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Structure Analysis of Synaptic Vesicles by Solution Small-Angle Scattering of X-Rays

Simon Castorph[1], Matthew Holt[2], Dietmar Riedel[2], Lise Arleth[3], Michael Sztucki[4], Reinhard Jahn[2], Tim Salditt[1].
[1]Universität Göttingen, Göttingen, Germany, [2]Max Plack Institut für Biophysikalische Chemie, Göttingen, Germany, [3]University of Copenhagen, Frederiksberg, Denmark, [4]European Synchrotron Radiation Facility, Grenoble, France.

The release of neurotransmitters from neurons, in response to stimulation, forms the basis of communication in the nervous system. Neurotransmitters are stored in small membraneous organelles, synaptic vesicles (SVs), within the presynaptic terminal. These vesicles undergo an elaborate cycle of fusion with the plasma membrane (releasing neurotransmitter), followed by retrieval and reformation and transport back to the plasma membrane for further rounds of fusion [1].

In recent years there has been enormous progress in our knowledge of the molecular composition and structure of synaptic vesicles [2]. However, we still lack a detailed view of the physical properties of this trafficking organelle as it proceeds through its life-cycle.

Here we use small-angle x-ray scattering (SAXS) to determine the average radial density profile rho(r) and the size polydispersity of SVs [3]. We show that SAXS can be used to study the supra-molecular structure of an entire functional organelle under physiological conditions. The profile rho(r) of SVs including structural parameters of the protein layers, as well as the polydispersity function p(R), are derived with no free prefactors on an absolute scale. The measured SV structure on length scales between the constituent biomolecules and the SV size confirms the main aspects of recent numerical modeling [2], which was based on the crystal structures of the constituent proteins and stoichiometric knowledge from biochemical studies. In addition, we present first evidence of a laterally anisotropic structure, indicative of larger protein clusters.

[1] T. Südhof, Annu. Rev. Neurosci. **27** (2004) 509.
[2] S. Takamori *et al,* Cell **127** (2006) 831.
[3] S. Castorph *et al,* in preparation.

1486-Pos

Implications of Criticality in Membrane Bound Processes

Benjamin B. Machta, Sarah Veatch, Stefanos Papanikolaou, James Sethna.
Cornell, Ithaca, NY, USA.

Recent work in giant plasma membrane vesicles (GPMVs) isolated from living cells demonstrates that these GPMVs can be tuned with a single parameter (temperature) to criticality, not far from in vivo temperatures [1,2]. Criticality requires the fine-tuning of two parameters suggesting important biological function, and its presence resolves many of the paradoxes associated with putative lipid rafts. Here we present a minimal model of membrane inhomogeneities. We incorporate criticality using a conserved order parameter Ising model coupled to a simple actin cytoskeleton interacting through fields which act as point-like pinning sites. Using this model we make a host of experimentally testable predictions that are in line with recent published findings. At low temperatures our 'actin' fields prevent macroscopic phase separation from developing. At physiological temperatures we find inhomogeneities in the form of critical fluctuations with a length scale of roughly 20nm. Individual constituents making up these liquid domains are mobile, but the correlated regions themselves can last as long as the cytoskeleton persists. We predict anomalous diffusion of components which strongly segregate into either phase, with a length-scale given by the size of actin compartments. In addition we predict that the instantaneous shapes of the correlated regions will be fractal on short distances and can conform to and feel the effects of the cytoskeleton at larger distances without positing any further superstructures. This is explained by an effective long ranged interaction mediated by the Ising order parameter. In general we find Ising criticality organizes and spatially segregates membrane components by providing a channel for interaction over large distances.

[1] Veatch et al., ACS Chem Biol. 2008 3(5):287-93
[2] Honerkamp-Smith, Veatch, and Keller, Biochim Biophys Acta. 2008 (in press)

1487-Pos

Structure and Dynamics of Lipid-Modified Antimicrobial Peptides in Anionic and Zwitterionic Membranes Investigated by Solid-State NMR

Holger Scheidt[1,2], Alexander Vogel[1].
[1]Martin Luther University Halle-Wittenberg, Halle, Germany, [2]University of Leipzig, Leipzig, Germany.

The increasing prevalence of antibiotic resistant strains of bacteria necessitates the development of new antibiotic drugs, preferably operating via novel pathways to avoid cross-resistance with drugs already in use. The group of Shai and coworkers has recently proposed a new set of very short lipid-modified antimicrobial peptides showing promising properties for possible application. We investigated two of these peptides, C16-KGGK and C16-KAAK in two different lipid environments, one more resembling mammalian membranes (POPC) and the other closer to bacterial membranes (POPE/POPG 2:1). Investigations were conducted on powder-type samples at a lipid/peptide ratio of 9:1 and a temperature of 303K. First, the host membranes were investigated using ^{31}P solid-state NMR clearly showing no influence of the peptides on the lamellar membrane phase state. Information about the chain dynamics and membrane packing properties was obtained using ^{2}H solid-state NMR. Order parameters of the lipids were slightly reduced upon addition of the peptide. However, the lipid modifications generally exhibit higher order parameters than the surrounding lipids meaning that the length of the peptide lipid modifications is larger than that of the lipid acyl chains. This is in agreement with ^{1}H NMR NOESY data exhibiting interactions between amino acid side chains and phospholipids suggesting a peptide backbone location in the headgroup region of the membrane. The dynamics of the lipid modifications were investigated by means of ^{2}H R_{1Z} relaxation rates. While other lipid-modified peptides exhibit square law plots that are bent the ones obtained for the antimicrobial peptides are linear and resemble that of saturated lipids. Therefore the lipid modifications of the antimicrobial peptides are less flexible and longer than that of other lipid-modified peptides allowing the peptide backbone to be located in the lipid headgroup region.

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Curvature Sensing by the Epsin N-terminal Homology (ENTH) Domain Measured on Cylindrical Lipid Membrane Tethers

Benjamin R. Capraro[1], Michael C. Heinrich[1], Youngdae Yoon[2], Wonwha Cho[2], Tobias Baumgart[1].
[1]Department of Chemistry, University of Pennsylvania, Philadelphia, PA, USA, [2]Department of Chemistry, University of Illinois at Chicago, Chicago, IL, USA.

The protein epsin is believed to be involved in generating the high membrane curvature necessary for vesicle formation in clathrin-mediated endocytosis. To assess the hypothesis that membrane curvature-dependent binding underlies this function, we quantify the curvature dependence of the area density of the epsin ENTH domain bound to cylindrical membranes of adjustable curvature. By fluorescence microscopy, we observe curvature-induced repartitioning of membrane-bound ENTH between flat and highly curved membranes, in cylindrical tethers pulled from micropipette-aspirated giant unilamellar vesicles. We analyze our measurements using a thermodynamic theory and determine the first Leibler curvature-composition coupling coefficient to be reported for an endocytic accessory protein. Thus our results clearly demonstrate and quantify the curvature sensing of epsin.

We believe our method will prove useful generally in relating molecular interactions to macroscopic cell membrane remodeling.

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Correlation Function Analysis Corrects Artifactual Self-Clustering and Reveals Significant Co-Localization of Fceri and Lyn in Resting RBL-2H3 Mast Cells

Sarah Veatch, Ethan Chiang, David Holowka, Barbara Baird.
Cornell University, Ithaca, NY, USA.

We use pair auto- and cross-correlation functions to quantify lateral heterogeneity within the plasma membranes of intact RBL-2H3 mast cells. 5nm and 10nm gold-antibody conjugates are used to specifically label plasma membrane proteins and lipids, and these are visualized using scanning electron microscopy with backscatter detection. An automated image-processing algorithm identifies positions of gold particle centers, enabling the processing of large datasets with high particle densities. Consistent with previous studies, we find that gold particles labeling a variety of plasma membrane lipids and proteins are highly self-clustered in resting cells. In contrast to previous studies, we find that this apparent self-clustering can be accounted for by multiple gold particles binding to single target proteins with Gaussian-shaped binding surfaces. This is demonstrated by imaging antibodies covalently conjugated to a silicon surface, by comparing correlation functions for a wide range of cell surface labels with varying surface densities, and by measuring cross-correlations between identical but distinguishable pools of either IgE-FcεRI or GM1 labeled with cholera toxin B on the cell surface. After correcting for artifactual clustering, we find that all (>5) proteins and lipids examined are not auto-correlated in resting cells at physiological temperatures, within experimental error bounds. In contrast, we find significant cross-correlation between IgE-FcεRI and the inner leaflet signaling protein Lyn in these unstimulated cells, and this co-clustering is only moderately modulated when membrane cholesterol levels are altered with

MβCD. Significant large-scale self-clustering of IgE-FcεRI occurs upon cross-linking with multivalent antigen, and we describe analytical methods to correct for multiple gold binding and quantify clustering in these stimulated cells. Our correlation function particle distribution approach is likely to have wide applicability in nanoscale image analysis.

1490-Pos

Design of a Biologically Relevant Supported Bilayer Platform for the Study of Membrane Active Peptides

Janice Lin, John Szymanski, Peter C. Searson, Kalina Hristova.
The Johns Hopkins University, Baltimore, MD, USA.

Membrane active peptides represent a class of soluble proteins that interact and disrupt the plasma membrane. Examples of these include antimicrobial peptides, cancer therapeutics, and cell-penetrating peptides. These peptides are amphipathic and, in a concentration dependent manner, can self assemble to destabilize the lipid bilayer. These peptides are rich in positively charged lysine and arginine residues and thus have a strong preference for negatively charged bilayers. In order to study the insertion mechanism and kinetics of these peptides, we have designed a negatively charged, supported bilayer platform on silicon. The negative charge serves to electrostatically drive peptides to bind to the lipid bilayer interface. Furthermore, this platform is electrically addressable through electrochemical impedance spectroscopy, which yields bilayer resistance, thickness, and structural heterogeneity data. This platform consists of an asymmetrical bilayer with 10 mol% negatively charged POPS, cholesterol, and POPC in the upper leaflet and DPhPC lipids in the lower leaflet, all supported by a PEG cushion on a silicon wafer. Resistances up to $2*10^4$ $\text{O}\eta\mu$ cm^2 and capacitances of 0.8 υF cm^{-2} have been measured for the platform. The high resistance allows for high accuracy in the detection of the activity of membrane active peptides of interest.

1491-Pos

***Staphylococcus aureus* Enriched in Ordered Lipids Present Resistance Towards the Antibacterial Agent sPLA$_2$-IIA: An Unusual Mechanism to Survive**

Hector Jackson Ocampo Ariza, Johanna Paola, Chavez Escobar,
Jorge L. Romero Becerra, Martha J. Vives, Chad Leidy.
Universidad de los Andes, Bogota, Colombia.

Bacterial membranes present solid-ordered/liquid-disordered (*so/ld*) cooperative melting event close to physiological temperature. The cellular advantage of this thermotropic melting event is yet to be determined. We show that this thermal behavior provides resistance towards a membrane active antibacterial agent.

Phospholipase A_2 type IIA (sPLA$_2$-IIA) is a hydrolytic enzyme which presents antibacterial properties towards Gram positive bacteria. The enzyme has higher activity in *ld* phase compared to *so* phase in anionic membranes. We show that the lipid phase behavior of Staphylococcus aureus (*S.aureus*) membranes as measured by FTIR modulates sPLA$_2$-IIA by inducing a sharp drop in activity below the melting temperature of the membrane (centered at 15.3°C).

The effects of sPLA$_2$-IIA treatment on cell viability are also investigated. While above the main melting event viability drops to 20% of the initial CFU after treatment, below the main melting event cell viability only drops to 60% under the same treatment. This strongly suggests that cells in the solid-ordered phase are better adapted to survive the enzymatic insult.

These results led us to explore if a subpopulation of *S.aureus* enriched in ordered lipids can be selected after repeated treatment with sPLA$_2$-IIA at 37°C. After selecting for resistance at 37°C we measured growth curves, membrane order, and cell viability as a function of treatment temperature. The results suggest that at 37°C there is a bacterial subpopulation with increased membrane rigidity that insures survival of the colony to an insult by sPLA$_2$-IIA. This subpopulation also presents a longer latency period which can be explained by the increased presence in ordered lipids, which are known to inhibit cell division. Even if the growth conditions are not optimal, the presence of this subpopulation ensures survival from the antibacterial insult.

1492-Pos

Lifetime of Hyaluronan Containing Tethers Obeys a Generalized Bell Model

Marius C. Staiculescu[1], Phillip Stein[1], Mingzhai Sun[2], Imre Derenyi[3], Gabor Forgacs[1].
[1]University of Missouri Columbia, Columbia, MO, USA, [2]Lewis-Sigler Institute for Integrative Genomics, Princeton, Princeton, NJ, USA, [3]Department of Biological Physics, Eotvos Roland University, Budapest, Hungary.

Hyaluronan (HA), an unbranched non-sulfated glycosaminoglycan, is an important component of the extracellular and pericellular matrix of various cell types. It has key roles in many biological processes such as wound healing, angiogenesis, embryonic development, tumor progression and invasion. HA is especially abundant around tumor cells in malignant gliomas where it is associated with high invasivity and a poor prognosis. However it is unknown how malignancy is correlated with the biomechanical properties of the cellular glycocalyx and the lifetime of the chemical bonds formed by HA with its ligands. Here we introduce a method applicable to the study of biophysical properties of cellular glycocalyx through tether extraction. Specifically, we reveal the extent of the cellular ECM of a glioma cell line (HB), we demonstrate that tethers formed through non specific binding can be pulled from the cellular glycocalyx and by using a magnetic tweezers we determine the lifetime of these tethers. To calculate lifetime we simultaneously extract multiple tethers under constant force using paramagnetic beads as force transducer. We demonstrate that the stochastic lifetimes of these tethers and thus the bonds they are associated with are exponentially distributed and can be parametrized by a generalized Bell model. We determine the maximum likelihood estimates of the relevant parameters, such as force-free dissociation constant and reactive compliance. We test the consistency of our approach using computer simulations. This method could be employed in the development of therapies which interfere with HA organization and HA-receptor binding.

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Analytical Derivation of Thermodynamic Properties of Bolalipid Membrabe

Sergei I. Mukhin, Boris B. Kheyfets.
Moscow Institute for Steel and Alloys, Moscow, Russian Federation.

A model of bilayer lipid membrane with bola-lipids is studied. The bola-lipid is modeled by linking tails of the hydrophobic chains in the opposite monolayers within bilayer. We use for analytical derivations a flexible string model of hydrocarbon chain (Mukhin, Baoukina 2005) with modified condition at the linked chains ends. Calculated lateral pressure profiles are asymmetrical due to different concentrations of the U-shaped bolalipids in the opposite monolayers, Fig. 1, and orientational order parameters for linked and regular chains differ significantly at the monolayers interface, Fig. 2.
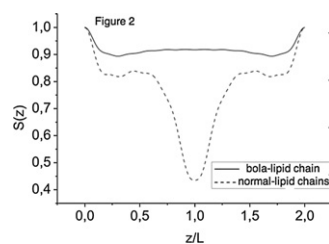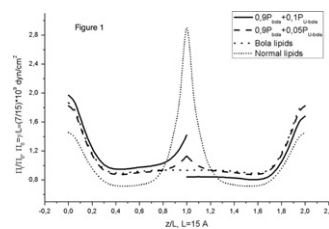
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Highly Stable Poly(Lipid) Bilayers for Long-Term Ion Channel Recordings

Benjamin A. Heitz, Robert P. Cordero, S. Scott Saavedra,
Craig A. Aspinwall.
University of Arizona, Tucson, AZ, USA.

Long-term ion channel (IC) screening using cell-based assays is currently limited by throughput and cell to cell variability. ICs isolated and reconstituted into suspended lipid membranes offer an isolated view into IC structure and function, but IC recordings are limited by the short lifetime of the bilayer. Polymerizable lipids (poly(lipids)) offers one potential strategy for long-term