spectroscopy, TEM and Zetasizer. To assess the cytotoxicity of these FGNPs, MTT assay was carried out with varied concentrations of FGNPs. In order to investigate the intracellular uptake of these FGNPs, ICP-MS analysis was done which revealed an increased uptake of FGNPs as compared to bare GNP. Moreover, in order to track the intracellular path of these FGNPs, ultra-thin sections of the nanoparticles treated cells were cut and analyzed after negative staining. Likewise the extent of DNA damage was assessed using the comet assay. We have also investigated the effect of these nanoparticles on human necrosis and apoptosis related genes by real-time PCR array analysis. A significant change of up to two fold was observed in the levels of expression of these genes in the treated cells as opposed to the untreated cells. The work is currently underway in order to further analyze the effects of these nanoparticles in terms of molecular and genomic effects and the pathways underlined therein.

**2974-Pos Board B744**

**Hollow Gold Nanoshells for Near Infra Red Cell by Cell Treatment**

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The temporal and spatial control over the delivery of materials such as siRNA or small molecular weight drugs into specific cells remains a significant technical challenge. We demonstrate the pulsed near-infrared (NIR) laser dependent release of siRNA or the chemotherapy drugs cisplatin and doxorubicin via the thermo-mechanical influence of 40 nm hollow gold nanoshells (HGN). Ligand-receptor interactions promote the cellular uptake of HGNs in targeted cells, resulting in HGN clusters within endosomes in the targeted cells. Non-targeted cells take up individual HGNs or no HGNs. The spatiotemporal silencing of a reporter gene (green fluorescence protein) was studied using photomasking to selectively irradiate targeted cells with NIR light to release chemically bound siRNA and rupture endosomes. GFP silencing followed siRNA release to the cytoplasm in NIR irradiated cells. The NIR laser induced release of siRNA from the nanoshells is found to be power and time dependent, through surface-linker bond cleavage, while the escape of the siRNA from endosomes occurs above a critical pulse energy attributed to local heating and nanobubble formation and collapse (cavitation). The same HGN could be used to selectively target the permeability of multi-drug resistant cancer cells to doxorubicin or cisplatin to enhance the efficacy and selectivity of conventional chemotherapy. NIR induced nanobubbles enhanced the chemotherapeutic efficacy of liposome encapsulated doxorubicin (Doxil®) 33 times, allowing a reduction in drug dose by an order of magnitude while reducing non-specific toxicity to 15% (from 100%) among normal cells in a model of drug resistant oral cavity squamous cell carcinoma.

**2975-Pos Board B745**

**Cell Type Dependent Effects of Nanowire Density on Cell Cultures**

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Semiconductor nanowires (NWs) are receiving a lot of attention for applications in cell biology. Their small size (sub-100 nm diameter and a few micrometers long) makes them ideal for probing cell behavior with applications ranging from electrical recordings to transfection. Here, we investigate the cell-NW interactions using ordered arrays of epitaxially grown gallium phosphide (GaP) nanowires with varying NW density ranging from 0 to 4 NW/µm². Normal-like human breast epithelial cells (MCF-10A) and human breast carcinoma cells (HMT-1) were cultured on the substrates for 24 h and 72 h. The Click-IT® technique was used to mark cells in S phase by labeling newly synthesized DNA, cells in S phase being a marker of cell proliferation. The results were evaluated using fluorescent microscopy and scanning electron microscopy (SEM).

For both cell lines, we found that the number of cells was much lower on the two highest nanowire densities (1 and 4 NW/µm²) compared to the lower nanowire densities where the cells approached confluency. The Click-IT® labeling revealed that the MCF-10A cells stopped synthesizing DNA on the denser regions but not on the lower NW densities. The JMT-1 cells did not show such behavior and cells in S phase were found on all NW density areas. The SEM investigations revealed that both types of cells spread normally on the low density NW areas and the plain GaP but adopted a rounded morphology on the denser areas with individual cells lined up on the border between high and low density regions.

These findings indicate that the nanowire density has an impact on cell survival and proliferation. Most importantly, there is a critical nanowire density above which one can observe cell-type specific behavioral changes. This could have an impact on lab-on-a-chip diagnostic technique development.

**2976-Pos Board B746**

**A Self-Assembling Polypeptide Nanoparticle Vaccine for Avian Influenza**

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While many influenza vaccines offer protection in high percentages, they have not been able to achieve optimal protectivity. M2 is a tetrameric influenza protein, found in small numbers on the viral surface, but not from the surface of infected cells. The external domain of M2 is a highly conserved sequence and has been shown to generate a protective immune response when presented in a tetrameric conformation.

Recently, we have shown that Self-Assembling Polypeptide Nanoparticles (SAPNs) are capable of eliciting a humoral immune response against a B cell epitope displayed on their surface (Kaba 2009). The basic architecture of a SAPN is an icosahedral structure composed of a linear peptide containing two coiled-coil oligomerization domains, connected by a short linker region. One of these domains is trimeric and has been de novo designed. Another can be either pentameric or tetrameric. The system can include both B-cell and T-cell epitopes, affixed to either the N- or C-terminal end.

In this study, we have developed several species of SAPNs. Animal testing was performed in chickens, who received three vaccinations of M2e SAPNs. Blood samples were collected pre and post-vaccination and antibody titers were evaluated using ELISAs against different conformations of M2e. We will report on the biophysical properties of these particles and discuss their immunogenicity and potential for the design of an avian flu vaccine.

**2977-Pos Board B747**

**Highly Oriented, Functionalized Nanoarray Based on Epitaxially Grown Aß25-35 Fibrils**

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The self-assembly properties of biomolecular systems are of fundamental appeal for nanobiotechnological applications. However, the lack of easy chemical access and nano- to micro-scale structural order often hinders the nanotechnological use of conventional biomolecules. We have previously shown that Aß25-35 forms a trigonally oriented network on mica by epitaxial growth mechanisms. Furthermore, a mutant form of the peptide, Aß25-34_N27C, in which the Cys27 is in principle chemically accessible, also forms an oriented network on mica. In the present work we explored whether the oriented amyloid-fibril network can be functionalized via sulfhdydryl chemistry. Oriented network of amyloid fibrils was generated by incubating mutant, Aß25-34_N27C peptides or mixtures of the mutant and wild-type peptides on mica in the presence of varying KCl concentration in order to fine tune the network’s structural order. To functionalize the fibrils we used either maleimidono-gold (1.4 nm), or maleimido-NTA followed by GFP-His6 binding. The structure of the functionalized network was investigated with AFM. In the nanogold-labeled amyloid network spherical particles of 1.4-nm diameter lined up along the oriented fibrils. In the GFP-functionalized network the surface of the fibrils was covered with spheroid particles of approximately 4-nm topographical height. The density of the particles could be adjusted by varying the ratio of the wild-type and mutant peptides. The functionality of the amyloid-bound GFP was tested with epifluorescence microscopy. We could demonstrate that the fluorescence properties of GFP were retained in the amyloid network. Thus, the oriented amyloid network may be used towards the construction of functional biomolecular nanowires.

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**Atomic Force Microscopy**

**2978-Pos Board B748**

**Direct Visualization of Cellobiohydrolase on Crystalline Cellulose using High-Speed Atomic Force Microscopy**

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Cellulases degrade crystalline celluloses through hydrolyzing β-1, 4-glucosidic linkage of cellulose producing soluble cellobiose saccharides. Although
the kinetics of crystalline cellulose hydrolysis by cellulases has been investi-
gated intensively so far, the mechanism of crystalline cellulose degradation still contains many mysteries. The main reason for the difficulty to understand the mechanism is the lack of analytical methods to monitor the enzymatic re-
action at a solid/liquid interface. We here use high-speed atomic force micro-
copy (HS-AFM) to reveal how the enzyme molecules behave on the substrate. When glycoside hydrolase family 7 cellobiohydrolase from Trichoderma reese-
sei (TrCel7A) was incubated with crystalline cellulose, many enzyme mole-
cules moved unidirectionally on the cellulose surface with the velocity of 7.2 ± 3.9 nm/sec but at some point the movement of individual molecules was halted, leading to appearance of traffic jams of enzyme molecules. The present results suggest that solving the traffic jams of productively bound cel-
lulose is a key to enhance the hydrolytic activity of cellulases on crystalline cellulose.

2979-Pos Board B749
Towards Tracking Moving Single Molecules in Atomic Force Microscopy
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The atomic force microscope (AFM) is an invaluable tool for observing bio-
logical systems, due in part to its incomparable resolution as well as its ability to observe systems in their physiological environments and to measure me-
chanical properties directly. Its slow imaging rate, however, greatly reduces its applicability in recording fast-changing mechanisms. Such studies are of critical importance. For example, investigating the dynamics of protein motors and other macromolecules is essential for understanding and treating a variety of genetic diseases. Motivated by this, we are developing an approach to AFM centering on tracking rather than imaging. The scheme is primarily designed to follow the motion of a single macromolecule moving along a biopolymer. In approaching this problem, we consider that the presence of the moving macromolecule on its track results in a change in the apparent width of the track. Based on this, we have developed a high-speed width detector that rapid-
dy determines the width by detecting the two edges of the sample during a fast scan. As a result, the motion of these single macromolecules is derived from direct tracking rather than a sequence of images. Such an approach promises a much higher temporal resolution than is achievable in time-lapse imaging.

2980-Pos Board B750
Encased Cantilevers and Alternative Scan Algorithms for Ultra-Galante High Speed Atomic Force Microscopy
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Live cells and many biological samples readily deform under the minimum force required to perform an AFM measurement precluding imaging at high temporal and spatial resolution. We reduced the force noise of the measurement by building a protective encasement around the cantilever. This keeps the can-
tilever is dry reducing the fluid viscosity and damping but allows the tip to probe the sample in solution. Encased cantilevers have exceptionally high res-

oneance frequency, Q factor, and detection sensitivity and low force noise en-
abling gentle high speed imaging. Present raster scan techniques are poorly matched to the instrument limitations of Atomic Force Microscopy making data collection slow. We have used advanced image processing tools such as inpainting to recover high-
resolution images from sparse quickly collected images to improve temporal resolution without applying more force or increasing bandwidth. We are also using spiral scanning to in-
crease temporal resolution by allowing higher tip velocities without distortion. Inpainting or interpolation is used to quickly create images from the nongridded data.

2981-Pos Board B751
Ultrasensitive Force Spectroscopy with Tuning Fork Based Frequency Modulation
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The standard method that is used with atomic force microscopy to monitor me-
chanical properties of materials such as elasticity and adhesion is based on beam bounce technology. With such an approach there are two major prob-
lems: one is jump to contact and the other is adhesion ringing. Numerous methods have evolved for trying to resolve these problems from methods call pulsed force mode to peak force. However, what is desired is a smooth approach and retract from a surface or molecule where force measurements need to be implemented. Over the last few years, it has been realized that the best method of force feedback in atomic force microscopy is based on tuning fork force modulation but there have been few studies implementing these advances into the realm of force spectroscopy. In this paper, force spec-
troscopic analysis is implemented based on tuning forks and it is demonstrated that there is close to single PN force sensitivity. These efforts use the pioneering theory of Sader and Jarvis that has shown theoretically that is possible to derive accurate formulas for the force versus frequency in such Frequency Modulations methods [J.E. Sader and S. P. Jarvis, “Accurate formulas for in-
teraction force and energy in frequency modulation force spectroscopy” Appl. Phys. Lett.84, 1801 (2004)]. It will be further shown that such normal force tuning fork based force spectroscopy can readily be integrated with other chemical and structural tools such as Raman microspectroscopy and Scanning Electron Microscopy.

2982-Pos Board B752
Measuring a Stabilization Constant Between Two Bio-Molecules using Atomic Force Microscopy
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Stabilization force constants are known to play an important role in biomolec-
ular functions that carry out delicate structural conformation changes along a re-
action coordinate during bio-molecular activation. Atomic force microscopy (AFM) has been used as a tool for probing protein-ligand interactions at the single molecular level. We developed a method that converts AFM force-distance curves into intermolecular force-distance curves. This method was applied to a model enzyme-inhibitor system of 5′ methylthioadenosine/S-
adenosylhomocysteine nucleo-sidase (MTN) and its transition state analogue homocysteinyl Immucillin A (HIA). Both the MTN and HIA molecules were attached to the sample and probe surfaces, respectively, through the flexible polymer polyethylene glycol. The stabilization force constant is found to be 0.235 N/m between MTN and HIA from the intermolecular force-distance curve, which is consistent with those measured by other techniques.

2983-Pos Board B753
Conducting Atomic Force Microscopy for Simultaneous Imaging of Structure and Ionic Current Through Nanopores
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Ionic currents through nanopores in both biological and synthetic materials play an important role in the function of the material. For biological systems, these pores are critical for normal physiological function and abnormalities lead to various disease states. The ability to measure the current through nano-
pores, while simultaneously relating their molecular and atomic structure, is currently limited in resolution. In order to perform structure-function measure-
ments using an atomic force microscope (AFM), conducting cantilevered tips capable of measuring ionic currents in fluid were designed and fabricated using various techniques. Insulated tungsten wires with conducting tips were fixed to steel supports to create cantilevers for AFM imaging. Gold films in fluid were imaged with simultaneous electrical current measurements by the conducting cantilevers to reveal the topography of the film. These simultaneous recording of the current and the 3D structure demonstrate the conducting capabilities of the cantilever. Ionic currents through membrane filters were successfully mea-
sured through 20 nm pores in the membrane. The results from this technology show promise for future structure-function imaging of macromolecules, such as ion-channels in health and disease as well as for the synthetic nanopores for energy and environmental applications.
We here use high-speed atomic force microscopy (HS-AFM) to reveal how the enzyme molecules behave on the substrate. When glycoside hydrolase family 7 cellobiohydrolase from Trichoderma reesei (TrCel7A) was incubated with crystalline cellulose, many enzyme molecules moved unidirectionally on the cellulose surface with the velocity of 7.2±3.9nm/sec but at some point the movement of individual molecules was halted, leading to appearance of traffic jams of enzyme molecules. The present results suggest that solving the traffic jams of productively bound cellulose is a key to enhance the hydrolytic activity of cellulases on crystalline cellulose. Discover the world's research. 20+ million members. cellulose-binding domain. atomic force microscopy. high performance liquid chromatography (HPLC). liquid chromatography. Access to Document. 10.1074/jbc.M109.034611. Link to publication in Scopus. Keywords = "Trichoderma reesei, cellobiohydrolase, hydrolyzing, cellulose, cellulose hydrolysis, cellulose-binding domain, atomic force microscopy, high performance liquid chromatography (HPLC), liquid chromatography", author = "Kiyohiko Igarashi and Anu Koivula and Masahisa Wada and Satoshi Kimura and Merja Penttilä and Masahiro Samejima", whereas high-speed atomic force microscopy allows tracking of motility of single cellulase molecules. (24. Igarashi K. Uchihashi T. Koivula A. Wada M. Kimura S. Visualization of cellobiohydrolase I from Trichoderma reesei moving on crystalline cellulose using high-speed atomic force microscopy. Methods Enzymol. 2012; 510: 169-182. Crossref. PubMed. Scopus (18). Google Scholar. Recent advances in high-speed atomic force microscopy (HS-AFM)... Direct and real-time visualization of single protein molecules is a powerful approach to understanding how they operate to function. Recent advances in high-speed atomic force microscopy (HS-AFM) provide a new opportunity to visualize dynamic events of label-free proteins in action under physiological conditions, at subsecond temporal and submolecular resolution. In this chapter, we first overview HS-AFM techniques used for fast and low-invasive imaging of proteins. Keywords. Atomic Force Microscopy Actin Filament Crystalline Cellulose Cellulose Surface Highly Order Pyrolytic Graphite. These keywords were added by machine and not by the authors. High-speed atomic force microscopy (HS-AFM) allows direct visualization of dynamic structural changes and processes of functioning biological molecules in physiological solutions, at subsecond to sub-100-ms temporal and submolecular spatial resolution. Unlike fluorescence microscopy, wherein the subset of molecular events that you see is dependent on the site where the probe is placed, dynamic molecular events unselectively appear in detail in an AFM movie, facilitating our understanding of how biological molecules function. Surface density of cellobiohydrolase on crystalline celluloses. A critical parameter to evaluate enzymatic kinetics at a solid-liquid interface. FEBS J. 273, 2869â€“2878 (2006).