triggers local Rac-GTP hydrolysis, thus reducing local actin polymerization required for filopodia formation. ArhGAP44 expression increases as the neurite network is established and the frequency of exploratory filopodia formation is diminished, suggesting that ArhGAP44 may facilitate the transition of neurons from a dynamic exploratory mode to a more mature static state, a hallmark of nervous system development. Together, our data reveals a local and receptor-independent auto-regulatory mechanism that limits initiation of exploratory filopodia in neurons via protein recruitment to nanoscale membrane deformations.

1238-Pos Board B189  
Role of Surface Tension in the Formation of Membrane Tubes  
Julian Hassinger1, George Oster2, Padmini Ranganami3  
1Biophysics, University of California Berkeley, Berkeley, CA, USA,  
2Molecular and Cell Biology, University of California Berkeley, Berkeley, CA, USA,  
3Mechanical and Aerospace Engineering, University of California, San Diego, La Jolla, CA, USA.

The formation of tubular structures is a fundamental morphological change that takes place in biological and reconstituted lipid membranes. Mechanical tension in biological membranes is thought to potentially regulate a number of cellular processes, including cell migration. Here, we explore the impact of surface tension on the formation of membrane tubes using elastic and viscoelastic continuum models of lipid bilayers. In the elastic framework, we demonstrate that application of a point load is sufficient to drive the formation of a tube from an initially flat patch of membrane which undergoes a tent-to-tube transition as a region of negative Gaussian curvature develops at the base of the tube. We generate force vs. displacement curves over several orders of magnitude in the surface tension that display a characteristic overshoot of approximately 13% in the force required to maintain a tube at constant length for all values of surface tension. Additionally, we observe a larger (smaller) linear deformation of the patch relative to the tube radius for a membrane under greater (lesser) tension. We also develop a viscoelastic framework that accounts for lipid flow on the membrane surface on a time scale set by the surface viscosity of the membrane. One key feature of this model is that it expressly allows for the local tension to vary as lipids flow within the plane of the membrane. Using this model we calculate lipid velocity as a function of curvature and local tension during tube formation. Additionally, we make comparisons between the force vs. displacement curves obtained from the viscoelastic model to those obtained via the elastic model.

1239-Pos Board B190  
Nanosystem Based on Phospholipids and Surfactants as Innovative Delivery System for Gene Therapy  
Michalina Skupin, Joanna Wolak, Maciej Kozak  
Department of Macromolecular Physics, Adam Mickiewicz University, Poznań, Poland.

Amphiphilic dicationic surfactants, known as Gemini surfactants, are currently studied for gene delivery purposes. The biggest advantages of these systems are that they are non-immunogenic and generally have low toxicity. One of the most important advantages of these systems is improved transfection efficiency. The aim of this study was to determine the possibility to use amphoterically surfactants (zwitterionic derivatives of sulfobetaine with carbohydrate moiety) and sulfobetaine/gemini surfactant mixtures as complexing agents for nucleic acids, with potential applications for gene delivery to reduce the toxicity and improved transfection. Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) were used to analyze influence of surfactants on the phase behaviour of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) bilayers with the presence of different DNA forms (small DNA oligomers, cDNA, low and high-molecular mass DNA).

The influence of different concentrations of sulfobetaine and sulfobetaine/gemini surfactant mixtures with the presence of DNA on creating stable complexes was investigated using circular dichroism (CD) spectroscopy and electrophoresis. A series of measurements of toxicity and transfection of these lipopolymers were performed in HeLa cells. These compounds appear to be excellent for creating complexes with DNA. Thanks to their construction, this DNA carrier molecules might be able to deliver genes to the cells of almost any DNA molecular size, unattainable when using viral gene delivery systems. The study was supported by research grant “GENERACJA PRZYSZŁOŚCI” from Ministry of Science and Higher Education (Poland) - decision: 12/POIG/GP/2013.
find that even small spontaneous curvatures have a very strong effect on the behavior of nanoparticles in contact with membranes. For a single nanoparticle interacting with a membrane, we predict four different stability regimes, leading to free, partially engulfed, or completely engulfed particles, as well as particles displaying bistability between the free and completely engulfed states. For the case of many nanoparticles in contact with a vesicle, we predict several distinct engulfment patterns, which should be observable in the optical microscope using fluorescently labeled particles. These patterns can be explored, e.g., by varying the particle size or the area-to-volume ratio of the vesicles.

1243-Pos Board B194 Membrane Fluidity in Cancer Cell Membranes as a Therapeutic Target: Validation using BPM 31510
Sumit Garg, Sirisha Dhavala, Katerina Krumova, Michael Kiebish, Vivek Vishnudas, Stephen Gesta, Ranaprasad Sarangarajan, Niren Narain, Berg LLC, Framingham, MA, USA.
Membranes in cancer cells are relatively more fluid compared to healthy cells. Higher membrane fluidity in cancer cells closely relates to their invasive potential, proliferation, and metastatic ability. Normalization of membrane fluidity in cancer cells represents a novel therapeutic modality, however, there are no strategies currently focused on targeting this modality as cancer therapeutics. This study describes the introduction BPM 31510, a proprietary CoQ10 based liposomal formulation that specifically targets cell membrane fluidity as one of the modalities influenced in cancer cell to effectuate a therapeutic end-point, i.e. decrease in cell proliferation. First, CoQ10 concentrations was systematically varied in the liposomal formulation and membrane rigidity protocols (dynamic light scattering) measured as function of temperature. Increasing concentrations of CoQ10 was associated with progressive and significant increase in rigidity of liposomal membranes followed by decrease at higher concentration. Interestingly, the concentration at which the local maxima in rigidity occurred matched with the composition of BPM 31510. Later, we demonstrate that BPM 31510 treatment temporarily increases cell membrane rigidity that orchestrates adaption in lipidome, proteome, and cell bioenergetics. To better understand differential response to BPM 31510, a spectrum of cancer and healthy cells were stratified based on intrinsic membrane rigidity, cell bioenergetics, and proliferation rates, and relative changes following treatment with BPM 31510 were compared. Collectively, the data provides novel insight into CoQ10 effect on cell membrane dynamics, suggesting an integration of biophysical, biochemical and molecular effects attributable to BPM 31510 mechanism of action in the treatment of cancer. Overall, the study provides compelling data in support of targeting of membrane fluidity, a biophysical characteristic of cell, as a novel target amenable to pharmacological manipulation in the treatment of cancer.

Biophysical Techniques for the Study of Protein-Lipid Interactions
1244-Pos Board B195 Using CW-EPR to Explore Substrate Binding and the Mechanism of TonB-Dependent Transport in BtuB
1Chemistry, University of Virginia, Charlottesville, VA, USA, 2Institute of Physical and Theoretical Chemistry, Frankfurt, Germany.
Outer-membrane TonB-dependent transporters function in the uptake of essential nutrients, and are important for the success of many pathogenic bacteria. These proteins consist of a 22 stranded β-barrel where the N-terminal 130 to 150 residues form a core domain that fills the barrel. During transport, these proteins undergo a cycle of binding and unbinding to the inner membrane protein TonB, through an interaction that is mediated by the Ton box, an energy-coupling segment near the transporter N-terminus. Over 30 high-resolution crystal structures have been obtained for 12 different TonB-dependent transporters, however the mechanisms of substrate transport remain unclear. During the coupling of the Ton box to TonB, transport is thought to involve a transient unfolding or rearrangement of the N-terminal core promoting the release of the substrate to the periplasm. Utilizing a combination of site-directed spin labeling (SDSL) and chemical denaturation we have examined the thermal stability of the core domain in the Escherichia coli vitamin B12 transporter, BtuB, as well as the thermodynamic and kinetic behavior of the substrate. The data indicate that core unfolding in a series of steps and that substrate, which alters the stability of the Ton box, also alters the thermal stability of the core. Pulse EPR methods are being used to determine the steps that occur during transport and to determine the position and binding sites for the substrate within the transporter. This work was supported by NIGMS, GM035215.

1245-Pos Board B196 Cytoplasmic Domain of Dengue Virus Protein NS4A Preferentially binds Highly Curved Membranes
Yu-Fu Hung1,2, Melanie Schwarten1, Silke Hoffmann1, Dieter Willbold1,2, Ella H. Sklan1, Bernd W. Koening1,2.
1ICS-6: Structural Biochemistry, Forschungszentrum Jülich, Jülich, Germany, 2Institut für Physikalische Biologie, Heinrich-Heine Universität, Düsseldorf, Germany, 3Clinical Immunology & Microbiology, Tel Aviv University, Tel Aviv, Israel.
Dengue virus (DENV) is a mosquito-transmitted virus that causes dengue fever, dengue hemorrhagic fever and dengue shock syndrome. There is no vaccine available against DENV and no specific treatment for dengue fever. DENV is believed to replicate its RNA genome in association with modified intracellular membranes. DENV non-structural protein 4A (NS4A) has been implicated in the formation of the viral RNA replication complex (RC). However, the details of RC assembly are incompletely understood. We have previously identified a conserved region in the N-terminal 48 amino acids of NS4A containing putative amphipathic helices (AH). Mutations (L6E; M10E) designed to reduce the amphipathic character of the predicted AH abolished viral replication and reduced NS4A oligomerization. [1] Solution state NMR spectroscopy was used to study the structure of recombinant wild type NS4A a.a. 1-48 peptide and a double mutant NS4A(1-48, L6E; M10E) in presence of pH 8 SDS micelles. The peptides are basically unstructured in aqueous buffer. However, two α-helical segments separated by a non-helical linker are observed for both peptides in presence of SDS micelles. Addition of liposomes induced formation of α-helical secondary structure in the wild type NS4A(1-48) but not in the mutant peptide. We used surface plasmon resonance, flotation assays, and circular dichroism spectroscopy to analyze the binding of recombinant NS4A(1-48) peptides to liposomes. We found that NS4A(1-48) binds to liposomes in a membrane curvature-dependent manner. The AH mutations reduced the affinity of NS4A(1-48) for lipid membranes. These results suggest that the two AHs in the N-terminus of NS4A may be crucial for membrane binding, curvature sensing and stabilization. Better understanding of the molecular details of the DENV RC formation might lead to novel anti-DENV strategies.[1] O. Stern et al. (2013) J. Virol. 87:4080-85