targeting riboflavin binding protein (RFBP), which acts as a model protein for the riboflavin receptor. A single molecule force spectroscopy study using Atomic Force Microscopy (AFM) was performed to examine the binding interactions between riboflavin-conjugated dendrimers and RFBP. Using multiple theoretical models, the apparent kinetic and thermodynamic parameters for the riboflavin-conjugated dendrimer system were estimated.

1957-Pos Board B687
Atomic Force Microscopy of DNA-CTAB Aggregates
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Past studies of DNA-surfactant aggregates involving UV-spectroscopy and gel electrophoresis show that when 20 to 100 base-length oligonucleotides interact with the surfactant CTAB in specific ratios of surfactant to oligonucleotide base, they form aggregates. The interaction between the oligonucleotide and surfactant molecule appears to involve both the hydrophilic head and the hydrophobic tail of CTAB. We characterized these aggregates using an atomic force microscope (AFM) by drying DNA-CTAB solutions on mica and silicon, reconstituting the dried aggregates in buffer and imaging them in fluid. Topographical data was collected that showed consistent, almost hexagonal, regular patterns of aggregates on the surface, and we studied the surface properties of the aggregates using force spectroscopy with functionalized AFM tips. The spring constants of the aggregates and information on the adhesion forces between the derivatized tips and the aggregate surfaces were obtained. Preliminary results show that tips functionalized with 1-dodecanethiol were found to have a lower adhesion to the aggregate surface than tips functionalized with mercaptoundecanoic acid implying that the surface of the aggregate is also hydrophilic.

1958-Pos Board B688
Energetics and Kinetics of SNARE Zippering
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SNARE proteins drive membrane fusion by assembling into a four-helix bundle in a zippering process. The energetics and kinetics of SNARE zippering have not been well characterized, but are critical to understand the functions of brains and other basic cellular processes, as well as their associated diseases. We used high-resolution optical tweezers to observe in real time a long-sought SNARE assembly intermediate in which only the membrane-distal N-terminal half of the bundle is assembled. Our finding supports the zippering hypothesis, but suggests that zippering proceeds through three sequential binary switches, not continuously, in the N- and C-terminal halves of the bundle and the linker domain. The half-zippered intermediate was stabilized by externally applied force which mimicked the repulsion between apposed membranes being forced to fuse. This intermediate then rapidly and forcefully zippered, delivering free energy of 36 kBT to mediate fusion. Thus, the synaptic SNARE complex seems perfectly evolved for precise regulation of neurotransmitter release: Slow assembly of the membrane-distal half allows control of vesicle priming by regulatory factors that can accelerate this step. The discrete pause in the half-zippered state in the presence of a force load enables clamping and thus regulation of release at this stage. Finally, the membrane-proximal domain zippers at the diffusion-controlled rate limit to allow neurotransmitter to be released.

1959-Pos Board B689
Dengue Virus Capsid Protein Interacts Specifically with Very Low-Density Lipoproteins
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Dengue virus (DENV) infects millions of people worldwide. With no specific treatment available, understanding its replication mechanisms is highly required to identify future therapeutic targets. In this study [1], mostly using AFM-based force spectroscopy [2], but also DLS, NMR and computational studies, we show that DENV capsid protein (C) binds specifically to very low-density lipoproteins (VLDL) but not to low-density lipoproteins (LDL). DENV C-VLDL binding is similar to DENV C interaction with lipid droplets (LDs), host intracellular structures essential for viral replication [3]. As on the DENV C-LDs binding, previously characterized by us [4-6], DENV C-VLDL interaction is K^+-dependent, involves the DENV C intrinsically disordered N-terminus, and is inhibited by pep14-23, a novel peptide drug lead against DENV [5,6]. As perilipin 3 (DENV C target on LDs [3]) is structurally similar to the VLDL protein ApoE, this protein may be the DENV C ligand on VLDL, explaining the similarities between the interactions of DENV C with VLDL and LDs. These data suggest the possibly of lipoviroparticles formation, as it occurs for other flaviviruses, such as the hepatitis C virus (HCV) [7]. This process may potentially be targeted, for DENV life cycle inhibition.
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1960-Pos Board B690
Characterizing the Interaction of Desmosomal Cadherins at Single Molecule Level
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Desmosomes are cell-cell adhesion complexes that are present in tissues that resist mechanical stress. They are mainly composed of two adhesive proteins, which are members of the cadherin superfamily of cell adhesion proteins, desmocollin (Dsc) and desmoglein (Dsg). However, the role of these proteins in desmosomal adhesion is unclear. Here, we use the single molecule force spectroscopy with an Atomic Force Microscope (AFM-FS) to characterize the interactions of type-2 isoforms of desmocollin (Dsc2) and desmoglein (Dsg2). We show that Dsc2 forms Ca^{2+} dependent homophilic bonds by swapping a conserved Tryptophan (Trp) residue between opposing binding partners; mutating this Trp inhibits Ca^{2+} dependent homophilic binding. In contrast, Dsg2 forms Ca^{2+} independent heterophilic bonds with Dsc2 via a mechanism that does not involve Trp strand-swapping.
Previous studies suggest that desmosome formation requires the presence of classical cadherins at the site of desmosome assembly. This suggests a cross-talk between desmosomal and classical cadherins at cell-adhesion contacts. We therefore used AFM-FS to test if Dsc2 and Dsg2 interact with E-cadherin, a classical cadherin present in the epithelium. Our data shows that while Dsc2 does not bind to E-cadherin in the presence of Ca^{2+}, Dsg2 forms Ca^{2+} independent complexes with E-cadherin. Using cadherin mutants we show that the interactions between Dsg2 and E-cadherin occur via a previously uncharacterized binding interface that does not involve either Trp strand-swapping or X-dimer formation (two well established classical cadherin binding mechanisms).

1961-Pos Board B691
Single Molecule Characterization of the Role of Divalent Ions in Prion Protein Aggregation
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Transmissible Spongiform Encephalopathies (TSEs) are a class of neurodegenerative disorders characterized by the accumulation of misfolded prion protein aggregates in the brain. In these diseases, normal cellular prion protein (PrP^C) misfolds into an infectious scrapie isoform (PrP^{Sc}), which results in PrP^C aggregation and neurotoxicity. Metal ions are known to play an important role in promoting PrP^C misfolding and aggregation, though the underlying mechanisms are poorly understood at molecular level.
Here we use single molecule Atomic Force Microscope-Force Spectroscopy (AFM-FS) and Confocal Fluorescence Microscopy (CFM) to determine how divalent metal ions mediate PrP aggregation *in vitro*. Using AFM-FS, we show that divalent metal ions enhance the aggregation of misfolded PrP by increasing the on-rate for aggregate formation; the lifetimes of aggregates are however unaffected. Increase in association rate is most pronounced when PrP is exposed to Cu^{2+} and less pronounced in the presence of Zn^{2+}, Ni^{2+} and Mn^{2+}. Using CFM we show that binding of metal ions to PrP induces a conformational change that makes the protein resistant to Proteinase-K digestion.Finally, we show that enhanced PrP aggregation is eliminated upon removing the octapeptide repeat, a major metal binding site on PrP.

1962-Pos Board B692
The Effects of Medically Relevant Compounds on the Physical Properties of Biofilms
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The adaptation of bacteria to form biofilms has been linked to many problems in the medical field including the rise of drug-resistant superbugs. Exopolymeric substances (EPS), including carbohydrates, proteins, and

polysaccharides, secreted by the bacteria provide protection from harmful chemical elements, such as chlorhexidine digluconate, commonly found in medical grade soap. Much is known about the biological and chemical mechanisms of chlorhexidine digluconate as an antiseptic, whereas the physical response of the bacterial biofilm to the chemical is poorly understood. In this investigation, we used force spectroscopy to analyze the physical effects of varied concentrations of chlorhexidine digluconate on a robust biofilm-forming Escherichia coli strain. Preliminary data suggest a correlation between the stiffness (spring constant) of the biofilm and the concentration of chlorhexidine digluconate.

1963-Pos Board B693

Increased Cytoskeletal Stiffness of Schlemm's Canal Endothelial Cells in Glaucoma

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The elevated intraocular pressure characteristic of glaucoma has been associated with a decreased pore density in the cells of the inner wall of Schlemm's canal (SC). SC cells form these pores in response to a transcellular pressure drop, facilitating the flow of aqueous humor across this endothelium. We hypothesize that impaired pore formation in glaucoma is due to an elevated stiffness of SC cells.

Atomic force microscopy (AFM) measurements of elastic modulus were performed using pyramidal or spherical (4.5 or 10 μm) tips on SC cells isolated from 6 healthy and 5 glaucomatous human eyes. Using finite element modeling and AFM experiments with latrunculin-A (an F-actin depolymerizing agent), we previously showed that sharp pyramidal tips characterize the cortex stiffness while larger, spherical tips characterize the stiffness of the subcortical cytoskeleton. The geometry of an SC cell was reconstructed based on electron microscopy images and used to model cell deformation under pressure (3-6 mmHg). When probed with spherical tips, the modulus of glaucomatous cells (1.36 ± 0.14 kPa) was significantly higher ($p<0.02$) than that of healthy cells (0.89 ± 0.10 kPa). No significant difference was detected between healthy and glaucomatous cells using sharp tips (7.40 ± 1.33 vs. 7.99 ± 0.97 kPa). The higher modulus measured on glaucomatous cells using spherical tips suggest that the altered stiffness is likely in the subcortical cytoskeleton and not in the cell cortex. Preliminary studies using finite element modeling predict a 34-40% decrease in cell deformation solely due to the increased cell stiffness measured on glaucomatous cells. This is consistent with our hypothesis that cell deformation is likely a precursor to the pressure-driven pore formation process and that increased cell stiffness may inhibit this process.

Acknowledgements: BrightFocus Foundation, NIH EY019696, NIH T32 EY007128.

1964-Pos Board B694

Cell Viscoelasticity as a Function of Substrate Stiffness

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While it is known that cells respond to the mechanical properties of their environment, limited information is available regarding mechanosensing and the means by which such stimuli are transduced. The cytoskeleton, a critical component used by cells to sense substrate stiffness, determines the viscoelasticity of cells. Information on cell viscoelasticity as a function of substrate stiffness and vimentin levels can lead to a larger composite model of the mechanism through which cells respond to external mechanical stimuli.

3T3 fibroblasts were grown on collagen-coated polyacrylamide gels, of which the stiffness was controlled by varying both the concentrations of acrylamide and bisacrylamide. An atomic force microscope (AFM) was used to perform dynamic micromechanical tests. The force-mapping mode was employed to obtain 24 by 24 maps of force curves. For each force curve, the AFM first indents a cell with a 700 pN force and then applies a 10 Hz sinusoidal strain to the cell. The storage, E', and loss modulus, E'', were calculated by fitting the sinusoidal portion of the applied force and resulting indentation to obtain the amplitude and phase offset.

Both E' and E'' increase with substrate stiffness. While the increase in E' is consistent with literature, we found that the ratio of E'' to E' decreases as substrate stiffness increases. This agrees with our hypothesis that cells become more solid-like as the elastic modulus of the extracellular matrix increases. Additionally, the value of E' was higher in vimentin null cells, suggesting that cells change their cytoskeletal composition to adapt to the loss of vimentin. This may involve an increase in the concentration of actin filaments, which contribute more to the elasticity than vimentin. Further experiments will investigate the potential link between vimentin expression and the mechanosensing ability of the cells.

1965-Pos Board B695

A Systematic, High Resolution Mapping of the Elastic Modulus of Mouse Cartilage Matrix

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Hyaline cartilage cushions bone at articular joints and is responsible for elongation and mechanical integrity of growing bones. The major components of cartilage matrix are the collagen mesh and the highly charged proteoglycan aggrecan entangled in and swelling the mesh. In growing cartilage, the composition and organization of these components varies across both growth plate and articular cartilage and in the vicinity of chondrocytes. Mice are extensively used as model system for a wide range of pathologies including arthritis, but there are limited studies mapping matrix nanomechanics across different regions. Nanomechanical studies, typically performed by indenting tissue sections, are subject to errors due to matrix collapse, surface roughness and diffusional loss aggrecan loss from the section surface. Moreover, there are often issues of data interpretation when the probe size and indentation depth are comparable to the mesh and aggrecan sizes.

We performed a high-resolution (~1um) nanoindentation elasticity mapping of extracellular matrix material of mouse growing cartilage across different regions of both cartilage types in physiological solution. We used an AFM with microsphere probes whose radii and indentation depth exceeded mesh and molecular sizes. We mildly fixed the cartilage to prevent aggrecan loss. We developed a new method for obtaining the Hertzian contact points on rough surfaces. We applied large-strain indentations and force corrections to remove the error due to surface roughness and to select the linear regime of the force data. The latter greatly improved the consistency of our elasticity results. Tissue collapse was partially corrected using the cartilage height mapping. Matrix regions were extracted by correlation with optical images. The matrix elasticity across the growing cartilage showed interesting difference between the two cartilage types and correlations with previous studies of matrix composition at the same locations.

1966-Pos Board B696

Cell Adhesion on Silicon Nanowires

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Understanding nanostructured materials - cell interactions is essential for the development of innovative substrates for cell culture, differentiation and precise electrical stimulation. In this framework, silicon nanowires (SiNWs), characterized by high aspect ratio and a broad range of mechanical, optical and electrical properties, are interesting nanomaterials for innovative cellular engineering. We investigated and quantified, using single cell force spectroscopy (SCFS), the interaction of murine embryonic fibroblast with two mechanically different SiNW substrates, and two reference substrates, collagen gel which is generally used for cell culture, and flat glass which has the same chemical reactivity of SiNWs. We observed comparable adhesion values on SiNWs substrates and on gel substrate, while flat glass generate a 10-times weaker interaction. A closer analysis of the adhesion curves revealed significant differences between soft and hard SiNW, suggesting that a different cell membrane organization is developed depending on the mechanical and geometrical properties of the substrate.

In conclusion SiNW substrates are compatible with cell engineering and thanks to their high range of mechanical and geometric properties, a fine tuning of cellular response can be achieved.

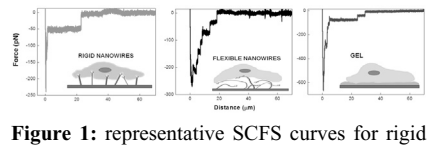

Figure 1: representative SCFS curves for rigid NWs, flexible NWs and collagen-coated coverslips.

1967-Pos Board B697

Characterization of Mechanotransensitivity of Articular Chondrocytes

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Articular cartilage is a protective soft connective tissue lining the ends of bones in weight-bearing joints. It sustains numerous cycles of mechanical loading during normal joint activity. Recent studies showed that mechanical factors not only play a critical role in the pathology of cartilage, e.g. initiation of degenerative joint disease such as osteoarthritis (OA); but also influence the metabolic response of chondrocytes, the only cellular element of cartilage. One of the early events implicated in chondrocyte mechanotransduction is a