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