amounts of stress-stiffening. In this study, we use two experimental systems to probe the effects of pressure on the wall stiffness. First, we study blebbing E. coli cells using atomic force microscopy to separate the contributions of the cell wall and turgor pressure to the overall cell stiffness. Second, we study the relationship between cell stiffness and external osmotic pressure which allows us to probe higher turgor pressures. Combining these two types of measurements, we find strong evidence of a power-law stress-stiffening in the E. coli cell wall such that the wall is significantly stiffer in live cells (E~5 MPa) than the relatively compliant sacculus stiffness (E~34 MPa).

2801-Plat
Ballistic Motion of Spirochete Membrane Proteins
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Borrelia burgdorferi spirochetes are pathogenic bacteria which can cause Lyme disease. *B. burgdorferi* use membrane proteins to anchor themselves inside ticks which act as intermediate hosts. Human immune cells that fight bacterial infections bind to bacteria to internalize them. This binding can be mediated by antibodies against bacterial membrane proteins. Therefore these proteins and their mechanical behavior in the membrane play a key role for the disease transmission and the immune response.
The mechanical behavior of proteins in bacterial membranes is still poorly understood. We investigated membrane protein dynamics in *B. burgdorferi* with microparticles coated with monoclonal antibodies against the spirochete outer surface protein A (OspA). We used holographic optical tweezers to attach the microparticles to membranes of live *B. burgdorferi* and we tracked the subsequent particle motion.
Surprisingly the particles were transported ballistically to one end of the bacteria. The average speed of this transport was 3.1 $\pm$ 0.2 um/s. To determine if the microparticle motion is driven by the proteins, we are using speckle microscopy with fluorescently labeled OspA. In addition, we measured the stall force of the transport and found that a few piconewtons were sufficient to inhibit the motion. Although *B. burgdorferi* have a symmetric morphology, the protein motion showed a preferred direction of transport towards one end in individual bacteria. The origin of this symmetry breaking still needs to be unraveled. Mutant *B. burgdorferi* which lack the flagella that are found in wild-type bacteria did not show directed transport of membrane proteins, but only diffusive motion. Therefore we hypothesize that the protein transport is enabled by the bacterial motility machinery. We will discuss the proposition that this transport of membrane proteins could be a novel type of bacterial defense mechanism against immune cells.
[HK and RB contributed equally]

Platform AX: Protein Folding & Stability II

2802-Plat
Protein Protein Interactions - the Effects of Cosolvents, Crowding and Pressure
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The effects of various kosmotropic and chaotropic cosolvents and salts on the intermolecular interaction potential of proteins (e.g., lysozyme, SNase) was evaluated at low to high protein concentrations by using synchrotron small-angle X-ray scattering in combination with liquid state theoretical approaches. The experimentally derived static structure factors $S(Q)$ obtained without and with added cosolvents and salts were analyzed with a statistical mechanical model based on the DLVO potential which accounts for repulsive and attractive interactions between the protein molecules. Different cosolvents and salts influence the interactions between protein molecules differently as a result of changes in the hydration level or solvation, in charge screening, specific adsorption of the additives at the protein surface, or increased hydrophobic interactions. Experimentally derived static structure factors were also obtained for the aggregation-prone protein insulin. The data reveal that the protein self-assembles into equilibrium clusters already at low concentrations. Striking differences regarding interaction forces between aggregation-prone proteins such as insulin in the pre-aggregated regime and natively stable globular proteins are found. Finally, the effects of crowding and pressure on the solvational properties and intermolecular interaction of the proteins were studied, and a tentative temperature-concentration-pressure phase diagram has been obtained.

2803-Plat
Markov State Models of Millisecond Folder ACBP Reveals New Views of the Folding Reaction
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A combination of large-scale distributed computing efforts and Markov State Model (MSM) approaches have recently been used to simulate the folding of proteins on the millisecond timescale. These models predict new views of the folding reaction–a complex set of metastable intermediates, multiple pathways, a hub-like network structure, and compact unfolded states with residual structuring–yet, experimental observables often report simple two-state kinetics. To study the events that precede folding, we used MSM approaches to model the folding reaction of ACBP (acyl-CoA binding protein), an 86-residue helix-bundle protein that folds on the ~10 ms timescale, with an ~80 μs collapse phase that can be probed both by simulation and experiment. Our combined results suggest that the fast kinetic phase is not due to barrier-limited formation of a well-defined intermediate, but rather the surprisingly slow acquisition of unfolded-state structure.

2804-Plat
Is the Serpin Folding Mechanism Conserved? The Folding Pathways of Human Alpha-1 Antitrypsin and Neuroserpin
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Inhibitory serpins are unusual because they fold to a kinetically trapped metastable state. In their lowest energy conformation serpins have a large 6 stranded central beta-sheet (sheet A), while in the metastable state this sheet is 5 stranded, and strand 4 is a flexible solvent exposed loop. How serpins avoid folding to their lowest energy conformation is an open question. We have used pulse labeling hydrogen/deuterium exchange coupled with mass spectrometry to probe the folding pathways of two serpins: alpha-1 antitrypsin (A1AT) and neuroserpin. Both proteins fold surprisingly slowly. Upon dilution from 6 M GuHCl, A1AT folds to completion in approximately 50 minutes, while nearly 90 minutes are required for neuroserpin. In A1AT the ''B-C barrel'' region, comprising much of the hydrophobic core and securing the C terminus of strand 4, folds first while beta-sheet A is among the last regions to fold. Most strikingly, beta strand 5 at the center of sheet A shows a lag phase of nearly 350 seconds before any protection from H/D exchange is seen. Strand 5A forms part of the insertion site for strand 4, and we propose that the separation of folding timescales in different regions of A1AT plays a role in guiding it to its metastable structure. Surprisingly, neuroserpin folds via a different pathway in which the center of beta-sheet A, including strand 5, is the first region to fold, securing the N terminus of strand 4, while the ''B-C barrel'' folds later. Our results indicate that multiple folding routes can lead serpins to be trapped in their metastable conformation. Apparently, securing either end of strand 4A (also called the reactive center loop) early in the folding process is a requirement for folding to the metastable state.

2805-Plat
Engineered Allosteric Activation of Kinases in Living Cells
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Studies of cellular and tissue dynamics benefit greatly from tools that can control protein activity with specificity and precise timing in living systems. Here, we describe a new approach to confer allosteric regulation specifically on the catalytic activity of kinases. A highly conserved portion of the kinase catalytic domain is modified with a small protein insert that inactivates the catalytic activity of the kinase, but does not affect other protein interactions. Catalytic activity is restored upon addition of rapamycin or its non-immunosuppressive analogs. We demonstrate the approach by specifically activating focal adhesion kinase (FAK) within minutes in living cells, thereby demonstrating a novel role for FAK in regulation of membrane dynamics. Molecular modeling and mutagenesis indicate that the protein insert reduces activity by increasing the flexibility of the catalytic domain. Drug binding restores activity by increasing rigidity of a specific region in the kinase that is critical for function. We further demonstrate the wide applicability of our approach by successful regulation of Src and p38 kinases in living cells.