no modification of cell surfaces is required, so that artifacts between the probe and the cell surface may be minimized compared with magnetic twisting cytometry and optical tweezers, in which microbeads are attached to the cell surface before measurements. However, the statistical properties of cell rheology, which are important to know the universality of cell mechanics, have not been so far investigated in the AFM method. In the present study, we measure a large number of cells in the force modulation measurements with a microarray technique. The amplitude and phase of fibroblast NIH3T3 cells ($n > 100$) were measured in the frequency range from sub-Hz up to 200 Hz, and $G^*(\omega)$ was estimated. The results indicated that the storage and loss moduli exhibited clearly a log-normal distribution Moreover, these moduli were well fitted to a power-law model, the so-called structural damping model, and the exponent was around 0.2 with a normal Gaussian. The relationship between the rheological properties and the internal structures of cells will be discussed.

2052-Pos Board B22

Single DNA Molecule Reaction Based On Dip-pen Nanolithography

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Controlling a reaction at the single molecule level has attracted substantial attention from chemical, physical and biological society for its scientific importance and potential applications. Some examples have been given using small molecules by scanning tunneling microscopy (STM). Challenges still remain in control of single-molecule reactions on the soft bio-molecules, which may reveal meaningful information on biological processes. Conventional techniques including magnetic tweezers and fluorescence microscopy have been used to study the enzymatic digests of single DNA molecules. Herein we report a new technique for localized digestion of single DNA molecules on solid substrate with nonspecific endonuclease, by employing an atomic force microscope (AFM) and the concept of Dip-pen nanolithography (DPN). We choose the solution containing DNase I with Mg2+ ions as the ink. As we known the DNase I can catalyze the hydrolysis of DNA strands without the requirement of specific sequence when Mg2+ ions or Mn2+ ions is presented. However, in our system, when DNase I ink solution was deposited on a local position of a single DNA molecule by an AFM tip, the followed digestion location of the DNA strands could be restricted with nanometer precision, so that the site cutting reaction with nonspecific endonucleases was achieved. This AFM site-specific reaction approach, although there is certain degree of shifting and broadening due to lateral diffusion of enzymes on surface, promises a deeper understanding of molecular surgery at the molecular scale and may become very important in the emerging field of nanobiotechnology.

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2053-Pos Board B23

High-speed, Thermo-chemical Nanolithography for Biological Applications

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Scanning probe-based chemical nanolithography has been recognized as an essential part of future nanofabrication processes. However, most of the present strategies still have significant limitations in terms of throughput, resolution and substrate variety. Recently, we have developed a new chemical nanolithography technique called thermo-chemical nanolithography (TCNL). TCNL utilizes a resistively-heated atomic force microscope tip to thermally activate a chemical reaction on an arbitrary organic or inorganic substrate surface. TCNL can write well-defined chemical features at a rate of mm/s, with sub-15 nm resolution [1]. In particular, we have demonstrated that carboxylic ester groups on a polymer surface can be deprotected by TCNL to give carboxylic acids and further modified to form anhydrides [2]. Therefore, TCNL can tune surface wettability with in situ write-read-overwrite capability. In addition, nanoarrays of TCNL-activated amine groups have been used as anchor sites to immobilize a variety of biological ligands [3]. This proves TCNL to be a powerful tool to control the physical placement of biomolecules and cells. It can be applied to a range of molecular cell biology studies such as ligand-receptor recognition and cell signaling.

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2054-Pos Board B24

Direct Mapping Of Surface-bound Liquid With Sub-nanometer Resolution

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At the solid-liquid interface molecules of the liquid adopt a particular arrangement which depends primarily on the interfacial energy and geometry. At the nanoscale this molecular arrangement is of central importance in a wide range of fields from molecular biology to surface physics, heterogeneous catalysis and electronics. In biology the role of interfacial liquid is further emphasized by the soft nature of most biomolecules whose conformation and dynamics depends on the surrounding medium. This is the case for protein function and folding (1), self-assembly processes and bio-electronics where a complex interplay between surface-bound liquid molecules and ions strongly affects any motion. Using amplitude modulation atomic force microscopy (AM-AFM) operated in liquid and in a particular regime, it is possible to simultaneously image the topography of the surface-bound liquid while measuring its adhesion energy to the solid investigated. We have used this method to map the binding energy of water to gold nanoparticles coated with mixed ligands, self-assembled in controlled patterns (2, 3). Such functionalized nanoparticles can mimic the typical surface of proteins (hydrophobicity, charge, surface domains) while allowing careful control of the domains' size and properties (2). Our results show that the average binding energy of water to the surface of the nanoparticles strongly depends on the spatial arrangement of the ligands molecules. The geometry as well as the size of the ligand domains both affect the local adhesion energy of the solvent in a non-linear fashion. Our findings provide experimental and quantitative insight into the interplay between solvent and surfaces in nanoscale biophysical processes.

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2055-Pos Board B25

Visualizing Lipid/Water and Lipid/Ion Interactions at the Biological Interfaces with Angstrom-Resolution by Frequency Modulation Atomic Force Microscopy

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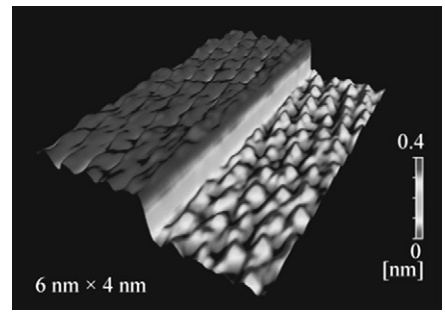
At the interface between biological membranes and their surrounding physiological solution, various interfacial phenomena take place through the interactions between lipid headgroups and water molecules (or ions) and hence have a great impact on the functions and structure of the biological membranes. However, it has been a great challenge to experimentally access such interfacial phenomena with Angstrom resolution.

Recently, we have developed an atomic force microscopy (AFM) technique that is capable of visualizing Angstrom-scale structures and phenomena at the solid/liquid interface with piconewton order loading forces. Here we apply this technique to the investigation of the lipid/water and lipid/ion interactions at the biological interfaces.

The figure shows an AFM image taken at the interface between the DPPC bilayer on mica and PBS solution. The image shows the molecular-scale corrugations of the lipid headgroups (lower half) and their primary hydration shells (upper half), revealing the existence of a stable hydration layer on the DPPC bilayer with nanometer-scale lateral extent.



Fluorescence Spectroscopy II

2056-Pos Board B26

Improved Global Spectral Analysis Method for Simulating Excitation-Emission Maps of Semiconducting Single-Walled Carbon Nanotubes

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Recent research breakthroughs in the field of semiconducting single-walled carbon nanotubes (SWCNTs) have centered strongly around the acquisition and analysis of photoluminescence excitation-emission maps (EEMs). EEMs have been shown to provide both qualitative and quantitative information of

SWCNT preparations including the helix-angle distribution, diameter distribution, length distribution, bundling properties and intensity distribution. To date quantitative analysis of the three dimensional EEMs has relied heavily on manual estimations and 2-dimensional profiling to deal with overlapping peaks and other features in the EEM surface. The global analysis software and method described facilitates a rapid and statistically robust simulation of the 3D EEM surfaces in either wavelength or energy units to yield crucial coordinate and line-width information on all identified PL peaks. The model parameter initialization is facilitated by derivatization of the surface to identify all major peaks coordinates and widths with adjustable amplitude discrimination. The program accepts EEM data in standard x-y-z columnar format in addition to matrix representation. An analytical form of the Voigt function is included to deconvolute the Lorentzian emission line shape from the Gaussian instrument response. The fitting functions can be fully constrained to ascertain physically realistic model parameterization using conserved themes for related data sets. Global linking/sharing of model spectral parameters is used to model excitation-emission peak coordinates relating the main energy levels (S3, S2 and S1) in addition to sidebands in the spectral emission. The model form can be adapted and constrained to yield information concerning anisotropic features, reabsorption phenomenon as well as energy transfer and quenching processes. The modeling routine also facilitates 3D surface simulations of Raman spectra of the radial-breathing modes of SWCNTs.

2057-Pos Board B27

Elucidating the Molecular Basis of Cellulase Synergism Through High Resolution Quantitative Fluorescence Microscopy

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Converting cellulose into fermentable sugars presents significant challenges to producing bioenergy from lignocellulose. Individual cellulases exhibit low rates and extents of hydrolysis. However, mixtures of cellulases and other cell wall degrading enzymes exhibit rates of hydrolysis that are much greater than would be predicted by summing individual rates. Thus, understanding the molecular mechanisms that give rise to synergistic behavior is essential for engineering more effective enzyme cocktails. Previous studies by the Walker Lab revealed mixtures of cellulases Cel9A, a processive endocellulase, and Cel6B, an exocellulase, exhibited higher extent of binding that would be predicted by summing the individual binding extents. A major question is whether this is driven by intrinsic cellulase binding kinetics or are changes in these two cellulases' diffusion rates into the cellulose macrostrucutre yielding this behavior.

In this study, bacterial microcrystalline cellulose fibrils were immobilized on a solid substrate using polymer lift-off. Cel9A and Cel6B were fluorescently labeled with either of two colors and purified into populations with known degree of labeling. These labeled cellulase populations were tested to validate the previous observation that labeling does not inhibit cellulose depolymerization. The binding of labeled cellulases on immobilized cellulose fibrils was observed using fluorescence microscopy for a period of 95 minutes, with images taken every minute for the first 10 minutes, every 2.5 minutes for the next 10 and every 5 minutes for the remainder. Individual binding curves were established for each enzyme in each color using different populations to characterize binding of enzymes with different numbers of labels. The effect of synergism was investigated by combining Cel9A and Cel6B, labeled in different colors, in varying molar ratios and observing effects of synergism and competition on diffusion and substrate binding in the system.

2058-Pos Board B28

Photophysical characterization of Dye-Encapsulated Calcium Phosphate Nanoparticles

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Organic dyes exhibit rapid photobleaching, low quantum yield, and random blinking under physiological conditions, limiting their utility in *in vivo* imaging. To address these photophysical shortcomings, the Adair group at Penn State has recently developed a novel method for synthesizing dye-encapsulated calcium phosphate (CP) nanoparticles based on a double microemulsion method. In this study, time-resolved single photon counting methods were used to characterize cy3-encapsulted CP nanoparticle size, dispersity, molecular brightness, and fluorescence lifetime (FL). Particle sizes measured using fluorescence correlation spectroscopy (FCS) confirmed the presence of highly mono-disperse 20 nm particles. The brightness of an individual nanoparticle measured using moment analysis was found to be 20 times higher than the free dye, due to a five-fold increase in quantum efficiency and encapsulation of 4 dye molecules per particle. FL of the encapsulated dye was independent of the solvent (water, PBS, DMSO, and 50% glycerol), suggesting that the dye was well-protected in the CP matrix. Furthermore, increased FL in CP nanoparticles compared to free dye suggests that the photoisomerization of cy3 was inhibited due to restricted mobility of the dye in CP matrix. Photostability increased 50-fold likely because the dye was protected from the photobleaching effects of dissolved oxygen. Finally, systemic administration of PEGylated CP nanoparticles in nude mice implanted with breast cancer tumors retained fluorescence signal in tumors even after 96 hours post-injection, demonstrating the utility of CP nanoparticles for long term *in vivo* imaging.

2059-Pos Board B29

Multi-exponential Luminescence Decay of Non-blinking CdTe Quantum dots upon one and two photon excitation

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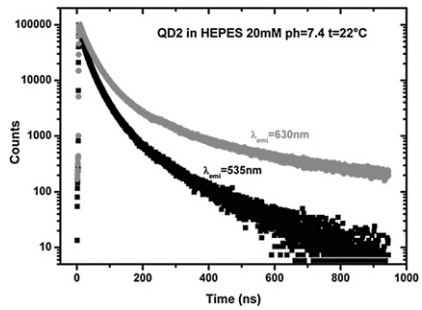
Quantum dots (QD) are semiconductor nanocrystals with quantum confinement of charges carried in limited spaces. They are expected to have high quantum yield, photo and thermal stability, and strongly size dependent emission wavelength. Non-blinking CdTe QDs have been synthetized in water phase by microwave irradiation with mercaptopropionic acid as a stabilizer. They exhibit high QY, good photo and chemical stability in water solution. QD with sizes from 2.1nm to 5nm have been studied. Time-resolved photoluminescence (PL) decays were measured by TCSPC technique upon one and two photon excitation (2PE). The effect of temperature and pH on PL decay was studied at several excitation wavelengths. Lifetime distribution, extracted from PL decay by Maximum Entropy Method of data analysis, display up to five components. PL decay of small QD is slower at longer wavelength (figure). The lifetime distribution of the larger QDs always exhibits three lifetime peaks around 15 ,37 and 100ns respectively , only relative contributions of each peak vary with size.



2060-Pos Board B30

Absolute Two-photon Absorption Spectra Of Orange And Red Fluorescent Proteins

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Two-Photon laser scanning microscopy, which makes use of genetically encoded fluorescent protein (FP) probes, is becoming a method of choice for studying biological systems from sub-cellular to the whole body level. However, reliable information on two-photon absorption (2PA) properties of FPs, specially for the more popular orange and red variants, is still very fragmentary. 2PA spectra, measured in absolute cross section values, will allow us to select the two-photon brightest FP variant with desired fluorescence properties and also to choose the optimum laser system and excitation wavelength. Here we study 2PA spectra of a large set of orange and red FPs, including DsRed2, mRFP, TagRFP, and mFruits series in a wide range of excitation wavelengths, 600 -1200 nm. We have found the the 2PA spectra and maximum cross sections are very sensitive to either changes in chromophore structure (mOrange vs mRFP) or to mutations in chromophore surrounding (DsRed and mFruits series). All red FPs show two pronounced 2PA transitions, the first peaking in the 1000 - 1100 nm region, and second - near 700 - 760 nm. We quantitatively describe the first transition within the framework of two-level model, and the second - within three-level model with strong resonance enhancement. Excitation in the longer wavelength region, accessible for Nd- and Yb-doped short-pulse lasers, has advantages of producing less two-photon autofluorescence