P.23 Imaging dynamic patterns in lipid membranes using molecular rotors

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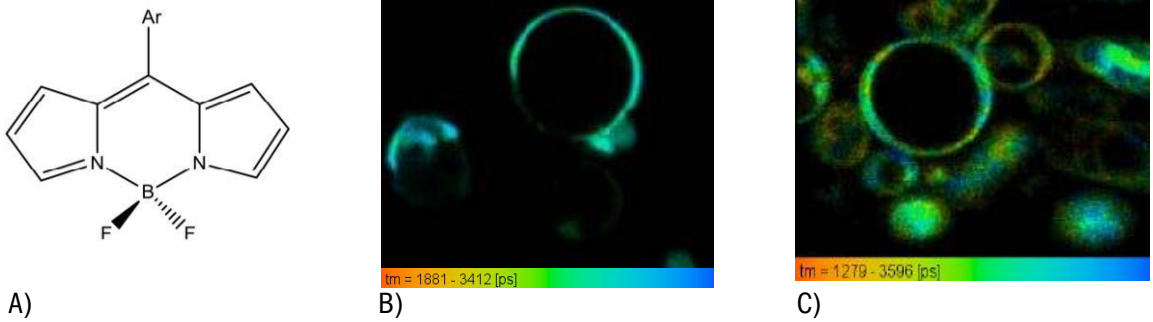
The existence of ordered lipid microdomains within the plasma membrane of live cells has remained a contentious idea within the research community, partially due to the difficulty associated with optical imaging below the diffraction limit of visible light. By using a new imaging approach based on the use of molecular rotors and unilamellar vesicles as model membrane systems, we have developed a method capable of giving detailed information about the phase behaviour of lipids within a membrane to a high degree of spatial and temporal resolution.

Molecular rotors are synthetic organic fluorophores whose fluorescence properties are related to the viscosity of the surrounding environment. In highly viscous environments the emission intensity and fluorescence lifetime of the rotor will increase significantly. The fluorescence lifetimes of rotors based upon a boron-dipyrrin (BODIPY) core (Figure 1A) have been shown to have a strong dependence on the viscosity of the surrounding environment,[1] meaning that fluorescence lifetime imaging (FLIM) can be used in conjunction with BODIPY rotors to give a spatially resolved map of microviscosities across a sample, independent of the local BODIPY concentration.

Here we report the use of molecular rotors based on BODIPY to investigate membrane viscosity and phase behaviour within unilamellar vesicles using FLIM (Figure 1 B-C). We investigate the effects of lipid composition and temperature on observed viscosity values. We also use several synthetic derivatives of the BODIPY rotors with various functional groups in order to change the membrane localisation of the probes, e.g. the new charged derivative[2]. We examine the relationship between the measured viscosity and the BODIPY structure.



A) B) C)

Figure 1 Generalised structure of a BODIPY rotor (A), lifetime images of a phase separating ternary GUV stained with a BODIPY rotor at 29°C (B) and 45°C (C). Viscosity decreases with increasing temperature, and phase separation can be seen to disappear as the system is heated beyond the transition temperature of the lipids.

[1] Kuimova, M. K. *Phys. Chem. Chem. Phys.*2012, *14*, 12671–86.C

[2] López-Duarte, I.; Vu, T. T.; Izquierdo, M. A.; Bull, J. A; Kuimova, M. K. *Chem. Commun.* 2014, Advance Article. DOI: 10.1039/c3cc47530a