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Article

A General Method to Improve Fluorophores Using Deuterated Auxochromes

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Reporter: Jin Li

Date: 2021-06-17

Biography of the author (Janelia Reaearch Campus)

We use modern organic chemistry to transform old dyes into tools for 21st century biology.

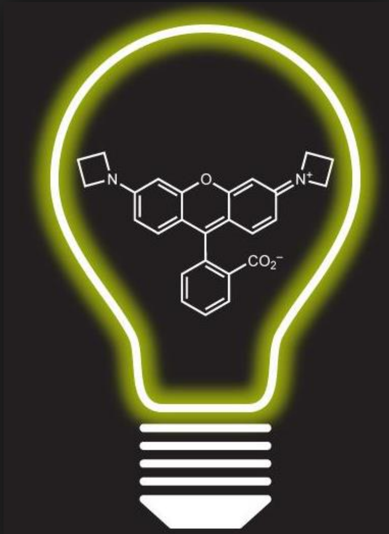
-Luke Lavis

Chemical biology and cell biology:

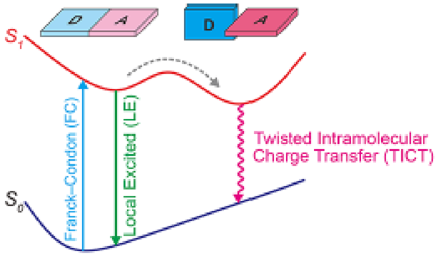
We work at the interface of chemistry and biology, assembling small molecule fluorescent dyes that facilitate sophisticated biological studies

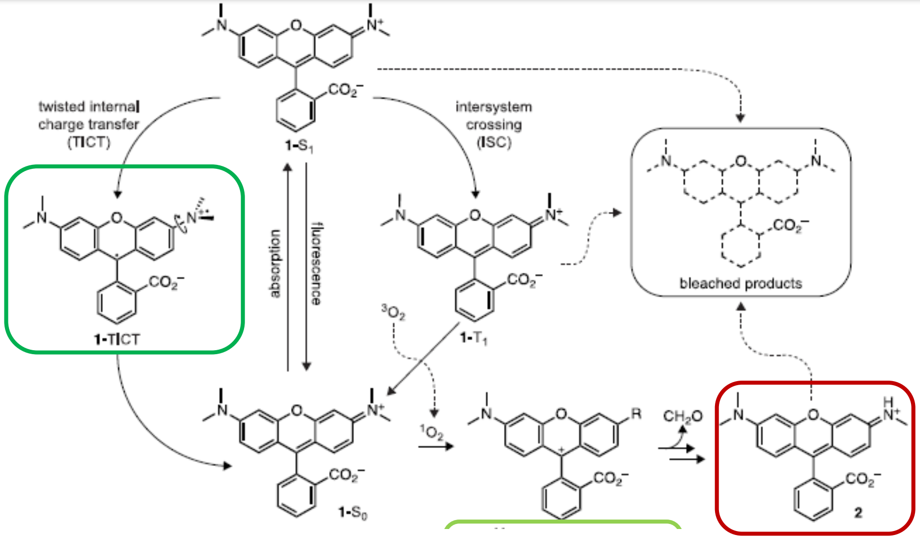
Current Research

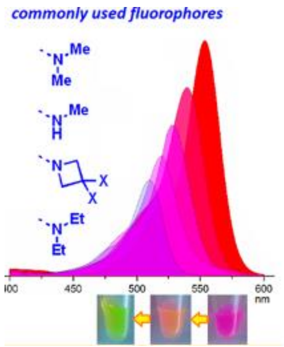
We are interested in designing and building small molecules to measure or manipulate biological systems. Our laboratory synthesizes bright fluorescent labels that enable the imaging of individual molecules in living cells. We also develop fluorogenic probes where the chemical and photophysical properties can be masked by assorted molecular functionalities and then unmasked by a user-designated process involving light, enzymatic activity, or environmental changes. This chemical masking suppresses unwanted fluorescence signals, thereby functioning as a filter for bioimaging and other experiments. Combining these novel compounds with advances in instrumentation, protein engineering, and genetic manipulation allows us to devise sophisticated ways to illuminate complex biological systems.



Photophysics of rhodamines and methods to improve rhodamine properties



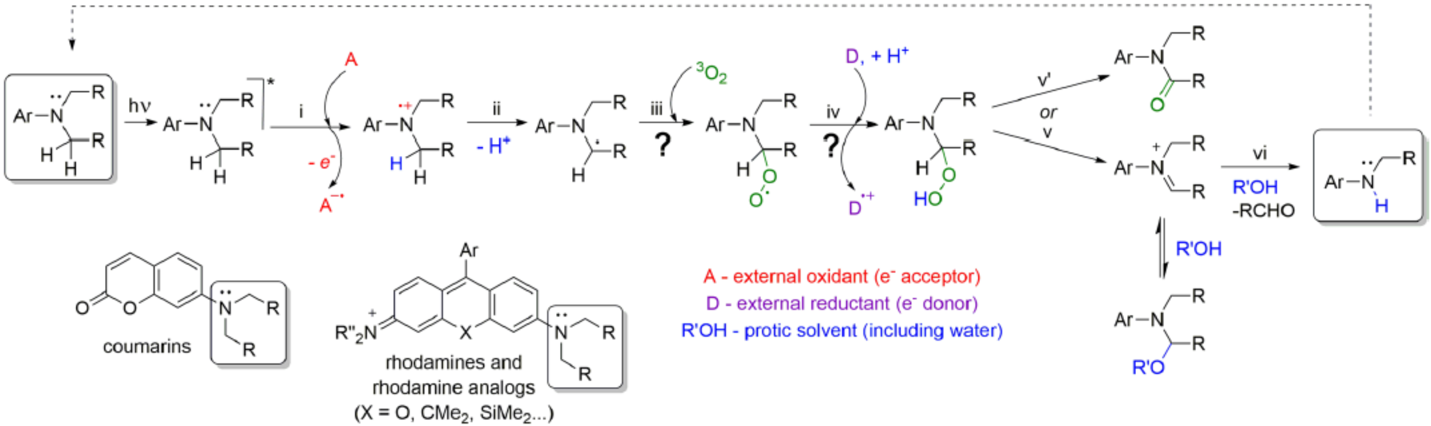




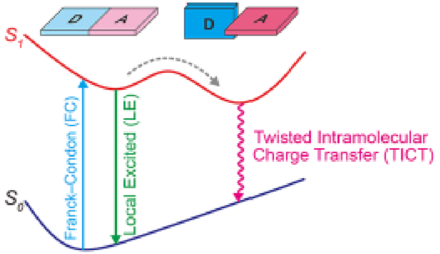
Φ_f

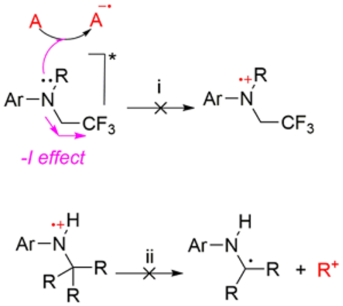
Photostability

Chromostability



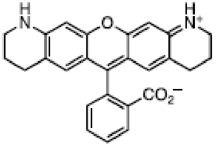
Strategies aimed at suppression of transitioning into the twisted intramolecular charge transfer (TICT) excited state and mitigation the dye photobluing



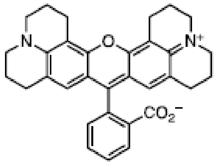


no α-H available for elimination
no fragmentation of radical cation

Structures of rigidified rhodamines:

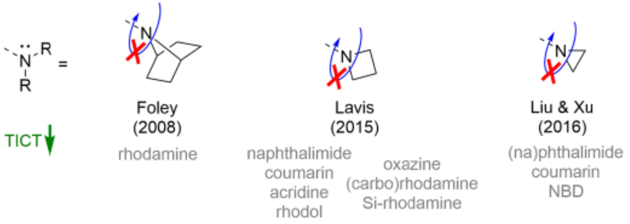


Q-rhodamine (3)
Φ_f = 0.83

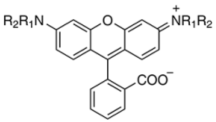
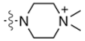
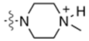


rhodamine 101 (4)
Φ_f = 0.90

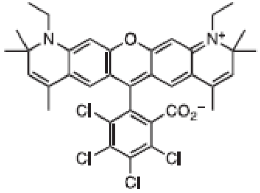
Structures of cyclic amine-containing rhodamines:



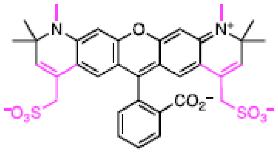
Structures of inductive effect rhodamines:

Core	Substituent (NR_1R_2)	Name	λ_{ab}[a] (nm)	λ_{em}[a] (nm)	Φ[a]	ε[a] (× 10^4 $M^{-1}cm^{-1}$)
		TMR	548	571	0.47	7.8[b]
		MPR	524	552	0.93	8.7
		Lyso-RH[c]	526	557	0.92	8.7

Structures of α-quaternary rhodamines:



ATTO Rho14 (7)

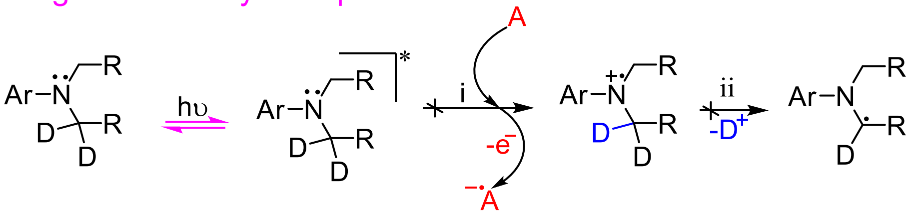


■+■: Alexa Fluor 594 (8)
■: di-t-butylrhodamine (9)

J. Am. Chem. Soc. **2008**, 130, 17652*; J. Am. Chem. Soc.* **2016**, 138, 6960; *Nat. Methods*, **2015**, 12, 244; *J. Am. Chem. Soc.* **2019**, 141, 981; *J. Am. Chem. Soc.* **2019**, 141, 14491

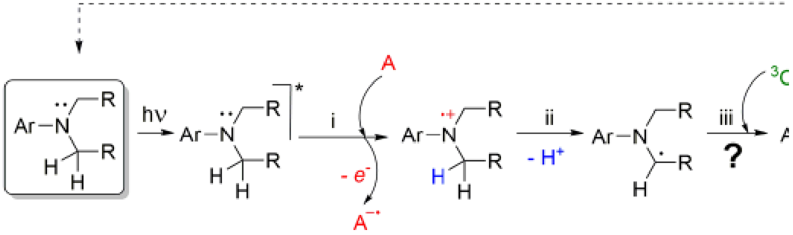
Photophysics of rhodamines and methods to improve rhodamine properties

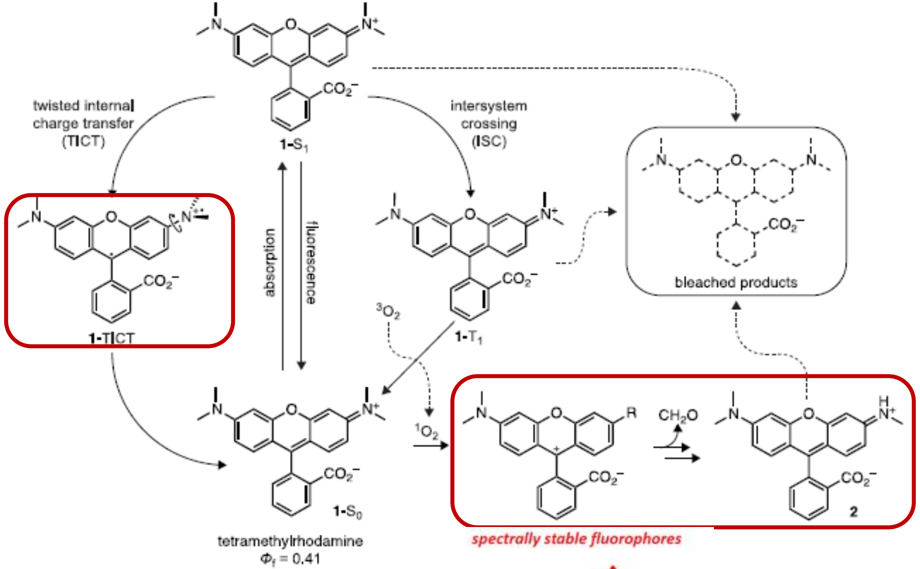
Large Secondary Isotope Effect



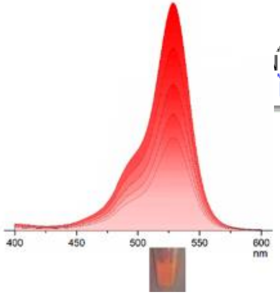
Primary isotope effect
&
Secondary isotope effect

Reduce the electron donor strength:
slow $^1O_2$-mediated oxidation;
Stronger C−D bond:
lower the rate of deprotonation.





Φ_f
Photostability
Chromostability



Deuterated tetramethylrhodamine and Spectral Properties of Rhodamines

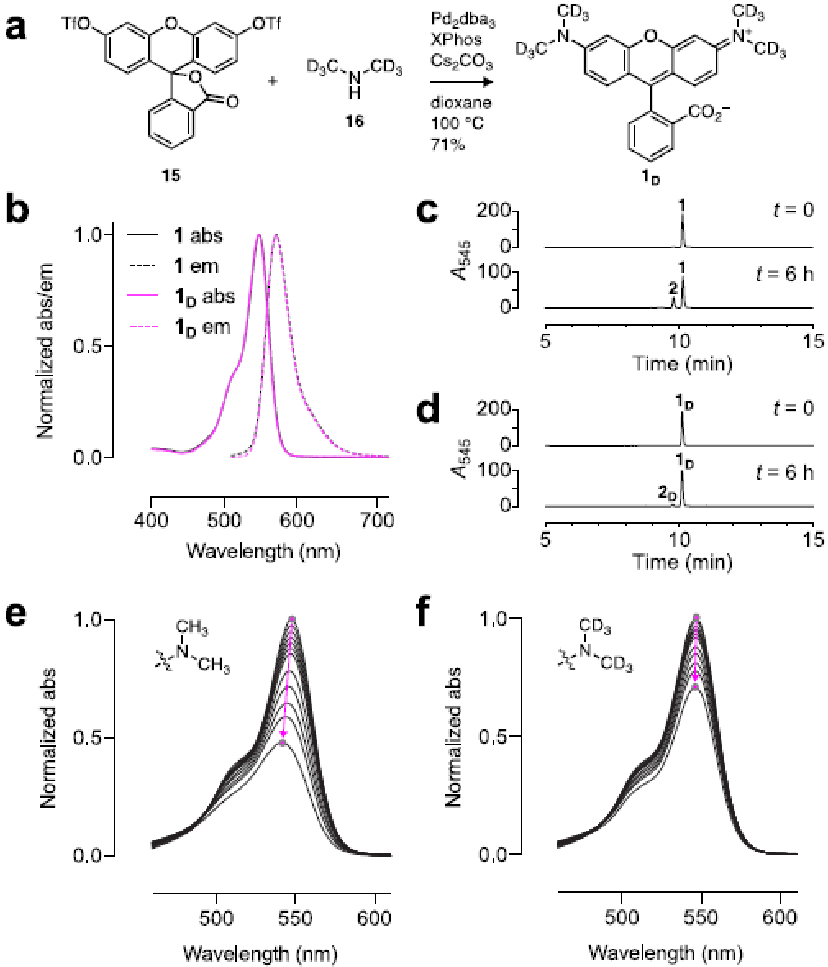
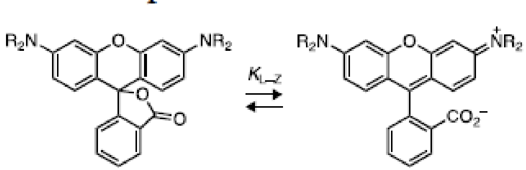
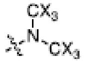
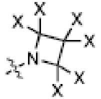
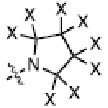
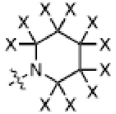
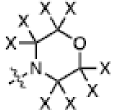


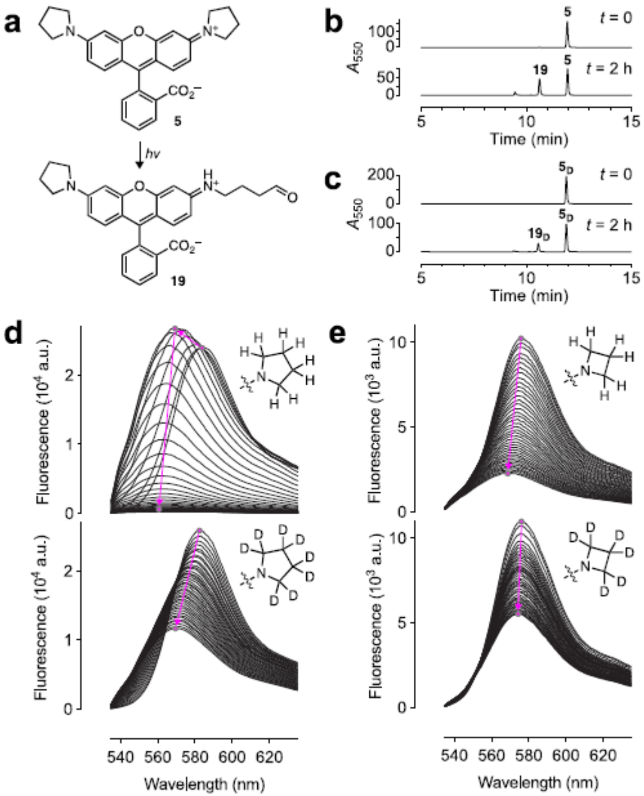
Table 1. Spectral Properties of Rhodamines[a]

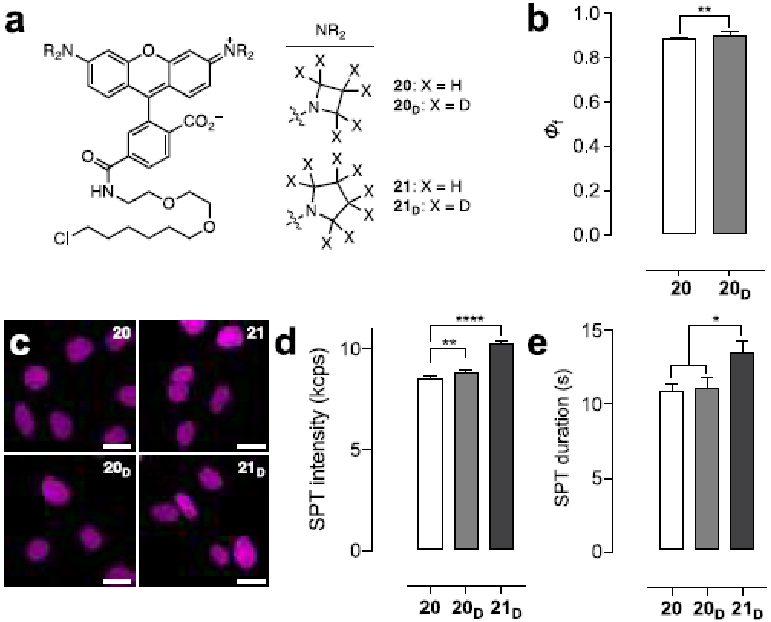


NR_2	dye	X	λ_{abs} (nm)	λ_{em} (nm)	ε ($M^{-1}cm^{-1}$)	K_{L-Z}	Φ_f
	1	H	548	572	78,000	3.96	0.41
	1_D	D	547	570	94,500	3.73	0.50
	6	H	549	571	101,000	3.47	0.88
	6_D	D	548	570	96,700	3.03	0.86
	5	H	553	576	76,000	4.50	0.70
	5_D	D	554	576	104,000	4.97	0.80
	17	H	560	586	80,000	2.85	0.08
	17_D	D	559	586	92,600	4.02	0.12
	18	H	545	574	84,000	0.14	0.11
	18_D	D	544	575	87,800	0.17	0.13

[a]All values are in 10 mM HEPES, pH 7.3 except for K_{L-Z}, which was measured in 1:1 v/v dioxane:H_2O.

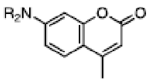
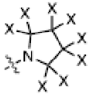
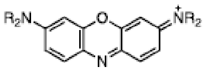
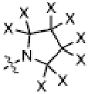
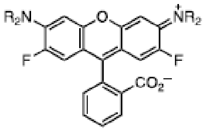
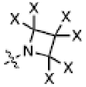
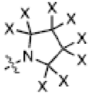
Photostability and chromostability of 5, 5D, 6, and 6D and Performance of rhodamine ligands





Spectral Properties of Other Deuterated Dyesa

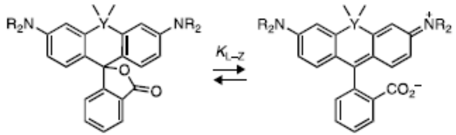
Table 2. Spectral Properties of Other Deuterated Dyes[a]

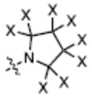
scaffold	NR_2	dye	X	λ_{abs} (nm)	λ_{em} (nm)	ε ($M^{-1}cm^{-1}$)	Φ_f
		**22**	H	381	472	19,800	0.46
		22_D	D	381	472	19,700	0.56
		**23**	H	655	671	85,000	0.16
		23_D	D	653	669	85,000	0.22
		**24**	H	552	575	95,200	0.83
		24_D	D	550	573	94,200	0.88
		**25**	H	561	585	81,000	0.30
		25_D	D	560	584	96,100	0.37

[a]All values in 10 mM HEPES, pH 7.3.

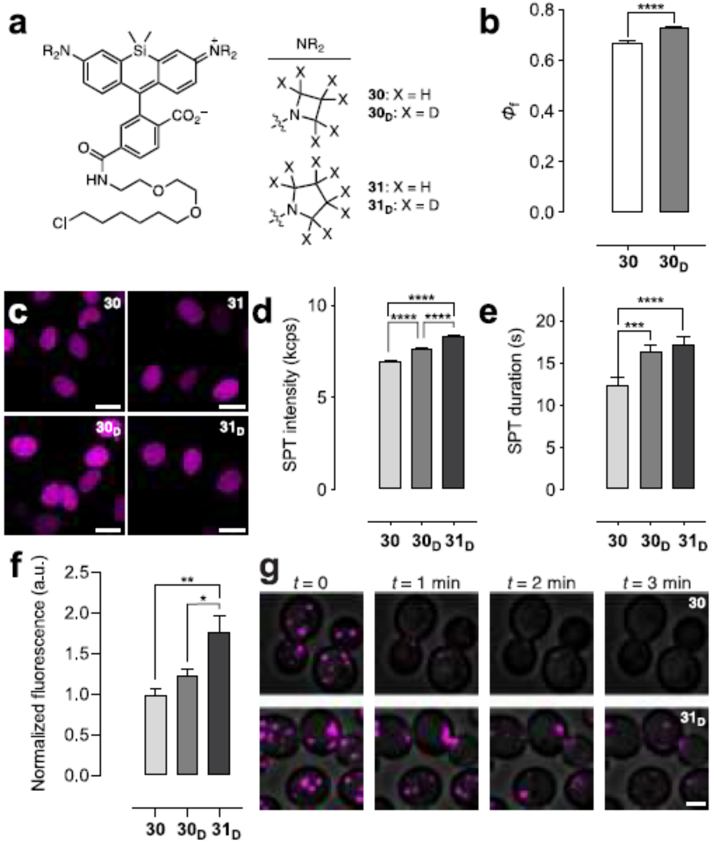
Spectral Properties of Red-Shifted Rhodamine Variants and Performance of Si–rhodamine ligands

Table 3. Spectral Properties of Red-Shifted Rhodamine Variants[a]

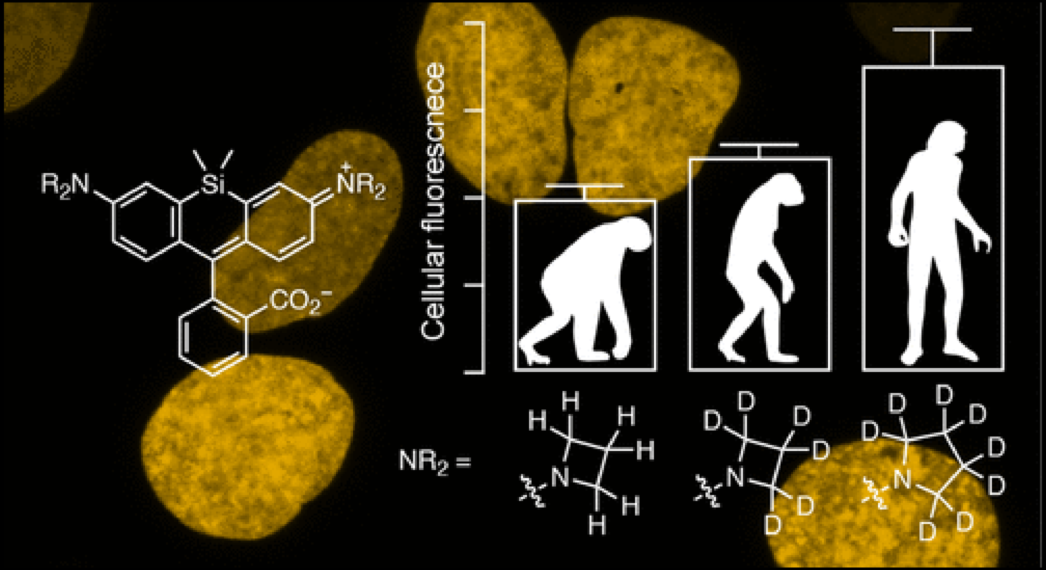


NR_2	dye	X	Y	λ_{abs} (nm)	λ_{em} (nm)	ε ($M^{-1}cm^{-1}$)	K_{L-Z}	Φ_f
	**26**	H	C	608	631	99,000	0.091	0.67
	26_D	D	C	608	628	111,000	0.14	0.74
	28	H	Si	646	664	5,600[b]	0.0014	0.54
	28_D	D	Si	645	662	8,600[b]	0.0013	0.54
	**27**	H	C	613	633	87,000	0.81	0.54
	27_D	D	C	612	633	130,000	0.88	0.70
	29	H	Si	652	668	12,600[b]	0.013	0.48
	29_D	D	Si	650	667	17,600[b]	0.015	0.53

[a]All values in 10 mM HEPES, pH 7.3 except for K_{L-Z}, which was measured in 1:1 v/v dioxane:H_2O. [b]ε > 150 000 $M^{-1}cm^{-1}$ in EtOH or TFE with 1% v/v TFA.



Conclusion



Chromostability