in the tube should adjust in order to minimize the bending free energy in the curved structures like tubes or small vesicles. Here we have systematically studied lipid sorting in membrane nanotubes of controlled diameter.
We designed an assay where nanotubes are pulled out of Giant Unilamelar vesicles made of Sphingomyelin (BSM), Cholesterol (Chol) and DOPC using optical tweezers. The tube radius is set via micropipette aspiration. The composition in the tube's membrane is measured by recording the fluorescence intensity of labeled lipids under a confocal microscope; simultaneously the force necessary to hold the tube is measured with optical tweezers.
We will show that curvature induced lipid sorting can occur, but only near a phase transition of the ternary system BSM:Chol:DOPC. We will show that the physical origin of sorting by curvature in pure lipid system is a reduction of the free energy of curvature of the membrane in the tube. We will present theoretical considerations supporting these observations.
Finally we will show that protein binding a specific lipid in the membrane can enhance sorting.
References
[1] F.R. Maxfield, T.E. McGraw, Nature Reviews (2004), 5, 121.
[2] Brügger, et al. JCB, vol. 151, 3 2000.

95-Plat
Stoichiometries and Energetics of Cationic Nanoparticle-Membrane Complexes
Christopher V. Kelly, Meghan G. Liroff, L. Devon Triplett, Douglas G. Mullen, James R. Baker, Bradford G. Orr, Mark M. Banaszak Holl.
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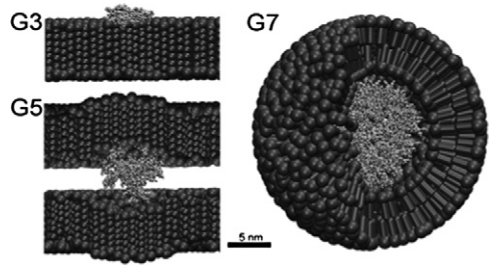
The nanoparticle-membrane interaction is essential to nanotheraputic design and nanotoxicity concerns. The equilibrium structure was determined for phospholipid membranes interacting with one type of nanoparticle, poly(amidoamine) dendrimers, at the atomistic and molecular scale via both experimental and theoretical approaches. The resulting dendrimer-phospholipid complex depends on both the number of primary amines per dendrimer and the dendrimer size. Large dendrimers (> 7 nm diameter) induce vesicle-encased dendrimers and significant membrane disruption. In contrast, small dendrimers (< 5 nm diameter) bind to the membrane surface without individually inducing significant membrane disruption. Techniques such as isothermal titration calorimetry (ITC), molecular dynamics (MD), and differential scanning calorimetry (DSC) were used for examination of the equilibrium structures and identifying the mechanisms of nanoparticle-induced membrane disruption.
Third-, fifth-, and seventh-generation poly(amidoamine) dendrimers (G3, G5, and G7, respectively) are shown here in complexes with phospholipids. The stoichiometries and dimensions of the dendrimer-lipid complexes indicate small dendrimers (G3) saturate with lipids on a planar membrane, medium-sized dendrimers (G5) induces local membrane curvature and/or binds to multiple bilayer surfaces, and each larger dendrimer (G7) becomes encased by a lipid vesicle. These understandings will guide nanoparticle design in both medical and industrial applications.



96-Plat
The Delivery of Lipidic Compounds to Model Membrane Interfaces by Non-lamellar Liquid Crystalline Nano-particles
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There is an increasing demand for methods to study processes at the lipid-aqueous solution interface, due to the importance of lipids and lipid self-assembly structures as regulators both for biological activity and for drug delivery vehicles. The biological membrane is one of the most important interfaces that the drug delivery vehicles encounter. The potential use of non-lamellar lipid structures delivery systems in pharmaceutical, food and cosmetic applications has invoked a number of studies of the assembly and interactions of cubic phases of these materials. One such system is a colloidal dispersion of the cubic liquid crystalline phase of glycerol monooleate. We will discuss some aspects of what happens when these liquid crystalline lipid nanoparticle encounters a lipid bilayer, consisting dioleoylphosphaticylcholine, either as supported bilayer or as a vesicle. Null ellipsometry and QCM-D provides kinetic information about the adsorption and triggerable release of the nanoparticles. Using contrast matching of the supported lipid bilayer, neutron reflectivity makes it possible to assess the exchange of material from one ordered lipid phase to another. Synchrotron Small Angle X-Ray Diffraction allowed us to in detail study the phase transition when non-lamellar glycerol monooleate based nanoparticle interact with phospholipid vesicles. Together the four techniques provide insight into the interaction mechanism and shows that the release of the particles are likely to be caused by phase transition of the lipid self-assembled structures.

97-Plat
Construction Of A Tethered-bilayer Lipid Membrane By Physiosorption Of Glycolipid GM_1 To A Hydrophilically Modified Gold Surface
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Interactions of membrane-bound molecules with their environment are difficult to study due to the complex and dynamic nature of biological membranes. The development of model membranes can provide insight into the function of membrane proteins in their natural environment. A phospholipid bilayer deposited at a gold surface offers a unique opportunity to investigate the mechanism of voltage-gated ion channel formation induced by surface-active proteins such as Colicin E1. Studies of voltage-gated phenomena involving transmembrane proteins require a model membrane that is supported at a gold electrode and has a water layer on either side of the bilayer. This can be achieved by creating a tethered lipid bilayer membrane (tBLM) with a hydrophilic spacer region separating the gold surface from the bilayer. We describe here the construction of a lipid bilayer membrane which is tethered from the gold surface using glycolipid, GM_1. The bilayer is composed of 1,2-dimyristoyl-sn-glycerol-3-phosphocholine (DMPC) and cholesterol. GM_1 is physiosorbed to gold by modifying the gold surface with a hydrophilic thiol, 1-thio-D-glucose. Due to the amphiphilic nature of GM_1 this is performed at the air/water interface using the Langmuir-Blodgett technique. The outer leaflet of the bilayer is deposited using the Langmuir-Schafer method. The quality of the bilayer formed at the gold surface was characterized using electrochemical methods, in which the capacitance of the tether bilayer is measured on a single crystal gold electrode (Au(111)). The tBLM was further characterized using Atomic Force Microscopy (AFM). The tBLM was depsited on a substrate composed of Au(111) terraces, force-distance curves were measured using AFM, the thickness of the bilayer was than extracted from these curves. These results will be presented for tBLMs constructed with varying GM_1 content (10, 20, and 30 mole percent).

Platform I: DNA, RNA Structure & Conformation

98-Plat
Computational and Experimental Determination of the tRNA-like Structure in the 3′UTR of the Turnip Crinkle Virus (TCV)
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Turnip crinkle virus (TCV) is a plant virus, which is not capped or polyadenylated. Being one of the smallest plus strand viruses makes it a useful system for studying translation and transcription. Its 3′ proximal region, together with the 5′ UTR, enhances translation. We have employed our massively parallel genetic algorithm, MPGAfold, to predict the secondary structure of the 3′ terminal 195 nt region. Compensatory mutagenesis analyses in vivo and in-line structure probing confirmed the existence of the key predicted features (stem-loop motifs an one H-type pseudoknot) and added another pseudoknot to the model. Based on this information, we employed our 3D molecular modeling software, RNA2D3D, to predict the 3D structure of the core three hairpins and two pseudoknots. Our model structurally resembled a tRNA. Experimental

data verified this structure, thus establishing the existence of the first internal tRNA-like structure discovered. Molecular dynamics simulations showed the stability of the entire structure as well as flexibility of some of its elements, in agreement with the in-line structure probing data. The model with its structure-implied functionality has led to further experimental determination of how the interconnected 3′ UTR sequence and structure elements participate in the processes of translation and replication. The structure element was found to bind the 60S ribosomal subunit, and thus act as a translation enhancer. This is the first such interaction in a 3′ UTR with the large subunit discovered. We also found that this tRNA-like element is a major part of a structural switch determining if the viral template is translated or replicated. Similar mechanisms may exist in other viruses and genomes, as the structural elements similar to the tRNA-like structure in the TCV have also been identified in several viruses.

99-Plat

Pre-stressed Tensegrity Structures built from DNA

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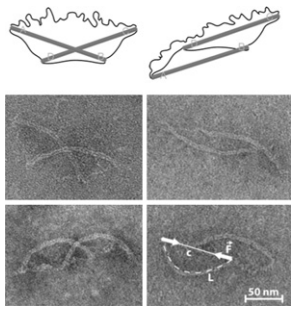
Tensegrity is a building principle that utilizes a network of tensed elements balanced by internal compression struts to create a self-equilibrated mechanical structure that requires tensile prestress for its mechanical stability. The concept of tensegrity has helped to understand the physical behavior of viruses, cells, tissues, organs and organisms [1] from the nano to the macro scale. This biological design principle also has been leveraged to construct artificial, static tensegrity structures using DNA nanotechnology approaches [2].

Here, we use the DNA origami approach [3] to engineer prestressed 2D and 3D nanoscale tensegrities assembled from compression-resistant DNA six-helix bundles connected by long sections of single-stranded DNA (several hundred bases long) that act as tensed entropic springs, which prestress the entire structure. The introduction of spring elements as building components for DNA nanostructures allows for the design of flexible nanostructures and nanoscopic lever spring balances. To prove the potential application of DNA tensegrity structures as force sensors and actuators, basic force experiments were performed with the 2D 'kite' structure.



1. Ingber, D.E., FASEB J. 20(7), 811-27, 2006.
2. Zhang et al., PNAS 105 (31), 10665-10669, 2008.
3. Rothemund, PWK, Nature 440, 7082, 287-302 2006.

100-Plat

The Role of Sequence-Dependent Mechanics in DNA Looping

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The formation of protein mediated DNA loops are a key component in many biological regulatory functions. The binding of LacI protein within the Lac Operon of E.coli serves as the canonical example in which loop regulated transcription is understood. This fundamental looping motif consists of one protein simultaneously bound to two DNA operator binding sites. We calculate the free energy cost of loop formation using a Hamiltonian constructed about the looped state as well as the intrinsically open state of the DNA. The shape of the inter-operator DNA loop in mechanical equilibrium with the protein, is determined using a non-linear mechanical rod model. Our rod model captures the effect of sequence dependent curvature, sequence dependent persistence length, including any anisotropic bending. The equilibrium DNA-protein binding orientations are inferred from LacI protein crystallized with DNA operator segments.

Our Hamiltonian describes the change in bending energy of the DNA due to linear perturbations about either the looped or open state. We now calculate the normal modes of the Hamiltonian in order to characterize thermal fluctuations of the loop. Comparing the change stiffness we then calculate the Stockmayer J-factor(looping probability), free energy as well as the entropic contributions, of loop formation. Our works shows that these entropic contributions can play a significant role in determining loop stability and formation.

101-Plat

Chromatin Organization in E.coli

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Prokaryotic organisms must strike a balance between DNA accessibility and condensation: facilitating the genetic processes of transcription, replication, and DNA repair while simultaneously enabling the structural and physical processes of chromosome condensation and segregation in rapidly dividing cells. Recent studies have revealed that prokaryotic chromosomes are intricately structured but the mechanism for this physical organization is not yet understood. In this study, we describe the construction of a large number of E.coli strains carrying three spectrally distinct, fluorescently labeled genetic loci. The origin and terminus of replication as well as a random position on the chromosome are labeled and tracked simultaneously in live cells. These labeled loci are identified, counted and tracked automatically using custom MATLAB software which enables the analysis of a large number of nucleoid conformations. Positions of the labeled loci are determined with respect to the cell body and used to compute both the distribution of locations of single loci within the cell (thus revealing "chromosome territories") as well as the correlations between fluctuations in the positioning of different loci. This data is analyzed in the context of a simple polymer model of the E.coli chromosome and it provides quantitative support for a nucleoid-centered mechanism of chromosome organization.

102-Plat

Structure and Dynamics of the Bacterial Chromosome

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Despite the great deal of studies on bacteria, and especially E coli, our understanding of the spatio-temporal organization of bacterial chromosomes is minimal, largely because their dynamics have been difficult to observe directly. Even more remarkable is bacteria's ability to control the topology of the 1.5 mm-long DNA in the confined environment of the cell. The objective of our project is to study dynamics of chromosome structure during the process of cell division at the single-cell level, in the bacterium E. coli. Using a unique microcolony growth technique, we track cell growth and with fluorescent-protein techniques we can monitor chromosome folding and quantify gene expression levels through fluorescence microscopy. We have developed a bacterial strain containing fluorescent gfp-fusion versions of a chromosome-folding protein, Fis, under inducible control. This strain is used to visualize bacterial chromosome conformation during cell growth and division. Our further objective will be quantitatively analyze the coupling of nucleoid protein level and chromosome folding to gene expression, and to directly examine nucleoid mechanical properties as a function of protein levels using micromanipulation methods.

103-Plat

DNA Strand Exchange on Liposome Surfaces

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The mechanism of DNA strand exchange, performed in vivo by proteins in the Rec A family, is despite extensive studies not understood in full detail. We therefore want to study the molecular parameters involved in the exchange using an in vitro platform. Positively charged liposomes have been widely used as non-viral gene-carriers, where the positive charges attract the negatively charged DNA. We here show that DNA strand exchange is significantly enhanced, both in rate and yield, on the surface of cationic liposomes.

We study the strand exchange reaction by adding an excess of non-labeled single strands to liposome bound DNA duplexes labeled with a FRET pair and monitor the exchange as an increase in donor emission. The fastest exchange is seen for liposomes containing 35% cationic lipid and when the positive charges on the outer leaflet of the liposomes equal the amount of negative charges on the added DNA. Both increasing and decreasing the amount of DNA slows down the exchange rate, either due to competition for the binding sites or due to that the single-strands on average are further away from the duplexes. By studying the exchange of mismatched DNA we conclude that the DNA opens in a ''zipper-like'' manner on the liposome surface, since a mismatched base-pair in the end of the sequence affects the exchange rate much more than a mismatch base-pair in the middle of the sequence. The liposome surface can be easily functionalized with lipids bearing specific functionalities and we intend to modify the surface to study the effects of hydrogen bonding and/or hydrophobic interactions.