

1655-Pos Board B499**Single-Molecule Dynamics of the Replication Sliding Clamp and Clamp Loader System**Cheng Liu^{1,2}, Xinghua Shi^{2,3}, Yi-Hsing Chen⁴, Isaac K. O'Cann^{2,4}, Taekjip Ha^{1,3}.¹Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA, ²Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA, ³Howard Hughes Medical Institute, Chevy Chase, MD, USA, ⁴Department of Animal Science, University of Illinois at Urbana-Champaign, Urbana, IL, USA.

The sliding clamp is a conserved, ring-shaped cofactor of DNA polymerases. It encircles DNA and plays a crucial role in orchestrating many replication-related processes. In all forms of life, the sliding clamp greatly enhances the processivity of replicative DNA polymerases. The ring-shaped sliding clamp is loaded onto DNA by a pentameric AAA+ enzyme complex called the clamp loader. In this study, we applied Förster resonance energy transfer (FRET) to an archaeal sliding clamp, *Methanosarcina acetivorans* (Mac) PCNA, and its cognate clamp loader, MacRFC, by labeling PCNA and DNA with fluorescent fluorophores. The FRET traces directly showed spontaneous diffusion of PCNA after it was loaded to the DNA. A closer look strongly suggested that the diffusion rate along the double strand DNA is much faster than the rate along the single strand. In addition, we found that this spontaneous diffusion is not random and has a preferred position at the junction where the single and the double strand DNA meet. We also developed a single molecule method to observe the clamp loading process in real time and detected a loading intermediate step of 0.34s duration likely corresponding to the ATP hydrolysis by RFC.

1656-Pos Board B500**Structural Fluctuations In Apomyoglobin Undergoing Transition To An Amyloid State**Huimin Chen¹, Margaret Stratton², Stewart N. Loh², Watt W. Webb¹.¹Cornell University, Ithaca, NY, USA, ²SUNY Upstate Medical University, Syracuse, NY, USA.

The β -sheet amyloid structure in proteins is implicated in many diseases like Alzheimer's and Parkinson's Diseases. Previous studies by Fandrich et al. have shown that apomyoglobin, while natively helical and not known to form amyloid structures, is able to convert to a β -sheet amyloid state under suitable conditions¹. This has led to the hypothesis that the amyloid structures are universal amongst proteins. We have observed structural fluctuations of apomyoglobin on the order of 2- 200 μ s under equilibrium conditions using Fluorescence Correlation Spectroscopy (FCS) at various stages of folding². We attributed the fluctuations to chain dynamics and have since extended our measurements of apomyoglobin to amyloid-forming conditions. Here we present initial results of FCS measurements of apomyoglobin chain dynamics as it undergoes the α -helical to β -sheet transition.

¹M. Fandrich, M.A. Fletcher & C.M. Dobson (2001) *Nature* **410**:165-166.²H. Chen, E. Rhoades, J.S. Butler, S.N. Loh & W.W. Webb. (2007) *PNAS* **104**(25):10459-10464.

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1657-Pos Board B501**Illuminating the Dynamic Changes Associated with Heterotropic Allosteric Communication in Phosphofruktokinase from *Bacillus stearothermophilus***

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Phosphofruktokinase from *Bacillus stearothermophilus* (BsPFK) undergoes dynamic changes in its otherwise rigid structure upon binding of ligands, fructose 6-phosphate (F6P) in the active sites, and allosteric inhibitor phosphoenolpyruvate (PEP) in the allosteric sites. PFK can exist in four distinct ligation states: Apo BsPFK, BsPFK-F6P, PEP-BsPFK, and PEP-BsPFK-F6P, however, the biophysical properties of each ligation state are poorly understood particularly with respect to their dynamic character. BsPFK is a homotetramer with four active sites and four allosteric sites, creating an intricate network of both homotropic and heterotropic interactions. The intrinsic tryptophan fluorescence emanating from hybrid enzymes in which a single tryptophan was used to report local side-chain dynamics upon ligand binding to sites involved in a single heterotropic interaction. Tryptophan was positioned by constructing conservative tryptophan-shift mutant enzymes in which one of the sixteen native phenylalanine and tyrosine residues in each subunit was substituted with tryptophan and the native tryptophan was changed to tyrosine. The first site of interest was a phenylalanine at position 230. Fluorescence intensity and steady-state anisotropy mea-

surements reveal no change in response to F6P binding, but a 10% increase in intensity and a slight increase in steady-state anisotropy for PEP alone. Time-resolved experiments indicated that rotational correlation time significantly increased (by greater than 1 ns) for the PEP-BsPFK and PEP-BsPFK-F6P ligation states, but only slightly increased in the presence of F6P alone when compared to the unligated state of BsPFK. These trends are opposite comparable measurements made with PFK from *E. coli* (EcPFK). These results may suggest a structural basis for the observation that allosteric communication in BsPFK is entropically dominated whereas it is enthalpically dominated in EcPFK. Funding is provided by NIH grant GM33216 and Welch Foundation grant A1543.

1658-Pos Board B502**Specific And Non-specific Interactions Of SfiI Restriction Endonuclease With DNA**

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The search by a site-specific protein of a specific region on the DNA template assumes that multiple interactions with non-specific regions occur before a specific site is found. Therefore characterization of both specific and non-specific interactions will provide an important information for understanding of the recognition process. Earlier, we¹ developed a method utilizing the AFM force spectroscopy for the characterization of interaction of site-specific enzymes requiring the binding to cognate sites on the DNA template (synaptic complex). The aim of this work was to use mentioned approach to characterize the interaction of the same enzyme with non-specific regions. We compared a pair of complexes of SfiI and DNA with and without recognition site (RS), flanked by 2 and 3 nucleotides at each side and with flexible T25 tether (T25-2RS3 and T25-2noRS3). Lifetimes were 225 ± 109 ms and 15 ± 15 ms for each of the complexes, respectively suggesting that the specificity is defined by an elevated dwell time of the protein on the DNA template. Other designs, containing both specific and non-specific DNA regions, have lifetimes shorter than those for the specific complex suggesting that both types of complexes are detected in these experiments. As SfiI is enzymatically active in the presence of magnesium ions, all mentioned experiments were performed in conditions where magnesium was replaced with calcium to study interaction while avoiding cutting of DNA. We show here that the lifetimes for non-specific complexes do not depend on the type of cation. Therefore, these results justify experiments with at least this system by using CaCl₂ instead of MgCl₂.

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¹Krasnoslobodtsev A.V., Shlyakhtenko L.S., Lyubchenko Y.L. *J Mol Biol.* 2007 Feb 2;365(5):1407-16.**1659-Pos Board B503****Nitrobenzyl Phototrigger for Fast Protein Folding Processes**

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Insights into the first steps of protein folding have increased tremendously over the years because of the many experimental and theoretical methods that made it possible to go beyond the millisecond time resolution of standard stopped-flow or rapid mixing techniques. Central to experimental investigations of the fastest steps of protein folding is the development of fast triggering methods. Although various approaches have been used, most of these suffer from significant drawbacks; in particular, the most widely used method of laser-induced temperature jumps generally triggers *unfolding*.

Alternative fast folding triggers make use of photochemical reactions. One promising approach is the photolysis of a nitrobenzyl group, which is based on the observation that incorporation of nitrobenzyl can affect protein structure and protein-protein interaction.¹ One main advantage of this method is that photolysis of the non-native nitrobenzyl results in the fully native protein, thus avoiding any potential distortion of the folding reaction by the phototrigger group itself, which cannot be ruled out for most other photochemical folding triggers. However, the time scale of the nitrobenzyl photolysis reaction, and thus the potential of this method for triggering fast folding reactions, had not been determined so far.

Recently, a peptide analogue backbone-caged with a photolabile nitrobenzyl group was reported.² We have used this model for studying the nitrobenzyl-peptide photolysis reaction using nanosecond time-resolved IR spectroscopy. Our experimental results show a time constant of approx. 100 ns for recovery of the uncaged peptide bond, which indicates that nitrobenzyl photolysis is highly suitable for kinetic studies of even the fastest folding processes.

[1] S.K. Nandy, R.S. Agnes, D.S. Lawrence, *Org. Lett.* **2007**, *9*, 2249.[2] H. Rhee, J. S. Lee, J. Lee, C. Joo, H. Han, M. Cho, *J. Phys. Chem. B* **2008**, *112*, 2128.