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# Posters

## Emerging Single Molecule Techniques I

**122-Pos Board B1**

**Single Molecule Immuno Pull Down Assay (SiMPull) for Studying Protein-Protein Interactions**

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Protein-protein interactions form the cornerstone for most biological pathways. Governed by numerous factors, same protein can associate with a host of different proteins and exhibit diverse functionality. This heterogeneity in complex composition is difficult to probe using bulk assays like immunoblot. Using TIRF microscopy, we have developed a *si*ngle *m*olecule immuno *pull* down assay (SiMPull) enabling direct visualization of protein-protein associations. Using fluorophore labeled antibodies we are able to visualize individual tethered molecules of protein of interest with high specificity. Surface bound antibodies are employed to specifically immobilize a target protein. We are able to pull down protein of interest (bait) from crude cell lysate, eliminating the need for protein purification. The bait protein co-precipitates its interacting partners. The identity of proteins bound to the bait is verified either by using fluorescent protein fusion constructs or through antibodies against anticipated targets. For a multimeric protein complex, fluorophore labeled antibodies against its subunits colocalize in the same diffraction limited spot. Using different dye labels for antibodies against different subunits and multicolor fluorescence colocalization, we are able to ascertain the molecular composition of these complexes. Individual photobleaching events provide us insights about the stoichiometry. SiMPull can be extended to single cell lysate analysis and provides a rapid, sensitive and robust platform for analyzing protein assemblies *in situ*.

**123-Pos Board B2**

**A Single-Molecule System for Detection and Quantification of Proteins with Robust Capture Units and Potential for High Multiplexing**

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We have developed a novel sensor technology with powerful high multiplexing detection potential and high sensitivity built around a specialized recombinant DNA molecule, Digital DNA. This digital DNA is constructed with unique identification patterns (barcodes), and serves as a scaffold for specific analyte-recognizing receptors, which can be antibodies, Fab fragments, or camelid nanobodies. The presence of the bound target is detected by binding of a uorescently-labeled secondary antibody. At the current parameters of our DNA-reading technology, the theoretical limit of multiplexing exceeds $10^5$. In addition, our microfluidic DNA reader requires a very small sample volume; many assays can be run on sub-microliter-sized samples. Assays have been developed for a half dozen proteins (glutathione-S-transferase, ovalbumin, botulinum toxoid, Venezuelan Equine Encephalitis virus coat, Follicle-stimulating hormone and pokeweed antiviral protein). Studies with these reagents have demonstrated low picomolar sensitivity and the capacity to detect multiple targets simultaneously, and illustrate the potential of this technology to provide solutions for applications in public health, clinical diagnostics, and biomedical research.

**124-Pos Board B3**

**Binding Specificity of Multi-Labeled PNA Probes Studied by Single Molecule Mapping**

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We evaluated a set of bisPNA probes carrying one or two fluorophores for their binding specificity to dsDNA by single molecule mapping using Direct Linear Analysis (DLA). In DLA, 50 to 250 kb-long DNA molecules are elongated in a continuous flow to their contour length and individually interrogated by laser light excitation of fluorescent tags. When averaged, optical traces of individual molecules present a physical map of PNA probes binding with a spatial resolution of 4 kb (x um). Average traces are directly related to the occupancies of binding sites, both exactly matched to PNA sequence and sites carrying mismatches. This analysis requires around $10^2$ molecules to be detected.
Here we report on specificity of multi-labeled PNA binding, defined as occupancy of the match site relative to the occupancy of a site with a single end mismatch, using a 185.1 kb-long bacterial artificial chromosome 12M9 and E. coli genomic DNA digest. We find that the type and position of the fluorophore on bisPNA determine its affinity and sequence specificity. Moreover, relative placement of fluorophores within the probe also affects probe brightness and consequently confidence of its detection in mapping studies. Fluorophore type and position has to be taken into account when developing probes for the whole genome mapping analysis.

**125-Pos Board B4**

**High Resolution Imaging Via SHREC And SHRImP For Ultra-High DNA/RNA Resolution**

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Single-molecule high resolution co-localization (SHREC) (Churchman et al., PNAS, 2005) and Single-molecule high-resolution imaging with photobleaching (SHRImP) (Gordon et al., PNAS, 2004) methods have been developed to measure distances between two fluorophores that are closer than Rayleigh limit ($\approx$250 nm for visible excitation). Combining the two techniques adds another dimension to the power of localization methodology and tens of distances could potentially be resolved by using several fluorophores of different colors each having multiple members.To apply this to DNA, we first stretched double-stranded DNA on a Polyacrylic acid and Polyallylamine coated surface, making the DNA relatively straight. To test SHRIMP, we made a DNA construct with a biotin followed by three Cy-3's at positions 475 bp, 172 bp, and 94 bp, corresponding to distances between Cy3 of 32nm, 58nm, and 90nm. We measured distances of 27 nm, 61nm, and 95 nm, in excellent agreement with the expected distances. To test simultaneously SHRIMP and SHREC, we placed Cy5 at position zero, and two Cy3's at position 94 bp and position 172 bp, and measured their positions using a dual-view imaging system. We determined the distances between Cy3-Cy5 pairs to be $37 \pm 5$ nm (32 nm expected) and $91 \pm 5$ nm (87 nm expected), and the distance between Cy3-Cy3 pair to be $56 \pm 3$ nm (58 nm expected). The agreement is excellent. The next step in this project is to study alternative pre-messenger RNA splicing and to quantify individual splicing variants.

**126-Pos Board B5**

**Dynamic Single-molecule Colocalization Imaging - A New Method For Examining Membrane Protein Association In Living Cells**

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Knowledge of the association state of cell membrane proteins is vital for understanding key processes such as signalling pathways and immunological response. However, at present there is a paucity of techniques that are able to accurately measure association on a molecule-by-molecule basis in live cells. One method to follow association on live cells is to label and track individual molecules of interest *in situ*. To this end, we have developed Dynamic Single-molecule Colocalization (DySCo), a technique which identifies the correlated movement of labelled proteins in two-colour channels. Proteins of interest are expressed at low levels with either a yellow or red fluorescent protein, and simultaneously imaged under a TIRF configuration. We then track molecules using a recently developed Bayesian approach, which is able to accurately recover tracks at low signal-to-noise ratios. Finally, we examine the inter-track distances between the two colour channels to determine the level of association. After validating the technique using control samples, we then applied it to the association state of T-cell receptors on the surface of living T cells.

**127-Pos Board B6**

**Characterizing The Equilibrium Blinking Behavior Of Fluorogens With Fluorogen Activating Proteins (FAPs)**

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We have recently developed FAPs (fluorogen activating proteins) that can specifically activate the fluorogenic dyes thiazole orange (TO) and malachite green (MG) with nanomolar affinities. Upon binding to FAPs, the otherwise dark fluorogens display thousands of fold increase in fluorescence intensity. The reversible interaction between the fluorogen and the FAP allows the same FAP to bind and activate fresh fluorogens in solution after one fluorogen dissociates from the FAP. Therefore, the binding and unbinding reactions lead to on-and-off (blinking) fluorescent signals at the single molecule level. As a result, this