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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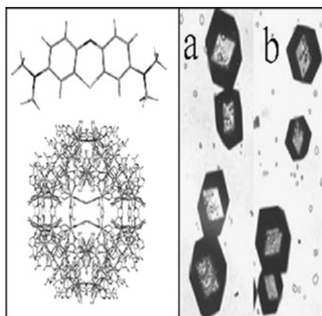
Zeolite-like metal organic frameworks (ZMOF) are of particular interest due to their application as gas storage material, drug delivery vehicles, and sensors. Stability, relatively straightforward synthesis and large internal cavities allow us to explore the possibility using of ZMOF material as a nanoreactors 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