Measuring the incorporation of fluorescently labelled lipid analogues into the membrane of giant unilamellar vesicles

I.L. Salvemini,[1] J. Reid[2] and P.D.J. Moens,[1] [1]Centre for Bioactive Discovery in Health and Ageing, School of Science & Technology, University of New England, Armidale, NSW 2351, Australia and [2]School of Science & Technology, University of New England, Armidale, NSW 2351, Australia.

The use of Giant Unilamellar Vesicles (GUVs) composed of fluorescently labelled lipid analogues has become an increasing popular model to study both structural and complex biophysical properties of bilayers. However, there is a common assumption that the number of probes incorporated into the membrane of the GUVs is proportional to the mole fraction (%) of these lipid molecules in the original solvent solution.

A commercial confocal laser scanning microscope (Nikon C1) was used to obtain single point fluorescence correlation spectroscopy (FCS) data. The time dependence of the spontaneous fluctuations and the number of molecules in the detection volume (point spread function) was calculated using the autocorrelation function of the fluorescent signals. From these data, the diffusion coefficient (D_1)and the number of fluorescent molecules (N) incorporated into the membrane was obtained.

We successfully measured the diffusion coefficient of two different labelled lipid analogues (1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate[$DiIC_{18}$] and BODIPY TMR-phosphatidylinositol (4,5) bisphosphate[TMR-PI(4,5)P2])incorporated into the membrane of GUVs (Moens, Gratton & Salvemini, 2010). The results obtained for these lipid analogs are in good agreement with previously published data (Golebiewska *et al.*. 2006; Golebiewska *et al.*. 2008; Gielen *et al.*. 2009).

We also show that the number of $DiIC_{18}$molecules incorporated into the membrane of the GUVs (formed by the electroformation method) is in agreement with the expected number of molecules calculated from the mole fraction of the organic stock solution. However, we find that the actual proportion of β-BODIPY-HPC,TR-PI(4,5)P2,and TMR-PI(4,5)P2 incorporated into the bilayer is significantly less than the proportion of these lipids in the organic solvent stock solution.

These findings draw attention to the need to quantitatively measure the incorporation of these probes for experiments in which the concentration is of importance to the parameter being investigated.

Gielen E, Smisdom N, vandeVen am, De Clercq B, Gratton E, Digman M, Rigo J-M, Hofkens J, Engelborghs Y, Ameloot M. (2009) *Langmuir*. **25(9):** 5209-18.

Golebiewska U, Gambhir A, Hangyás-Mihályné G, Zaitseva I, Rädler J, McLaughlin S. (2006) *Biophysical Journal* **91(2):** 588-99.

Golebiewska U, Nyako M, Woturski W, Zaitseva I, McLaughlin S. (2008) *Molecular Biology of the Cell* **19(4):** 1663-9.

Moens PD, Gratton E, Salvemini IL. (2010) *Microscopy Research and Technique* Aug 23. [Epub ahead of print] http://dx.doi.org/10.1002/jemt.20919