensive flexibility is confined to its extremities, where GTPase binding occurs. The SNARE- binding module is rigidly attached to the core, therefore, ideally positioned between the membranes to catalyze fusion. Our results explain HOPS dual functionality and unravel why tethering complexes are an essential part of the membrane fusion machinery.

The unusual secretion mechanism of Tc toxins

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Disease-causing bacteria use a variety of secreted toxins to invade and subjugate their hosts. While the machinery responsible for secretion of many smaller

toxins has already been established, it remains enigmatic for larger ones such as Tc toxins from human and insect pathogens, which approach the size of a prokaryotic ribosome. Here we combine targeted genomic editing, proteomic profiling and cryo-electron tomography to finally reveal the mechanism of Tc toxin secretion and visualize it in unprecedented detail.

Structural insights into the catalysis of the [FeFe] hydrogenase from Desulfovibrio desulfuricans

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Hydrogen is considered to be an ideal primary energy carrier for a future society based on renewable energy due to its high energy density and clean combustion. At present H2 is produced mostly from fossil fuels or by water electrolysis using expensive noble metal catalysts. Nature has provided us with a series of enzymes that efficiently produce hydrogen with active sites containing only Fe or Ni and Fe. [FeFe] hydrogenases are the most active H2-converting catalysts in nature, but their extreme oxygen sensitivity is a major problem still to overcome. The [FeFe] hydrogenases from the sulfate-reducing bacterium Desulfovibrio desulfuricans (DdHydAB) is the most active H2-producing bio-catalyst, and it can be isolated in an O2-stable, inactive state called Hinact. We have obtained insights into the mechanism of O2- protection, the activation of the Hinact state, the catalytic mechanism of DdHydAB, and its inhibition by CO using a combination of X-ray crystallography and spectroscopy. Our results provide snapshots of the enzyme in different states and contribute to a better understanding of the function of [FeFe] hydrogenases.

Effects of in vivo conditions on protein aggregation: computational approaches

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The aggregation of proteins into β-sheet structures has been extensively studied in vitro under conditions that are far from the physiological ones. There is need to extend these investigations to in vivo conditions where protein aggregation is affected by a myriad of biochemical interactions. As a hallmark of numerous diseases, these self-assembly processes need to be understood in detail to develop novel therapeutic interventions. The aim of our work is to elucidate the effects of various in vivo components and conditions, such as the presence of metal ions, oxidative stress, an acidic environment mimicking tissue inflammation, the presence of cell membranes and the brain extracellular matrix on the conformational dynamics and aggregation of the Alzheimer's disease-related amyloid-β peptide. To this end, we develop multiscale simulation approaches, perform large-scale molecular dynamics simulations, and establish novel analysis tools allowing us to unravel the aggregation pathways under varying external conditions. The most recent and enlightening results from these simulations will be presented in my talk.

Interactions between DNA repair and transcription

Ingrid Tessmer

The base excision repair (BER) glycosylase hOGG1 (human oxoguanine glycosylase 1) is responsible for repairing oxidative lesions in the genome, in particular oxidised guanine bases (oxoG). In addition, a role of hOGG1 in transcription regulation by recruitment of various transcription factors has been reported. Here, we investigate the mechanism of recruitment of the medically important oncogene transcription factor Myc that is involved in transcription initiation of a large number of genes including inflammatory genes, by hOGG1 under oxidative stress conditions using

single molecule atomic force microscopy (AFM) in combination with biophysical and biochemical assays.

What 2000 macromolecular structures tell us about SARS-CoV-2

Andrea Thorn

During the COVID-19 pandemic, scientists rushed to solve the structures encoded by the SARS- CoV-2 genome in order to understand the viral infection cycle and to enable drug design. Over 2000 macromolecular structures were released in a short time span, which immediately were used to understand how the virus hijacks human cells, for drug and vaccine design. However, errors occur in even the most careful structure determination - and may be even more common among these structures. The Coronavirus Structural Task Force [1] has responded to this challenge by rapidly evaluating and reviewing all of these structures. In addition, we provided improved models for key structures online, set up a website (www.insidecorona.net) and data base. We also engaged in outreach activities, writing blog posts for scientists and the public alike, refining structures live on Twitch and offering a 3D printable virus model. We were an ad hoc collaboration of 26 researchers across nine time zones, brought together by the desire to fight the pandemic. Still, we were able to rapidly establish a host of COVID-19 related research, forge friendships and collaborations across national boundaries, spread knowledge about the virus and provide improved models for drug discovery projects. Now, after more than two years, we have consolidated our collective knowledge about the virus, and can leverage this insight for the question: What is next?

[1] Croll et al. (2021) NSMB 28, 404–408
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Lysine-cysteine redox switches in proteins

Kai Tittmann

Disulfide bonds between cysteine residues are important post-translational modifications in proteins with critical roles for protein structure and stability, as redox-active catalytic groups in enzymes or allosteric redox switches that govern protein function. Regulatory redox switches between two amino acids other than disulfides have not been demonstrated. We recently discovered a crosslink between a cysteine and a lysine with an unprecedented N-O-S bridge that serves as an allosteric redox switch in the enzyme transaldolase from N. gonorrhoeae. High resolution protein crystallography of the protein in the oxidized and reduced state reveals a loaded-spring mechanism with a structural relaxation upon redox activation that is propagated from the allosteric redox switch at the protein surface to the active site. A survey of the protein structure data base discloses that the NOS bridge is existing in diverse protein families across all domains of life. NOS redox switches are found in diverse structural motifs and chemical variants. In several instances, lysines are observed in simultaneous linkage with two cysteines forming a SONOS bridge with a trivalent nitrogen, which constitutes an unusual native branching crosslink. In many proteins, the NOS switch contains a functionally essential lysine with direct roles in enzyme catalysis or binding of substrates, DNA or effectors, linking lysine chemistry and redox biology as a regulatory principle. NOS/SONOS switches are frequently found in proteins from human and plant pathogens including SARS-CoV-2 but also in many human proteins with established roles in gene expression, redox signaling and homeostasis in physiological and pathophysiological conditions.

Redox-regulated chaperones in cell stress responses

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Protein unfolding and aggregation have long been associated with premature aging and disease development. Oxidative stress is a major cause of protein unfolding. Due to the concomitant drop in cellular ATP levels, the function of ATP-dependent chaperones and proteases is impaired. To counteract the severe risk of protein aggregation under these conditions, cells immediately activate a pool of highly abundant proteins as ATP-independent chaperones that bind unfolding protein intermediates and prevent their aggregation. We recently discovered how the highly conserved, eukaryotic protein Get3, which

functions as an ATP-dependent targeting factor for tail-anchored membrane proteins under non-stress conditions, becomes activated as a molecular chaperone upon oxidative stress. The chaperone function is tightly regulated by its nucleotide-binding state and the redox status of two conserved cysteines. Nucleotide-binding determines the accessibility and/or reactivity of the redox-sensitive cysteines. Thiol oxidation locks the protein in a nucleotide-free state, causes local unfolding and the formation of chaperone-active oligomers. Accordingly, chaperone inactivation depends on the reduction of Get3's cysteines followed by ATP-binding, which is essential for the transfer of client proteins to the ATP-dependent Hsp70/Hsp40 system for refolding as soon as non-stress conditions have been restored. Manipulating this highly orchestrated switching mechanism prevents client release and causes a growth defect and increased sensitivity towards oxidative stress in yeast cells. We recently found that the functional switch is conserved in the human homolog Asna1/TRC40, which protects the cells against oxidative stress.

To unlock sensitive molecular switches in live cells using high throughput FRET

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Keywords:
high throughput FRET, single-cell, time-resolved FRET, CD95, CTLA4

Introduction:
The field of molecular biology is rapidly evolving from studying binary yes/no relationships to probing complex signal transduction mechanisms. To study a system where the outcome depends quantitively on the concentration of input signaling molecules, a method is required that can measure interactions with high sensitivity, cover large concentration ranges and gather sufficient statistics to study the natural variability in live cells. Here we present high throughput FRET (htFRET), which uses EGFP-mCherry lifetime information from cellular data to perform FRET sensitized donor decay (ε(t)) fits1 to detect FRET fractions as low as 1%. Using our highly automated acquisition and data processing pipeline we can measure > 3000 cells in one experiment while obtaining phenotype information such as cell fate and protein expression level.

Application:
For the CD95 protein in presence of its ligand, we convert FRET fractions to oligomer fraction using Accessible Volume Simulations to reveal that 10 % oligomer formation in median is sufficient to trigger apoptosis. Furthermore, we study dimerization of the CTLA4 membrane receptor protein and find that the dimerization fraction depends on concentration, enabling us to obtain the dimerization constant Kd. An upper limit in the concentration accessible by FRET is posed by unspecific interaction due to proximity FRET. We quantify and discuss strategies to overcome this limit. Our method is easily transferable to measure molecular interaction < 10 nm in any cellular organelle.

1Greife, A., Peulen, T.O., et al. Functional FRET imaging in living cells with sub-nanometer resolution, in preparation

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Structural insights into sarcomere organisation by cryo-electron tomography

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Sarcomeres are force-generating and load bearing devices of muscles. A precise molecular picture of how sarcomeres are built underpins understanding their role in health and diseases. Here, we determine the molecular architecture of native skeletal sarcomeres and structures of sarcomeric proteins using cryo-focused-ion-beam milling (cryo-FIB) and electron cryo-tomography (cryo-ET). Our three-dimensional reconstruction of the sarcomere reveals molecular details in the A-band, I-band and Z-disc and demonstrates the organisation of the thin and thick filaments and their cross-links. Various in situ structures determined within sarcomeres with sub-tomogram averaging reveal unexpected interactions

between actin, myosin, tropomyosin, troponin and α-actinin. The native structure of the previously "nebulous" protein, nebulin, determined at 4.5 Å demonstrates the molecular mechanism underlying its role in stabilising thin filament, in regulating myosin binding and as a "molecular ruler". The structure establishes the molecular basis for the pathogenicity of nemaline myopathies mutations. In addition, myosin in the rigor-state sarcomere exhibits unique "double-head" and "split head" arrangements. The different conformations of the myosin neck domain, both within one and among different "double-head", highlight the inherent structural variability of myosin in muscle. Our structures of both the entire sarcomere and individual sarcomeric proteins after cryo-FIB and cryo-ET represent an expansion in capability for in situ structural biology and serve as a foundation for future investigations of muscle diseases.

Interplay between phase separation and reversible aggregation

Christoph Weber

Interactions among proteins in living cells can lead to coexisting phases and aggregates of different sizes. Both processes play an essential role in the spatial organization of cells and the regulation of biological function and dysfunction. A key challenge is understanding the interplay between aggregation and phase separation. Here, we study how phase coexistence influences aggregation equilibrium and how, in turn, aggregation affects the properties of coexisting phases.To this end, we propose a kinetic theory for a multicomponent mixture that contains aggregates of different sizes. Aggregates can nucleate, grow, shrink, and phase separate from the solvent. At thermodynamic equilibrium, we find that the size distributions of aggregates are significantly different between the dilute and the dense phase. We also show how size distributions by molecular interactions

at unsaturated conditions. Moreover, we find a gelation transition of the dense phase that coexists with a dilute phase mainly composed of small aggregates. We then study the aggregation kinetics of coexisting phases initially composed of monomers. We show that depending on the molecular interactions, the dense phase can contract or expand in volume.

Our theory can explain how aggregation kinetics affects the properties of coexisting phases and is consistent with recent experimental observations of densification and volume changes of protein droplets.

Exploiting the intrinsic structural dynamics of biomolecules as design feature - from antimicrobials to novel biosensors

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The ability of biomolecules to respond to a wide range of input signals is essential for life. Molecular switching and recognition events are critical for the cellular machinery regulating essential biological processes. Molecular switches are therefore of great interest for synthetic biology, biomedical, and protein engineering applications. In contrast to their fundamental role, a detailed understanding of the design principles and biophysical parameters underlying their switching-mechanism is lacking. We have developed a combined experimental biophysics and computational analysis platform that allows us to investigate the role of intrinsic structural dynamics changes for biomolecular decision making and identified these properties as a previously overlooked contributor to antibiotic action (Girodat,[...], Wieden, JACS 2019; Girodat, [...], Wieden, JMB 2020) or as a parameter for the rational design of biomolecular devices including custom biosensors (Smith,[...],Wieden, Biosens Bioelectron 2022; Smith,[...],Wieden, Sensors 2022).
Here we report a Molecular Dynamics (MD) guided study revealing for the first time how the Human cytomegalovirus (HCMV) pentamer exploits the intrinsic structural dynamics of host Neuropilin 2 (Nrp2) to gain access into the host cell (Dhalla, Smith & Wieden 2022, PNAS under review). We provide an overview of how computer aided description of structural dynamics and their underlying biophysical properties can be matched with experimental strategies such as single molecule FRET (Morse,[...],Wieden, PNAS 2020) and rapid kinetics to provide a multiscale description of switching and biomolecular recognition events in a wide range of biological systems.

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