exploit glycosylation as a strategy for tuning cellulase efficiency and stability, which can serve to enhance the cost-competitiveness of second-generation biofuels.

2634-Pos Board B64
Single-Molecule Studies of Multidomain Protein Folding
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Proteins are synthesized as linear polymers that must fold into specific three-dimensional structures to become functionally active. How proteins find their native structures is still not well understood, particularly for multi-domain proteins that account for a large fraction of proteomes. It has become evident that the way proteins are synthesized directly affects their folding: The ribosome synthesizes proteins through the addition of single amino acids to the C-terminus of the growing nascent chain. Folding can begin while elongation is still proceeding. Thus, folding and synthesis are kinetically coupled, and modulations in elongation rates can affect the folding outcome. Molecular chaperones interact with nascent polypeptides and begin to guide their folding as soon as they emerge on the ribosomal surface. We are using single-molecule force spectroscopy with optical tweezers to study the folding of multi-domain proteins in the context of the translation machinery and molecular chaperones. Our measurements are revealing how the process of translation and interactions with molecular chaperones shape the folding energy landscapes of nascent proteins and efficiently guide newly synthesized proteins toward their native structures.

2635-Pos Board B65
Folding and Unfolding Dynamics of Titin Immunoglobulin Domain Under Constant Forces
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The mechanical stability of force-bearing proteins is crucial for their functions. However, slow transition rates of complex protein domains have made it challenging to investigate their equilibrium force-dependent structural transitions. Using ultra-stable magnetic tweezers, we report the first equilibrium single-molecule force manipulation study of the classic titin I27 immunoglobulin domain. We found that individual I27 in a tandem repeat unfold/fold independently. We obtained the force-dependent free energy difference between unfolded and folded I27, and determined the critical force of ~ 5.2 pN at which unfolding and folding have equal probability. We also determined the force-dependent free energy landscape of unfolding/folding transitions based on measurement of the free energy cost of unfolding.
Our results suggest that the conformations of titin immunoglobulin domains can be significantly altered during low force, long duration muscle stretching. The ultra-stable magnetic tweezers can be used to study the folding/unfolding dynamics of other complex proteins.

2636-Pos Board B66
Exploring the Stability Limits of Actin and its Suprastructures
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Actin is the main component of the microfilament system in eukaryotic cells and can be found in distinct morphological states. G-actin is able to assemble into highly organized, supramolecular cellular structures, filamentous (F) and bundled (B) actin. To evaluate the structure and establish the stability of G-, F- and B- actin over a wide range of temperatures and pressures, FTIR spectroscopy in combination with differential scanning and pressure perturbation calorimetry, small-angle X-ray scattering, laser confocal scanning and transmission electron microscopy was used. On the one hand, our analysis was designed to provide new insights into the stabilizing forces of actin self-assembly and to reveal the stability of the actin polymorphs, including conditions encountered in extreme environments. On the other hand, our data help understand the limited pressure stability of actin self-assembly observed in vivo. G-actin is not only the least temperature-stable, but also the least pressure-stable actin species. At abyssal conditions, where temperatures as low as 1-4°C and pressures up to 1 kbar are reached, G-actin is hardly stable anymore. The supramolecular assemblies of actin are stable enough to withstand the extreme conditions usually encountered on Earth, however. Beyond ~3-4 kbar, filamentous structures disassemble, and beyond ~4 kbar, complete dissociation of F-actin structures is observed. Between about 1 and 2 kbar, some disordering of actin assemblies commences already, in agreement with in vivo observations. The limited pressure stability of the monomeric building block seems to be responsible for the suppression of actin assembly in the kbar pressure range.

2637-Pos Board B67
Thermal Stabilization of Adenylate Kinases by Optimizing Local Structural Entropy
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Local structural entropy (LSE) is an empirical descriptor for the extent of conformational variability in short protein sequences, computed from structural information derived from structures in the Protein Data Bank. Reducing the LSE of a protein sequence by mutating amino acid residues can result in fewer conformational states and thus a more stable structure. Here, we report a series of LSE optimization experiments designed to stabilize mesophilic and thermophilic adenylate kinases (AKs). The crystal structures of the LSE-optimized AK variants reveal that stabilization by LSE reduction may result from the optimization of local hydrophobic contacts. The limitations of the LSE optimization are likely due to ignorance of interactions connecting distant regions of the polypeptide. Our results illustrate the effectiveness and limitations of LSE optimization as a protein stabilization method and highlight the complementarity of local and global structural features in protein thermal stability.

2638-Pos Board B68
Mos1 Transposase Thermodynamic Stability and Flexibility
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DNA transposons are mobile DNA elements that can move (or transpose) from one DNA molecule to another and thereby deliver genetic information into human chromosomes in order to confer a new function or replace a defective gene. This process is catalyzed by a transposase enzyme. The reaction of transposition occurs in several steps, during which two or more transposase enzymes bind to the terminal inverted repeats on the transposon DNA, bring them together to form a synaptic complex, excise the gene flanked by the terminal inverted repeats, and catalyze strand transfer to insert the excised gene at a new location. Thus, transposases must be sufficiently flexible to allow conformational rearrangements of their domains to bind the transposon DNA and to supply a catalytic site during each step of transposition. Here, we investigate the dynamics, thermodynamic stability, and flexibility of Mos1 transposase, a member of the Tc1/mariner family of transposases. We use a computational model called the minimum Distance Constraint Model (mDCM) and the analysis of quantitative stability/flexibility relationships (QSFR). With these tools, we determine the free energy landscape and the flexibility and mechanical coupling of secondary structure elements or residues in Mos1. Our data provide an insight into how Mos1 is structured and how it functions and are applicable to Tc1/mariner transposases in general.

2639-Pos Board B69
Stabilization and De-Stabilization of (Membrane-)Proteins by Microbial Glycolipid and Lipopeptide Biosurfactants - *in-vivo* Relevance and Industrial Applications
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Glycolipid and lipopeptide biosurfactants are secondary metabolites which are produced and secreted by a wide range of both pathogenic and non-pathogenic microorganisms. Under optimal conditions production of biosurfactants can reach 400 g/L. In-vivo, biosurfactants interact with a wide range of functional proteins and biosurfactants also has the potential to substitute non-sustainable chemical surfactants in detergents where they interact with enzymes. It is therefore relevant to understand how biosurfactants *in-vivo* interact with functional extracellular proteins and also how biosurfactants interact with industrial detergent enzymes.
Biosurfactants can denature globular proteins such as myoglobin and α-lactalbumin. Denaturation kinetics is however an order slower when compared to classical chemical surfactants such as SDS. Commercial detergent enzymes are not denatured and inactivated by biosurfactants. This is in contrast to chemical surfactants such as SDS. Biosurfactants are thus promising eco-friendly alternatives to chemical surfactants.