we monitor the energy required to overcome the adhesion between the bacteria and substrate. Generally, increased contact time leads to stronger adhesion due to bond strengthening. However, the magnitude of this effect varies greatly among substrates with different surface potentials and hydrophobic properties.

2990-Pos Board B760
Investigating the Surface Structure and Antibody Recognition Forces of Tannerella forsythia by Scanning Probe Microscopy
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T. forsythia is a Gram-negative oral anaerobe which is one of the major contributors to periodontal disease in humans [1]. It possesses a glycosylated S-layer consisting of two regularly arrayed subunits as outermost cell envelope layer. The S-layer of Tf was shown to be a virulence factor, capable of delaying the bacterium’s recognition by the innate immune system [2] and mediating adhesion and invasion of host cells [3]. In this study, we used scanning probe microscopy to probe the S-layer surface of live Tf cells by investigating the nano subunit structure so as to characterize the surface structure and the interactions of Tf S-layer proteins. High-resolution imaging of living Tf wild-type cells has shown a periodic square lattice structure with about 10 nm dimension, while on the surface of an S-layer single mutant no periodic structure was visible. In addition, single-molecule force spectroscopy using a Tfsa S-layer protein specific antibody immobilized onto AFM tips revealed specific interaction forces with the S-layer structure on living bacteria and to the understanding of their subunit arrangement with nano-meter resolution.

2991-Pos Board B761
Receptor Arrays for the Selective and Efficient Capturing of Viral Particles, Proteins and Nanoparticles
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We describe microarrays of receptor molecules that capture viral particles with high specificity and at high density. Patches of viral receptors were generated by first modifying microscale gold squares on glass substrates with alkanethiol derivatives and then immobilizing the His-tagged very-low-density lipoprotein (VLDL) receptor ligand binding domain via metal-chelate complexes to the gold surfaces. Free glass areas surrounding the gold squares were passivated with a dense film of poly(ethylene glycol) (PEG). As assessed by atomic force microscopy, human rhinovirus particles were captured onto the VLDL-receptor patches with a high surface coverage but were effectively repelled by the PEG layer, resulting in a 330 000-fold higher density of the particles on the gold as compared to the glass surfaces. The metal chelate-based coupling strategy was found to be superior to two alternative routes, which used the covalent coupling of viral particles or viral receptors to the substrate surface. The high density receptor arrays were employed for sensing and characterizing viral particles with far unprecedented selectivity.
Furthermore, an alternative route to create arrays in the nanometer range for the site specific binding of proteins, nanoparticles and pathogens is shown. These arrays are results of AFM based nanolithography performed on Mica substrates passivated by repellent protein- or PEG films. We could show that with the help of this method we are able to create nanometer-sized structures in the range of an AFM tip radius. The structures created in this manner were subsequently refilled with proteins which serve as a basis for the specific binding of other proteins, particles or pathogens of interest and could furthermore be analyzed by AFM under near physiological conditions.

2992-Pos Board B762
Characterization of Enhanced Monovalent and Bivalent Thrombin DNA Aptamer Binding using Single Molecule Force Spectroscopy
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Thrombin aptamer binding strength and stability is dependent on sterical parameters when used for atomic force microscopy sensing applications. Sterical improvements on the linker chemistry were developed for high-affinity binding. For this we applied single molecule force spectroscopy using two enhanced biotinylated thrombin aptamers, BFF and BFA, immobilized on the atomic force microscopy tip via streptavidin. BFF is a dimer composed of two single-stranded aptamers (aptabody) connected to each other by a complementarity sequence close to the biotinylated end. In contrast, BFA consists of a single DNA strand and a complementary strand in the supporting biotinylated part. By varying the pulling velocity in force-distance cycles the formed thrombin-aptamer complexes were ruptured at different force loadings allowing determining the energy landscape. As a result, BFA aptamer showed a higher binding force at the investigated loading rates and a significantly lower dissociation rate constant, koff, compared to BFF. Moreover, the potential of the aptabody BFF to form a bivalent complex could clearly be demonstrated.

2993-Pos Board B763
Characterization of Glucocorticoid Receptor-hsp90 Chaperone Machinery: Heterocomplex Assembly using Atomic Force Microscopy
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Atomic force microscopy (AFM) has demonstrated ability to provide direct visualization of individual molecules of proteins as well as multimeric protein-protein and DNA-protein complexes in near real-time and in buffered solutions. Here, we use tapping-mode AFM to visually analyze the interaction of the essential eukaryotic molecular chaperone protein hsp90 with the glucocorticoid receptor (GR), a well-established hsp90 client protein. An issue of debate within the hsp90 community has been to what extent hsp90 works independently of its molecular chaperones, such as hsp70, or as part of a multiprotein machinery. Endogenous hsp90 was isolated from rabbit reticulocyte lysate using size exclusion chromatography, both as an individual protein and in a complex with hsp70 and the hsp90/hsp70 co-chaperone Hop. AFM imaging showed that the hsp90 complexed with hsp70 and Hop (referred to as the hsp90/hsp70-based chaperone machinery) preferentially interacts with the GR. We demonstrate the extent to which hsp90 can be positively identified in AFM images of heterogenous protein mixtures using an anti-hsp90 monoclonal IgM antibody. Additionally, novel software was developed to calculate the molecular mass of AFM-imaged protein particles in a semiautomated process using the spherical-cap model for mica-adsorbed proteins and protein complexes. Molecular mass calculations and AFM-imaged antibody detection were used in tandem to predict protein particle composition. Eight highly purified and well-characterized proteins of known molecular weights, ranging from 44 – 623 kDa, were used to construct a standard curve relating calculated particle volume (nm3) to approximate protein complex molecular mass (kDa).

2994-Pos Board B764
Alzheimer’s β-Amyloid All-D-Enantiomers and Native All-L-Enantiomers Exhibit Similar Pore Structures in Lipid Bilayers: Atomic Force Microscopy
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Alzheimer’s disease (AD) is characterized by a build-up of β-amyloid (Aβ) peptide in senile plaques inside the brain, leading to neuropathologies exemplified by uncontrolled neurodegeneration and memory loss. One hypothesis for the AD pathology is the disruption of cell ionic homeostasis, mediated by the interaction of globular Aβ with cell membranes. Recent results by us and others suggest that the Aβ peptide directly inserts into cell membranes creating ion conductive pores that destabilize intracellular calcium ion homeostasis. However, Aβ may also destabilize ion homeostasis via interactions with cell membrane receptors. To investigate the mechanism of toxicity we took advantage of the biochemical tenet that ligand-receptor interactions are stereospecific; all L-proteins but not all D-proteins bind to cell membrane receptors. Using all D-enantiomer and all L-enantiomers of the full length Aβ1-42, we probed the nanoscale pore and fiber formation as well as oligomer stability using atomic force microscopy (AFM) imaging. We report similar morphological properties for both stereoisomers. The AFM images display similar channel-like structures when embedded in bilayers and both enantiomers show comparable fiber structures. These results are supported by electrophysiology studies that show similar electrical conductances for D-Aβ and L-Aβ.
Both isomers appear to interact with lipid bilayers in a similar fashion. Our results suggest that Aβ mediated disruption of ionic homeostasis may occur by a direct pathway of ion channel formation and may not need to rely on interactions with membrane receptors. Understanding the mechanism of peptide-membrane interaction and insertion at nanoscale resolution is essential for therapeutic design aiming to control and prevent Aβ pore formation. Funded by NCI Contract HHSN261200800001E (RN) and NIH (National Institute on Aging AG028709) extramural program (RL).

2995-Pos Board B765
Scanning Probe Microscopy of Serpin Polymers
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Vibrational Spectroscopy

2996-Pos Board B766
Deep-Uvrr Spectroscopy Studies of Amyloidogenic Transthyretin Fragments
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The protein transthyretin (TTR) has been implicated as the pathogen in several amyloid diseases. Normally a transport protein for thyroxine, amyloidosis occurs when the protein aggregates and deposits in organ tissue as β-sheet structured amyloid fibrils. The formation of amyloid fibril deposits associated with TTR diseases is poorly understood. In order to study amyloid fibril formation several amyloidogenic fragments of TTR have been studied in aqueous solutions. It has been suggested that the amyloidogenic fragments TTR(10-20) and TTR(105-115) contain portions of the protein essential to aggregation and amyloid fibril formation, however, neither have been studied in the presence of cell-like lipid membranes. Here, we present the first studies comparing aqueous and model membrane solution studies of TTR(10-20) and TTR(105-115) via deep-ultraviolet resonance Raman spectroscopy. Initial results suggest a change in peptide secondary structure upon interaction with lipid membranes.

2997-Pos Board B767
In Vivo Molecular Labeling of Halogenated Volatile Anesthetics via Intrinsc Molecular Vibrations using Nonlinear Raman Spectroscopy
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Halogenated volatile anesthetics are frequently used for inhaled anesthesia in clinical practice. No appropriate biological method has been available for visualizing their localization in action. Therefore, despite their frequent use, the mechanism of action of these drugs has not been fully investigated. We measured coherent anti-Stokes Raman scattering (CARS) spectra of sevoflurane and isoflurane, two of the most representative volatile anesthetics, and determined the low-frequency vibrational modes without nonresonant background disturbance. Molecular dynamics calculations predict that these modes are associated with multiple halogen atoms. Because halogen atoms rarely appear in biological compounds, the entire spectral landscape of these modes is expected to be a good marker for investigating the spatial localization of these drugs within the intracellular environment. Using live squid giant axons, we could detect the unique CARS spectra of sevoflurane for the first time in a biological setting.

2998-Pos Board B768
Cell Surface Protein Detection using Surface-Enhanced Raman Scattering (SERS) Gold Nanoparticles
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Surface enhanced Raman scattering (SERS) Au nanoparticles have been used as novel cell surface receptor labels for the identification of markers of interest in chronic lymphocytic leukemia (CLL) and lung cancer. Biocompatibility of the particles was improved using multiple coating and particle protection strategies. Each of these strategies facilitated different methods for the inclusion of Raman active reporter molecules, as well as for different types of targeting moieties. Characterization of the SERS nanoparticles was undertaken including quantification of the number of antibodies bound to the surface. Long-term stability of both the nanoparticle Raman signal intensity and monodispersity was assessed under standard storage conditions, as well as conditions suitable for in vitro biological experiment. The SERS labeling platform has been demonstrated as being compatible with traditional pathology protocols including flow cytometry, and stains such as giemsa. SERS detection using these particles has been adapted to models for both adherent and circulating malignancies, in addition to patient cell samples in the example of CLL. The narrow vibrational spectra of SERS particles used in this study greatly increase the multiplexed labeling potential over traditional fluorescence-based technologies. Preliminary multiplexed labeling of CLL has also been demonstrated.

2999-Pos Board B769
Micro-Raman of Cancer Cells: Toward Label-Free Sorting of Circulating Tumor Cells from Whole Blood
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Even in the early stages of cancer, circulating tumor cells (CTCs) can travel from a primary tumor site to other organs through the blood and lymphatic system. Detecting and isolating CTCs from the blood has great potential for early cancer detection and studies of metastasis. However, they are notoriously rare (a few CTCs per mL of blood) and difficult to distinguish from epithelial non-tumor cells and leukocytes. Attempts to analyze CTC genetic or protein changes in response to treatment have been hampered by the difficulty in isolating intact clonogenic cells. Toward the goal of developing a rapid, non-invasive tool for detecting and isolating live CTCs from the blood, we measured the Raman spectra of live, unlabeled single cells. Using principal component analysis, we can distinguish 98% of breast cancer cells, and tumorigenic ovarian cancer cells (A2780, OVCAR2, CaOV3) from non-tumorigenic ovarian cancer cells (OV429). Adherent cell lines and suspended (laser trapped) cells from cancer patient fluid samples were measured. A micro-fluidic platform with pressure control has been implemented to transport such cells single-file through the Raman laser focus. Custom microfluidics are in development and will sort the live, unmarked cancer cells into separate reservoirs for cell culture. Each Raman spectrum requires ~1-2 minutes, so we are concurrently developing a faster, coherent anti-Stokes Raman scattering (CARS) microscope for higher throughput analysis. Rapid detection and sorting of live CTCs will give prognostic information and allow for observation of the effect of targeted cancer treatments via a minimally invasive blood test and possibly well before the availability of response data.

3000-Pos Board B770
Study of Energetic Particle Induced Biological Effect through FTIR and Raman Micro-Spectroscopy
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Energetic particles exist ubiquitously in nature and may cause varied biological effects which have been found useful applications such as radiotherapy of...