

2283-Plat**Microvesicles : What's Plasma Made of?**

Alain R. Brisson, Nicolas Arraud, Romain Linares, Sisareuth Tan, Celine Gounou.

University of Bordeaux, Pessac, France.

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 μ m, 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 them expose phosphatidylserine (PS)?; 4) How many of them 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 μ m in diameter, tubular vesicles, of 1-5 μ m in length, and large membrane fragments, 1-8 μ m wide. The sub-population of pro-coagulant MVs that expose PS, identified by labeling with Annexin-A5-conjugated gold nanoparticles, was found to form a minority 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 Jobl¹, Michael F. Brown^{1,2}.

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 using NMR spectroscopy generally require isotopic labeling of the lipids, thereby precluding investigations of complex lipid systems. Combining natural-abundance solid-state ¹³C magic-angle-spinning (MAS) NMR with a statistical mean torque (MT) model [2] enabled us to obtain the average area per lipid $\langle A \rangle$ and volumetric bilayer thickness D_B for lipid bilayers. The 2D separated local field (SLF) NMR experiment DROSS gives isotropic chemical shifts in the F2 frequency dimension and site-specific dipolar Pake doublets in the F1 dimension [1]. By analyzing the Pake patterns we extracted ¹³C-¹H residual dipolar couplings (RDCs) and calculated segmental $|S_{CH}|$ order parameters. The $|S_{CH}|$ order parameters carry the structural information that aid calculation of $\langle A \rangle$ and D_B using the MT model. Experiments were conducted with DMPC, POPC, and EYSM lipids as single component (liquid-disordered, l_d) lipid bilayers as well as with cholesterol. EYSM is more ordered in the l_d phase and hence experiences less structural perturbation upon adding cholesterol to form the liquid-ordered (l_o) phase relative to POPC. We infer that the cholesterol stiffening effect on lipid bilayers is limited by the maximum volumetric hydrocarbon thickness [2]. Knowledge of structural parameters like $\langle A \rangle$ and D_B is important for molecular dynamics (MD) simulations and provides information about the balance of forces in membrane lipid bilayers [3]. Our studies demonstrate the applicability of solid-state ¹³C NMR spectroscopy to site-specific structural investigations of complex lipids, where isotope labeling may be prohibitive, allowing structural studies of biologically relevant membrane systems. [1] A. Leftin *et al.* (2013) *JMB* **425** 2973-2987. [2] H. I. Petrache *et al.* (2000) *BJ* **79** 3172-3192. [3] A. Leftin *et al.* (2011) *BBA* **1808** 818-839.

2285-Plat**Binding of Neurotransmitters to Lipid Membranes**

Günther H. Peters¹, Mikkel Werge¹, Maria N. Elf-Lind¹, Chunhua Wang², Nicolaj Cruys-Bagger², Gustavo F. Velardez¹, Jesper J. Madsen¹, Peter Westh².

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 neurotrans-

mitters (NTs) 5-hydroxytryptamine (5-HT), γ -aminobutyrate (GABA), glycine (GLY), acetylcholine (ACH) and glutamate (GLU) as well as the amidated / acetylated γ -aminobutyrate (GABA^{neu}) 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 \ll GABA < GLY \ll GABA^{neu} \ll 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 Lelimosin¹, 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 aggregates. 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.

2287-Plat**Reshaping Biological Membranes: From Molecular Interactions to Macroscopic Mechanics**

Mijo Simunovic^{1,2}, Patricia Bassereau², Gregory A. Voth¹.

¹The University of Chicago, Chicago, IL, USA, ²Institut Curie, Paris, France.

The remodeling of cell membranes is deeply rooted in many biological processes. We combine theoretical modeling with experimental biophysics to study the driving force underlying remodeling mediated by BAR proteins. These proteins are key regulators of membrane dynamics, taking part in endocytosis, communication between cells, division, infection, and immune response.

By combining coarse-grained simulations with field-theoretical and continuum methods, we simulate the large-scale behavior of BAR proteins on the membrane at molecular resolution. We complement simulation techniques with fluorescence microscopy to elucidate the

