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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