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Lipid Membrane Domains Promote In-Vitro Presynapse Formation

Gopakumar Gopalakrishnan, Patricia T. Yam, Isabelle Rouiller, David R. Colman, R. Bruce Lennox.

Regeneration of damaged neurons has been one of the main challenges in the attempt to cure brain damages occur from neurodegenerative diseases and head injuries. Successful formation of functional synapses on artificial substrates is a very important step in the development of engineered *in vitro* neural networks. We have recently shown that spherical supported bilayer lipid membranes (SS-BLMs) can be used as a novel substrate to achieve presynaptic accumulation at an *in vitro* synaptic junction.[1] The results indicate that lipid membrane domains may play a role in the observed phenomenon, in addition to the chemical and electrostatic interactions between the neurons and SS-BLMs.[2] With the help of domain specific fluorescent dyes, micron-sized lipid phase domains were specifically labeled on Giant Unilamellar Vesicles (GUVs) and on silica beads. Experiments, *in vitro*, clearly show that specific membrane domains play a role in the synapse formation. These results as well as Cryo-TEM based molecular level confirmation of presynapse formation on artificial substrates will be presented.The aspect of lipid membrane domains in synapse formation is key in developing many neuroengineering approaches to design functional artificial synapse formation as well as for synaptogenesis studies *in vivo*.

1. Lipid Bilayer Membrane- Triggered Presynaptic Vesicle Assembly'' Gopakumar Gopalakrishnan,Peter Thostrup, Isabelle Rouiller, Anna Lisa Lucido, Wiam Belkaïd, David R. Colman, and R. Bruce Lennox,*ACS Chem. Neurosci.***2010**, *1*, 86-94

2. Rapid Assembly of Functional Presynaptic Boutons Triggered by Adhesive Contacts'' Anna Lisa Lucido, Fernando Suarez Sanchez, Peter Thostrup, Adam V. Kwiatkowski, Sergio Leal-Oritz, Gopakumar Gopalakrishnan, D. Liazoghli, Wiam Belkaid, R. Bruce Lennox, Peter Grutter, Craig C. Garner, and David R. Colman *J. Neurosci.***2009**, *29*, 12449-12466

2755-Pos Board B741

Electroformation of Super-Giant Unilamellar Vesicles Containing Cationic Lipids

Christoph Herold, Petra Schwille, Eugene P. Petrov.

Super-giant unilamellar vesicles (SGUVs) with sizes exceeding 100 μm and thus a negligible curvature on the scale of tens of microns represent a convenient free-standing lipid bilayer system. In particular, SGUVs proved to be very useful in studies of dynamics and conformation of macromolecules interacting with the freestanding membrane by means of single molecule fluorescence microscopy [1]. Electroformation on indium tin oxide (ITO) coated glass slides is a standard method to produce giant unilamellar vesicles (GUVs) [2]. When applied to lipid mixtures containing cationic lipids (e.g. DOTAP, EDOPC, etc.), the standard electroformation method frequently produces GUVs with sizes not exceeding 10-20 μm, which are additionally surrounded by a dense network of lipid tubules. We demonstrate that annealing of ITO slides at t ~ 150 °C before the electroformation procedure allows one to reliably produce samples containing cationic SGUVs with diameters of 100-300 μm not contaminated by lipid tubular structures.

[1] C. Herold, P. Schwille and E.P. Petrov, Phys. Rev. Lett. 104, 148102 (2010).

[2] M. I. Angelova, S. Soleau, P. Meleard, J. F. Faucon and P. Bothorel, Prog. Colloid Polym. Sci. 89, 127 (1992).

2756-Pos Board B742

Phase Separation and Near-Critical Fluctuations in Two-Component Lipid Membranes: Monte Carlo Simulations on Experimentally Relevant Scales

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By means of lattice-based Monte Carlo simulations, we address properties of two-component lipid membranes on the experimentally relevant spatial scales of order of a micrometer and time intervals of order of a second, using DMPC/DSPC lipid mixtures as a model system. Our large-scale simulations allowed us to obtain important results previously not reported in simulation studies of lipid membranes. We find that, within a certain range of lipid compositions, the phase transition from the fluid phase to the fluid-gel phase coexistence proceeds via near-critical fluctuations, while for other lipid compositions this phase transition has a quasi-abrupt character [1, 2]. The line tension characterizing lipid domains in the fluid-gel coexistence region is found to be ~ 2 pN. When approaching the critical point, the line tension, the inverse correlation length of fluid-gel spatial fluctuations, and the corresponding inverse order parameter susceptibility of the membrane vanish [2]. All these results are in agreement with recent experimental findings for model lipid membranes. We observe transient subdiffusive behavior of lipids in the presence of near-critical fluctuations, which is a new result important for understanding the origins of subdiffusion in cell membranes. The effects of the interaction of the membrane with the cytoskeleton will be discussed as well.

[1] J. Ehrig, E. P. Petrov, and P. Schwille, arXiv:1010.1207.

[2] J. Ehrig, E. P. Petrov, and P. Schwille, arXiv:1009.4860.

2757-Pos Board B743

A study on the Mechanisms of Amphotericin B Pore Formation

Javier Iñiguez-Stahl, Iván Ortega-Blake, Armando Antillón-Díaz.

After more than fifty years since it was first used as an anti fungal drug, Amphotericin B is still the drug of choice for the treatment of systemic fungal infections. Late research about this drug consist essentially in pharmacological tests or *in-vivo* studies, as well as the development of new derivatives and mechanisms of drug administration. Nevertheless, all this studies are made without having certainty on the mechanism of action at a cellular level, and a detailed description of its structure. Previous results determined the number of molecules involved in the different types of channels, using the equation $g=a[AmB]^n$. These conductances were determined by single channel studies and showed that the most accepted mode of action of the drug, which consists in a barrel-like structure, needs a more complex arrangement to reproduce the experimental results. These results also suggest the existence of a lower conductance AmB channel. Low noise experiments and the development of a new software to analyze the experimental data shows the existence of this lower conductance channel and allows the addition of more experimental data to test the model proposed previously. This results were also used to test new models of AmB channel which require an extra degree of freedom for the molecules involved in the formation of the pore.

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Oligonucleotide-Based Amphiphilic Block Copolymers Interacting with Cell Membrane Models at the Air-Water Interface

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Oligonucleotides have exceptional molecular recognition properties, which are involved in biological mechanisms such as gene silencing or cell-surface receptor recognition. In many cases, the cell membrane structure is barrier to investigate their use in human therapy for drug or gene delivery. However, this can be obviated by grafting a hydrophobic tail to the oligonucleotide. In this present work, we demonstrate that two oligonucleotides, one consisting of 12 guanosine units (G12), and the other one consisting of five adenosine and seven guanosine (A5G7) units, when functionalized with poly(butadiene), namely PB-G12 and PB-A5G7, can be incorporated into Langmuir monolayers of dipalmitoyl phosphatidyl choline (DPPC), which served as a cell membrane model. With surface pressure and surface potential-area isotherm, we observed that PB-G12 and PB-A5G7 affect the DPPC monolayer, even at high values of surface pressure. The effects from PB-G12 were consistently stronger, particularly in reducing the surface compressibility of the DPPC monolayers, which may have important biological implications. Multilayers of DPPC and nucleotide-based copolymers could be transferred onto solid supports, in the form of Y-type Langmuir-Blodgett films, in which the molecular-level interactions led to lower energies in the vibrational spectra of the nucleotide-based copolymers. The successful deposition of solid films opens the way for devices to be produced which exploit the molecular recognition properties of the nucleotides.

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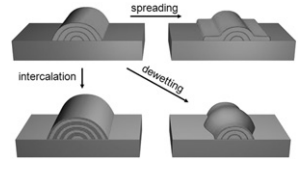
Adhesion of Lipid Multilayer Micro- and Nano-Structures Fabricated by Dip-Pen Nanolithography

Steven Lenhert.

Cellular and membrane adhesion in biology is a highly complex and ubiquitous process, where specific and non-specific interactions mediate biological functions. Model systems such as liposomes can provide a means of testing biophysical hypotheses. Dip-pen nanolithography (DPN) is a promising method of printing lipid droplets onto solid surfaces at length scales relevant to biological cells, and can be carried out in parallel and with high throughput. Phospholipids are a particularly useful as inks for DPN because of their physicochemical properties as well as innate biofunctionality.(1-3)

Here the dynamic adhesion properties of lipid multilayer structures fabricated by DPN is studied. Short-timescale kinetics of lipid spreading and other shape changes within the micro-and nanostructured lipid multilayers is studied. By forming the lipid multilayers into optical diffraction gratings, a label-free read out and biosensing mechanism is developed based on these shape changes within the fluid and adherent lipid multilayers.(4)



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