1612-Pos Board B456
Multivariable Parameter Optimization Of Microfluidic Post Arrays For DNA Fractionation
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Length-based fractionation of long DNA is a fundamental process in genomic analysis. Traditional methods, such as pulsed-field gel electrophoresis or capillary electrophoresis, are slow and not easily coupled to downstream analytical processes. Microfluidic devices containing arrays of micron-scale posts have previously been described for length-based fractionation of kilobase length DNA. [1] Here, we present experimental and computational studies defining and optimizing a broad array of parameters in order to maximize mass throughput and isolation of DNA $>$ 150 kb. Parameters explored include post field geometry, electric field intensity, and field oscillation timing. Experimental studies were performed by analyzing of the mobility of single YOYO-1 intercalated DNA molecules in PDMS post arrays. Computational studies simulated kilobase length DNA as a worm-like chain model and investigated the interaction of such molecules with post arrays of various geometries. Optimization of our system results in the ability to isolate 165 kb DNA from 125 kb DNA and process 5 ng of DNA within 30 minutes of operation. This research was supported by the Department of Homeland Security, Science and Technology Directorate. [1] Huang et al.., Nature Biotechnology, 2002, 20, 1048.

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Nanopore Sequencing with MspA
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Nanopores may provide the basis for a high-speed and inexpensive de novo DNA sequencing technique that could revolutionize medical and biological science. In this technique, single-stranded DNA is electrophoretically translocated through a pore with inner dimensions similar to that of DNA. The co-passing ion current is recorded to obtain sequence information. Since its inception, nanopore sequencing has had promising results with only one bacterial pore α-Hemolysin and various solid-state pores. The geometry of another bacterial pore, MspA, found in the outer membrane of Mycobacteria Smegmatis, appears to be ideally suited for nanopore sequencing. We used site-directed mutagenesis on MspA to produce mutants that allow DNA translocation. These mutants can resolve small chains of the nucleotides A, C, and T when a duplex region of hairpin DNA arrests translocation. Additionally, DNA interaction with the mutant MspA is significantly and predictably altered with further mutations to the MspA structure. Our results introduce MspA as a promising and engineerable framework for nanopore sequencing technology.

1614-Pos Board B458
Mechanotransduction in Single Cardiac Myocyte Studied Using Laser Tweezers and FRET
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Deletion or mutation of a variety of proteins localized at cell-matrix and cell-cell junctions, such as vinculin and its splice-variant metavinculin, can lead to dilated cardiomyopathy in mice and humans, leading some to hypothesize that these molecules are involved in mechanotransmission or mechanotransduction in the heart.
To investigate cardiac mechanotransduction mechanisms in single cells, we have combined laser tweezers with a fluorescence resonance energy transfer (FRET) biosensor to apply localized forces and probe localized signaling events in isolated mouse ventricular myocytes.
Isolated murine ventricular cardiac myocytes were transfected with a focal adhesion kinase (FAK) FRET reporter to monitor integrin-mediated activation events. An integrin ligand-coated microsphere was adhered to the cell surface. Then laser tweezers were used to apply localized piconewton forces parallel or normal to the image plane either cyclically or statically. To ensure a constant force application on the microsphere, 10υM of blebbistatin was added to the imaging media. In conjunction with force application, a pulsed Ti:sapphire infrared laser was used for two-photon excitation of the FRET reporter.
Phase contrast and fluorescent images were captured simultaneously, allowing quantification of applied forces and FRET ratio changes. Results indicate that piconewton level forces can be applied to the microsphere and FRET ratio changes validate that integrin-mediated events are being activated by the locally applied forces. This data suggests that the use of laser tweezers combined with FRET provides a means to study integrin-mediated events in cardiac mechanotransduction. Future studies include varying the type of integrin ligand-coated microsphere and FRET biosensors, as well as studying genetically manipulated murine lines in isolated adult cardiac myocytes.

1615-Pos Board B459
Engineering Polymeric Drug Delivery Systems For Cancer Therapeutics Using Multi-scale Modeling
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Polymers are of particular interest as drug delivery vehicles due to their ability of targeting drugs to tumors while simultaneously decreasing drug exposure to normal tissues. The classical method of designing polymer-drug conjugates invokes trial-and-error testing of chemical substances on animals and subsequently matching apparent effects to treatments. While effective, this procedure can be time-consuming and expensive. In our study, we use an *ab initio* approach to elucidate certain physicochemical properties of polymer-drug therapeutics that cannot as readily determined by traditional experimental methods: bottom-up atomistic-to-mesoscale computational modeling.
Our polymeric DDS is poly-L-glutamyl-glutamine (PGG) covalently bound to Paclitaxel, a widely used anticancer therapeutic. Physicochemical properties of polymer-drug conjugates that have been shown to potentially affect the delivery and targeting of drugs to tumors are particle size and shape. The size and shape of polymer-drug conjugates have been shown to affect their abilities of adhering to tumor endothelium, being endocytosed by tumor cells, and diffusing through fenestrations of leaky tumor vasculature. We have developed coarse-grained models of PGG Paclitaxel in effort to achieve a variety of sizes and shapes by varying the Paclitaxel % weight loading (18%, 24%, 37% of total wt) and distribution (even, random, clusters, middle, side, ends) on PGG. Parameterization of PGG and PGG Paclitaxel was accomplished using the MARTINI force field, and simulations were run in GROMACS in explicit water solvent in 310 K for ~1us. The aggregation of PGG Paclitaxel molecules into different sizes and shapes were then observed.
We plan to demonstrate multi-scale modeling as a novel tool that allows us to successfully engineer a polymer-drug cancer therapeutic. With this model we expect to suggest optimal physicochemical properties of PGG Paclitaxel for future synthesis and testing.

1616-Pos Board B460
Impact Of Oxysterols On Endothelial Elastic Properties, Contractility And Morphogenesis
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Our previous studies, have shown that exposing bovine aortic endothelial cells (BAECs) to oxLDL resulted in an increase in cell stiffness, force generation, and endothelial network formation. The mechanisms responsible for these effects, however, were completely not clear. Since all three effects could be simulated by cholesterol depletion, we suggested that oxLDL may be mediated changes in the membrane cholesterol. However, the total amount of the cholesterol remains the same after exposing oxLDL to BAECs. To resolve this discrepancy, we tested whether oxLDL-induced effects can be reversed by supplying a surplus of cholesterol. To achieve this goal, we used the MBCD complexed with cholesterol, a known cholesterol donor. In all the experiments, cells were exposed first to oxLDL for one hour and then subsequently to MBCD-cholesterol for an additional 1 hour. Our observations show that after treating with MBCD -cholesterol, cell elastic modulus, force generation and network formation were back to the normal level as compared to control cells. It suggests that cholesterol plays an important role in oxLDL induced cell mechanics. Furthermore, we show that the impact of oxLDL on endothelial biomechanics and morphogenesis can be simulated by specific oxysterols, known components of oxLDL. Here, we show that specific oxysterols have distinct effects on endothelial biomechanical properties. Specifically, we tested four different oxysterol species that are present in oxLDL: 7B- hydroxycholesterol , 7-ketocholesterol , 25-hydroxycholesterol and 27-hydroxycholesterol to study their mechanic properties. The results show that 7k and 27HC are potent factors to increase cell elastic modulus. However, only 27HC raises the force