pre-miRNAs. A three-way junction building block, which was obtained from the RNAjunction database, was attached to three human let-7 pre-miRNAs. This highly stable RNA nanoparticle is expected to enable the binding of the dicer protein for the efficient cleavage of the pre-miRNA in the cytoplasm. As a result, the mature let-7 miRNAs can enter into the targeted cell cytosol, and be protected from degrading interactions while performing its specific regulatory functions.

2079-Pos

Surface-Charge Influences Voltage-Dependent Pore Formation in Lipid Membranes by Quantum Dots

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Semiconductor nanocrystals, such as quantum dots (QDs) have many biomedical applications. It is of great interest to understand the mechanism by which these nanoparticles cross the cell membrane. Here, QDs, such as cadmium selenide / zinc sulfide core shell (CdSe/ZnS) nanoparticles are shown to interact with lipid bilayers painted on picoliter microelectrode cavities and to produce rapid current bursts with half widths in the range of tens of microseconds. These bursts are voltage dependent and are observed in both polarities. The voltage-dependence of the burst frequency is strongly influenced by external solution parameters like pH and salt valency, charge of the lipids, bilayer size and also nanoparticle size and charge. Correlating these findings with results of dynamic light scattering (zetasizer), we present evidence that electrostatic interactions play a pivotal role in generating the current bursts. Future experiments will focus on optical characterization in order to obtain more insight into the mechanism of interaction.

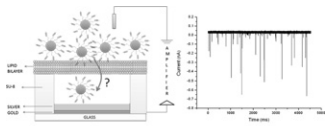

Figure 1. (A) Scheme of the measurement set up. (B) Current bursts measured across the bilayer formed over 10 um electrode in the presence of QDs at - 200 mV.

2080-Pos

Retrieval of a Metabolite from Cells with Polyelectrolyte Microcapsules

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To monitor cellular processes in individual cells, it is an important goal to measure the concentrations of intracellular components in real time, and to retrieve them for analysis. We report here the use of functionalized polyelectrolyte microcapsules as intracellular sensors for in vivo reporting. When capsules were loaded with streptavidin and introduced into Vero fibroblasts by electroporation, they were initially inaccessible to cytosolic biotin-fluorescein (BF), but after several hours, they bound and accumulated BF. Our work demonstrates the utility of polyelectrolyte microcapsules for intracellular sensing and suggests that they can autonomously escape from an endocytic compartment, making them ideal carriers for intracellular investigations.

2081-Pos

Monofunctional Quantum Dot Probes for Single-Molecule Imaging

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Recently, it has been shown that the optical properties of quantum dot (QDs) nanoparticles enable novel experiments at the single molecule level in live cells, thereby opening new prospects for the understanding of cellular processes. One difficulty with these experiments is that the complex biological environment imposes stringent design requirements on QD probes, necessitating the development of smaller, low valency and more biocompatible QDs. In this work, we present our efforts towards minimizing the size and controlling the surface functionality of QDs. We show that an engineered peptide surface coating and a purification method based on gel electrophoresis are sufficient to produce compact monofunctional QDs covalently conjugated to streptavidin (SAV), biotin and antibodies. To prove the monofunctionality of the QD-SAV probes, we apply novel single-molecule assay following complexation of the QDs with a fluorescent dye biotin derivative. Counting the photobleaching steps of the fluorescent dye gives us direct access to the number of binding sites present on the QD surface. We then apply these QD probes to the targeting and tracking of individual biotinylated membrane proteins expressed in living HeLa cells. We analyze the diffusion properties of these membrane proteins and compare the measurements to those obtained using commercially available QD probes. Overall, these monofunctional QD probes should be useful for studying a wide-range of biophysical phenomena, down to the single molecule level in live cells.

2082-Pos

Quantification of Functional Binding Sites Per Quantum Dot

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Antibody conjugated quantum dots have become increasingly useful in the study of proteins both *in vitro* and *in vivo* and are particularly valuable in single molecule experiments due to their narrow emission spectra and photostability. Despite their wide range of uses, it has been difficult to determine the number of functional binding sites per quantum dot. Previous studies focused on the characterization the total number of antibodies conjugated to quantum dots rather than the quantification of functional antibodies per quantum dot. Understanding the later is especially important to interpretation of data obtained in single molecule experiments using quantum dot labeled proteins. Additionally, since the number of functional binding sites per quantum dot may vary depending on the antibody conjugation method and also between quantum dot preparations, it is necessary to develop a simple and rapid method to test this experimentally. In this study, we use a direct read-out from the protein itself to determine the average number of proteins bound per quantum dot. By using radio-labeled enhanced green fluorescent protein (EGFP) constructs conjugated to common affinity tags used for protein labeling, we will quantify the number of functional binding sites per quantum dot and the specificity of quantum dot labeling *in vitro*. This method will be extremely useful in the interpretation of data obtained using quantum dot labeled proteins.

2083-Pos

Quantitative Study of the Protein Corona on Engineered Nanoparticles

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Nanoparticles are finding a rapidly expanding range of applications in research and technology, finally entering our daily life in medical, cosmetic or food products. Colloidal inorganic nanoparticles rendered water soluble by highly ordered organic shells hold great promise as powerful tools for applications in biotechnology and biomedicine. However, their ability to invade tissues, cells and even subcellular compartments may result in biological hazards if nanoparticle incorporation and migration within the body cannot be tightly controlled.

As yet, little known about the detailed mechanisms by which objects on the nanoscale interact with living organisms. Upon incorporation via the lung, gut or skin, nanoparticles become exposed to biological fluids containing dissolved biomolecules, especially proteins. Quantitative studies of the interactions between nanoparticles and biomolecules, which depend on the nature of the nanoparticle surface, are still scarce, and even less is known as to how such a 'protein corona' affects nanoparticle uptake by living cells.

Here we have employed fluorescence methods, especially FCS, to investigate the adsorption of human serum albumin and (apo-)transferrin on polymer-coated FePt nanoparticles of ~5 nm radius. Both proteins form a monolayer on the surface of these nanoparticles and bind with micromolar affinity. We have also studied the effect of the protein corona on the nanoparticle uptake behavior by HeLa cells (1, 2).

1. Röcker, C. et al., Nature Nanotechnology 4, 577-580 (2009).
2. Jiang, X. et al., J. R. Soc. Interface, published online, doi:10.1098/rsif.2009.0272.focus

2084-Pos

Nanocandles: Developing Optical Probes for the Cell Interior

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As knowledge of the bulk behavior of biological systems continues to grow, there is an increasing demand for knowledge of cellular processes at the single-molecule level. This presents a unique challenge, a combination of the dynamic nature of the system and the inability to modulate the concentration of