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.

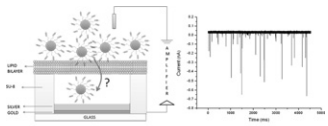

Figure 1. (A) Scheme of the measurement set up. (B) Current bursts measured across the bilayer formed over 10 um electrode in the presence of QDs at - 200 mV.

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

the target species. A significant limitation of previous low-penetration methods arises from the very character that provides their utility: the low penetration depth also means they can only probe molecular events very close to the substrate surface. We have fabricated vertical silicon dioxide nanopillars which, at a height of up to one micron, carry that low penetration depth up into the cell environment where the relevant molecular processes occur. The pillars can also be specifically functionalized with molecules of interest for either delivery into the local environment or study while tethered in the observation volume. With single molecule detection at biologically-relevant concentrations and biologically-applicable locations, these nanopillars provide a template on which to study a multitude of biological processes in a controlled, dynamic, and localized fashion.

2085-Pos

Evaluation of Photophysical Properties of Methylene Blue Incorporated within ZMOF Framework

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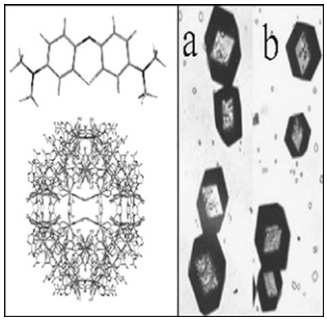
Zeolite-like metal organic frameworks (ZMOF) are of particular interest due to their application as gas storage material, drug delivery vehicles, and sensors. Stability, relatively straightforward synthesis and large internal cavities allow us to explore the possibility using of ZMOF material as a nanoreactors for various chemical and photochemical reactions. Here we present a synthesis and photophysical characterization of the ZMOF framework functionalized by encapsulation of a photosensitizer, methylene blue. Our data show that encapsulation of methylene blue within the ZMOF framework facilitates fluorophore self-aggregation as evident from the red-shift of methylene blue emission spectra, anisotropy increase, and decrease of the fluorescence lifetime. Interestingly, the fluorescent properties of methylene blue incorporated within zeolite-like methyl organic framework differ significantly from those reported previously for methylene blue aggregates in aqueous medium indicating strong interactions between the fluorophore and the framework.



Biotechnology & Bioengineering I

2086-Pos

Intracellular Effects of Nanosecond, High Field Electrical Pulses

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Mitochondria, which play a crucial role in apoptosis, release several apoptosis-inducing factors into the cytoplasm, presumably through the mitochondrial permeability transition pore (MTP). Under certain conditions, nanoelectropulses can manipulate mitochondrial structure and permeabilize the mitochondrial membrane without permanent damage to the plasma membrane. In this work we investigate the effects of nanoelectropulses on mitochondrial membrane permeabilization, assess changes in mitochondrial transmembrane potential, and monitor plasma membrane integrity under the same pulse conditions. 4 ns electrical pulses were applied to living human Jurkat T lymphoblasts in an electrode microchamber on a microscope slide. Changes in mitochondrial transmembrane potential were evaluated with rhodamine 123 (R123), a lipophilic cationic fluorescent dye that is accumulated within mitochondria. For assessing MTP opening, a calcein-cobalt quenching method was used. Calcein-AM is an anionic fluorochrome that enters cells freely and labels cytoplasmic as well as mitochondrial regions following esterase removal of the acetoxymethyl group. Because cobalt ions do not readily pass through mitochondrial membrane, mitochondria can be specifically identified by the cobalt quenching of cytoplasmic, not mitochondrial, calcein fluorescence, and MTP opening can be recognized by the decrease of calcein fluorescence within mitochondria. Finally, cell membrane integrity was evaluated with propidium iodide (PI), which is excluded from the cell interior by intact cell membranes. When the cell membrane is permeabilized, PI enters the cell, binds to double-stranded nucleic-acid molecules, and exhibits red fluorescence. The effects of different pulse amplitudes and pulse numbers on mitochondrial membrane permeability will be reported, providing a framework for an analysis of pulse doses and exposure conditions which lead to mitochondrial modifications while minimizing effects on the plasma membrane. We will also discuss the interpretation of data from fluorescence microscopic imaging analysis using R123 and cobalt-quenched intracellular calcein fluorescence intensity and the influx of PI.

2087-Pos

Hydrodynamic Trap for Single Cells and Particles

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Particle trapping and micromanipulation techniques have revolutionized biological sciences during the last two decades. Proteins, enzymes and cells have been studied extensively through manipulation methods based on optical, magnetic and electric fields. In this work, we present an alternative trapping method called the hydrodynamic trap which is based solely on hydrodynamic forces generated in a microfluidic device. The hydrodynamic trap is based on a purely extensional flow field created at the junction of two perpendicular microchannels where opposing laminar flow streams converge. The flow field in the vicinity of the microchannel junction can be described as a potential flow with a semi-stable potential well and a stagnation point. We implement an automated feedback-control mechanism to adjust the location of the stagnation point, thereby actively trapping arbitrary particles in free solution. Using the hydrodynamic trap, we successfully demonstrate trapping and manipulation of single cells and single particles with micron and sub-micron dimensions for arbitrarily long observation times. Brownian dynamics simulations show that the trap stiffness is comparable to alternative trapping techniques including magnetic traps. Overall, this new technique offers a venue for observation of biological materials without surface immobilization, eliminates potentially perturbative optical, magnetic and electric fields, and enables the ability to vary the surrounding medium conditions of the trapped object in real-time.

2088-Pos

Atomic and Photonic Force Microscope: from Nanonewton to Piconewton

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Cell differentiation and organization is influenced from chemical and mechanical characteristics of the extracellular matrix which determine its fate. Cellular compartmentalization can be explained as mechanical equilibrium of tensed and compressed cables which is continuously changing during cell motility, intracellular transport and cell division. Chemical composition of subcellular compartments determine the function they accomplish in the cell. Change in the chemical composition of subcellular structure produce not only change in their function but create also a change in their mechanical properties.

Dynamic behavior of the cell is obtained by continuous modulation of chemical composition and local recruitment of molecules in cell compartments: cell membrane vary its stiffness during endocytosis and exocytosis, cell refractive index change during cell division, actin and tubulin persistence length is modulate by assembly proteins during cell protrusions formation. Moreover single molecule mechanical characterization is becoming an important tool to study the molecule properties in different condition.

On the other side during pathologies cell mechanical characteristics change too: Brownian motion of trapped healthy cell is different from malignant one, membrane elasticity is changed in cell presenting abnormal organization of cytoskeleton.

Cell mechanics is becoming an emerging field to understand cell organization in healthy state and represent an additional way to analyze the onset of pathologies. Therefore we are developing a setup which combine AFM and Photonic force microscope to apply force spectroscopy measurement either in the piconewton and nanonewton range.

2089-Pos

Mechanotransductive Engineering of Neural Stem Cell Behavior

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Neural stem cells (NSCs) play important roles in learning and memory in the adult mammalian brain and may also serve as a source of cells in cell