2587-Pos Board B573
Rapid Two-Photon Imaging with Nanometer Accuracy of Individual Quantum Dots in a Biological Environment
Ruobing Zhang, Eli Rothenberg, Gilbert Fruhwirth, Ido Golding, Tony Ng, Ward Lopes, Paul R. Selvin.
Two-photon (2P) excited fluorescence microscopy is particularly advantageous for imaging biological specimens since it offers reduced scattering, deep sample penetration, and intrinsic confocality when excited with point excitation. Here we report the first 2P microscopy of individual quantum dots in biological (water-based) environment. Near-complete suppression of blinking and of photobleaching was achieved. Three schemes of 2P excitation are shown: 1) widefield, 2) diffraction-limited spot scanning with a single rastered spot, or 3) a multi-point excitation scheme employing a 9 × 9 matrix hologram that increases the scan rate by 80 fold. An array detector (EMCCD camera) was also used as a detector for 2P scanning microscopy, providing faster whole image acquisition and superior detection capabilities compared to a conventional single point detector (a PMT). We also resolved the step size of individual Myosin V motors in vitro and imaged the three-dimensional (3D) distribution of LamB receptors on live E. coli cells and epidermal growth factor (EGF) receptors on fixed breast cancer cells. We obtained 3D localization at < 3 nanometer accuracy and fast 3D imaging at confocal resolution, while doing no harm to protein activity and cell samples.

2588-Pos Board B574
Wavelet Shrinkage to Resolve Single Molecule FRET Structural Landscape of the Isolated Ligand Binding Domain of the AMPA Receptor
Christy F. Landes, J. Nick Taylor, Anu Rambhadran, Vasanthi Jayaraman.
Single molecule fluorescence resonance energy transfer (smFRET) was used to examine the conformational landscapes explored by the agonist binding domain of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor in its apo and agonist bound forms for wild type and T686 mutant proteins. Although the average conformation for each receptor form was found to be similar to those reported by ensemble measurements, the smFRET data reveal several new points. First, the glutamate-bound form explores a wide range of conformations. The distribution of conformations was so wide as to be obscured by measurement shot-noise. By employing novel data analysis techniques, it was determined that each protein form comprised multi-state, sequential equilibria. Rate constants were extracted for the equilibrium conformational interconversions in the denoised data. These results illustrate that the extent of activation is dependent not on a rigid closed cleft, but instead on the probability that a given subunit will occupy a closed cleft conformation, which in turn is not only determined by the lowest energy state but by the range of states that the protein explores. Also, the results emphasize both the need for and the utility of advanced data processing techniques to quantify structure and dynamics in heterogeneous systems.

2589-Pos Board B575
Time-Resolved Three Dimensional Tracking of Individual GFP Molecules in Solution at Biologically Relevant Transport Rates
Jason J. Han, Peter M. Goodwin, James H. Werner.
Many important cellular functions such as intracellular signaling and protein trafficking involve three dimensional molecular motion. Monitoring these processes at the single molecule level requires the ability to track individual fluorescent emitters in high background environments at biologically relevant diffusion rates, and over several microns in all three dimensions. We have previously reported a custom confocal 3D tracking fluorescence microscope design capable of tracking single quantum dots (QDs) over several microns in X, Y, and Z in high background environments (*Anal. Chem*., **2008**, *80*, 9830-9834), and followed QD labeled antibodies bound to membrane receptors on live cells (*Proc. of SPIE*, **2009**, *7185*, 71850Z). While QDs are useful for obtaining long trajectories, their use as biological labels is generally limited to receptors that can be externally labeled or to permeabilized cells. GFP labeling circumvents these limitations, and as such, is the fluorescent label of choice for many intracellular fluorescence microscopy experiments. While CCD-based methods for 2D tracking of individual GFPs in live cells are well established, 3D tracking of single GFP labeled intracellular proteins in live cells will represent a tremendous technical achievement. Towards this end, here we show our 3D tracking methods can follow the Brownian diffusion of single GFPs in glycerol-water mixtures at rates comparable to intracellular protein traffic (D ~ 1 um^2/s). Trajectory durations are limited by GFP photostability, with some trajectories lasting for seconds. Unlike CCD-based tracking schemes, the arrival time of each photon is recorded with ~400 picoseconds accuracy, enabling time-resolved spectroscopy to be performed on the molecules tracked. In particular, we demonstrate fluorescence correlation spectroscopy (FCS) of individual GFPs, with these FCS curves being obtained as we follow individual molecules moving through 3 dimensional space.

2590-Pos Board B576
Holliday Junction as Single Molecule System for Studying Protein Binding
Matthew V. Fagerburg, Syam P. Anand, Grant Schauer, Saleem A. Khan, Sanford H. Leuba.
We are using single-pair Förster resonance energy transfer (spFRET) to characterize the conformational kinetics of single Holliday junctions (HJs). By comparing such kinetics in the absence and presence of various HJ-binding proteins we aim to gain some understanding of how HJ structures are recognized and processed in vivo as well as how processes such as HJ branch migration are regulated. Addtionally, we plan to use our system to make quantitative measurements of protein binding energies.

2591-Pos Board B577
Dual-Color Fluorescence Microscopy for Imaging DNA Loops in Living Cells
Joshua Milstein, Mike Chu, Jens-Christian Meiners.
The formation and breakdown of protein-mediated loops in DNA is an important regulatory motif in gene expression. Unfortunately, direct microscopic observations of this mechanism within living cells have remained elusive because of a lack of in vivo techniques with sufficient spatial and temporal resolution. To address this deficiency, we have developed a two-color fluorescence microscopy technique designed to witness DNA looping in living cells. Our method involves the simultaneous tracking of two different colored quantum dot fluorophores conjugated to opposite ends of a strand of dsDNA. By analyzing the spatio-temporal correlations of the motion of the two fluorophores, we are able to determine when a loop has formed. We illustrate the technique by analyzing the motion of model constructs of varying lengths that are transfected into Dictyostelium discoideum cells.

2592-Pos Board B578
Unmasking Preferred Structures and Structural Dynamics in Dimers of Amyloid-Beta Peptide
Robin K. Lammi, Chelsea Russell, Abigail Bradner, Rebecca Mitchum.
The neurodegeneration of Alzheimer's disease is causally linked to the self-association of amyloid-β peptide (Aβ), a protein of 39-43 amino acids that is found in brain plasma and cerebrospinal fluid. Although insoluble Aβ fibrils have garnered much attention due to their prevalence in the extracellular senile plaques characteristic of Alzheimer's, recent studies confirm that Aβ oligomers as small as dimers are linked to disease symptoms, including impaired long-term potentiation and synapse loss. We have used FRET measurements to probe structures of Aβ40 dimers, both in bulk solution and in single, surface-tethered species. Results of bulk fluorescence lifetime studies and single-dimer measurements reveal two characteristic FRET efficiencies, consistent with two characteristic dimer structures. Single-dimer investigations also uncover structural dynamics occurring in approximately one-third of the observed assemblies. These insights into preferred structures of Aβ dimers may afford improved understanding of peptide association and new avenues for the prevention and treatment of Alzheimer's disease.

2593-Pos Board B579
Roles of Cytoskeletal Components on the Membrane Movement of Individual Bk_{Ca} Channels in Live COS-7 cell
Sehoon Won, Sulgi Kim, Chul-Seung Park.
Membrane dynamics of the large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca} channels) was investigated in COS-7 cells by co-expressing the α subunit of the channel tagged with an acceptor peptide for biotin at its extracellular N-terminus and a genetically modified *E. coli* biotin ligase. We were able to visualize individual BK_{Ca} channels that had been biotinylated metabolically and then expressed on to the surface of the cells using quantum dots (QDs) coated with streptavidin. The time-lapse imaging on hundreds of homomeric BK_{Ca} channel revealed that the channels in live COS-7 cells exhibited a 'confined diffusion' in an area of 1.915 μm^2 with an initial diffusion coefficient of 0.033 μm^2/s. In order to understand the roles of cytoskeleton on the diffusional characteristics of the channel, we monitored the channel movement in the presence of drugs known to disrupt the filamentous actin and the microtubule, and quantified their effects on channel dynamics. It was found that both diffusion coefficient and the area of diffusion are differentially affected by such drug treatments. We are currently investigating the domain(s) of the channel responsible for cytoskeletal influence on the membrane dynamics of BK_{Ca} channel. Using this QD-based, single-molecule tracking technique, we wish to understand the cellular players influencing the diffusional movement of BK_{Ca} channel and their mechanism of action.
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