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but they spontaneously migrate to the SLG areas and eventually form an ordered interconnected neuron pattern perfectly superimposed to the pattern design. Surface functionalization was then more effective on the SLG, and resulted in notably aligned neural network. The described technique could be considered a valuable candidate to realize a new generation of highly specialized biosensors. To gain further insight into the preferential positioning of neurons onto SLG, the distribution of focal adhesion proteins on the patterned SLG was investigated; Stochastic Optical Reconstruction Microscopy (STORM) was employed in order to localize the cellular components for focal adhesion. Super resolution imaging qualitatively confirms that the distribution of vinculin molecules tagged with Alexa 647 has more affinity towards the SLG regions compared to the ablated ones.

1 Lorenzoni et al, Scientific Reports 3:1954, DOI:10.1038/srep01954.

1063-Pos Board B818

Cell-Permissive Protein-Resistant Substrates for Interrogating Neuronal Guidance Cues

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Current in vitro tools for evaluating neuronal guidance cues suffer from several drawbacks, including difficult substrate preparation and hard-to-interpret results. Microcontact printing of an alkanethiol self-assembled monolayer (SAM) provides a robust method for producing high-resolution protein patterns by protein adsorption from solution. However, cell and protein resistant glycol terminated monolayers are typically employed in the background, which prevents the evaluation of potential neuronal guidance cues. We have developed zwitterionic background monolayers that are protein-resistant, but remain cell-permissive. Using surface plasmon resonance imaging (SPRi) and cell-culture studies, we have demonstrated that these zwitterionic monolayers provide well-defined, non-receptor mediate cellular attachment through interactions with cell-surface glycosylation. Exploiting these properties, we have created a monolayer based stripe assay, where the interactions between neurons (cell bodies and neurites) and extracellular matrix (ECM) proteins or guidance cues may be observed and quantified. This system goes beyond current technologies, such as direct protein patterning and microfluidics, and is even capable of evaluating neuronal response to ECM protein, such as laminin.

1064-Pos Board B819

Conductive Milieu on Cellular Electromechanics

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Cellular polarity and alignment are significant biophysical factors in tissue architectures and tissue-to-organ level functions. Multiple biophysical contributors are addressed as cellular alignment factors in mechanical, biochemical and electrical aspects; however, passive electric conductance has not been discussed. Herein, we report the spontaneous alignment of cardiomyocytes to predefined electric conductivity environments as example. Two to three days after passaging iPSCs-derived cardiomyocytes on thin gold strips (i.e., 10-nm thickness, 10s-μm width and 10s-μm period) patterned on a nonconductive substrate (i.e., glass), the most single-cell cardiomyocytes adhere on the nonconductive area and align themselves parallel to the conductive pattern, without any external stimuli. From control experiments, we can exclude any mechanical cues as the reasons of the spontaneous alignment, such as surface groove and mechanical stiffness. Currently, we hypothesize diamagnetic effect or Fröhlich electromagnetic effect as the physiological response of cardiomyocyte, which may be induced from the electric field coupling between cardiomyocytes and the gold pattern. Along with the further understanding, this observation will highlight passive electric elements as important biophysical aspects since many cellular components possess a level of electric properties.

1065-Pos Board B820

Contractility of Neonatal Cardiomyocytes is Altered with Different Densities of Laminin Covalently Attached to Microposts

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Heart disease is the number one killer in the USA. The collective contractility of the muscle cells of the myocardium - cardiomyocytes - generates the necessary force for the function of a healthy beating heart. Laminin interacts in vivo with cardiomyocytes. Changes in the extracellular concentration and organization of laminin relate to different types of heart disease. Arrays of polydimethylsiloxane (PDMS) microposts measure forces generated by adhesive mammalian cells and were here used to characterize the contractility of single neonatal cardiomyocytes. We used two types of organosilanes to bind laminin to the surface of PDMS microposts: 3-glycidoxypropyltrimethoxysilane and 3-aminopropyltriethoxysilane. We acquired videos of contracting cardiomyocytes at two different days after cells started to beat and functionally characterized the contractility of single cells. More specifically, we calculated generated forces, beating rate, time of contractions and speeds of contraction and relaxation. These parameters varied in time as a function of organosilane surface stability and cardiomyocyte biological changes when cultured in vitro. Higher forces are generated by cardiomyocytes cultured on laminin covalently attached to PDMS microposts relative to laminin physisorbed to oxidized PDMS. We obtained higher laminin density with 3-glycidoxypropyltrimethoxysilane, which correlated to higher generated forces. We also observed higher beating rate at the day 1 and a considerable decrease at day 2. Compared to 3-glycidoxypropyltrimethoxysilane, higher stability of laminin covalent attachment was observed with 3-aminopropyltriethoxysilane. The beating rate and speeds of contraction and relaxation increased and time of contractions decreased at day 2 for neonatal cardiomyocytes cultured on these PDMS micropost surfaces. Our results shed light on the potential of in vitro biomechanical systems to model extracellular disease conditions of heart pathologies. Future work will test the contractility of cardiomyocytes with mutations known to originate cardiomyopathies.

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1066-Pos Board B821

Flow Injection of DNA in Nanopores : Direct Optical Visualization of a Pressure Threshold

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In the regime of pores smaller than the radius of gyration, the flow injection of polymers and biopolymers exhibit a flow threshold independent of the pore and of the polymer itself. We have developed a new combination of near field optics (zero mode wave guide) and image analysis in order to revisit this phenomenon. Working at constant pressure we are able to control and observe directly the transport of individual biomolecules with a time resolution of 5 ms. In the case of DNA, we show that the forced transport through the pore can be described as an energetic barrier only dependent on the injected flow. Further application to biological systems of this barrier measurement will also be discussed.

1067-Pos Board B822

Designing Hydrophobic Gates into Biomimetic Nanopores

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The use of nanopores is fast being a major scientific tool in molecular analysis and detection due to their ability to detect polynucleotides, proteins and small molecules. Biomimetic modelling of pores allows for a specific function to be incorporated into the molecular structure of the nanopore, based on amino acid motifs found in existing protein structures.

An initial beta barrel model was built computationally, based on the transmembrane domain of 14 stranded beta-barrel pore, alpha-hemolysin. Hydrophobic and hydrophilic residues were built in a specific arrangement within the structure to replicate an hourglass shape cavity with a central constriction. From this, pore conductions were observed via Molecular Dynamics (MD) and selected models were transformed into hybrid pores in which the location of hydrophobic residues differed to give constricting regions surrounded by hydrophilic residues. From All Atom MD simulations, a hydrophobic gating mechanism has been established within these toy models with intermittent water currents through the pore giving an insight into possible biomimetic motifs which could be biochemically integrated into the wild type protein.

1068-Pos Board B823

Developing a Broadband Amplifier for Analysis of DNA Structural and Molecular Characteristics

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The recent emergence of DNA-based diagnostics increases the need for rapid DNA sequencing technologies. One method to achieve this is to pass DNA through a nanopore, recording the trans-membrane current with a low-noise current amplifier. The challenge presented in this method is that the bandwidth of commercially available current amplifiers is limited to 100kHz, which is not

fast enough to resolve the signals present in DNA sequencing. To overcome this problem, researchers have developed a custom amplifier that has reached a bandwidth of approximately 1MHz, being able to reveal additional information during the DNA translocation events through a nanopore.
We will demonstrate a design of a custom amplifier that offers a wider bandwidth than the current designs, enabling the study of DNA translocation without the need to limit the speed of translocation. In addition, an amplifier with a bandwidth larger than 1MHz allows discoveries to be made about information that might be present in a higher range of frequencies, enabling measurements at a higher time resolution than what was previously possible. The amplifier will be designed to allow direct integration of a micro- or nanopore sensing area on the same physical substrate, eliminating the need for external electrode wiring. The outcomes from this research open up the possibility of an integrated high-speed DNA sequencer chip enabling rapid disease diagnostics.

1069-Pos Board B824
Diffusion and Trapping of Single Particles in Pores

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Passage of single molecules and particles through pores is the basis of resistive-pulse sensing. We introduced an additional control of the particle transport by modulation of the driving voltage during the translocation process. Balancing all forces acting on the particles allowed us to observe diffusion of single particles in the pore, and quantifying their diffusive coefficient. The developed method for measuring diffusion coefficient in pores is applicable to particles of difference sizes, does not require fluorescence labeling, and is entirely based on ion current recordings. Application of a modulating voltage signal together with rising edge triggers enabled transporting the same particle back and forth within the pore without letting the particle leave the pore. This method is especially useful for the analysis of species present in a solution in low concentrations where statistics on an ensemble of particles/molecules has to be replaced by the statistics based on one particle studied many times. The experiments were performed with negatively charged polystyrene particles passing through single 11 μm long pores in a polyethylene terephthalate (PET) film. The pores were prepared by the track-etching technique, which when applied to PET films leads to pores with undulating diameter along the pore axis. The pore topography is reflected in the pulse shape. Passage of particles through narrower parts of a pore causes a larger change of the transmembrane current compared to the case when the particles pass through wider pore segments. Each particle 'follows' the same pore topography thus all current pulses for a given pore look alike. We used the ion current pulse substructure as reference points for particle position along the pore axis, which facilitated the determination of diffusion coefficient of translocating particles.

1070-Pos Board B825
Controlling Motion of DNA in a Nanochannel with Transverse Alternating-Electric-Voltages

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One key requirement for fast and accurate DNA sequencing in nanopores or nanochannels is to control the DNA position and modulate the speed, which, however, still remains a big challenge. Because of drastically changed electric potentials across a nanochannel, the DNA motion is too rapid for a sensor to detect each DNA nucleotide. A nanofluidic channel, with a pair of perpendicularly aligned nanoelectrodes, is proposed to electrostatically control the motion of DNA molecules. Using all-atom molecular dynamics simulations, we studied electrostatic responses of a charged DNA molecule in the nanochannel and investigated optimized operating conditions for controlling the motion of the DNA. When the transverse electric field was periodically turned on and off, the DNA molecule was correspondingly immobilized on and released from the channel surface. Under simultaneously applied longitudinal biasing and transverse trapping electric fields, the DNA molecule moved forward in a ''ratchet''-like fashion. It is expected that achieving the controlled motion of DNA in the channel can advance studies and applications of a nanochannel-based sensor for analyzing DNA (e.g., DNA sequencing).

1071-Pos Board B826
Disentangling Steric and Electrostatic Factors in Nanoscale Transport through Confined Space

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The voltage-driven passage of biological polymers through nanoscale pores is an analytically, technologically, and biologically relevant process. Here we exploit an experimentally tunable system to understand and quantify the relative importance of electrostatic and steric interactions in nanopore analytics. The approach provides understanding of previously unresolved fundamental aspects of pore transport particularly of biopolymers which vary in charge and volume along their sequence. Our tunable experimental system is based on a common DNA oligonucleotide of 27 nucleotides which carries at an internal base position a positively charged oligoarginine tag of three, five, or seven residues or a negatively charged hexa-aspartate tag. An applied voltage was used to drive these modified oligonucleotides through the inner constriction of an alpha hemolysin pore embedded in a lipid bilayer causing a measurable drop in ionic current. Statistical analysis of large numbers of event durations provided the characteristic translocation time (t*) in each case. A biophysical model was then developed to describe t* as a function of voltage and arginine/aspartate tag lengths. Through best fit analysis this model generated reasonable oligonucleotide charge and basal hopping rate values. Further, this model allowed us to predict the impact of only steric or electrostatic effects providing insight on the relative importance of each. This new, fundamental framework facilitates the understanding of how complex biopolymers are transported through confined space and indicates how their translocation can be slowed down to enable future sensing methods.

1072-Pos Board B827
A Simple, Single-Carbon-Nanotube Nanofludic Platform for Fundamental Transport Studies

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Fundamental understanding of ionic and molecular transport phenomena in a simple model nanopore is critical for elucidating function mechanisms of much more complex biological systems, and for advancing technological areas such as membrane separation, energy harvesting/storage, and single molecule detection. For this goal, carbon nanotubes (CNTs) offer key advantages as model nanofluidic channels due to their simple chemical composition and structure (known with atomic precision), robustness, facile length and diameter control, and straightforward local functionalization at their open rim. CNTs have also very interesting fluidics properties such as enhanced pressure-driven fluid transport rates, unusually high electroosmotic flow, and ionic selectivity.
Here, we present our work toward developing and validating a novel nanofluidic platform featuring an individual carbon nanotube (CNT) as the flow channel in an advanced Coulter Counter. To fabricate the CNT nanofluidic device, vertically aligned single-walled CNTs are synthesized directly on a suspended silicon nitride membrane and then bound in a solid matrix before an individual CNT is opened by focused ion beam milling. Single-molecule translocation studies with small molecular size analytes suggest the successful fabrication of a Coulter Counter with a-few-nm wide CNT nanochannel. Our initial ionic conductivity studies indicate a power-law increase of conductance with KCl concentration in CNT channels, a dependence that seems to be unique of CNT pores.

1073-Pos Board B828
Double Occupancy of a Protein Pore as an Intermediate State of Competition at the Single Molecule Level

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Alpha-hemolysin nanopores are used to detect single oligomers of poly(ethyleneglycol) (PEG) which under high salt conditions reside in the pore for extended periods of time (up to several ms) suggesting binding to the pore's inner wall. We study the interaction of two species of PEG of degree of polymerization 28 and 32 which, following sequential entry, simultaneously reside in the pore. This doubly occupied (DO) state can result in direct replacement of the first occupant by the second. Analyzing the dwell times of the DO states, we ask whether binding as a first or second pore occupant is equivalent in terms of stability. We find that the DO state lifetimes are shorter than would be predicted by simple superposition (see Figure). We conclude that binding in the pore as the second occupant is highly unstable, suggesting that the more stable primary binding state cannot be attained by more than one PEG molecule. The doubly occupied state thus represents an intermediate state with possible general relevance for competitive interactions at binding sites to which access is gained through long channels, such as in some enzymes.