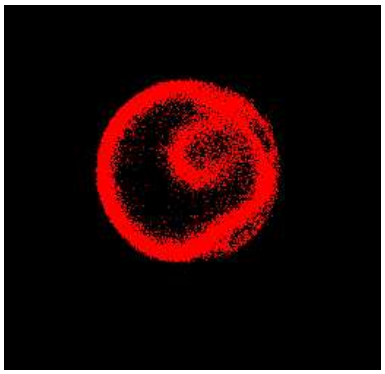
Shedding light on liposomes: Using lipid-mimetic metal complexes for fluorescent labeling

A. Mechler, M. S. Huda, E. Doevan and C. Hogan, Department of Chemistry, La Trobe University, VIC 3086, Australia .

For the study of biophysical systems involving phospholipid membranes, such as membrane lysis by antimicrobial peptides, supported membrane deposition, or liposome size and shape studies, it is often desired to apply a fluorescent label to the lipid phase. However, lipid membranes are dynamic systems that are highly dependent on the strength of headgroup interactions, internal pressure and lipid mobility. If either of these factors is changed, the properties of the membrane (surface tension, liposome diameter, ability to attach to surfaces) might change as well, compromising the measurements. Unfortunately, fluorescent labeled lipids and membrane-specific dyes typically do change the physicochemical properties of the membrane. Our aim was to develop a fluorescent dye that is similar to the lipid molecules in shape and size, and to use it for liposome imaging.

Two metal complexes, (Ru(2,2-bipyridine)$_2$(4,4-dinonyl-2,2-dipyridyl)(PF6)$_2$) and (Ir(2-phenylpyridine)$_2$(4,4-dinonyl-2,2-dipyridyl)(PF6)) have been synthesized, both with the same molecular structure: a "head group" made of the metal coordinated by bpy and ppy, respectively, and a "tail group" with two alkane chains. The lipid-mimetic complexes have been successfully reconstituted into DMPC liposomes. Importantly, when making the liposomes, extrusion was omitted; only a gentle vortexing and 30 s weak sonication was employed and the liposome size distribution was left to evolve. As a result, liposomes of a relatively broad size distribution were created, tending towards a bimodal distribution containing small unilamellar liposomes (SUV; ~100nm of diameter) and large (up to micron size) liposomes, as revealed by DLS measurements. Importantly, the size distribution of the liposomes labeled with the metal complexes is very similar to DMPC liposomes without the fluorescent label, thus the presence of the complex does not alter significantly the physicochemical properties of the membrane. It is regularly assumed that if larger liposomes form they must be multilamellar "onion" structures with an SUV core. However, confocal microscopic imaging of the metal complex labeled liposomes shows hollow structures with occasional encapsulated smaller liposomes. The figure shows an example of a confocal microscopic cross-section picture of a large liposome enclosing a smaller one. Remarkably, the metal complexes suffer minimal, practically negligible photobleaching, making longer time lapse studies feasible. The two metal complexes fluoresce at different wavelengths opening the door for dichroic measurements with this new labeling method.



A liposome- in- liposome system, labeled with Ru(bpy)$_2$dinone. The diameter of the outer liposome is ~3.5μm.