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
Samir M. Hamdan, Joseph J. Loparo, Masateru Takahashi, Charles C. Richardson, Antoine M. Vanoijen.
Harvard Medical School, Boston, MA, USA.
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
Alexander Schug[1], Yann Gambin[2], Ashok Deniz[2], Jose Onuchic[1].
[1]CTBP, UCSD, San Diego, CA, USA, [2]The Scripps Research Institute, San Diego, CA, USA.
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
Dirar M. Homouz[1], Michael Perham[2], Antonios Samiotakis[1], Margaret Cheung[1], Pernilla Wittung-Stafshede[3].
[1]Univ of Houston, Houston, TX, USA, [2]Rice University, Houston, TX, USA, [3]Umeå University, Umeå, Sweden.
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
James M. Mottonen, Donald J. Jacobs, Dennis R. Livesay.
UNC-Charlotte, Charlotte, NC, USA.
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.
[1] D.R. Livesay, et al. *FEBS Lett.* 576, 468-476 (2004), and D.J. Jacobs and S. Dallakayan, *Biophys. J.* 88, 1-13 (2005).
[2] J.M. Mottonen, et al. *PROTEINS* In press (DOI: 10.1002/prot.22273).

2925-Plat
Configuration Entropy Modulates the Mechanical Stability of Protein GB1
Hui-Chuan Wang, **Yi Cao**, Hongbin Li.
The University of British Columbia, Vancouver, BC, Canada.
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
Eva Y. Chi[1,2], Amy Winans[2], Shelli L. Frey[2], Kinlok Lam[2], Jaroslaw Majewski[3], Kristian Kjaer[4], C. Lee Ka Yee[2].
[1]University of New Mexico, Albuquerque, NM, USA, [2]University of Chicago, Chicago, IL, USA, [3]Los Alamos National Laboratory, Los Alamos, NM, USA, [4]Max Planck Institute of Colloids and Interfaces, Am Muhlenberg, Germany.