En Route for Visualizing Membrane Lipid Domains

P. Greimel[1], Y. Harada[2], Y. Senoh[1], M. Oshima[2], T. Kobayashi[1]
[1] RIKEN, Lipid Biology Laboratory, Japan
[2] University of Tokyo, Department of Applied Chemistry, Japan

The cell membrane is the most essential border in the biological world. This frontier acts not only as a barrier, but also plays a crucial role during metabolic processes. The emerging key themes indicate that membranes are patchy, with lipid regions varying in thickness and composition. It is envisioned, that segregated regions rich in saturated lipids and cholesterol float in a sea of unsatured lipids poor in cholesterol. While both regions are considered to be in the liquid phase, the region high in cholesterol – which tends to order the lipids – is referred to as a liquid-ordered phase. The coexistence of two liquid phases in the membrane causes phase separation, leading to a lateral variation in lipid composition. It is postulated, that these lateral variations play a key role in many cellular events. Unfortunately, it has been difficult to probe membrane composition at sufficient resolution in the region of tens or hundreds of nanometers.

In order to gain a better understanding of phase separation processes in membranes, we investigated artificial membranes by scanning transmission X-ray microscopy (STXM). We conducted near edge x-ray absorption fine structure (NEXAFS) analysis at the carbon K-edge of dried 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). The DPPC spectrum exhibited the characteristic absorption maxima at about 287.2eV and 287.8eV of aliphatic carbonyls and alkyl chains, respectively. DOPC exhibited an additional absorption maximum at around 285eV due to the presence of an unconjugated double bond from its oleic acid residue. Consequently, this difference allows the unambiguous identification of DOPC in the presence of DPPC in artificial membranes.

In a separate experiment, we studied a mixture of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1',3'-bis[1,2-dimyristoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (TMCL). While DMPC and TMCL are fully miscible in membranes at room temperature, phase separation was induced by the addition of calcium, a divalent ion. Dried liposomes as well as liposomes suspended within a water layer were visualized by STXM. The different domains were unambiguously identified by their specific carbon, nitrogen and oxygen as well as calcium profiles.