1485-Pos
Structure Analysis of Synaptic Vesicles by Solution Small-Angle Scattering of X-Rays
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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
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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
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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.

1488-Pos
Curvature Sensing by the Epsin N-terminal Homology (ENTH) Domain Measured on Cylindrical Lipid Membrane Tethers
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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.

1489-Pos
Correlation Function Analysis Corrects Artifactual Self-Clustering and Reveals Significant Co-Localization of Fceri and Lyn in Resting RBL-2H3 Mast Cells
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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