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.

Biosurfactants can promote and stabilize protein structure. Biosurfactants fold and stabilize outer membrane proteins. The thermal stability is however perturbed in anionic biosurfactants compare to non-ionic dodecyl maltoside which is often used for membrane protein stabilization.
Upon infections, human proteins may be exposed to secreted microbial biosurfactants. Lysozyme, a defensive and antimicrobial human enzyme, is highly protease resistant. However in the presence of anionic rhamnolipid, produced by the opportunistic pathogen *Pseudomonas Aeruginosa,* the positive surface potential is neutralized and lysozyme becomes easily susceptible for both human and bacterial proteases. Rhamnolipid also promotes fibrillation of FapC, the main protein in *Pseudomonas Aeruginosa* functional amyloids. Rhamnolipid induces instant fibrillation and also morphological changes in FapC fibrils. Biosurfactants may thus play an important role in microbial biofilms and infections.
In general, biosurfactants can interact with proteins in multifarious ways with fundamental, medical and industrial relevance.

2640-Pos Board B70
Stability Analysis of CFTR via Tryptic Digestion
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Cystic fibrosis (CF) is caused by malfunctioning cystic fibrosis transmembrane conductance regulator (CFTR). When the flow of chloride ions through the CFTR channel becomes hindered or ceases, mucus begins to accumulate within the body causing a plethora of respiratory, digestive, and reproductive complications. From the 1,500 different CFTR mutations that exist, a genetic mutation resulting from the deletion of a phenylalanine residue at the 508 position (ΔF508) has been identified as the most common. For that reason, the ΔF508 mutation has become a target for initial treatment options. Due to the transmembrane nature of CFTR and a length of 1480 residues, the crystal structure and consequently the functionality of CFTR, are still vastly unknown. The objective of this research is to more thoroughly understand the relative stability of CFTR, the ΔF508 mutant and the treated ΔF508 mutant via limited tryptic digestion and the quantification of western blots. The ΔF508 mutant was treated with an energy source in which it typically binds (ATP), a non-hydrolysable ATP analogue (AMP-PNP), and with an available cystic fibrosis treatment option (lumacaftor/VX-809). To adequately represent both of the nucleotide binding domains (NBDs) in CFTR, antibodies 660 (IGg1) and 769 (IGg2b) were used. The quantification of results indicated that the induced stability of the AMP-PNP treated and ATP treated ΔF508 CFTR were significantly greater than the ΔF508 mutant alone, but not significantly greater than the VX-809 treated CFTR.

2641-Pos Board B71
Modulation of Cytochrome C Stability during Chemical and Thermal Denaturization by Addition of Hofmeister Ions
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The ferric cytochrome *c* (Cyt *c*) (un)folding mechanism in the presence of ions from the Hofmeister series is examined. Unfolding was initiated both thermally and with chemical denaturants. Hofmeister ions were added singly and in pairs to alter the stability of the native folded state, the unfolded state, and two partially folded intermediates. Protein stability was characterized by either the midpoint of the chemical denaturization curve or by the melting temperature in the thermal studies. UV/VIS absorption spectroscopy and a basis spectra fitting analysis were used to determine the populations of each protein conformation along the folding pathway. These species can be differentiated by their axial heme ligands. Four species exist in solution: the native HM state (His18/Met80), the partially folded HW (His18/water) and HH (His18/His33) intermediates, and the 5C (water) unfolded state. The results indicate that the thermal and chemical denaturization pathways are not the same and that both involve significant backbone rearrangement. The relative populations of the conformational states depends on how the protein is denatured. Additionally, it was found that addition of multiple ions changed the protein's stability in an additive manner. For example addition of guanidinium, which destabilizes folded structure, can be countered by addition of phosphate, which stabilizes folded structure. Both the (un)folding kinetics and the accessible conformations were found to depend on the identity of ions present. These results are discussed in terms of the hydrophobic effect, partitioning of the ions to the protein surface, and an altered water structure around the protein.

2642-Pos Board B72
Theoretical Prediction of Thermal-Stability Changes upon Mutations of a Protein
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Maintaining the structure and function of a protein at a temperature above the denaturation temperature of its native state is a principal objective of researchers in a variety of fields. One of the methods of enhancing the thermal stability is the mutation. In the present study, we report the results of the first attempt to develop a theoretical approach for predicting the thermal-stability changes upon mutations of a protein without using any parameters fitted to the experimental data. The approach is based on our recently developed free-energy function wherein the water-entropy effect is treated as an essential factor. Protein folding is driven by a large gain of water entropy. The gain is ascribed to an increase in the number of accessible configurations of water which is brought primarily by an increase in the total volume available to the translational displacement of the water molecules in the bulk. This water-entropy effect can be characterized by the following: It is reasonably taken into account only by a molecular model for water; not only the water near the protein surface but also the water in the bulk makes a substantial contribution to the effect (i.e., the effect cannot be considered in terms of the water-accessible surface area alone); and the protein-water-water triplet and higher-order correlations play critical roles. Our theoretical approach can also be distinguished from the previously reported approaches in the respect that the water-entropy effect is taken into account to its full extent. Its performance is compared with that of FOLD-X, one of the most popular approaches using the fitting parameters, for ten proteins and single and multiple mutations. Ours is shown to be superior to FOLD-X.

2643-Pos Board B73
Tuning of Alpha-Synuclein Aggregation by Small Molecules and Bacterial Proteins
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Parkinson's disease affects a growing number of the population and involves motor complications due to the death of dopamine neurons. Cytosolic inclusions containing amyloid fibrils of α-synuclein are a hallmark of the disease and it is believed that the aggregation process (going from monomers to amyloid fibers) of alpha-synculein somehow causes neurodegeneration. The synuclein-rich inclusions share structural characteristics with amyloid fibers found in many other neurodegenerative disorders. In addition, many organisms employ amyloid structures for mechanical or biological functions; for example, amyloid fibers are the major component of microbial biofilms. Mature amyloid fibers of alpha-synculein may not be the source of cytotoxicity; instead, transient oligomeric structures may be most dangerous to the neuronal cells. To investigate molecular pathways leading to alpha-synculein amyloid fibers, and thereby get hints for how to combat Parkinson's disease in vivo, we have taken a unique approach that involves purified proteins, biophysical experiments in vitro, and small-molecule tools. We have found that strategic ring-fused 2-pyridone compounds (mimics of small peptides), can tune alpha-synuclein aggregation such that either inhibitory or templating oligomers accumulate. Moreover, a fine balance between templation and inhibition processes is evident since one particular 2-pyridone inhibits bacterial amyloid formation but promotes alpha-synuclein amyloid fibers. In analogy with the small molecule tools, we found that bacterial proteins can cross-react with alpha-synculein and inhibit as well as promote amyloid fiber formation at sub-stoichiometric levels. Direct interactions of alpha-synculein with bacterial proteins and/or natural metabolites may play a role in controlling Parkinson's disease in humans.

2644-Pos Board B74
Ezrin is a Potent Modulator of Alpha-Synuclein Oligomerization
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Alpha-synuclein oligomers are associated with toxicity in Parkinson's disease. Different forms of alpha-synuclein (aS) have been described, some of which can destabilize lipid bilayers, and seed the formation of fibrillar assemblies. Therapeutic interventions such as molecular chaperones and small molecule