and change conformation in solution in the presence of $Ca^{2+}$. To test this prediction and determine the configuration of the two domains, double cysteine mutations were engineered into a water soluble fragment of sytI C2A-C2B and derivatized with the methanethiosulfonate spin label. Four-pulse DEER (Pannier et al., J. Mag. Res., 2000, 142, 331) was used to obtain distance measurements between tandem C2A and C2B in solution and with membranes. The data obtained thus far indicate that there are no direct interactions between the two domains, and that $Ca^{2+}$ does not significantly alter the relative orientation of the domains in solution. Models are being generated by means of simulated annealing for both solution and membrane-bound forms of sytI, using DEER-derived distance constraints and depth constraints from EPR power saturation.

The work was supported by NIGMS grant GM 72694.

## 2481-Pos The Mechanism of Ribonucleotide Triphosphate Reductase Reaction, as Studied with Rapid Freeze-Quench Electron Paramagnetic Resonance and Electron Nuclear Double Resonance

Julia Manzerova, Gary J. Gerfen, Vladimir Krymov

*Albert Einstein College of Medicine, Bronx, NY, USA.*

**Board B595**

Ribonucleotide reductases convert ribonucleotides to their corresponding deoxyribonucleotides. Ribonucleotide tripshosphate reductase (RTPR) from *L. leichmanii* is an adenosylcobalamin-dependent allosterically regulated monomeric enzyme that relies on the radical generation for catalysis. The catalytically relevant intermediates are all spin $^1/_2$ species, which makes the enzyme amenable to study with electron paramagnetic resonance (EPR). We capture the intermediates using rapid freeze-quench (RFQ). This technique arrests the catalytic cycle at predetermined timepoints and delivers samples in the form of ice powder. Samples quenched on the order of seconds have been studied, and substrate radical has been detected and characterized by high-frequency EPR and Electron-Nuclear Double Resonance. To obtain samples at faster quench times, a commercially available RFQ apparatus has been transformed in our lab: to facilitate sample collection, the sample is sprayed onto liquid nitrogen-cooled copper wheels; to enhance contact of the solutions, a 250 lines per inch mixing grid is being used; consistent freezing of the solution at the copper surface is ensured through the use of precision blade scrapers. The performance of the mixer/RFQ/EPR sequence has been tested and a dead time of 15 +/− 5 msec has been demonstrated.

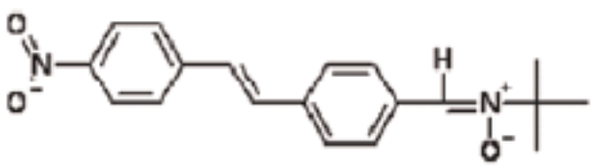
## 2482-Pos Novel Fluorescent Spin Traps

Stefan Hauck, Yvonne Lorat, Matthias Schneider, Wolfgang E. Trommer

*Kaiserslautern Technical University, Kaiserslautern, Germany.*

**Board B596**

A fluorescent nitrone composed of a nitrostilbene moiety and the t-butyl-nitrone has been synthesized. Upon addition of short-lived radicals (ROS) a relatively stable nitroxide is formed which quenches the fluorescence. Simultaneously, the fluorescence maximum is shifted to shorter wavelength due to the shorter conjugated system. Hence, by means of confocal laser microscopy the formation of ROS may be followed with subcellular resolution and their nature eventually even be determined by EPR spectroscopy.



**Single Molecule Biophysics - I**

## 2483-Pos Single-Molecule Protein Folding Studies on Barnase

Kambiz M. Hamadani, Shimon Weiss

*UCLA, LA, CA, USA.*

**Board B597**

The mechanistic details of protein folding reactions are complex and often involve numerous intermediates through which a protein must pass before reaching its final folded state. Single molecule methods allow the direct resolution of such intermediates from more stable states such as the completely folded or unfolded populations. Here we present single molecule FRET studies conducted under a variety of equilibrium conditions on Barnase, a protein which has such an intermediate (1). Using a capillary-based continuous-flow single molecule mixing device (2), we plan to resolve the major protein folding phases of Barnase under non-equilibrium folding conditions using single molecule FRET as our reaction coordinate. A comparison between the equilibrium and non-equilibrium data sets will reveal the degree to which protein folding mechanisms can change under these widely different experimental conditions.

### References

1. Dalby PA, Oliveberg,M, Fersht AR. 1998. "Movement of the Intermediate and Rate Determining Transition State of Barnase on the Energy Landscape with Changing Temperature". Biochemistry *37*:4674–4679
2. Hamadani KM, Weiss S. "Non-equilibrium Single Molecule Protein Folding in a Co-axial Mixer". (in preparation).

## 2484-Pos Dynamics of Single Annexin V Molecules on Supported Lipid Bilayers

Jason J. Han, Chan Hoo Park, Seung Han Park, Doo Wan Boo

*Yonsei University, Seoul, Republic of Korea.*

**Board B598**

We report on the interactions of single annexin V (A5) molecules with planar supported lipid bilayers (SLBs) containing phosphatidylserine. The diffusion and aggregation dynamics of dye-labeled A5 was investigated on both glass and mica SLBs at picomolar to low nanomolar A5 concentrations in a calcium rich environment. On both glass and mica substrates, A5 monomers are the dominant stable species and reversibly bind to fluid SLBs, while diffusing rapidly across fluid bilayers in the membrane-bound state. A5 monomers and smaller diffusive aggregates can be immobilized by utilizing saturated lipids and proper temperature control between the bilayer liquid and gel phases. Such immobilization of A5 allows high-resolution AFM imaging of monomers and smaller aggregates that are normally impossible to image due to diffusion, and also facilitates the use of force spectroscopy to probe the membrane binding interaction between single A5 monomers and the membrane binding sites.

## 2485-Pos Single Molecule Study on the RecA Filament Dynamics

SUNG HYUN KIM[1], Chirlmin Joo[2], Jeehae Park[2], Kaushik Ragunathan[2], Doseok Kim[1], Taekjip Ha[2,3]

[1] *SOGANG UNIVERSITY, SEOUL, Republic of Korea,*
[2] *University of Illinois at Urbana-Champaign, Urbana, IL, USA,*
[3] *Howard Hughes Medical Institute, Urbana, IL, USA.*

**Board B599**

E. coli RecA protein plays important roles in DNA repair via the homologous recombination. RecA proteins form a helical filament on a single-stranded DNA that searches for a homologous sequence during the recombination. In this study, we used a single molecule FRET (fluorescence resonance energy transfer) assay to characterize the end-dependent filament dynamics. The association and dissociation rates of RecA monomers to the filament ends were directly obtained from the FRET time-trajectories of single RecA filaments. To investigate the nature of the filament dynamics, the kinetic rates were measured under various chemical conditions. Our results revealed that the high stability of the RecA filament at low pH is due to the increase in the association rate and the moderate decrease in dissociation rate. Next, the dissociation rate was strongly correlated with the ATPase activity of RecA and also related to the DNA sequences. Finally, the effect of nucleotide cofactors was studied to quantitatively characterize each step of the ATP hydrolysis cycle.

## 2486-Pos Single Molecule Identification Via Nanofluidic Immunospectroscopy

Jun Kameoka[a)], Nan Jing[a)], Chin B. Su[a)], Chao-Kai Chou[b)], Mien-Chie Hung[b)]

[a)] *Department of Electrical Engineering, Texas A&M University, College Station, Texas 77843-3128*
[b)] *Department of Molecular and Cellular Oncology, University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030-4095*

**Board B600**

The targeted single protein detection in real samples without any cleaning process after cell lysate has been demonstrated by using the nanofluidic immunospectroscopy. Targeted MAX proteins were conjugated to polyclonal anti MAX antibodies and hybridized to the secondary antibodies with quantum dots. Their photon burst counts were monitored by the single molecule detection system that consists of the nanochannel and the laser source focused into the nanochannel with 0.5 μm spot size. Whenever molecules pass through this spot, the photon counts were monitored. Due to the small dimension of the detection volume in nanofluidic channels, photon burst counts strictly from single molecules can be obtained. In addition, because a few quantum dots were hybridized to targeted MAX proteins, they were distinguished from the unbound individual quantum dots by comparing their photon counts by plotting into two-dimensional photon burst diagram (photon burst width vs photon burst counts). From this diagram, the existence of MAX proteins can be detected.

## 2487-Pos Direct Observation of a Molten Globule Ensemble Using Single Molecule Force-Clamp Spectroscopy

Sergi Garcia-Manyes[1], Lorna Dougan[1], Hui Lu[2], Frauke Grater[1], Anuradha Mittal[2], Carmen L. Badilla[1], Bruce J. Berne[1], Julio M. Fernandez[1]

[1] *Columbia University, New York, NY, USA,*
[2] *University of Illinois, Chicago, IL, USA.*

**Board B601**

We use force-clamp spectroscopy to dissect the complete folding pathway of the small protein ubiquitin, starting from the fully stretched state and ending with the native state topology. A single polyprotein is first unfolded under a constant high force, which is subsequently quenched to a low value to trigger protein collapse to its folded length driven by the hydrophobic effect. The recovery of mechanical stability is then probed by (re)stretching the collapsed protein. In contrast to the expectation that mechanical stability would recover gradually, we observe an abrupt all-or-none transition between a heterogeneous set of unstable conformations and the fully stable protein. The fraction of ubiquitins that have regained their mechanical stability increases exponentially with the elapsed time. The mechanically weak forms of ubiquitin feature a broad distribution of lengths spanning up to the size of a single monomer. These unstable conformations unravel 40 times faster than the native form, yielding a distance to the transition state of $\sim 2$ Å, which is surprisingly similar to the unfolding of the native state. We propose that these unstable forms correspond to an ensemble of molten globules that constitute an *en route* intermediate stage in the folding pathway of ubiquitin. The physicochemical properties of such conformations have been characterized using solvent substitution, revealing that the molten globule represents a distinct chemical species different from both the native state and the extended polypeptide chain. These results demonstrate that force clamp spectroscopy provides unprecedented access to intermediate conformations in the folding energy landscape of a single protein.

## 2488-Pos Probing TRiC-Mediated Folding of Actin *in vitro* with Bulk and Single-Molecule Fluorescence Measurements

Whitney C. Duim[1], Jian Cui[1], Erik J. Miller[1], So Yeon Kim[1], Dmitriy Gremyachinskiy[2], Klaus M. Hahn[2], Robert J. Twieg[3], Judith Frydman[1], W. E. Moerner[1]

[1] *Stanford University, Stanford, CA, USA,*
[2] *University of North Carolina at Chapel Hill, Chapel Hill, NC, USA,*
[3] *Kent State University, Kent, OH, USA.*

**Board B602**

The eukaryotic chaperonin TRiC is required for the folding of actin, an essential component of the cellular cytoskeleton. The mechanism by which correct folding of actin is achieved by TRiC has yet to be elucidated. We use bulk anisotropy and fluorescence lifetime measurements combined with single-molecule imaging using environment-sensing fluorescent probes to characterize the folding of actin by TRiC *in vitro*. Single-molecule fluorescence measurements allow us to probe the potentially heterogeneous mechanisms by which TRiC folds its substrate proteins, providing a level of detail not attainable in bulk studies. Dyes obtained through collaborations with Klaus Hahn and Robert Twieg have proven to be bright, highly-sensitive reporters at the single-molecule level when covalently attached to proteins. G-actin has been labelled at the Cys374 position with these environmentally-sensitive fluorophores, a location which is believed to be near denatured actin's binding site to TRiC. Analysis of bulk and single-molecule fluorescence data will shed light on the mechanism by which TRiC mediates the folding of actin.

## 2489-Pos The quantitative determination of ligand-protein interactions at single molecular levels using Diffracted X-ray Tracking (DXT)

Takuma Sagawa[1], Takachika Azuma[2], Yuji C. Sasaki[1]

[1] *SPring-8/JASRI JST/CREST Sasaki-team, Sayo-Gun, Hyogo, Japan,*
[2] *Research Institute for Biological Sciences, Tokyo University of Science, Noda, Chiba, Japan.*

**Board B603**

Protein-ligand interactions are essential for most cellular processes, including signal transduction, gene regulation, and enzyme reaction. Recently, various single-molecular detection systems were applied to biomolecules interaction analysis, such as atomic force microscopy (AFM) and Fluorescence Correlation Spectroscopy (FCS). Those methods provide us with attractive information such as binding forces and Brownian translational diffusion. However it was not well understood the relationship between molecular structural fluctuation and interaction aspects.

In this study, we investigated the effects of ligand-binding to the structural fluctuation of a single protein molecule using Diffracted X-ray Tracking method. This is a new method for observing the movement of single molecule with X-rays, which is monitored the rotating motions of a labeled gold nanocrystal. In this experiment, antigen-antibody interactions were investigated. The gold nanocrystal was bound to Fab fragment through Cys128 of heavy chain constant domain or Cys214 of light chain constant domain. In the 3D structural model of antibodies, these Cys residues occur far from antigen binding site in variable domains. We used the white X-ray mode (Laue mode) of beam line BL44B2 (RIKEN Structural Biology II, SPring-8, Japan) to record Laue diffraction spots from labeled gold nanocrystals. We found that the structural fluctuations of Fab fragments were negatively regulated by antigen-binding. In addition, we clarified that ratio between antigen-binding condition and no-binding one in observed structural fluctuations are extremely relative to the binding-affinity or Gibbs free energy change. These results indicate that the phenomena of protein-ligand interactions considered as stable states can be defined as results of dynamical processes at the single-molecule level. Such new quantifications from angstrom-level structural fluctuations can be applied to various biological science and biotechnologies. Now we are investigating the relationship between structural fluctuations and thermodynamic aspects.

## 2490-Pos Single-Molecule Studies of Calmodulin: Conformations and Dynamics

E. Shane Price, Matthew S. DeVore, Carey K. Johnson

*University of Kansas, Lawrence, KS, USA.*

**Board B604**

Calmodulin (CaM) is a calcium signaling protein that binds and activates a wide range of targets. These targets have a diverse set of binding site geometries and sequences, requiring CaM to be a very flexible ligand. To explore the flexibility of CaM we make use of Förster resonance energy transfer (FRET) and fluorescence cross correlation spectroscopy (FCCS). Previous studies in our lab have demonstrated multiple conformations of

CaM in solution (Slaughter, B. D. et al. *Biochemistry* **2005**, *44*, 3694–3707). Conformational studies are presented using mutants of CaM labeled with a FRET pair with data collection performed through dual channel time binned burst measurements. The bursts are identified and FRET efficiencies calculated for each bin to generate a histogram of efficiencies for distance distribution analysis. Dynamics measurements are performed using the same FRET pair labeled CaM and the data is auto-correlated and cross-correlated between the donor and acceptor channels to generate four correlation curves. The correlations are fit globally giving more reliable values for determining the timescale of the FRET dynamics.

## 2491-Pos Monitoring Oligomerization in Amyloid-β Peptide, One Peptide at a Time

Robin K. Lammi, Kyle Dukes, Cassie Rodenberg

*Winthrop University, Rock Hill, SC, USA.*

**Board B605**

Amyloid-β (Aβ) is a peptide of 39–43 amino acids that is implicated in the pathology of Alzheimer's disease: its misfolding results in the formation of a diverse array of toxic aggregates. Recent work shows that soluble oligomers - not larger, fibrillar species - are the most cytotoxic of these. Therefore, detailed understanding of the earliest peptide-association steps is crucial to Alzheimer's prevention and treatment. We have utilized single-molecule fluorescence spectroscopy (SMS) to monitor oligomer formation in individual copies of dye-labeled Aβ (1–40) tethered to the surface of functionalized cover slips. Since each dye exhibits one characteristic fluorescence intensity, the number of Aβ monomers in each peptide species is easily deduced from the number of discrete intensity levels it displays prior to photo-bleaching. By investigating tens of tethered peptides, one at a time, we have directly determined the distributions of monomers and small oligomers present in heterogeneous solutions near physiological concentrations. We have compared the effects of sample aging, acidic pH, and zinc coordination, three known promoters of Aβ aggregation, on oligomer distributions. Fresh samples at pH 7.4 consist primarily of monomers and dimers, with a small percentage of trimers. After aging the stock solution for several days, the proportions of dimers and trimers are only slightly increased. In contrast, acidic pH (pH 5.8) and metal coordination each result in rapid oligomer formation: fresh samples prepared under these conditions are dominated by trimers and tetramers, with fewer dimers and larger species. These results agree well with expectations based on published bulk-solution studies and illustrate the power of SMS for characterizing small Aβ oligomers. The effects of aggregation inhibitors, including β-sheet-breaker peptides and zinc chelators, will also be presented.

## 2492-Pos Single Molecule Fret Study On The SRC-1 Coactivator / Estrogen Receptor Interaction

Alvaro Ortega, Emmanuel Margeat, Cedric Godefroy, Catherine Anne Royer

*Centre de Biochimie Structurale, Montpellier, France.*

**Board B606**

Nuclear receptors are ligand dependent transcription factors. In the presence of a hormone they can bind a specific sequence on the DNA to promote the transcription of a gene as a response to this signal. In most of the cases, they will dimerize, change their localization in the cell, and bind a number of coactivators that will enhance the process. One of the best studied coactivators is the steroid receptor coactivator 1 (SRC-1), that interacts with the dimer of the estrogen receptor (ER) in the presence of different steroid hormones in a stoichiometry that corresponds to one SRC-1 molecule per ER dimer.

The regions of the SRC sequence that interact with the hydrophobic surface created on each ER upon binding of the hormone are known. Those include three well conserved nuclear receptor (NR) boxes that appear on the receptor interaction domains (RID) of the coactivators, each of them composed by a LLXXL aminoacid sequence.

We present a study on the conformation and precise interaction between the specific RID of the SRC-1 and the ER ligand binding domain (LBD). We use ensemble and single molecule Förster Resonance Energy Transfer (sm-FRET) to characterize accurately the distances and distances changes upon interaction between two probes (donor and acceptor) placed at different specific positions on the NR/coactivator couples.

### References

Moras D., Gronemeyer H. Current opinion in Cell Biology. *10*, 384–391. 1998.

Margeat E., Poujol N., Boulahtouf A., Chen Y., Muller J.D., Gratton E., Cavailles V. and Royer. C.A. J. Mol. Biol. *306*,433–454. 2001.

Lee N.K., Kapanidis A.N., Wang Y., Michalet X., Mukhopadhyay J., Ebright R.H., Weiss S. Biophysical Journal. *88*, 2939–2953. 2005.

## 2493-Pos Single Molecule Studies of beta-Amyloid (1–40) Oligomers Interacting with Planar Lipid Membranes

Hao Ding, Ari Gafni, Duncan G. Steel

*University of Michigan, Ann Arbor, MI, USA*

**Board B607**

Recent evidence supports the hypothesis that the early oligomers of beta-Amyloid peptide, rather than the fibrils are the origin of cellular toxicity in Alzheimer's disease (AD). It has been further suggested that these small oligomers, containing possibly only a few monomeric units, interact with lipid membranes and permeabilize them by forming ion-conducting pores. However, the mechanism behind this process still remains unclear, partially due to the lack of methodologies that are able to characterize these early oligomers. The task is challenging because the oligomers are extremely heterogeneous, metastable, and form at very low physiological concentrations (nM). In this study, single molecule spectroscopy (SMS) is successfully applied for the characterization of beta-Amyloid (1–40) oligomer species. The evolution of these early oligomer species on lipid membranes was monitored from a few hours to a few days. The effect of different lipid compositions and charges on protein-lipid interaction was also studied.

(Supported by NIA R21 AG027370)

## 2494-Pos Adressing Plasma Membrane Structure at the Nanometer Length Scale

Stefan Wieser[1], Michael Cooper[2], Hannes Stockinger[3], Gerhard Schütz[1]

[1] *Biophysics Institute, Johannes Kepler University Linz, Austria,*
[2] *GE Healthcare, The Maynard Centre Cardiff, United Kingdom,*
[3] *Department of Molecular Immunology, Center of Biomolecular Medicine and Pharmacology, Medical University of Vienna, Austria.*

**Board B608**

We employed single molecule fluorescence microscopy to study protein diffusion in the plasma membrane at sub-wavelength reso-

lution, both on the cell body and on tunneling nanotubules connecting cells. In general the cellular plasma membrane can be described as a two dimensional liquid, where its constituents - lipids and proteins - are free to diffuse. Recently, a refined picture of the plasma membrane emerged, where the underlying cytoskeleton, but also lipid heterogeneities modify the molecular localisation and mobility.

Here, we report a novel strategy to detect confinements to free diffusion of CD59, a GPI-anchored protein, in the plasma membrane of living T24 (ECV) cells. The lateral motion of this single fluorescence labeled molecule was imaged on a millisecond time scale down to 50μs exposure time to avoid time averaging during illumination. We provide an analytical expression for diffusion in a meshwork of squares and on a cylindrical shell including the effect of finite illumination times.

Within the experimental errors, no indications for confined diffusion for CD59 on the cell body in T24 cells have been found [1]. Furthermore, by separating longitudinal and transverse mobility, we found isotropic diffusion in the plasma membrane of tunneling nanotubules, rendering direct influences of the membrane skeleton unlikely. This method provides a first in vivo measurement of the nanotubule radius, yielding a narrow size distribution around an average value of 66nm.

## References

1. S. Wieser, M. Moertelmaier, E. Fuertbauer, H. Stockinger, and G. J. Schutz, Biophys. J. ***92*** (10), 3719 (2007).

# 2495-Pos Single-Molecule Motions of Oligoarginine Cell-Penetrating Peptides on the Plasma Membrane of CHO Cells Imply Multiple Entry Mechanisms

Hsiao-lu Lee[1], Elena Goun[1], Hanshin Hwang[1], Alexander Semyonov[2], Hui Wang[1], Lisa Jones[1], Robert Twieg[2], Paul Wender[1], W.E. Moerner[1]

[1] *Stanford University, Stanford, CA, USA,*
[2] *Kent State University, Kent, OH, USA.*

**Board B609**

To further our understanding of the interactions between cell-penetrating peptides (CPPs) and live biological membranes, fluorescently labeled oligoarginine conjugates were imaged interacting with the plasma membrane of Chinese hamster ovary (CHO) cells at the single-molecule level. The lateral diffusional behavior of fluorophore-labeled octaarginines (a model guanidinium-rich CPP) on the plasma membrane is compared to that of labeled tetraarginine (negative entry control), lipid analog fluorophores (passive diffusion model), and fluorescently labeled Transferrin proteins (endocytosis model). Single-molecule high-contrast imaging of the CPPs was enabled by the use of a new fluorophore in the dicyanomethylene-dihydrofuran (DCDHF) family that brightens upon interaction with the plasma membrane. This study shows that the motions of the octaarginine conjugate single molecules are highly heterogeneous, and cannot be described as Brownian motion with a single diffusion coefficient. As such, the mode by which octaarginine penetrates the cell membrane appears to either be a multi-mechanism uptake process or a mechanism different from unimodal passive diffusion or endocytosis.

This work was supported in part by the National Institutes of Health Grant Nos. HG003638 (W.E.M., R.J.T.) and CA 31841 and 31845 (P.A.W.).

# 2496-Pos Real Time Single-Molecule Power Stroke Measurements of the E. coli F1-ATPase to Determine Origins of Torque-Generation

Wayne D. Frasch, James Martin, David Spetzler, Tassilo Hornung, Robert Ishmukhametov

*Arizona State University, Phoenix, AZ, USA.*

**Board B610**

The rate of rotational 120 degree transitions of the F1-ATPase gamma subunit was measured in single molecule experiments using data acquisition with a time resolution as small as 2.5 microseconds. This was accomplished by monitoring changes in intensity of red light scattered from a single gold nanorod attached to the rotating gamma subunit as observed through a polarizing filter with a photon counter. Data acquisition rates comparable to at least 40,000 fps were required to obtain an accurate measure the rotational transitions. Data acquisition at 50 fps with a color camera distinguished ATP-dependent nanorod rotation via a Strobe effect and provided evidence that as many as 60% of the F1-ATPase molecules in a field of view were rotating in a given experiment. The contribution of substrate binding energy to the generation of torque was measured as a function of solution viscosity by varying the PEG 400 concentration to increase viscous drag on the nanorod. Without PEG, no differences in the rate of rotational transitions were observed when ATP, ITP, GTP, or ATPγS was used as a substrate even though ITP and GTP have much lower affinity for the catalytic site. However, as the viscosity of the solution increased, the rate of ITP and GTP rotational transitions became significantly slower than the other nucleotides. These results indicate that the energy of substrate binding contributes indirectly to torque generation, and that the pi-bonding between the nucleotide and betaY345 is more important than the salt bridge formed between the nucleotide gamma-phosphate oxygen and the arginine finger or the P-loop lysine in torque generation.

# 2497-Pos Detection and Tracking if Single Calmodulin Molecules in Live Cells

Carey Johnson[1], Gregory Harms[2]

[1] *University of Kansas, Lawrence, KS, USA,*
[2] *Rudolf Virchow Center, University of Würzburg, Würzburg, Germany*

**Board B611**

Calmodulin is a molecular switch that responds to calcium and regulates a broad array of cellular processes. We detected single molecules of fluorescently labeled calmodulin in HEK and COS cells and probed the mobility of calmodulin in cells. We will describe introduction of fluorescently labeled calmodulin into cells

and report wide-field imaging and single-molecule tracking of calmodulin in live cells. Analysis of single-molecule trajectories reveals a wide range of translational mobilities of calmodulin in cells with diffusion coefficients spanning several orders of magnitude, from roughly 0.01 $\mu m^2\ s^{-1}$ to 10 $\mu m^2\ s^{-1}$, consistent with interaction of calmodulin with a variety of target proteins. Mobilities were sensitive to the calcium level in cells. Calmodulin was labeled with tetramethylrhodamine, Texas red, or Alexa Fluor 647.

## 2498-Pos Characterization of Prestin Stoichiometries Using Single Molecule Fluorescence Microscopy

Ramsey I. Kamar[1], Laurent Cognet[2], Robert M. Raphael[1]

[1] *Rice University, Houston, TX, USA,*
[2] *Centre National de la Recherche Scientifique, Bordeaux, France*

**Board B612**

Prestin is the putative motor protein that drives outer hair cell electromotility. Several groups have shown evidence of prestin-prestin interactions and prestin oligomerization using biochemical techniques and optical imaging. However, it is unknown what role prestin-prestin interactions or oligomerization play in electromotility or what molecular motifs mediate these interactions. Ensemble optical measurements of prestin self-association cannot provide the molecular level details of prestin-prestin interactions, nor can they measure the oligomeric states they produce. We have thus developed our ability to detect individual prestin-YFP molecules using single molecule fluorescence (SMF) microscopy. We have applied SMF microscopy to measure the distribution of intensities emitted by individual prestin clusters in the HEK cell membrane. The distribution displays peaks spaced at multiples of the unitary intensity which one would expect for a distribution of noninteracting fluorescent emitters. We have resolved the stoichiometries up to tetramers; however it is clear that higher populations also exist. We will explore the effect of membrane cholesterol depletion on the oligomerization of prestin using SMF microscopy to asses whether this treatment dissociates prestin oligomers or simply removes a bulk population of intact oligomers from microdomains. Our development of SMF microscopy for the study of prestin creates the opportunity to investigate previously unexplored aspects of prestin structure that could not be studied using bulk optical methods.

## 2499-Pos Two-state Single-particle Trajectories On Sculpted Energy Landscapes: Accurately Predicting Higher Order Fluctuations Using Maximum Caliber

David Wu[1], Kingshuk Ghosh[2], Mandar Inamdar[3], Heun Jin Lee[1], Scott Fraser[1], Ken Dill[2], Rob Phillips[1]

[1] *California Institute of Technology, Pasadena, CA, USA,*
[2] *University of California, San Francisco, San Francisco, CA, USA,*
[3] *Indian Institute of Technology, Mumbai, India.*

**Board B613**

We explore characterizing the trajectory space of two-state dynamics in a single-particle system. In our experiments, a single 1-micron bead makes Brownian-activated hops back and forth between two energy wells, A and B, which we establish using laser traps. We can control the well depths and barrier height, so we are able to 'sculpt' arbitrary energy landscapes. We model the distributions of trajectories of the particle on these landscapes using a dynamical partition function method, derived from the principle of Maximum Caliber. The theory gives the probability distribution of quantities such as the mean and variance of the A-dwell or B-dwell times and the switching rates. The experimentally measured moments are in good agreement with the theory, which has no free parameters, as well as higher moments. In addition, the theory predicts a hierarchy of new dynamical reciprocal relationships, resembling the Onsager relationship of coupled transport coefficients and the Maxwell relations of equilibrium thermodynamics, also in good agreement with the experiments. This work shows that the dynamical partition function method is useful for characterizing the distributions of trajectories in single-particle two-state dynamical systems, and should be applicable to systems such as oscillations in protein or nucleic acid oligomer conformations.

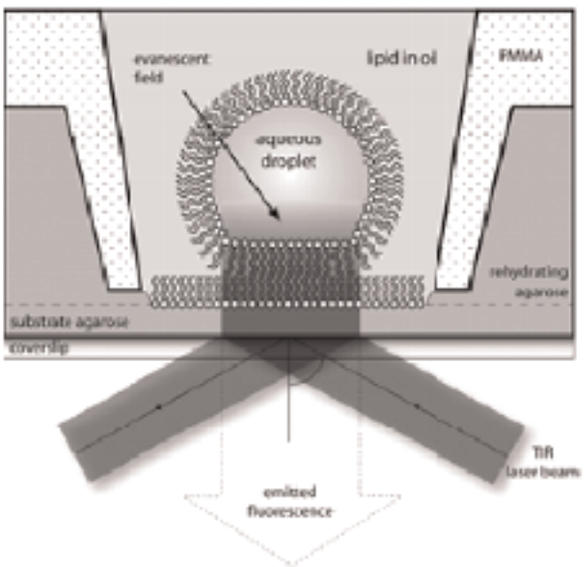
## 2500-Pos Single-molecule Fluorescence Imaging Of alpha-hemolysin Assembly Using Water-in-oil Droplet On Hydrogel Bilayers

James R. Thompson, Andrew J. Heron, Yusdi Santoso, Mark I. Wallace

*University of Oxford, Oxford, United Kingdom.*

**Board B614**

We have developed methods to create artificial lipid bilayers suitable for simultaneous single molecule fluorescence imaging and single-channel electrical recording using water-in-oil droplets supported on a hydrogel. We have used these methods to study the assembly mechanism of the pore-forming toxin, α-hemolysin. α-hemolysin is secreted by *Staphylococcus aureus* as a water soluble monomer which binds to lipid bilayer membranes, whereupon it oligomerises to form a heptameric protomer that spontaneously inserts a β-barrel pore through the bilayer. We are able to observe the kinetics of different stages in α-hemolysin assembly at the single-molecule level using TIRF microscopy. We present our progress towards simultaneous electrical and fluorescence measurement of pore protein assembly and insertion. We also characterise droplet on hydrogel bilayers using single particle tracking and report similar fluidity to unsupported lipid bilayers.




## 2501-Pos Distinct Classes of Amyloid Pores Formed by Abeta1-40 on Lipid Bilayers are Revealed by Simultaneous Single Molecule Fluorescence and Conductivity Measurements

Joseph A. Schauerte, Pamela Wong, Kathleen Wisser, Duncan Steel, Ari Gafni

*University of Michigan, Ann Arbor, MI, USA.*

**Board B615**

Alzheimer's disease is characterized by the existence of amyloid fibrils within extracellular senile plaques in brain tissue. It has been increasingly demonstrated that the mechanism of toxicity of human Abeta may not directly relate to the fibrils, but rather occur through the effects of smaller intermediate species in the fibrillization pathway. Abeta cell toxicity has been suggested to result from its ability to associate with membranes and form pores that disrupt ion balance in cells. In order to characterize Abeta pore formation, fluorescently labeled human Abeta 1–40 was used to monitor the interaction of the peptide with planar Black Lipid Membranes (BLM) while simultaneously monitoring membrane conductivity. Single molecule scans of the BLM surface indicated two classes of pores were formed with membrane conductances on significantly different scales. One class of pores is represented by small localized pores (<0.5uM, diffraction limited) with low ion conductance (<25pS/pore) that are relatively stable in time. However at higher concentrations, Abeta is capable of forming extended structures carpeting the lipid surface resulting in much higher pore conductivities. The extended pores are highly dynamic and are prone to significant enlargement resulting in disintegration of the membrane.

(supported by NIA R21AG027370)

## 2502-Pos Catalyzing The Translocation Of Polypeptides Through An Engineered Transmembrane Pore

Aaron J. Woolfe[1], Mohammad M. Mohammad[1], Stephen Cheley[2], Hagan P. Bayley[2], Liviu Movileanu[1]

[1] *Syracuse University, Syracuse, NY, USA,*
[2] *Oxford University, Oxford, United Kingdom.*

**Board B616**

Translocation of polypeptides through a protein pore is a ubiquitous and fundamental process in biology. Here, we employed rational protein design and single-channel electrical recordings to obtain detailed kinetic signatures of polypeptide translocation through the *staphylococcal* alpha-hemolysin (αHL) transmembrane pore, a robust, tractable, and versatile β-barrel protein. Acidic binding sites comprised of negatively-charged aspartic acid residues, engineered within the pore lumen, produced dramatic changes in the functional properties of the αHL protein, catalyzing the translocation of cationic polypeptides from one side of the membrane to the other (Wolfe et al., 2007). When two electrostatic binding sites were introduced, at the entry and exit of the β barrel, both the rate constants of association and dissociation increased substantially, diminishing the free energy barrier for translocation. However, more hydrophobic polypeptides exhibited a considerable decrease in the rate constant of association to the pore lumen, having to overcome a greater free energy barrier due to the hydrophilic nature of the pore interior.

### References

Wolfe AJ, Mohammad MM, Cheley S, Bayley H, Movileanu L. 2007. Catalyzing the translocation of polypeptides through attractive interactions. J Am Chem Soc in press.

## 2503-Pos Stepping of Individual RecBCD Molecules

Ashley R. Carter[1], Hsiu-Fang Fan[2], Hung-Wen Li[2,3], Thomas T. Perkins[1]

[1] *JILA, National Institute of Standards and Technology and University of Colorado, Boulder, CO, USA,*
[2] *McGill University, Montreal, QC, Canada,*
[3] *National Taiwan University, Taipei, Taiwan.*

**Board B617**

RecBCD is a processive, DNA-based motor with both helicase and nuclease activities. Understanding the molecular mechanism of any motor activity involves determining the elementary step size with which it moves. Previously two different methods (crystallography and pre-steady state kinetics) yielded two proposed step sizes for super family I helicases, of which RecBCD is a member. From crystallographic studies of the enzyme-DNA complexes in different nucleotide states, a 1 base-pair (bp) step size is inferred. Pre-steady state unwinding assays, on the other hand, measure the number of base-pairs per rate-limiting step or the "kinetic" step size of 4 bp. To directly measure RecBCD's physical step size, we built an optical trapping instrument capable of resolving 1 bp steps at moderate

forces (6 pN) and low frequencies (0.1–5 Hz) in a surface coupled assay. With this enhanced resolution, we note three significant results. First, RecBCD takes distinct steps of 1–6 bp, occasionally larger. The distribution of steps shows a peak centered at 4 bp, while the distribution of dwell times indicates that each mechanical translation represents a single rate-limited step. Second, the "noise" in the traces is enhanced (2-fold) when ATP is added, suggesting that the enzyme undergoes substantial conformational changes when moving. Third, we see backward steps that are similar in size to forward steps at moderate force (6 pN) and low ATP levels (2 μM). Recovery of forward motion is comparable in both size and kinetics to normal forward steps. We interpret these backward steps as reannealing of the unwound DNA coupled with a backwards motion of the enzyme along the template. In summary, our results support a model in which RecBCD has a variable step size; the average of which (4.5 bp) quantitatively agrees with the previously determined "kinetic" step size.

## 2504-Pos Force propagation patterns in proteins from Molecular Dynamics simulations

Wolfram Stacklies[1], Frauke Graeter[1,2]

[1] *CAS-MPG Partner Institute for Computational Biology (PICB), Shanghai, China,*
[2] *Max Planck Institute for Metals Research, Stuttgart, Germany.*

**Board B618**

A protein's behavior under external pulling forces can be studied by monitoring its force-induced transitions such as unfolding or enzymatic function, using an atomic force microscope, optical tweezers or complementary force-probe molecular dynamics simulations. How the applied force propagates within proteins determines their mechanical behavior, yet hitherto remains largely unknown. How do forces flow through protein scaffolds and how is this related to protein mechanical function and regulation?

We perform molecular dynamics simulations of the well-studied titin immunoglobulin (IG) domain as a test system. We monitor alterations in average molecular forces between pairs of atoms upon pulling at different constant forces, which are low enough to keep the protein in the folded state. We observe that the externally applied force is anisotropically distributed throughout the protein scaffold as reflected by statistically significant changes in pair wise forces in major force-bearing structural elements. The obtained patterns of force propagation can be used to explain resistance to mechanical stress and may contribute to understand the effect of external perturbations on proteins in general, including allostery.

## 2505-Pos Quantifications of Simplified X-ray Radiation Pressure Force on Individual Gold Nanocrystal

Yuji C. Sasaki, Takuma Sagawa

*Biomedical Group, SPring-8/JASRI (JST/CREST Sasaki-team), Sayou-gun, Japan.*

**Board B619**

Recently, we succeeded to observe x-ray radiation pressure force on individual single gold nanocrystal using an x-ray single molecular methodology. The observed force is estimated at about 0.13–0.63 atto-Newton. In the future, we will be able to control and measure dynamics of micro- or nano-crystalline materials using x-ray radiation pressure force. However, we must study quantitative analysis of x-ray radiation pressure forces on the nanocrystals in details before we applied ones. For example, in my previous research, we utilized the adsorbed protein molecules, and the gold nanocrystals are linked to the adsorbed ones. Because we can not observe free Brownian motions of the individual gold nanocrystals in aqueous solutions. Therefore, this system is very complexly. Additionally, we utilized white x-ray probe to track the diffraction spots from the linked gold nanocrystals. Thus, phenomena of high-order x-ray diffraction are included in the first observations of x-ray radiation pressure forces.

In order to do a detailed and more accurate analysis of x-ray radiation pressure force, we utilized both quasi-monochromatic x-rays and non-adsorbed free-standing gold nanocrystals. In this experiment, the free-standing gold nanocrystals are distributed in organic solvent (for example, polycarbonatediol: PCD)) of high viscosity to slow down the movements of the individual gold nanocrystals. Additionally, in these experiments, we controlled the size of gold nanocrystals and the energy of quasi-white x-rays. The averaged exposure time in single shot was 100μsec.-1 msec.

In simplified DXT observations, we can obtain observed mean square displacement (MSD) curves from the free-standing gold nanocrystals. According to experimental results of the observed MSD curves, we confirmed that the observed gold nanocrystals exhibit not normal simple Brownian motion but the directed ones.

## 2506-Pos $F_1$-ATPase Rotates By An Asymmetric, Sequential Three-site Mechanism

Takayuki Ariga[1], Eiro Muneyuki[2], Masasuke Yoshida[3]

[1] *Kyoto University, Kyoto, Japan,*
[2] *Chuo University, Tokyo, Japan,*
[3] *Tokyo Institute of Technology, Yokohama, Japan.*

**Board B620**

$F_1$ is the catalytic part of $F_oF_1$-ATP synthase, which utilizes energy from the proton gradient to produce ATP. Isolated $F_1$, or $F_1$-ATPase, can hydrolyze ATP by rotating the central γ subunit, causing it to rotate in 120° steps within the $\alpha_3\beta_3$ cylinder, each step consuming a single ATP. However, how the catalytic activity of each β subunit coordinates with the other two β subunits to drive rotation remains unknown. To clarify this issue, we have shown that hybrid $F_1$ containing one or two mutant β subunits with altered catalytic kinetics rotates in an asymmetric stepwise fashion [1]. Analysing the rotations revealed that for any given β subunit, the subunit binds ATP at 0°, cleaves ATP at ~200° and carries out the third catalytic event at ~320°. This elucidates the concerted nature inside the $F_1$ complex where all three β subunits participate to drive each 120° rotation of the γ subunit with a 120° phase difference, that is, a "sequential three site mechanism". Here we report further analysis

of hybrid $F_1$ with various mutants and discuss candidate models for each elementary event while considering other recent reports.

## References

[1]. Ariga, T., Muneyuki, E. & Yoshida, M. *Nat Struct. Mol. Biol.* ***14***, 841–846 (2007)

# 2507-Pos ATP-driven Rotation of $F_oF_1$-ATP Synthase Reconstituted into Supported Membrane

Ryota Iino, Kazuhito V. Tabata, Hiroshi Ueno, Rie Hasegawa, Hiroyuki Noji

*Osaka University, Osaka, Japan.*

**Board B621**

$F_oF_1$-ATP synthase ($F_oF_1$) is a rotary molecular motor that reversibly catalyzes ATP hydrolysis/synthesis reaction coupled with the proton translocation across the cell membrane. Rotation mechanism of $F_oF_1$ (or isolated $F_1$ part) driven by ATP hydrolysis has been extensively studied by single molecule techniques. However, rotation driven by protonmotive force, generated by the membrane potential and the difference in proton concentration across the membrane, has not been directly observed yet. Although we have addressed this issue using the planar membrane method (Ide and Yanagida, 1999), even the ATP-driven rotation of $F_oF_1$ embedded in planar membrane has been rarely observed.

In this study, we tried supported membrane method as an alternative. $F_oF_1$ from *Escherichia coli* was reconstituted into the large supported membrane (>10 μm in diameter) formed on the NiNTA-modified coverglass, and immobilized via histidine-tags introduced into c-ring of $F_o$. Rotation was observed by streptavidin-coated 200nm latex beads attached to the biotinylated β subunits of $F_1$. The number of rotating particles (~5) found in a single observation chamber increased significantly as compared with that found in the planar membrane (<0.1). Furthermore, the rotational speed (>10Hz) was much faster than that observed in the planar membrane (<1Hz) at high ATP concentration. These results indicate that planar membrane, formed in the presence of an organic solvent such as squalene, may interfere with $F_oF_1$ rotation, presumably due to its thickness. To drive the reverse rotation of $F_oF_1$, we are trying to apply protonmotive force across the supported membrane.

**Single Molecule Biophysics - II**

# 2508-Pos Single Molecule TIRF Imaging And Analysis Of Nonspecifically Labeled Fibrinogen - A Molecular Calibration

Alina L. Popescu Hategan, Katie Gersh, Dan Safer, John W. Weisel

*University of Pennsylvania, Philadelphia, PA, USA.*

**Board B622**

Single molecule imaging studies can in principle yield information on molecular processes not obtainable by any other method, but its usefulness depends on accurate quantitative characterization of the labeling. The size of fibrinogen and the complexity of its structure makes available on its surface a large number of amino groups to which fluorescent dyes can attach nonspecifically. The bleaching behavior of fluorescently labeled fibrinogen molecules observed in total internal fluorescence microscopy (TIRF) together with theoretical calculations of labeling probability were used to determine the number of active fluorophores attached to each fibrinogen molecule and characterize the uniformity of nonspecific labeling of this molecule. Two different dyes (Tetramethyl Rhodamine and Alexa 488) in bulk dye/fibrinogen ratios ranging from 0.3 to 4.2 were used with similar results. Whereas the predominant labeling was shown to be one active dye molecule per fibrinogen, with increasing bulk labeling ratios, two or more active dyes per fibrinogen start to be significant. From the intensity distribution of the bleaching steps and the probability of active labeling of fibrinogen molecules, a single molecule intensity calibration was obtained. Such calibration is necessary for further studies of fibrin fibers formed from fluorescent fibrinogen, to provide information at the molecular level on the structure of the fibers and their growth kinetics.

# 2509-Pos Cell-based Single-molecule Detection of a Fluorescent Unnatural Amino Acid Incorporated into the Nicotinic Receptor

Rigoberto Pantoja[1], Erik A. Rodriguez[2], Mohammed I. Dibas[1], Dennis A. Dougherty[2], Henry A. Lester[1]

[1] *California Institute of Technology, Division of Biology, Pasadena, CA, USA,*

[2] *California Institute of Technology, Division of Chemistry and Chemical Engineering, Pasadena, CA, USA.*

**Board B623**

Fluorescent unnatural amino acids (fUAAs) represent an attractive strategy for labeling ion channel proteins. Here we report the first successful incorporation of a fUAA, Lys(BODIPYFL), into the muscle nicotinic acetylcholine receptor (nAChR). *Xenopus* oocytes were injected with the frameshift suppressor tRNA aminoacylated with Lys(BODIPYFL) (YFaFS$_{ACCC}$-Lys(BODIPYFL)) and nAChR (α/β19′GGGU/δ/γ) mRNAs. Two-electrode voltage-clamp recordings confirmed the presence of functional surface-expressed nAChRs with respective ACh $EC_{50}$ and Hill coefficient of 37.8 ± 1.84 μM and 1.13 ± 0.05 (n = 5). We measured fluorescence from oocytes expressing the nAChR β19′GGGULys(BODIPYFL) using time-lapse total internal reflection fluorescence (TIRF) microscopy. Under conditions of relatively low expression (<0.1 receptors/μm$^2$), puncta with discrete decrease in fluorescence intensity consistent with single-molecule photobleaching were detected. The puncta displayed a Gaussian distribution of intensities; the average single-molecule signal-to-background (ΔF/F ± SD) was 0.23 ± 0.01. Puncta densities were much lower in oocytes injected with YFaFS$_{ACCC}$-Lys(BODIPYFL) (~0.007 puncta/μm$^2$). To confirm that the puncta originated from Lys(BODIPYFL) incorporated into