Deuteration improves fluorophores for sensitive imaging strategies

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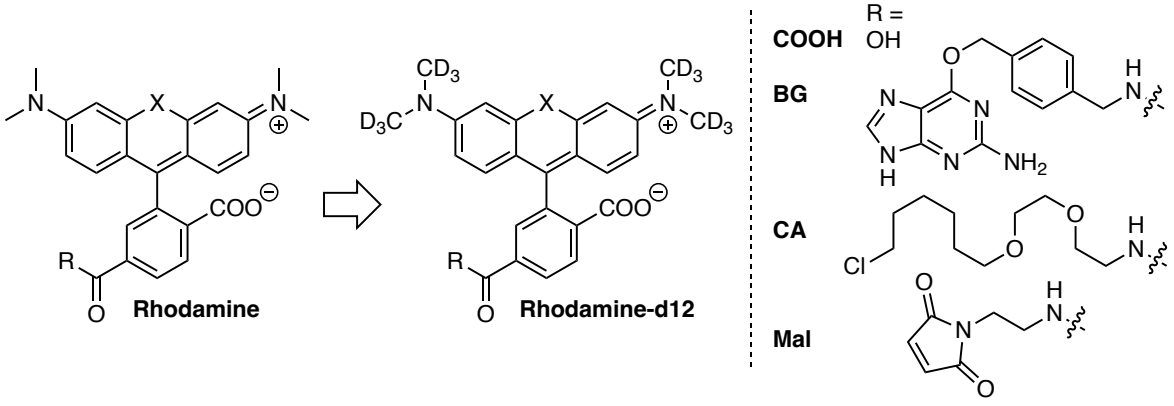
Modern fluorescence microscopy relies on bright and robust dyes for best performances. We report a general method to improve the photophysical (*i.e.* enhanced brightness, lifetime) and chemical (*i.e.* reduced bleaching) properties of fluorophores by incorporating deuterium on the *N*-methyl groups on several dye classes. We highlight these findings with tetramethyl(silicon)rhodamine congeners in several applications. When SNAP- and Halo-tags were labelled in live cells, increased intensities were observed by fluorescence activated cell sorting (FACS), which translated to findings in ensemble and single molecule Förster Resonance Energy Transfer (FRET). In addition, deuterated rhodamine congeners displayed longer fluorescence lifetimes, and yielded improvements in live cell confocal and stimulated emission by depletion (STED) microscopy on SNAP- and Halo-tag protein labelling in terms of intensity with retained super-resolution, highlighted on Halo-tagged Tubb5 microtubules. Mechanistic investigations by transient absorption spectroscopy and temperature-dependent fluorescence lifetime microscopy (FLIM) suggest increased fluorescence quantum yields by suppression of twisted intramolecular charge transfer (TICT). Our strategy provides a general method to improve chromophores.



	X	ε (M^{-1} cm^{-1})	$\lambda_{Ex / Em}$ (nm)[c]	ϕ[a]	ϕ x ε	bleaching k (10^{-3} s^{-1})[a]	labelling k (10^{-3} s^{-1})[d]	lifetime τ (ns)[e]
TMR	O	78,000[a]	549 / 576	0.43	34	3.24	52.1	2.3
TMR-d12	O	90,000[a]	549 / 577	0.51	46	1.80	56.8	2.6
SiR	$SiMe_2$	141,000[b]	648 / 669	0.35	49	n.d.	29.5	2.9
SiR-d12	$SiMe_2$	156,000[b]	652 / 670	0.46	72	n.d.	23.6	3.5

[1] Roßmann, K.; Akkaya, K. C.; Poc, P.; Charbonnier, C.; Eichhorst, J.; Gonschior, H.; Valavalkar, A.; Wendler, N.; Cordes, T.; Dietzek-Ivanšić, B.; Jones, B.; Lehmann, M.; Broichhagen, J., *N*-Methyl deuterated rhodamines for protein labelling in sensitive fluorescence microscopy, *Chem. Sci.* **2022**, accepted.