this work, we demonstrate ligand-targeted delivery with a novel magnetic microsphere by conjugating the drug carriers with a folic acid ligand that preferentially binds to HeLa cells overexpressing folic acid receptors. The microspheres used in this study are produced in-house and contain magnetite nanoparticles (~10 nm) distributed uniformly throughout an amine-functionalized silicone core. The sphere diameter is scalable from 0.5 to 2.0 microns, and the concentration of magnetic nanoparticles can be varied up to 50% wt. The silicon matrix of this carrier facilitates compatibility with lipophilic drugs, the high magnetic content allows the potential for magnetically-stimulated drug release, and an abundance of protein binding sites within the matrix enables surface functionalization with a variety of ligands. Microspheres in this study were conjugated with folic acid using an EDAC reaction and tagged with a fluorophore. The spheres were incubated with HeLa cells, which overexpress folate-binding protein, and the degree of binding after 30 minutes was analyzed with fluorescence microscopy. We show a five-fold increase in bound spheres per cell relative to a control sphere without folic acid, indicating a high degree of specific binding. The preferential binding of ligand-conjugated magnetic microspheres gives insight into the utility of these drug carriers for targeted drug delivery studies.

2685-Pos Board B704
The Styrene-Maleic Acid Copolymer Extracts Active Complexes from Native Biomembranes
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Amphiphilic polymers have been widely used to maintain the solubility of membrane proteins and complexes following detergent solubilization. However, their ability to extract proteins directly from lipid bilayers has remained largely unexplored. Here we show that a copolymer composed of styrene and maleic acid pendant groups (SMA) extracts proteins from native membranes and reconstitutes them into polymer-bound lipidoprotein particles. First, we found that the SMA copolymer disrupted the membranes of intact mitochondria in a concentration-dependent and saturable manner. This was evidenced by the collapse of the transmembrane electric field of the inner mitochondrial membrane and by the solubilization of mitochondrial membrane proteins, both of which were mediated by the SMA copolymer in a manner similar to that mediated by nonionic detergents. Second, following incubation of the SMA copolymer with mitochondrial membranes and chromatographic separation, we observed by transmission electron microscopy that the resulting polymer-bound particles were a monodisperse population of discoids. The dimensions of these particles were similar to those previously reported for particles derived from liposomes or proteoliposomes of synthetic phospholipids (Lipidosis®).
Finally, using mitochondrial respiratory Complex IV (cytochrome c oxidase) as a model enzyme, we demonstrate that the SMA copolymer can extract even large, multicomponent complexes from native lipid bilayers and maintain them in a fully functional state amenable to solution-based biophysical studies. This novel approach to membrane protein reconstitution obviates the requirement for detergents and is therefore better suited to preserving native annular lipids and protein stability in comparison with traditional solubilization techniques.

2686-Pos Board B705
Hierarchical Accessibility of Double-Helical RNA and DNA Processing Signals in Highly Dense Self-Assemblies
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The ability to accurately detect biomolecules in single-cell amounts is an important goal of basic and translational research. The inherent capability of nucleic acids to self-assemble allows the spontaneous formation of highly dense, self-assembled monolayers (SAMs). These assemblies can provide the basis for development of nano-based devices with programmable, single-molecule detection capability. Double-stranded(ds) RNA is an important biomarker of viral infection and certain cancers. While dsRNA behavior in solution has been extensively characterized by diverse physicochemical approaches, the properties of highly dense assemblies of dsRNA are largely unknown, and may be qualitatively different from those in solution. Here we demonstrate that dsRNA-specific RNase III of Aquifex aeolicus (Aa-RNase III) and restriction endonuclease BamHI, each having a recognition site in the dsRNA and dsDNA segments, respectively. We also show that the reactivity of the BamHI cleavage site, which is proximal to the gold surface, can be controlled by SAM density, and that access to the BamHI site is dependent upon the prior action of RNase III at its site in the center of the ds-chimera. These results reveal novel properties of protein-nucleic acid interactions within a high-density array environment that are relevant to nanoscale detection methodologies.
2687-Pos Board B706
A Kinesin Driven Microfluidic Concentrator Device for Ultrasensitive Detection of Analyte
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The discovery of a vast array of biomarkers has spurred the demand for diagnostic assays with lower detection limits for early disease detection. Microfluidics makes it possible to work with small sample volumes and has played a significant role in creating more sensitive diagnostic tools. Our goal is to adapt our previous biomolecular motor (kinesin) based concentrator (NanoLett. 8:1041) and integrate antibody-functionalized microtubes into the device. Transforming this device into an immunoassay platform allows a variety of proteins or biomarkers to be actively captured and concentrated for detection. We believe this concentrator can improve typical ELISA assays by integrating two key features. First, concentrating the analyze-carrying microtubes into a small 625μm2 concentrator region increases the signal to noise ratio allowing for more sensitive fluorescence measurements. Second, by ensuring that the binding capacity of these functionalized microtubes is high, we allow for a large number of antibodies and antigen to be concentrated. To achieve these goals we have developed a protocol to covalently link a high density of monoclonal, polyvalent or f(Ab)2 antibodies onto microtubes without significantly affecting the motility of the complex. Motility is critical for the device since the microtubes with captured and fluorescently-labeled analyte are rapidly transported by kinesin into the concentrator region. The intensity of the resulting fluorescent signal in the concentrator region directly corresponds to the concentration of analyte in the initial sample. Our results show that the fluorescence intensity of individual anti-BSA coated microtubes allows the detection of sub-picomolar concentrations of TMR-BSA by integrating the fluorescence signals along microtubes. In conclusion, our data suggest that integrating functionalized microtubes and raising the signal to noise ratio by concentration in this device improves the detection limits of a typical ELISA assay while significantly reducing the assay time.

2688-Pos Board B707
Towards Nucleotide Differentiation with Solid-State Nanopores
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Nanopores have made significant progress toward a viable “$1000 genome” since their discovery just over a decade ago. To date, however, solid-state nanopores have not demonstrated the resolution and signal power necessary to discriminate between different nucleotides or short polymer chains. Here we report on the detection and discrimination of short chains of nucleotides. We use ultra-thin membranes(1), reproducibly fabricate sub-2nm nanopores, and exploit our proven ability to sample at high frequencies with low noise(2). Combining these experimental features enables measurements with high-sensitivity, high-signal, and low signal-to-noise, which allow us to detect different molecules through our nanopores. 1. Wanunu, M., Dadosh, T., Ray, V., Jin, J., McReynolds, L., and Drndic, M. 2010. Rapid electronic detection of probe-specific microRNAs using thin nanopore sensors. Nature Nanotechnology 5:807-814. 2. Rosenstein, J.K., Wanunu, M., Merchant, C.A., Drndic, M., and Shepard, K.L. 2012. Integrated nanopore sensing platform with sub-microsecond temporal resolution. Nature Methods 9:487-U112.
2689-Pos Board B708
A Binary Molecular Gate Made of DNA and Protein
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Many proteins have evolved the ability to recognize and bind to specific sequences of DNA. This specificity is quite high and often is the basis for genetic switches which can repress or induce transcription in cells. One of the best known DNA-binding proteins is the lac repressor protein which binds tightly to the lac operator but loses its affinity for this site upon the binding of lactose. We have used this lac repressor protein and the lac operator sequence to
construct a binary molecular gate which can be controlled by the effector molecule lactose. The device is constructed of three DNA strands which form an elongated hairpin structure of two dsDNA arms with a short ssDNA hinge at one end, and two ssDNA regions on the opposite end. A fourth ssDNA molecule (the ‘locking’ strand), complementary to the two ssDNA regions, binds by complementary base pairing to seal the gate in the closed configuration. Addition of lact repressor protein to the device causes a displacement of the locking strand by binding to two lac operators embedded in the double-stranded hairpin structure. Binding of the proteins is rapid and sufficiently strong to cause a displacement of the locking strand, switching the gate into the open conformation. Addition of lactose quickly causes a release of the lac repressor protein from the operators allowing the gate to be closed by the binding of the locking strand. The opening and closing of this binary gate was detected by gel shift assays and by fluorescence resonance energy transfer spectroscopy of two dyes located near the opening of this gate. The gate could be cycled repeatedly, responded uniquely to lactose, and may be useful as a device for moving or holding structures on a molecular scale.

2690-Pos Board B709

Nanoparticle Surface Charge Directs the Cellular Binding of Nanoparticle-Protein Complexes

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Nanoparticles are commonly utilized in biological systems as imaging probes, drug delivery agents and in vivo sensors. In most biological applications, the nanoparticle is exposed to a complex mixture of extracellular proteins that adsorb non-specifically to the nanoparticle surface. The resulting “protein corona” may alter the interactions of the nanoparticle with the cellular membrane. We have focused on the role of nanoparticle surface charge in the cellular binding of nanoparticles in the presence of extracellular proteins. Cationic, amine-modified polystyrene nanoparticles and anionic, carboxylate-modified polystyrene nanoparticles were studied as a model system. The cellular binding of cationic and anionic nanoparticles is distinctly different, determined from fluorescence microscopy experiments. For cationic nanoparticles, the cellular binding is increased in the presence of serum proteins. In comparison, anionic nanoparticle binding is inhibited by the presence of serum proteins. Competition assays performed with flow cytometry allowed us to quantify differences in binding and to identify the cellular receptors used by the nanoparticle-protein complexes. We have determined that complexes formed with anionic nanoparticles bind to native protein receptors, while those formed with cationic nanoparticles bind to scavenger receptors. These results indicate that for nanoparticles used in biological applications, the initial surface charge of the nanoparticle mediates cellular binding. Currently, we are extending our studies to nanoparticles used for drug and gene delivery.

Single Molecule Techniques III

2691-Pos Board B710

Quantitative Imaging of Protein Complexes using TIRF Microscopy

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The dynamic composition of protein complexes can have a profound impact on their function. However, conventional pull-down assays cannot provide dynamic information, and sample preparation may affect complex stability. Here, we combine total internal reflection (TIRF) imaging with quantitative image and data analysis to examine the stoichiometry of protein complexes as well as the dynamics of protein associations. Fluorescently labeled proteins are immobilized on glass coverslips, and perfused with other fluorescently tagged protein or small molecules. Complexes are imaged on a TIRF microscope to selectively illuminate bound proteins. We determine copy number and relative positions of fluorescent proteins inside complexes by counting photobleaching events, using computational super-resolution and mixture-model fitting algorithms for spot detection. We then statistically correct the results for experimental artefacts, such as expression levels and pre-bleaching of fluorophores, which can reach levels of 20%. We further determine binding kinetics by measuring dwell time of fluorescently labeled proteins on immobilized substrates using the same computational approach. Together these methods have allowed us to determine that CENP-A exists in octameric nucleosomes throughout the cell cycle, that myosin adopts multiple conformations on the cortex of the C. elegans zygote that are differentially sensitive to perturbations, and that the interaction of the catalytic domain of CENP-A in a domain depends on its phosphorylation and its Myb-like domain structure.

2692-Pos Board B711

Superresolution Imaging of RNAP and Ribosomes in Live E. Coli

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Superresolution fluorescence imaging is used to locate and track ribosomes, RNA polymerase, and various DNA loci in live E. coli. The strong degree of DNA-ribosome segregation indicates that most protein production occurs on free mRNA transcripts that have diffused into the ribosome-rich regions, not by co-transcriptional translation. The predominant ribosome diffusion coefficient (about 80% of the population) is $D_{\text{Ribosome}} = 0.04 \mu m^2 s^{-1}$, attributed to free mRNA being translated by multiple 70S ribosomes (polysomes). A smaller, faster population is attributed to 3OS subunits searching for a translation initiation site. New quantitative estimates of ribosome and RNAP copy numbers show that ribosome concentration is more tightly controlled than RNAP. The RNAP diffusion is heterogeneous. About half the population sub-diffuses very slowly (like DNA loci) and half diffuses freely with $D_{\text{RNAP}} = 0.15 \mu m^2 s^{-1}$. We attribute the slow component to copies that are transcribing or stalled while specifically bound to DNA and the fast component to “hopping and sliding” copies. In long RNAP trajectories we observe transitions between slowly and rapidly diffusing states, suggestive of initiation or termination of transcription events. These data provide a more detailed, quantitative picture of how the components of the transcription/translation machinery work together in space and time.

2693-Pos Board B712

Movement on Uneven Surfaces Displays Characteristic Features of Hop Diffusion

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Diffusion in cell plasma membranes is reported to be appreciably slower than diffusion in artificial membranes. We suspect that this is largely an artifact resulting from 2D interpretation of 3D movement over an uneven surface, which leaves underestimation of the net movement. Transient anchorage and/or confinement in domains can easily be explained by topographical trapping. The most serious flaw in the current methods of diffusion measurement is the assumption that the membrane is smooth. When the membrane is uneven, the straight-line-distance between two points requires that the particle leaves the surface, which underestimates the distance travelled. Clearly this is an illegitimate move since both lipids and proteins are restricted to movement within the plane of the membrane. We have therefore developed a new method for analyzing tracks that confines the path to the surface - the shortest within surface distance (SWSD). Simple diffusion was simulated over smooth and uneven surfaces created on a 3D orthogonal grid using an array of connected voxels starting a conditional dilation at the first location that spreads over the surface for a defined number of steps. Notably, after a few steps the diffusion coefficient was higher than after more steps - a characteristic feature of the hop diffusion. We conclude that the minimum requirement for accurate diffusion measurements on the plasma membrane is high resolution topographical images that can be used to assess the effect of local topography on simple diffusion. Only if there is a discrepancy between the measured diffusion coefficient and that expected from Brownian motion within the plane of the membrane after accounting for cell topography is it relevant to invoke anomalous diffusion.


2694-Pos Board B713

Single-Molecule Super-Resolution Microscopy at Video-Rate using Novel SCMOS-Specific Localization Algorithms

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Single molecule localization based super-resolution microscopy relies on precise and accurate localization of a large number of single molecules. However, the necessity of accumulating large numbers of localization estimates limits the time resolution to typically seconds to minutes. Two of the major limitations are the acquisition speed and the photon budget. Replacing EMCCD cameras which are usually implemented in such experiments with recently introduced SCMOS cameras results in a leap in both acquisition speed and effective quantum efficiency. However, the intrinsic pixel-dependent Gaussian noise of the SCMOS cameras introduces localization artifacts and greatly reduces the reliability of the results. Here, we present a set of specially designed statistics-based algorithms that allows for the first time to fully characterize an SCMOS camera and perform unbiased and precise localization analysis. Using this method we demonstrate...