Brightness enhancement of red- and near-infrared-emitting fluorophores in heavy water and applications in conventional and single-molecule fluorescence microscopy

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ABSTRACT: Organic dyes and fluorescent proteins (FPs) are key tools in many fluorescence imaging applications. Their brightness is determined by the extinction coefficient and fluorescence quantum yield. Fluorophores emitting in the red and near-infrared (NIR) spectral range are well suited for imaging in biological samples due to little background signal from scattering and autofluorescence. Unfortunately, those fluorophores exhibit low quantum yields in aqueous media.

We investigated the fluorescence quantum yields of 46 commercially available fluorescent dyes and six FPs and found an overall increase when water (H_2O) was replaced by heavy water (D_2O). Interestingly, we observed an enhanced effect for fluorophores with spectra shifted further towards red wavelengths. The spectroscopic results were verified by imaging representative red- and NIR-emitting fluorophores in a biological environment with conventional fluorescence microscopy. The improved imaging contrast obtained in D_2O is illustrated in Figure 1. We also successfully demonstrated single-molecule localization microscopy in dye-specific switching buffers with increased photon yields [1, 2].

Replacing H_2O by D_2O is a useful tool for various fluorescence applications, since it is simple, cost-efficient and can be used in combination with all water-based imaging buffers, e.g. oxygen-scavenging and photoprotection buffers [3]. By making less bright red/NIR dyes accessible, it offers new opportunities for multicolor imaging.

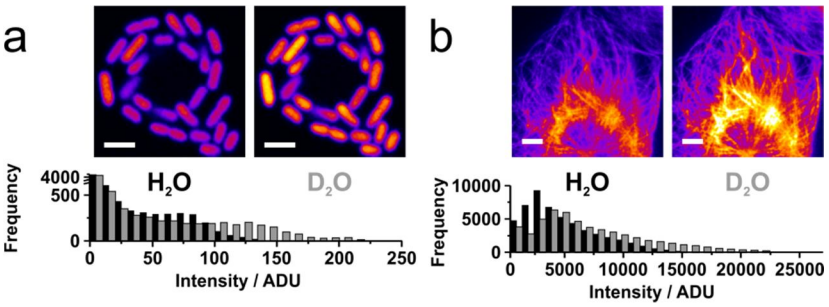

Figure 1. (a) Confocal images of mNeptune expressed in *E. coli*. Scale bar: 2 µm. (b) Widefield-TIRF images of microtubule in U2-OS cells labeled via immunofluorescence with Cy7. Scale bar: 5 µm.

REFERENCES: [1] S. F. Lee; Q. Vérolet, and A. Fürstenberg, "Improved super-resolution microscopy with oxazine fluorophores in heavy water", *Angew. Chem. Int. Ed.*, **52**, 8948-8951 (2013). [2] K. Klehs; C. Spahn; U. Endesfelder; S. Lee; A. Fürstenberg, and M. Heilemann, "Increasing the Brightness of Cyanine Fluorophores for Single-Molecule and Superresolution Imaging", *ChemPhysChem*, **15**, 637-641 (2014). [3] J. Vogelsang et al., "A reducing and oxidizing system minimizes photobleaching and blinking of fluorescent dyes", *Angew. Chem. Int. Ed.*, **47**, 5465-5469 (2008).