Src-uniRapR expressed in HeLa cells treated with rapamycin displayed rapid induction of Src-mediated phenotypes, including protrusion and polarized spreading. Remarkably, uniRapR maintains its switch functionality in whole organisms, enabling the investigation of Src in key developmental processes inaccessible by cell culture studies. Here, we demonstrate that activation of Src kinase in the epidermal tissue of zebrafish leads to morphological changes and loss of cell-cell contacts. The rational creation of uniRapR exemplifies the power of computational protein design, and offers a powerful means for targeted activation of many pathways to study signaling in living cells and organisms.

100-Plat
Effect of Different Lysine Linkages on Polyubiquitin Chain Structure and Function
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Polyubiquitination is a critical post-translational modification of proteins. Polyubiquitination signals for a wide variety of cellular events including proteasomal degradation, DNA repair, cell cycle regulation, T-cell activation, etc. The molecular basis of the diversity of polyubiquitin signaling lies in the different structural and dynamical properties of polyubiquitin chains, linked between the C-terminus of one ubiquitin (Ub) and the epsilon-amine of a lysine side chain (K6, K11, K27, K29, K33, K48 or K63) on a second Ub. The canonical K48-linked and K63-linked chains have been well characterized with biophysical techniques. Owing to a lack of linkage-specific Ub-conjugating enzymes, little is known about the structural or functional properties of the other polyubiquitin chains. We devised a chemical ligation method to assemble polyubiquitin chains comprised of every lysine linkage, of specific length, and with selective isotopic labeling. Solution NMR measurements, including chemical shift perturbations, 15N relaxation measurements and residual dipolar couplings, in conjunction with small angle neutron scattering (SANS) measurements, have enabled us to describe the structural and dynamical properties of free polyubiquitin chains of every lysine linkage for the first time. We observed that a few of these chains (particularly K6-linked chains and K11-linked chains at high salt) can adopt compact conformations. Furthermore, binding studies demonstrated that K11-linked chains bind to Ub receptors with differential affinities than either K48-linked or K63-linked chains. In any case, these studies highlight the importance of determining the conformational ensemble of each of these polyubiquitin chains. Our analyses will provide a foundation for future work with polyubiquitin chains of any desirable length and linkage composition.

101-Plat
Cysteine-Shotgun Mass Spectrometry (CS-MS) for Probing Nuclear Lamin Conformation during Mechanical Stress
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Nuclear envelope proteins including the various lamins are expressed in all adult tissues, but lamins that are implicated in aging, cancer, and various dystrophies often affect specific tissues for unknown reasons. We have shown that the level of lamin-A,C and additional components of the nuclear envelope proteome that link the nucleus to the cytoskeleton scale systematically with tissue elasticity, while physical manipulation has demonstrated that nuclear stiffness scales with lamin-A,C. For example, brain tissue, which is relatively unaffected in the lamin-A,C-based aging disease progeria, has an elasticity about ten-fold softer than striated muscle with proportionately less lamin-A,C and a dominant amount of constitutive B-type lamins. Evidence suggests a mechano-sensitive regulation of the lamina, but the process of mechanical stimulation of molecular processes is poorly understood. Cysteine-shotgun mass spectrometry (CS-MS), a method capable of mapping the exposure of cysteine residues as proteins are stressed in complex biological systems such as isolated nuclear or whole cells, allowed us to identify stress sensitive proteins. We discovered a number of stress-sensitive proteins in isolated nuclei - including lamin-A,C - consistent with cell and tissue evidence that the nucleus transduces physical stress. Further work enquires whether substrate stiffnesses representative of soft and stiff tissue are reflected in changes to protein conformation.

102-Plat
Conformational Dynamics in Designer Cellulosomes Studied by Single-Pair Fret with Mfd-Pie
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Cellulose is the most abundant biopolymer on earth and when broken down into oligosugars, it can be used for the production of bioethanol. As such, cellulose holds great potential as a novel energy source. The anaerobic thermophilic bacterium Clostridium thermocellum expresses an extracellular multi-enzyme complex that degrades cellulosose: the cellulosome. Cellulosomes, in general, consist of a scaffoldin domain harboring binding sites for multiple cellulases. These enzymes interact via their dockerin domain with complementary cohesin domain on the scaffoldin. An emerging research field aims at engineering 'designer cellulosomes' by genetically coupling different types of cohesins. These novel minimal cellulosomes should provide similar, if not better, enzymatic activity as the wild-type. Little is known about the exact role of the linker peptides between cohesin moieties. To address this, we have site-specifically labeled a designer cellulosome consisting of two cohesin subunits connected by either a natural or shortened peptide linker and performed single pair Förster resonance energy transfer experiments using pulsed interleaved excitation and multi-parameter fluorescence detection. We observe different conformational states of the cohesin dimer, suggesting the presence of conformational dynamics. Relating the conformational dynamics of such designer cellulosomes with their activity will be of great help for understanding and improving their function.

103-Plat
G Protein Activation: A Protein Unfolding Event?
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Ric-8A, is a guanine nucleotide exchange factor for the alpha subunits of class i, q and 13 heterotrimeric G proteins. The biochemical activity of Ric-8A is analogous to that of G protein-coupled receptors (GPCRs), in that it activates Gα by catalyzing the exchange of GDP for GTP at the Gα guanine nucleotide binding site. Unlike GPCRs, Ric-8A acts on Gα•GDP monomers, rather than G protein heterotrimers composed of Gα•GDP and Gβγ. Ric-8A is required to support asymmetric cell division in *C. elegans*, *Drosophila* and mouse. Beyond its putative regulatory functions, Ric-8 is essential for Gα biogenesis and membrane localization, and inhibits ubiquitination and degradation of Gα. We have used several biophysical techniques, notably heteronuclear NMR, hydrogen-deuterium exchange, double electron-electron resonance, and Förster resonance energy transfer to characterize the spatial dimensions and kinetics of structural changes that occur in Gαi1 upon binding to Ric-8A, an event that is accompanied by GDP release. Ric-8A induces large inter-domain rearrangements in Gαi1, together with a globally dynamic state in which the nucleotide-binding Ras-like domain in particular appears have diminished tertiary structure. It appears that Ric-8A catalyzes nucleotide release by inducing or stabilizing a structurally heterogeneous, partially unfolded state of Gα.

104-Plat
Through the Ramachandran Haze: Ca-Parameters Reveal Secondary Structure at Low Resolution
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CaBLAM (Calpha-Based Low-resolution Annotation Method) addresses the challenge of producing stereochemically accurate models from rough chain tracings at low resolution (3-4Å). Much current excitement in biological crystallography centers on large complexes and ''molecular machines'', but the resulting models can be subject to artifacts arising from inherent properties of low-resolution electron density maps.
Even at poor resolution, chain tracing can produce full backbone models, achieving the primary objective of locating the amino-acid residues in 3D space. However, structural details like peptide orientation are very often distorted by misleading or ambiguous density. Mismodeled backbone wreaks havoc with standard ways of identifying secondary structure, placing side

chains, and refining a reasonable model. CaBLAM uses carbonyl oxygen placement to diagnose commonly occurring patterns of correctable backbone errors, then uses contours derived from a high-quality dataset in a novel parameter space of overlapping Cα pseudo-dihedrals to identify the secondary structures disguised by those errors.
In addition to continuous secondary structures like alpha helix and beta sheet, CaBLAM can identify non-continuous secondary structures such as helix caps, tight turns, and beta bulges, even in low-resolution models. The power to distinguish between modeling errors and these real irregularities will provide refinement with a more realistic, more detailed protein backbone from which to produce a reliable structural model.

105-Plat
Automated Circular Dichroism Spectroscopy for Medium-Throughput Quantification of Protein Conformation
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Circular dichroism (CD) spectroscopy is a powerful method for monitoring conformational changes of biomolecules. For proteins, it is highly sensitive to changes in secondary structure, which, in turn, are influenced by amino acid composition, posttranslational modifications, solution conditions (e.g., temperature, pH, salts, detergents, denaturants, excipients, etc.), and ligand binding. The CD signal is directly linked to protein structure, the analyte is in solution and label-free, the technique requires low sample amounts, and data analysis is straightforward. However, CD spectroscopy has remained a low-throughput method because it imposes high requirements on the optical quality of sample cells and thus cannot be performed in microplate-reader format. Here, we introduce an automated CD spectrometer that is equipped with a flow-through cell and coupled to a 3-axis robotic liquid handler. This enables completely unattended CD measurements on up to 384 samples, including sample transfer from 96-well plates into the flow-through cell, data acquisition, and cell cleaning. We demonstrate that the accuracy, precision, and data quality of the automated CD spectrometer are as good as those of a conventional, manually operated instrument and exemplify how the advantages offered by automated CD spectroscopy can be exploited in protein-unfolding experiments using chemical denaturants.

Platform: Membrane Active Peptides & Toxins

106-Plat
Probing the Membrane Interaction of Antimicrobial Agents In Vivo with Intact Bacteria by 2H NMR
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The cell membrane is an important biological interface often targeted by bioactive molecules such as antimicrobial peptides (AMPs) which can disrupt the bacterial lipid barrier. Identification and study of AMPs is a dynamic research field motivated by the emergence of antibiotic-resistant pathogens. To develop new efficient therapies, the action mechanism of membrane-disrupting antimicrobial agents should thus be studied. 2H solid-state NMR is a useful method to monitor changes in organization and dynamics of phospholipids in bilayers. Usually carried out with model systems, the complex composition of bacterial membranes - especially the presence of lipopolysaccharides (LPS) - encourages such studies on intact organisms. We have therefore developed a protocol to 2H-label phospholipids in Escherichia coli membranes without mutating. About 75% of the phospholipids had deuterated acyl chains. We have thus investigated in vivo the interaction mechanism of antimicrobial agents, i.e., the antibiotic polymyxin B (PxB), the detergent cetyltrimethylammonium chloride (CTAC) and fullerenol nanoparticles. 2H NMR spectra and spectral moment analysis support the insertion of the PxB's lipid tail in the bacterial membrane. Our results also suggest that membrane rigidification could play a role in the biocide activity of the detergent. The use of intact bacteria seems important in cases where the antibiotic action relies on an interaction with LPS. This is shown with fullerenol nanoparticles for which a different effect is seen on intact cells as compared to model DPPC/DPPG membranes, as will be detailed. This is further demonstrated with the blue pigment marennine produced by marine microalgae which would also interact with LPS. A different perturbation of the bacterial membranes by the intra- and extracellular forms of this pigment was also evidenced, thus shedding light on the action mechanism of this potential natural antibiotic.

107-Plat
Molecular Modeling of the Attachment of the Dengue II Envelope Protein into Host Endosomal Membranes
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Dengue virus is a world health threat responsible for 50-100 million infections per year with a 0.077% mortality rate. Its outer envelope is composed of an icosahedral shell of 180 copies of a class II fusion peptide, responsible for cell recognition and for escape from the host endosome via pH-induced rearrangement into a trimer. The lipid makeup of the endosomal membrane can block viral association and release, with homologous members of the flavivirus family requiring different key factors. We report results of the first molecular dynamics calculations on the complete envelope protein trimer at the membrane interface (21-aa segments were studied in Biochem. 49:287, 2010). The insertion energy profile and pattern of host membrane deformation upon viral association indicate that the insertion process is not highly energetically favorable. Instead, it is hypothesized that membrane association and viral fusion are mediated by binding of individual lipids to the tip of the viral envelope protein trimer. Our results explain the fusogenic activity of the isolated fusion peptide fragment, and suggest an alternative mechanism for the experimentally observed specificity to membrane composition.

108-Plat
Bacterial Biofilm Formation Induces Strong Shifts in Lipid Composition Resulting in Increased Resistance Towards Antimicrobial Peptide Activity
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Bacteria that interact with surfaces under hydrated conditions can form aggregated structures known as biofilms. Biofilms are characterized by having increased resistance to a variety of antibacterial agents. This resistance is responsible for the generation of persistent chronic infections, and represents a serious threat to human health. Several antimicrobial agents, including hydrolytic enzymes such as PLA2-IIA and antimicrobial peptides (AMPs) such as Magainin-2, act by disrupting bilayer membrane integrity. Since these antimicrobial agents require physical disruption of the bilayer membrane, their activity is likely to be sensitive to lipid packing.. In this work we show, by measuring generalized polarization of Laurdan incorporated into lipid extracts, drastic changes in the level of lipid packing in Staphylococcus aureus during biofilm formation. When analyzing lipid composition we find a significant reduction in the level of branched lipids in the biofilm membranes. A strong reduction in the level of carotenoids is also observed during biofilm formation. Additionally, we present evidence that this shift in the melting temperature modulates resistance towards magainin-2 at 37C where bacterial lipids are in the liquid-crystalline phase. These results point to a mechanism by which bacterial membranes can generate resistance towards membrane active antibacterial agents through the modulation of lipid composition during biofilm formation.

109-Plat
Antimicrobial Peptide Activity in a Competitive Membrane Lipid Environment
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Mastering the structure-activity relationship and specificity of antimicrobial peptides (AMP) against bacterial lipid membranes is required for the therapeutic development of membrane-active peptides. Correlation of physiological observations with in vitro studies, including high resolution structural work, can provide the required understanding of the mechanism by which AMP kill bacteria. For instance, maculatin 1.1 is an antimicrobial peptide that serves as part of the innate immune defences of an Australian tree frog, and has shown promising activity against methicillin-resistant Staphylococcus aureus but which also has appreciable haemolytic activity. Against that common assumption that lipid composition is often assumed to be the regulative mechanism,