Applications of water-quenched fluorophore for sensing hydration in biomolecular environments

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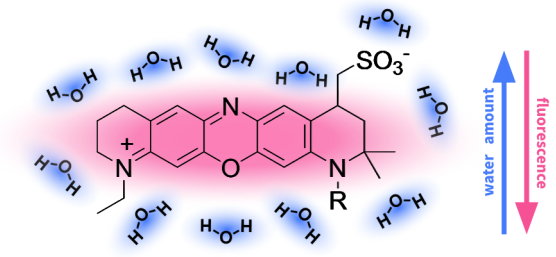
Red-emitting fluorophores are widely used to study biosystems as the red part of the spectrum is less photodamaging and better transmitted in biological samples. At the same time, these fluorophores are known to be quenched by surrounding water molecules [1]. Quenching by water reduces both the fluorescence quantum yield and lifetime of such dyes, so that the dye's direct hydration can be assessed by monitoring changes in its fluorescence lifetime [2]. In this work, we develop and use derivatives of water-sensing red-emitting fluorophore, Atto655, to target purified proteins, model membranes, and membrane organelles in living cells.



ATTO 655

Hydration-related changes in fluorescence lifetime are detected in bulk by time-correlated single photon counting (TCSPC) or, when relevant, mapped in 2D via fluorescence lifetime imaging microscopy (FLIM). For purified proteins, site-specific sensing of hydration has the potential to report conformational rearrangements. In the case of model membranes and cellular organelles, FLIM enables visualization of local variations in the probe's hydration. Differences in hydration can then be used for lifetime-based imaging contrast or to monitor environmental changes in cellular processes.

[1] J. Maillard, K. Klehs, C. Rumble, E. Vauthey, M. Heilemann, A. Fürstenberg, Chem. Sci., **2021**, 12, 1352–1362
[2] J. Maillard, C. A. Rumble, A. Fürstenberg, J. Phys. Chem. B, **2021**, 125 (34), 9727-9737