Binding of Neurotransmitters to Lipid Membranes

Peters, Günther H.J.; Werge, Mikkel; Elf-Lind, Maria Northved; Wang, Chunhua; Cruys-Bagger, Nicolaj; Velardez, Gustavo; Madsen, Jesper Jonasson; Westh, Peter

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Microvesicles (MVs) are cell-derived membrane fragments which are present in plasma and other body fluids. In plasma, MVs participate in physiological processes of hemostasis and inflammation. MVs contain cell-specific molecules and are present at elevated levels in various diseases, which has raised the hypothesis of their potential application as disease biomarkers. The characterization of MVs is however hampered by their small size, estimated from 50 nm to 1 μm, and by limitations of the methods currently used for their analysis. Our aim is to provide a comprehensive description of MVs from plasma of healthy individuals and answer basic questions concerning MVs: 1) What do MVs from plasma look like? 2) What is their size distribution? 3) How many of these MVs derive from erythrocytes?, from platelets? or 5) What is their concentration? By combining cryo-Electron Microscopy (EM) and receptor-specific gold labeling, the morphology, size and phenotype of MVs from normal plasma were characterized. MVs present three morphologies, consisting of spherical vesicles, of 40 nm – 1 μm in diameter, tubular vesicles, of 1-5 μm in length, and large membrane fragments, 1-8 μm wide. The sub-population of pro-coagulant MVs that we identified by labeling with Annexin-A5-conjugated gold nanoparticles, was found to form a majority of MVs, about 25%, in contrast with the current theory of MV formation. MVs derived from the main blood cell populations, erythrocytes, platelets and leukocytes, were identified by immuno-gold labeling. Finally, concentrations of MVs were determined by a novel quantitative approach based on MV sedimentation on EM grids.

This study (in revision) provides a detailed description of MVs from normal plasma, novel insights on mechanisms of MV formation, and will serve as a reference for further studies of MVs in pathological situations.

**2284-Plat**

**Area Per Lipid of Membranes from Natural Abundance Solid-State 13C NMR Spectroscopy**

Trivikram R. Molugu1, Avigdor Leftin1, Constantin Job1, Michael F. Brown1-2.

1Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ, USA, 2Department of Physics, University of Arizona, Tucson, AZ, USA.

The properties of liquid-crystalline membranes vary according to the molecular composition of the lipid bilayer [1]. Structural investigations of lipid membranes by NMR spectroscopy generally require isotopic labeling of the lipids, thereby precluding investigations of complex lipid systems. Combining cryo-electron microscopy (EM) and receptor-specific gold labeling, the morphology, size and phenotype of MVs from normal plasma were characterized. MVs present three morphologies, consisting of spherical vesicles, of 40 nm – 1 μm in diameter, tubular vesicles, of 1-5 μm in length, and large membrane fragments, 1-8 μm wide. The sub-population of pro-coagulant MVs that we identified by labeling with Annexin-A5-conjugated gold nanoparticles, was found to form a majority of MVs, about 25%, in contrast with the current theory of MV formation. MVs derived from the main blood cell populations, erythrocytes, platelets and leukocytes, were identified by immuno-gold labeling. Finally, concentrations of MVs were determined by a novel quantitative approach based on MV sedimentation on EM grids.

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**2285-Plat**

**Binding of Neurotransmitters to Lipid Membranes**

Günther H. Peters1, Mikkel Werge1, Maria N. Elf-Lind1, Chunhua Wang2, Nicolaj Crøys-Bagger1, Gustavo F. Velardez3, Jesper J. Madsen1, Peter Westh1.

1Department of Chemistry, Technical University of Denmark, Kgs. Lyngby, Denmark, 2NSM, Research Unit for Functional Biomaterials, Roskilde University, Roskilde, Denmark.

We have performed a series of thermodynamic measurements and molecular dynamics (MD) simulations to study the interactions between the neurotransmitters (NTs) 5-hydroxytryptamine (5-HT), γ-aminobutyrate (GABA), glycine (GLY), acetylcholine (ACH) and glutamate (GLU) as well as the amidated / acetylated γ-aminobutyrate (GABA™) with a dipalmitoylphosphatidylcholine (DPPC) bilayer. This study was motivated by recent research results that suggested that neural transmission may also be affected by nonspecific interactions of NTs with the lipid matrix of the synaptic membrane. Our results revealed that dependent on the nature of NTs, some of the NTs penetrate into the bilayer. We found that membrane affinity can be ranked with increasing affinity as follows: ACH > GLU < GABA < GLY < GABA™< 5-HT. The latter three penetrated the bilayer at most with the deepest location being close to the glycerol backbone of the phospholipids. It is surprising that hydrophilic solutes can deeply penetrate into the membrane pointing to the fact that membrane affinity is governed by specific interactions. Our MD simulations identified the salt-bridge between the primary amine of NTs and the lipid phosphate group as the most important interaction by which the NTs are anchored to the membrane.

These distinctive interactions could be related to nonspecific effects of these neurotransmitters and could point to a bilayer-mediated modulation of nerve transmission. However, due to the strong variability in affinity observed for the different NTs, this attraction is not an inherent property of all neurotransmitters.

**2286-Plat**

**In Silico Studies of Asymmetric Membranes Perturbations Caused by Dynamic Aggregation of a Cell-Penetrating Peptide**

Jean Helie1, Mickael Lelimouins1, Charlotte M. Deane1, Francesca Milletti2, Mark S.P. Sansom1.

1Oxford University, Oxford, United Kingdom, 2Roche, Nutley, NJ, USA.

Membrane active peptides are therapeutically relevant for a variety of purposes. However a better understanding of their mechanisms of interaction with lipid bilayers is needed in order to maximise both efficiency and selectivity. In the case of cell-penetrating peptides (CPP) it is particularly important to avoid membrane toxicity while maintaining translocation across the plasma membrane. Experimental in vitro studies based on light microscopy and dye release have shown CPP can be internalised via both endocytic and energy-independent pathways. However, uncertainties remain concerning the mechanisms involved in membrane translocation and perturbation. In silico studies using molecular dynamics (MD) simulations have hitherto mainly focused on the interactions of peptides with relatively simple lipid bilayer models. Here we present coarse-grained simulations of the interactions between transportan, a CPP known to perturb cell membranes, and large bilayers with biologically relevant lipid composition. We observe that transportan forms dynamic, unstructured and transient clusters that catalyse the formation of local defects such as bilayer thinning, lipid redistribution and decrease of the lipid tail order. We present a novel analytic approach which shows that the extent of the membrane perturbations induced by CPP clusters depends on their size and varies in time. In particular, anionic lipid flip-flops are consistently observed above a certain cluster size. The importance of asymmetric lipid composition in the bilayer is also investigated and found to impact the stability of the peptide aggregate. We also apply our approach to extended bilayer systems that contain approximately 50,000 lipids and hundreds of transportan peptides and thus allow comparison with high-resolution light microscopy results.