control of replication timing. We also show that variations in the spatial distribution of origins have minimal effect on the accurate control of replication times. Finally, we compare the replication program in *Xenopus* to the program that minimizes the use of certain replicative proteins; we find them to be similar.

2921-Plat
Dynamics Of DNA Replication Loops Reveal Temporal Control Of Lagging-strand Synthesis
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In all organisms, the protein machinery responsible for the replication of DNA, the replisome, is faced with a directionality problem. The antiparallel nature of duplex DNA permits the leading-strand polymerase to advance in a continuous fashion, but forces the lagging-strand polymerase to synthesize in the opposite direction. By extending RNA primers, the lagging-strand polymerase restarts at short intervals and produces Okazaki fragments. At least in prokaryotic systems, this directionality problem is solved by the formation of a loop in the lagging strand of the replication fork to reorient the lagging-strand DNA polymerase so that it advances in parallel with the leading-strand polymerase. The replication loop grows and shrinks during each cycle of Okazaki-fragment synthesis. Here, we employ single-molecule techniques to visualize, in real time, the formation and release of replication loops by individual replisomes of bacteriophage T7 supporting coordinated DNA replication. Analysis of the distributions of loop sizes and lag times between loops reveals that initiation of primer synthesis and the completion of an Okazaki fragment each serve as a trigger for loop release. The presence of two triggers may represent a fail-safe mechanism ensuring the timely reset of the replisome after the synthesis of every Okazaki fragment.

Platform BE: Protein Folding & Stability II

2922-Plat
The Rop-Dimer: A Folded Protein Living Between Two Alternate Structures
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A central assumption in protein folding is that a protein's native state is unique and stable. The Rop-dimer (Repressor Of Primer) shows strong changes in its folding kinetics and binding ability to RNA upon mutation of its hydrophobic core. Computer simulations investigated the possibility of two competing conformations to explain these results. Given an equivalent energetic bias, both conformations show different kinetic accessibilities in these simulations. Thus Rop's mutational behavior was explained by a preference of the kinetically less favored Wild-Type conformation for slow (un)folding mutants. Faster (un)folding mutants should prefer the kinetically favored conformation. For specific mutants it was suggested that the protein's native state is constituted by two competing conformations. Inspired by these simulations, single-molecule FRET-measurements verified the suggestion of two competing conformations constituting the native ensemble. Despite the need of a large-scale conformational change to get from the one conformation to the other, it shows that for a specific mutant the same dimer can adopt both conformations over time without disassociation of its monomers or changes in environmental conditions.

2923-Plat
Crowded, Cell-like Environment Induces Shape Changes In Aspherical Protein
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Protein dynamics in cells may be different from that in dilute solutions in vitro since the environment in cells is highly concentrated with other macromolecules. This volume exclusion due to macromolecular crowding is predicted to affect both equilibrium and kinetic processes involving protein conformational changes. To quantify macromolecular crowding effects on protein folding mechanisms, here we have investigated the folding energy landscape of an α/β protein, apoflavodoxin, in the presence of inert macromolecular crowding agents using in silico and in vitro approaches. By coarse-grained molecular simulations and topology-based potential interactions, we probed the effects of increased volume fraction of crowding agents (fc) as well as of crowding agent geometry (sphere or spherocylinder) at high fc. Parallel kinetic folding experiments with purified Desulfovibro desulfuricans apoflavodoxin in vitro were performed in the presence of Ficoll (sphere) and Dextran (spherocylinder) synthetic crowding agents. In conclusion, we have identified in silico crowding conditions that best enhance protein stability and discovered that upon manipulation of the crowding conditions, folding routes experiencing topological frustrations can be either enhanced or relieved. The test-tube experiments confirmed that apoflavodoxin's time-resolved folding path is modulated by crowding agent geometry. We propose that macromolecular crowding effects may be a tool for manipulation of protein folding and function in living cells.

2924-Plat
Assessing Mechanical And Thermodynamic Response Upon Allosteric Perturbation
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Allosteric regulation involves changes of protein activity upon ligand binding or covalent modification at a location distinct from the active site. We investigate the underlying mechanisms of allosteric proteins using a minimal Distance Constraint Model (mDCM) [1]. We recently employed the mDCM to assess how relationships between mechanical and thermodynamic descriptions affect intramolecular communication [2]. Here, both the mechanical and thermodynamic response upon allosteric perturbation is assessed. Constraints are introduced at every residue position to mimic the binding of allosteric ligands, and the resulting changes are analyzed using a wide array of response functions. We apply the methodology to several proteins, including calmodulin, CheY, and ras. Interestingly, application of a small number of constraints in one domain of the extended calmodulin structure is sufficient to cause substantial changes in the other, despite the propagation path being channeled through a long connecting helix. Residues identified as important to the binding and function of these dynamic protein systems show acute changes in thermodynamic response. In addition to quantifying changes in free energy, thermodynamic response is decomposed into component enthalpies and entropies. Allosteric response is also quantified by induced changes within mechanical properties, such as flexibility along the backbone, cooperativity correlation between residue pairs, and global rigidity characteristics. Taken together, the mechanical and thermodynamic responses provide insight into the fundamental mechanisms of allosteric communication. This work is supported by NIH R01 GM073082.
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2925-Plat
Configuration Entropy Modulates the Mechanical Stability of Protein GB1
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Configurational entropy plays important roles in defining the thermodynamic stability as well as the folding/unfolding kinetics of proteins. Here we combine single molecule atomic force microscopy and protein engineering techniques to directly examine the role of configurational entropy in the mechanical unfolding kinetics and mechanical stability of proteins. We use a small protein GB1 as a model system and constructed four mutants that elongate loop 2 of GB1 by two, five, twenty four and forty six flexible residues, respectively. These loop elongation mutants fold properly as determined by far-UV circular dichroism spectroscopy, suggesting that loop 2 is well tolerant of loop insertions without affecting GB1's native structure. Our single molecule AFM results reveal that loop elongation decreases the mechanical stability of GB1 and accelerates the mechanical unfolding kinetics. These results can be explained by the loss of configurational entropy upon closing an unstructured flexible loop using classical polymer theory, highlighting the important role of loop regions in the mechanical unfolding of proteins. This study not only demonstrated a general approach to investigate the structural deformation of the loop regions in mechanical unfolding transition state, but also provides the foundation to use configurational entropy as an effective means to modulate the mechanical stability of proteins, which is of critical importance towards engineering artificial elastomeric proteins with tailored nanomechanical properties.

2926-Plat
Air/water Interface Induced Folding And Self-assembly Of Amyloid-beta Peptide
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The amphipacity of the natively unstructured amyloid-beta (Ab40) peptide may play an important role in its aggregation into beta-sheet rich fibrils that is linked to the pathogenesis of Alzheimer's disease. Using the air/water interface as an ideal hydrophobic interface, we characterized Ab's surface activity and the structure, assembly, and morphology of Ab adsorbed to the air/water interface. Ab dissolved in water readily adsorbed to the air/water interface to form a contiguous film with a surface pressure of approximately 14 mN/m and showed an apparent critical micelle concentration of about 100 nM. Adsorbed Ab was composed of a single molecular layer extending approximately 20 Å into the aqueous subphase with in-plane ordering that gave rise to X-ray diffraction peaks. Analysis of the diffraction peaks showed that the air/water interface induced the otherwise unstructured Ab peptides to self-assemble into nano-size clusters with Ab peptides folded in a beta-sheet conformation. The presence of these clusters was further confirmed by imaging the morphology of the Ab film using atomic force microscopy. The formation of these ordered clusters was not affected by solution pH, ionic strength, or the presence of cosolutes sucrose and urea at concentrations that are known to stabilize and denature native proteins in solution, suggesting that the hydrophobic interface-driven Ab folding and assembly is robust and strongly favorable. Furthermore, Ab adsorbed at the air/water interface can seed fibril growth in solution when re-introduced into the bulk. Our results implicate that that interface-induced Ab folding and self-assembly may serve as a mechanism by which Ab aggregation occurs in vivo.

2927-Plat

Thermodynamic and Kinetic Characterization of MST1 and Rassf5 conserved Sav/Rassf/Hpo (SARAH) Domain Interactions

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The molecular switch Ras exhibits its biological function- control of growth, differentiation and apoptosis through the interaction with a multitude of different effectors. It is apparent that growth-inhibitory properties of Ras are mediated via noncatalitic polypeptides of Rassf (Ras Association Domain Family). Tumour suppressor Rassf5 (also termed Nore1) binds directly to active Ras via the Ras Binding Domain (RBD). It is also known to form self-associated complex as well as heterodimers with the proapoptotic serine/threonine Mammalian Sterile 20-like kinase (MST1), the human ortholog for Hippo (Hpo), through their common conserved C-terminal Sav/Rassf/Hpo (SARAH) domains [1, 2]. This unique interaction motif connects the proteins involved in the recent discovered pathway mediated by proteins of the MST family, which promotes apoptosis and restricts cell proliferation [3-6].

For a better understanding of MST1 and Nore1 homo- and hetero- interactions via the SARAH domains, we have investigated the thermodynamics and kinetics of association/dissociation as well as the unfolding mechanism of this domain by use of different biophysical and biochemical methods, such as Differential Scanning Calorimetry (DSC), size-exclusion chromatography, artificial chemical cross-linking, Isothermal Titration Calorimetry (ITC), Circular Dichroism Spectroscopy (CD). MST1 and Nore1 SARAH domains are shown to form not only homodimers, but also higher oligomers. Nevertheless, the heterodimers rather than homodimers are preferentially formed. Finally, we propose a possible mechanism for the thermal unfolding of MST1 and Nore1 SARAH homo- and heterocomplexes.

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2928-Plat

Chromophore Isomerization Has Large Effects On The Residual Structure Of The Fully Unfolded State Of The Blue Light Receptor Photoactive Yellow Protein

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Protein folding occurs between a well-defined fully folded native state and a structurally much less studied fully unfolded state. We use denaturant *m* values and changes in heat capacity ΔC_p to probe folding transitions in photoactive yellow protein (PYP). PYP is a bacterial photoreceptor that exhibits rhodopsin-like photochemistry based on the *trans* to *cis* photoisomeriation of its covalently attached *p*-coumaric acid (pCA) chromophore. We report strong effects of the isomerization state of the pCA on the residual structure of the ''fully unfolded'' state of PYP by comparing the unfolding of two partially unfolded states of PYP: the acid-denatured state pB_{dark}, which contains *trans*-pCA, and the partially unfolded pB photocycle intermediate, which contains *cis*-pCA. Our characterization of pB_{dark} by circular dichroism spectroscopy and quenching of aromatic fluorescence indicates a strong loss of tertiary structure in pB_{dark}. Despite its low tertiary structure content, pB_{dark} retains considerable cooperativity for unfolding. As expected, the unfolding of pB_{dark} is associated with values for denaturant *m* value and ΔC_p that are smaller than those for the native pG state of PYP. A range of published studies show that the pB state is partially unfolded. We characterize the pB state based on its specific cold denaturation. Despite its partially unfolded nature, we find that the denaturant *m* values and ΔC_p values for unfolding of the pB state are essentially the same as those for the native pG state. These results provide experimental evidence that pCA *trans* to *cis* photoisomerization causes significant loss of residual structure in the ''fully unfolded'' state of PYP. Such large changes in the residual structure of the fully unfolded states have important implications for describing and understanding protein folding.

2929-Plat

Pressure Induced Denaturation in Proteins: Stability and Kinetics

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Intricate interplay of temperature and pressure on protein folding leads to interesting phase diagram. In addition, kinetics of pressure induced folding exhibit complex behavior. Here, we propose a simple mesoscopic model, a combination of landscape theory and microscopic details based on polymer physics to investigate this interesting phenomenon. The model is applied to experimental data to understand physical principles of pressure induced denaturation.

Platform BF: Exocytosis & Endocytosis

2930-Plat

Massive Endocytosis (MEND) Activated by Ca and Polyamines in Fibroblasts and Cardiac Myoyctes: Dependencies on nucleotides, PIP_2, cholesterol, clathrin, and other factors

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We describe four protocols that result in internalization of ~50% of the surface membrane of BHK fibroblasts and cardiac myocytes within <1 min. To do so, we use patch clamp with large pipette tips for cell dialysis and Na/Ca exchangers to evoke cytoplasmic Ca transients (5 to 200 μM Ca^{2+}) for 1-5 s. Endocytosis is monitored via capacitance and/or optically by standard membrane dyes. In the first protocol, ATP is depleted from the cytoplasm, a Ca transient is evoked, and MEND is then activated by replenishment of ATP and GTP. GTP alone is not sufficient, Ca transients are required, and AMPPNP does not substitute for ATP. Second, when membrane cytoskeleton is stabilized with phalloidin, MEND is made 'available' for 1 to 3 min, and it occurs within 5 s during a Ca transient without ATP depletion. Third, high ATP concentrations (4 to 8 mM) promote MEND to occur within 20 to 60 s after (but not during) a Ca transient. Fourth, polyamines, spermine or spermidine, at physiological concentrations (1 mM) cause MEND to occur within <5 s during Ca transients without ATP depletion. MEND is not blocked by protein domains and other interventions that block chathrin-dependent endocytosis or by tyrosine kinase inhibitors. MEND is blocked by cholesterol depletion, GTPgS, and PIP_2 phosphatases, and MEND is promoted by perfusion of PIP_2 into cells when ATP and GTP are depleted. In neonatal myocytes, transient GTPgS perfusion substitutes for Ca transients in permissive steps leading to MEND activation upon ATP perfusion. We conclude that MEND is a regulated and massive cell stress response that can remove large fractions of the cell surface of multiple cell types by clathrin-independent mechanisms.

2931-Plat

Distinct Dynamics Of Endocytic Clathrin Coated Pits And Coated Plaques

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Clathrin is the scaffold of a conserved molecular machinery that has evolved to capture membrane patches, which then pinch off to become traffic carriers. These carriers are the principal vehicles of receptor-mediated endocytosis and are the major route of traffic from plasma membrane to endosomes. We report here the use of *in vivo* imaging data, obtained from spinning disk confocal and total internal reflection fluorescence microscopy, to distinguish between two modes of endocytic clathrin coat formation, which we designate as ''coated pits'' and ''coated plaques''. Coated pits are small, rapidly forming structures