P.22 Dual-mode microviscosity measurements in lipid monolayer and bilayer systems with a molecular rotor

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Viscosity of the cellular membrane has a significant impact on cellular processes, including on the diffusion of biomolecules within or through the membrane. Although measurement of viscosity on a microscopic scale is a challenging task, we have demonstrated[1] that it can be achieved with the aid of molecular rotors, which are fluorophores with viscosity dependent fluorescence intensities and lifetimes. Microviscosity measurements are usually performed via Fluorescence Lifetime Imaging Microscopy (FLIM), which produces a spatial map of the lifetime of a molecular rotor in the object of interest. Alternatively, ratiometric imaging can be used if the molecular rotor exhibits a viscosity dependent intensity ratio between two peaks in its fluorescence spectrum.

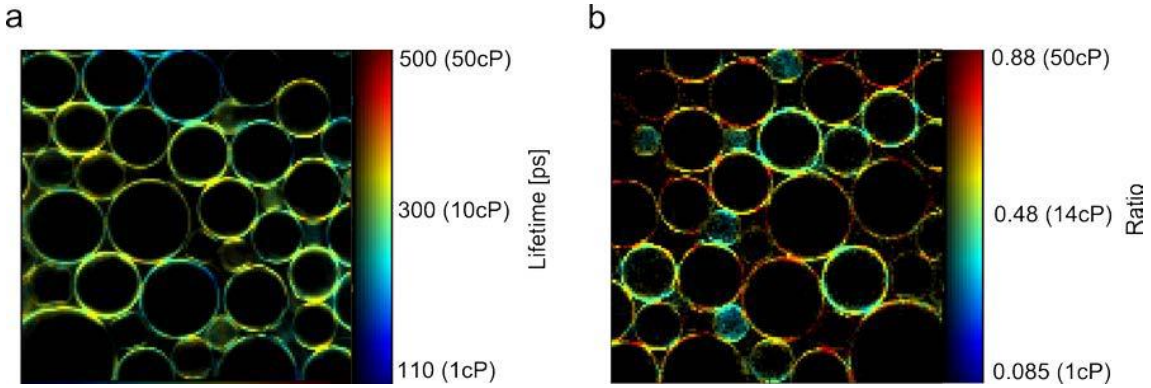

Figure 1. FLIM (a) and ratiometric (b) images of lipid monolayers around water droplets in dodecane. Viscosity values corresponding to the lifetimes and ratios given in the colourbar are shown in brackets.

In this work we have examined a molecular rotor MB, constructed as a porphyrin dimer, which is capable of measuring viscosity via both of the methods described above. Only a few such molecular rotors are reported in the literature, however, this provides a useful opportunity as it allows us to independently double check measured viscosity values. We performed the calibration of the rotor in methanol/glycerol mixtures of varying viscosity using both FLIM and ratiometric imaging (Figure 2). MB was then employed for measuring viscosity in several lipid-based systems, such as (i) lipid monolayers made by coating water droplets in dodecane with the lipid DOPC (Figure 1); (ii) large and giant unilamellar vesicles (LUVs and GUVs), which are used as model system for cell membranes.

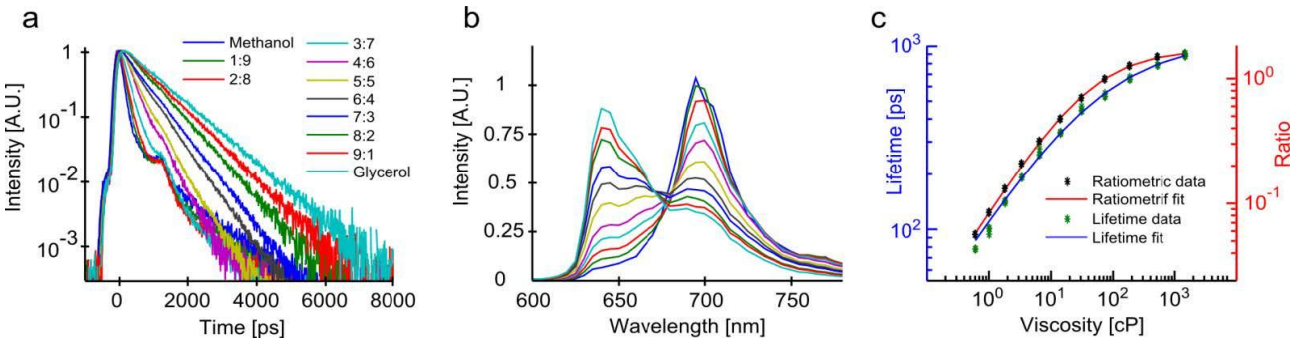

Figure 2. Calibration data of MB in methanol/glycerol mixtures. Normalized fluorescence decays (a) and normalized fluorescence spectra (b) in solvent mixtures of different viscosities. The volume ratios of glycerol to methanol are shown in the legend of (a). (c) Lifetime (blue) and ratiometric (red) calibration curves obtained from the data in (a) and (b).

[1] Kuimova, M. K. Mapping viscosity in cells using molecular rotors. *Phys. Chem. Chem. Phys.* 14, 12671–12686 (2012)