

**3888-Pos****Effect of Trifluoroethanol on the Tryptophan Side Chain Orientation in the Hydrophobic Core of Troponin C Studied by NMR and Time Resolved Fluorescence**Guoli Wang<sup>1</sup>, Olivier Julien<sup>2</sup>, Abel Jonckheer<sup>1</sup>, Brian D. Sykes<sup>2</sup>, Yves Engelborghs<sup>1</sup>.<sup>1</sup>Biomolecular Dynamics, University of Leuven, Celestijnenlaan, Leuven, Belgium, <sup>2</sup>Department of Biochemistry, University of Alberta, Edmonton, AB, Canada.

Several NMR studies have shown that co-solvent trifluoroethanol (TFE) does not perturb the overall three-dimensional structure of proteins. We have inserted a single tryptophan (F77W) in the hydrophobic core of the N-domain of cardiac troponin C (cTnT), and determined the structure of the mutant with and without TFE (Julien et al., Protein Sci. 2009 18:1165-74). Interestingly, the position of the tryptophan side chain orientation was shown to be in opposite directions. We have monitored the effect of TFE on the tryptophan rotamer population using <sup>13</sup>C-HSQC spectra and used a full lineshape analysis to quantify the rate of the conformational exchange.

To further characterize this phenomenon, we have used Time Correlated Single Photon Counting experiments as a function of the TFE concentration. The time dependence could be fitted very well with three lifetimes in the wavelength region from 320 to 380 nm, and global analysis was further used. Addition of TFE (up to 19%) causes a gradual decrease of the lifetimes, due to dynamic quenching with very low quenching constants between  $k_q = 0.1$  to  $0.01 \text{ M}^{-1} \text{ ns}^{-1}$ . The amplitude fractions of the lifetimes change upon addition of TFE. At 340 nm, the amplitude fraction of the long lifetime (5.9 ns) increased from 13 to 29%, while that of the middle lifetime (3.3 ns) decreased from 63 to 50%. The short lifetime changed only to a limited extent. These data indicate that the change in the tryptophan indole position (different rotameric state) upon addition of TFE, as observed in NMR, is reflected mainly in the amplitude fractions of the different lifetimes. This is consistent, in this case, with the interpretation of lifetimes linked to rotameric states of tryptophan.

**3889-Pos****Single Molecule Studies of Polymerase  $\eta$  DNA Interaction with TIRF Microscopy**

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All cellular organisms depend on DNA molecules for the long-term storage of their genetic information. One rather obvious prerequisite for preserving such large amounts of information is a mechanism able to make fast and error proof duplicates despite the presence of damages. The importance of DNA damage repair for a cell's healthy growth becomes evident when considering that there are between 1000 and 1000000 induced DNA lesions in a cell per day.

Failure to correct those lesions can lead to serious medical conditions such as Xeroderma Pigmentosum. There for a special class of Polymerases capable of transcribing through such a lesion evolved. One such polymerase is Polymerase  $\eta$ . However, in the case of cancer treatment where such lesions are intentionally induced by chemotherapeutic agents to suppress cancer growth it would be desirable to block the repair mechanism of the low fidelity error prone Polymerase  $\eta$ .

There for, to gain insight into the dynamics of the underlying process of lesion-replication, we performed single molecule Förster Resonance Energy Transfer (FRET) experiments with an Total Internal Reflection Fluorescence Microscope (TIRFM). In the assay used, we avoid the difficulties of protein labelling by measuring the induced conformational changes of the template DNA by protein binding. Through these experiments, we were able to obtain information about single molecule binding kinetics of Polymerase  $\eta$  in the presence or absence of nucleotides and also about processivity on different DNA templates. The findings will be contrasted with other high fidelity polymerases such as the Klenow fragment.

**3890-Pos****Investigations of Calmodulin Conformations Resolved by Single Molecule Microscopy**

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Measurements of the distance between two dye molecules covalently linked to the calcium signaling protein calmodulin (CaM) have been previously performed by our group to investigate the conformations of CaM in solution. It was shown that calmodulin exists in a wide range of distinct conformations

whose amplitudes depend upon free calcium concentrations (1). Currently, we are investigating affects that the choice of dye pair or labeling site has on measured conformations. This is done using an alternating laser excitation (ALEX) single molecule microscope system that has been custom built in our laboratory. Time correlated single photon counting in bulk samples is used to determine the time resolved anisotropy of the dye pair and the orientational mobility of each dye. Analysis of burst measurements using interphoton time burst selection criteria and the probability distribution analysis reveal a wide range of CaM conformations. Conformational analysis is performed using both discrete states and the maximum entropy method. The maximum entropy method reveals the most probable underlying conformational distribution that fits our data. Finally, we are investigating fluorescence fluctuations within CaM conformations using conformationally sorted fluorescence correlation spectroscopy.

1. Slaughter et al., J. Phys. Chem. B, 2004, 108, 10388-10397

**3891-Pos****A FRET-FLIM Study Reveals the Interaction between ALCAM and Actin as a Potential Regulator of ALCAM Binding Activity**Cicerone Tudor<sup>1</sup>, Agnieszka Esseling-Ozdoba<sup>2</sup>, Carl G Figdor<sup>2</sup>, Hans Kanger<sup>1</sup>, Vinod Subramaniam<sup>1</sup>.<sup>1</sup>University of Twente, Enschede, Netherlands, <sup>2</sup>Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.

Interactions between T cells and antigen-presenting cells represent the first step in the induction of an adaptive immune response. CD6 is a cell surface receptor expressed on mature T cells that specifically binds to activated leukocyte cell adhesion molecule (ALCAM). It has been shown that CD6 and its ligand ALCAM are actively recruited to the antigen-induced DC-T cell contact zone and that CD6-ALCAM interactions are also required during the proliferative phase of the T cell response. The molecular mechanism controlling ALCAM mediated interactions still remains unclear. Specifically, how the cytoskeleton dynamically regulates ALCAM binding activity at the cell-cell contact remains unknown. Transient cotransfection with Actin-RFP of a K562 cell line stably transfected with ALCAM-GFP was performed in order to investigate by FRET-FLIM the interaction between ALCAM and Actin. By measuring the donor fluorescence lifetime (GFP) in the absence and the presence of acceptor (RFP) the FRET efficiency and the distance between donor- and acceptor- labeled proteins were estimated. This FRET-FLIM study demonstrates the interaction between ALCAM and Actin (Figure 1) and opens the door for further investigation of the role of ALCAM-Actin interactions in the formation and stabilization of the immunological synapse.

**3892-Pos****Surface Diffusion of Cellulases on Cellulose Fibrils Studied through Fluorescence Spectroscopy**

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Cellulases are enzymes that can depolymerize cellulose, producing soluble sugars. Depending on their activity, cellulases can be classified into endocellulases, which randomly break intermediate glucosidic bonds, or exocellulases, which cleave bonds at the ends of cellulose chains. In addition, cellulases can be processive and cleave multiple bonds in succession. The current view of the mechanism for cellulase binding, diffusion along cellulose, and catalytic activity has been mostly derived from experiments performed in bulk. This has led to the generalized assumption that cellulases have multiple binding states: weak binding where the enzyme surveys the cellulose surface without catalysis, strong binding with or without catalysis, and processive displacement. Because the topography of lignocellulosic substrates is highly heterogeneous, the types of binding are further convolved by the availability of binding sites and reduced accessibility due to pore structure. Thus, experiments that elucidate surface diffusion behavior can significantly contribute to understanding how cellulases depolymerize cellulose and to improve the efficiency of saccharification processes by targeting limiting steps in binding, diffusion, and catalysis.

To study the characteristic interactions between cellulases and cellulose, we have employed fluorescently-labeled cellulases in conjunction with labeled or unlabeled cellulose and various fluorescence microscopy methods. In particular we have studied long-range surface diffusion characteristics for *Thermobifida fusca* cellulases Cel5A, Cel6B, and Cel9A through confocal FRAP and short-range displacement through single molecule tracking. In order to simplify the interpretation of results, we have used bacterial microcrystalline cellulose fibrils immobilized on glass surfaces through "molecular combing" and incubated with the different cellulases. The experiments presented have