3145-Pos
Targeted Optimization of a Molecular Motor for Controlling Movement in Biohybrid Devices
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The construction of biohybrid devices in which motor proteins are integrated as biomechanical components for powering nano- to microscale movement holds great potential for a wide range of nanotechnological applications, ranging from basic research to diagnostic lab-on-a-chip technologies. However, to operate motor proteins efficiently in regard to cargo transport, sorting, and assembly processes, it is important to control parameters such as velocity, motile activity, force production, directionality, and processivity of movement tightly. Long-term stability of the motor protein on synthetic environments is an additional prerequisite for their successful integration in biohybrid devices. Here we describe a structure-based molecular engineering approach leading to the design and generation of two myosin constructs that maintain their motile activity when immobilized on glass surfaces over greatly extended periods. Direct functional assays and single molecule experiments show that important motor properties of the engineered nanomotors can be modulated by controlled changes in buffer conditions. We show that the motile behavior of the dimeric M5P construct can be switched between processive and non-processive modes of movement and the motor activity of the monomeric M5S construct can be turned on and off in a controlled, continuous, and reversible manner by coordinated changes in the concentration of $MgCl_2$ and KCl. The parametric control is achieved accurately and with great ease. The resulting effects on motor function can be used for applications ranging from organising directed transport with targeted accumulation of cargo as well as assembly and sensing functions on the nano- to micro-scale levels.

3146-Pos
Immobilization and Incorporation of Antigenic Peptide P17-1 from HIV-1 P17 Protein in Nanostructured Films
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The immobilization of antigenic peptides in nanostructured films is promising for the development of highly specific immunosensors. In this work, we analyze the peptide p17-1 (LSGGELDRWEKIRLRPGG), derived from the HIV-1 p17 protein, immobilized into Layer-by-Layer (LbL) films and incorporated into Langmuir monolayers of phospholipids. The LbL film was assembled using different polyelectrolytes but only poly(allylamine) hydrochloride (PAH) was efficient for the peptide immobilization. The intensity in the UV-Vis. spectra of PAH/p17-1 films increased exponentially with the number of layers, which may indicate that the peptide can be reorganized in each bilayer adsorbed. Fluorescence and circular dichroism (CD) spectra indicated that the interaction with the film did not induce an alpha helix conformation in p17-1, analogously to what occurs in an aqueous solution and in contrast to the organized peptide in a methanol solution. The maximum emission for p17-1 fluorescence occurred at 340 nm in methanol, compatible with tryptophan residue buried in the solvent, while for p17-1 in an LbL film the maximum appeared at 355 nm. This red shift is consistent with the tryptophan being exposed to the environment. The CD spectra confirmed these results showing the random structure for p17-1 in the LbL films and an α-helix structure in methanol solution. The lack of structure is the probable reason for the low sensitivity toward anti-p17 observed in amperometric sensors made with PAH/p17-1 LbL films. With regard to the Langmuir monolayers, p17-1 was found to affect the surface pressure isotherms of dipalmitoyl phosphatidyl glycerol (DPPG), even at a concentration as low as 0.5 mol%. This cooperative interaction of p17-1 and DPPG may perhaps be exploited in designing new architectures for producing immunosensors based on antigenic peptides.

3147-Pos
Engineering Hamlet-Like Proteins
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It has been shown that a folding variant of alpha-lactalbumin containing oleic acid (HAMLET: Human Alpha-lactalbumin Made LEthal to tumor cells) induces apoptosis in tumor cells whereas healthy cells remain resistant. Furthermore, the apoptotic activity of HAMLET seems related to fundamental physicochemical features, such as the ability to populate partially unfolded conformations, likely associated to a marginal folding/unfolding free-energy barrier. Here, we explore the possibility of using protein engineering to create HAMLET-like behavior in proteins other than alpha-lactalbumin. To this end, we have introduced in a suitable protein model system mutations that are expected to affect the thermodynamic folding barrier and we have probed the oleic-acid binding capability of the variants thus obtained. Finally, the tumoricidal activity of the resulting protein/oleic-acid complexes has been characterized.

3148-Pos
High Throughput Methods for Biophysical Characterization of Monoclonal Antibodies
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New high throughput methods of biophysical characterization of monoclonal antibodies were developed to accelerate the process of pharmaceutical drug development. The methods, thermostability screening, detection of aggregates and viscosity measurements, can be performed in multi-well plate format, and require low amounts of protein sample. A wide range of protein concentrations including concentrations typically used in pharmaceutical formulations can be studied. Case studies are presented and the results compared between new and older techniques. We have shown that the new methods are fully comparable with previously used techniques such as differential scanning calorimetry, size exclusion chromatography and viscosity measurement by the cone and plate method. The new methods have advantages of efficiency and high throughput capability and could be widely applied in the biopharmaceutical industry for formulation and process development and characterization.

3149-Pos
Asymmetric Giant Unilamellar Vesicles
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The lipid composition of the eukaryotic plasma membrane is asymmetric; that is, if the bilayer is considered to consist of two leaflets, the outer-facing leaflet contains different lipids at different concentrations than the inner-facing leaflet. While there has been much speculation as to the physiological purpose of this asymmetry, it has been notoriously difficult to study in in vitro systems, since synthetic artificial bilayer are difficult to form. This is especially true for giant unilamellar vesicles (GUVs), which have been essential tools in studying lipid mechanics and phase separation, but which are made from inherently symmetric bilayers.
Here, we present a microfluidic technology that allows for the formation of asymmetric GUVs. The vesicles are assembled in two independent steps. In each step, a lipid monolayer is formed at a water-oil interface.. The first monolayer is formed inside of a microfluidic device with a multiphase droplet flow configuration consisting of a continuous oil stream in which water droplets are formed. Control over the flow parameters allows for control of droplet and, ultimately, vesicle size. Droplets are dispensed into a vessel containing a layer of oil over a layer of water. The second lipid monolayer is formed by transferring the droplets through this second oil-water interface using a spontaneous transfer method. A density difference between the droplet interior and the aqueous subphase drives this transfer. My dissolving different lipid compositions in the different lipid phases, and asymmetric membrane can be fabricated.
This method produces GUVs with controlled size, high stability , and compositional asymmetry. Asymmetry is demonstrated with a fluorescent quenching assay, in which a membrane-impermeable chemical quenching agent is used to quench fluorophores on only the exterior of the bilayer.

3150-Pos
Design Concepts For a Biocushion to Comfort Lipid Membranes
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Polyelectrolyte multilayers (PEMs) are promising materials for obtaining stable and potentially functional supports for various biomimetic systems. Such films may serve as highly hydrated cushions to comfort deposited biomembranes. In this study we report new results from a continuous investigation of a polyelectrolyte multilayer system that is composed of alternating layers of chitosan and

heparin. A set of methods was applied: Surface Plasmon Resonance (SPR) was chosen to continuously monitor the self-assembly process of physi-sorption of subsequent PE layers and to report about deposition efficiency, dynamics and stability of the PEM film. Atomic force microscopy (AFM) was used to visualize and characterize the surface topology obtained after subsequent steps of the layer-by-layer deposition. The multi-layer structure of the composite film and its hydration were studied with neutron reflectometry. The knowledge gathered so far on this system is now being applied for the deposition of lipid bilayers and other biomimetic systems for subsequent biophysical studies.

3151-Pos

Analysis of Lipid Compositional Changes During Alcoholic Fermentation in Industrial Yeast Strains with Varying Ethanol Tolerance

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In model lipid bilayers, ethanol is known to alter the mechanical and thermodynamic properties of the membrane. The extent to which alcohol affects these properties depends upon the lipid composition of the bilayer. Our group has demonstrated that increased ergosterol levels in model membrane systems mitigate the membrane thinning effect of ethanol - a phenomenon known as interdigitation. Perturbations to the yeast biomembrane due to increasing ethanol levels has been implicated in reduced sugar utilization and cell viability in Saccharomyces sp. However, variations in fermentation conditions and analytical methods have not yielded a comprehensive picture of how yeast biomembranes adapt to increasing levels of ethanol. In this work, we analyzed the partial lipidome of 30 industrial yeast strains at different stages of fermentation using high-resolution mass spectrometry. Quantification of selected lipid species was performed using high performance liquid chromatography coupled online to quadrupole ion-trap mass spectroscopy. Multivariate statistical analysis of the quantitative data was performed to determine any correlations between changes in lipid composition and ethanol tolerance in the different yeast strains. Information regarding how yeast biomembranes adapt to greater ethanol concentrations will be used to construct biophysical models to analyze the complex physical properties of lipid biomembranes in an alcohol milieu.

3152-Pos

Automated Lipid Bilayer Formation using a PDMA Gasket

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Planar lipid bilayer membranes(BLMs) play important roles in studying ion channels, as well as in potential applications for drug discovery. Despite the importance of many practical applications using BLMs, membrane formation is still based on conventional methods invented by Montal and Mueller in 1960s. Although membranes can be simply reconstituted using the conventional technique, membranes should be created where experiments are conducted due to their mechanical instability. In our recent work a membrane formation technique using high melting temperature solvent mixture was devised by Jeon, et al. (Lab on a Chip, 8(9): 1742 (2008)) Briefly, 2:8 mixture of n-decane and hexadecane was spread over a small aperture and froze before its spontaneous self-assembly process to a bilayer membrane. Since the membrane precursor can be created in a central facility and shipped to any place, it can be transported to any place and thawed when a membrane is needed, widening the usability of artificially created lipid bilayer membranes. Nevertheless the main drawback of the conventional technique was not completely ameliorated in this work due to the membrane support. Since a membrane precursor was deposited in a small aperture on a plastic sheet, the membrane formation process by self-assembly was unchanged, resulting in variations in membrane formation time with a range of ~30 minutes to 24 hours. In our work a PDMS gasket was used to support membrane structure in place of a plastic sheet. Since organic solvent can be extracted into the PDMS gasket, a lipid bilayer membrane can be formed within ~30 minutes in a controlled manner. Ion channels incorporated into a membrane formed in a PDMS gasket functioned as in a conventional membrane. Furthermore, we will show the broad applicability of our membrane formation technique.

3153-Pos

Fabricating a New Stabilized Lipid-Based Platform for Handling and Presenting GPCRs

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G protein-coupled receptors (GPCRs) are members of large family of signaling molecules. They have a key function in neuronal biology and play a critical rule in transmitting extracellular signals in eukaryotic cells. GPCRs include receptors for many neurotransmitters like serotonin. The serotonin receptor $5HT_{1A}$ has an important role in mental disorders like anxiety, and is the target of some anti anxiety drugs. Here we constructed a novel biomimetic lipid membrane-based platform to be used for screening molecules that interact with GPCRs. Since fragility and short life time limit the applicability of liposomes that are normally used to present membrane proteins, we have developed a platform based on nanoscale liposomes with enhanced longevity and stability. We developed two approaches to make stable liposomes: liposomes containing a UV-initiated poly(ethylene glycol) (PEG) hydrogel and conjugated hydrogel liposomes made from lipids covalently anchored to the hydrogel network. Stability of nanoscale liposomes was confirmed by addition of high concentration of sodium dodecyl sulfate (SDS) to the liposome suspension. The liposome/detergent micelle mixture was passed over a size exclusion chromatography (SEC) column, separating intact liposomes from micelles. Using dynamic light scattering (DLS), we could confirm the existence of intact 160 nm liposomes. This result was in good agreement with the SEC result for polymerized liposomes without detergent. Since our goal is making GPCR-bearing liposomes, serotonin receptor $5HT_{1A}$ was incorporated into liposomes using a detergent-mediated method. GPCR incorporation was confirmed by binding labeled HTR_{1A} antibody to liposomes containing biotinylated lipids and subsequently separating them from bulk with streptavidin-coated magnetic beads. We are currently undertaking further studies on antibody-liposome binding by fluorescence anisotropy to validate our approach.

3154-Pos

In Vitro Enhancement of Collagen Deposition in Fully Biological Bioprinted Constructs

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Engineering new tissues, ideally from the patient's own body cells to prevent rejection by the immune system, is a rapidly growing field that rests on three pillars: cells, supporting structures (or scaffold) and stimulating biological environment. As the cells grow and depose their own extracellular matrix (ECM), the scaffold degrades slowly. After implantation, remnants of the scaffold can trigger chronic inflammation and create mechanically weak zones by interfering with extracellular matrix assembly. Over the last few years, we developed an alternative, scaffold-free method. Our technique exploits well-established developmental processes (such as tissue fusion, spreading and cell sorting). Conveniently prepared bio-ink units (multicellular spheroids or cylinders composed of single or several cell types) are delivered into the bio-paper (a hydrogel support material) to build tubular constructs. Structure formation takes place by the post-printing fusion of the discrete units and maturation in bioreactor. The slow buildup of cell-produced ECM needed for adequate mechanical strength before implantation, however remains an issue. Collagen deposition is hindered *in vitro* due to the slow conversion of de novo synthesized procollagen to collagen before its secretion.

Here we explore different strategies to enhance collagen deposition in the bio-ink units and the post-fusion construct. Collagen deposition could be observed throughout spheroids obtained by seeding human aortic smooth muscle cells in 96 well plates and culturing them in presence of ascorbic acid. However the process still took 3 weeks. Boost in collagen production by the bio-ink units was observed when negatively charged dextran sulfate (DxS, 500 kDa) was added to the medium. After high content bio-ink units fuse into a tube, ECM reorganization is expected under mechanical stimulation in the bioreactor.

In conclusion, DxS could fasten the production of ECM in tissue engineering applications.

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