such as a protein, across a lipid bilayer. To create a membrane, two sub-microliter, lipid-encased aqueous droplets are contacted - termed a droplet interface bilayer (DIB). The peptides adsorb to the protein cargo non-covalently and somehow ''carry'' the protein from one droplet to the other through the membrane. We then assay the translocated cargo through a fluorogenic assay. The DIB method recapitulates the findings of earlier studies involving Pep-1, including the dependence of protein transport on voltage and membrane charge, while also contributing new insights. Specifically, we found that the symmetry of the bilayer membrane may play a role in Pep-1-mediated protein translocation. In addition, we used a newly developed peptide transduction domain mimic (PTDM) as a protein carrier, which exhibited distinct differences compared to Pep-1's mechanism. We've also used the DIB system to monitor the translocation of proteins through pores, such as the anthrax toxin. We anticipate that the DIB method may be useful for a variety of transport-based studies; in particular those which must make use of tiny quantities of purified species.

1058-Pos Board B813

Cobaltabisdicarbollide Macroanion is able to Diffuse across the Lipid Membrane; Study of Kinetics and Transport

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The ability to form membranes is not restricted to polar lipid molecules or even to organic molecules, as the boron-based molecule cobaltabisdicarbollide, $[Co(C_2B_9H_{11})_2]^-$, (commonly known as cosan) can also form vesicles and membranes. Here we show that cosan and its derivatives can interact with phospholipid membranes.

Cosan is a monoanionic metallabisdicarbollide, which is soluble in both water and oils. Structurally, cosan has no similarity to the polarized lipid molecules that make up biological membranes. It comprises a cobalt atom sandwiched by two carboranyl clusters [1-3]. Although these clusters are hydrophobic, the metal ion imparts a dispersed negative charge spread over the whole molecule (fig.1). As a consequence of this, the exposed B-H and C-H bonds of the carboranyl clusters possess weak dipoles and form intermolecular attractions that give the molecule its unusual physico-chemical property of being simultaneously hydrophobic and hydrophilic. This duality allows cosan to interact with lipid membranes.

Using membrane electrophysiology recordings and direct measurements of COSAN concentrations by inductively coupled plasma mass spectrometry (ICP-MS) we show that COSAN transits cell-free artificial lipid membranes. We study the kinetics of the transport and try to get insights on the mechanism by which COSAN crosses planar phospholipid membranes.

[1] C.E. Housecroft et al., Encyclopedia of Inorganic Chemistry. John Wiley & Sons; 2008.
[2] M.F. Hawthorne et al, J. Am. Chem. Soc 1968;90:879-896.
[3] R.N. Grimes, Carboranes. Academic Press: Burlington, MA; 2011.

1059-Pos Board B814

Experimental Observation of Surface Charge Inversion in a Biological Nanopore in Presence of Monovalent and Multivalent Cations

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The electric double layer formed at the pore surface produces the exclusion of coions and the accumulation of counterions. A particularly captivating situation occurs when interfacial charges attract counterions in excess of their own nominal charge, thus leading to an effective charge inversion of the system. This phenomenon has been reported in such diverse systems like lipid vesicles, colloids, Langmuir monolayers, membranes, flexible polyelectrolytes and other synthetic nanodevices that are in contact with an aqueous solution containing multivalent ions. Here, we report experimental evidence of charge inversion in the bacterial channel OmpF of E. coli, not only in presence of multivalent cations but also in presence of monovalent ones. We perform reversal potential experiments in different conditions of pH and salt concentration to analyze both the effect of cation type and size in the selectivity changes.

1060-Pos Board B815

Influences on Cellular Adhesion of Nanoparticles under Blood Flow-Like Conditions

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Nanocarrier-mediated drug and gene delivery are novel strategies to treat diseases, for example cancer, neurological disorders, infectious and cardiovascular diseases. Sophisticated drug delivery and gene therapy systems are typically equipped with targeting ligands to improve the specific binding to diseased cells within the human body. These targeting moieties are usually small molecules, peptides or proteins which are able to specifically bind receptors on the cell surface. In many diseased cells certain receptors are overexpressed and therefore represent suitable targets.

In our experiments we focus on the specific adhesion of nanocarriers to target cells. Therefore, we use fluorescent labeled polystyrene beads as model particles, shield them with PEG (polyethylene glycol) and mount a ligand - the transferrin receptor (TfR) binding peptide B6 - to mimic the surface of therapeutical nanocarriers. The binding of these targeted beads is directly compared to the adhesion of non-targeted beads on TfR overexpressing HuH7 cells. As non-targeted beads we use three different types, one with hydroxyl groups on the surface, another with scrambled B6 peptide (same amino acids, but different order) and a third type with modified B6 peptide (all positively charged amino acids are replaced by neutral ones). To include dynamics and determine the impact of shear stress, the binding study is performed under laminar flow conditions, i.e. the beads are flushed over a cell monolayer within a microfluidic channel. After fixation of the cells, highly-sensitive fluorescence widefield microscopy is performed to analyze the adhesion of beads on a single cell level. With this approach we are able to directly analyze the effect of the targeting ligand. In addition, the influences of electrostatics and shear stress on cellular particle binding are investigated.

1061-Pos Board B816

Surface Interactions in Suspensions of Swimming Cells

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Interactions between swimming cells and surfaces are essential to many microbiological processes, from bacterial biofilm formation to human fertilization. However, despite their fundamental importance, relatively little is known about the physical mechanisms that govern the scattering of flagellated or ciliated cells from solid surfaces. A more detailed understanding of these interactions promises not only new biological insights into structure and dynamics of flagella and cilia but may also lead to new microfluidic techniques for controlling cell motility and microbial locomotion, with potential applications ranging from diagnostic tools to therapeutic protein synthesis and photosynthetic biofuel production. Due to fundamental differences in physiology and swimming strategies, it is an open question of whether microfluidic transport and rectification schemes that have recently been demonstrated for pusher-type microswimmers such as bacteria and sperm cells, can be transferred to puller-type algae and other motile eukaryotes, because it is not known whether long-range hydrodynamic or short-range mechanical forces dominate the surface interactions of these microorganisms. Here, using high-speed microscopic imaging, we present direct experimental evidence that the surface scattering of both mammalian sperm cells and unicellular green algae is primarily governed by direct ciliary contact interactions. Building on this insight, we predict and experimentally verify the existence of optimal microfluidic ratchets that maximize rectification of initially uniform Chlamydomonas reinhardtii suspensions. Because mechano-elastic properties of cilia are conserved across eukaryotic species, we expect that our results apply to a wide range of swimming microorganisms.

1062-Pos Board B817

Selective Growth of Neural Networks on Micro-Patterned Graphene

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Single crystal graphene is the ideal candidate for next generation of electronic devices and of biosensors. Interfacing graphene with neural cells could be highly advantageous in exploring their electrical behaviour and promising for several biomedical applications, including neural regeneration and artificial retina. Here, we present a straightforward fabrication technique based on laser ablation to obtain patterned substrates promoting ordered neuron growth. Chemical vapor deposition (CVD) single layer graphene (SLG) was machined by means of single pulse UV laser ablation technique at the lowest effective laser fluence in order to minimize laser damage effects (1). The obtained patterned substrates, with alternating micro-sized stripes of ablated and not-ablated SLG, were uniformly coated with poly-D-lysine; primary embryonic hippocampal neurons were cultured on the functionalized substrates. As monitored by time-lapse imaging, neurons adhered on both regions of the pattern,

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