

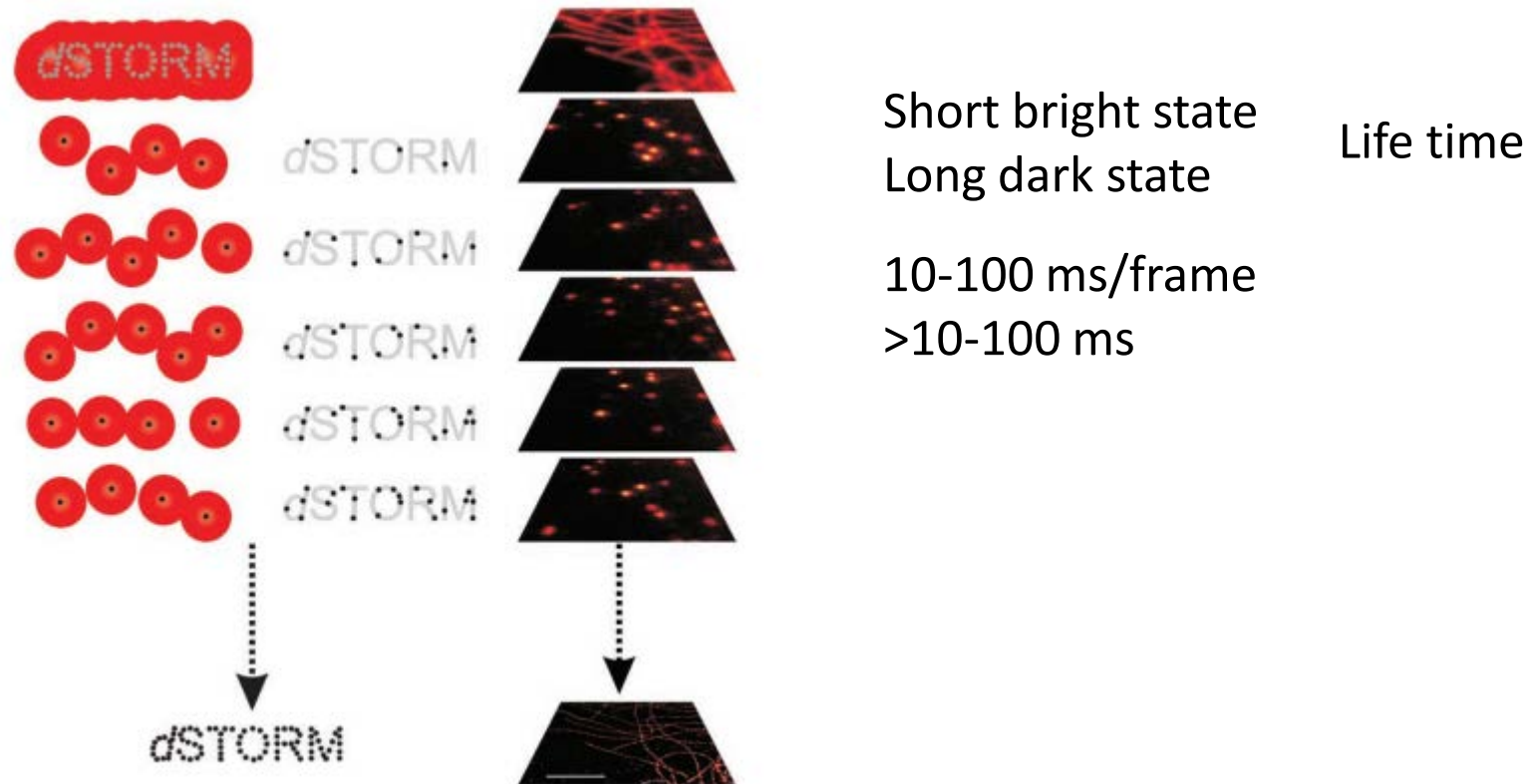
A spontaneously blinking fluorophore based on intramolecular spirocyclization for live-cell super-resolution imaging

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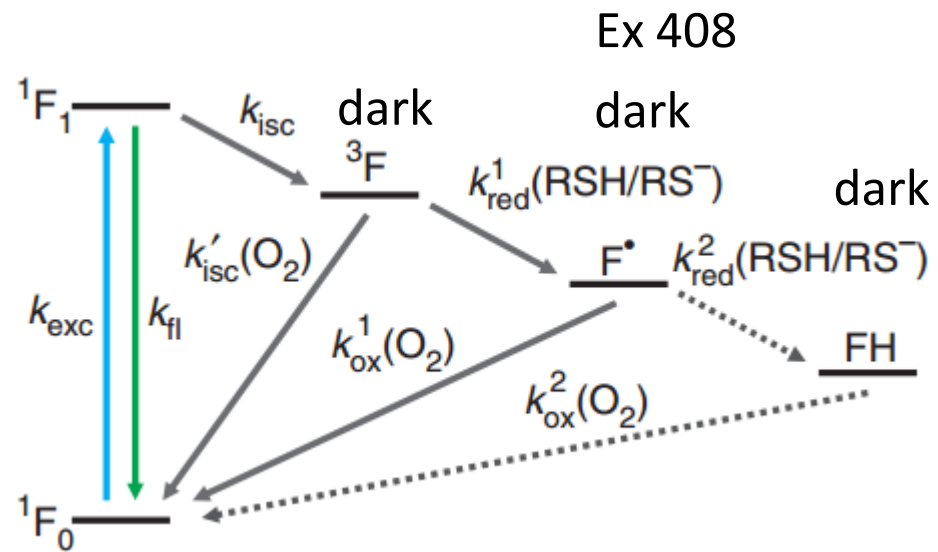
¹Graduate School of Medicine, The University of Tokyo, Tokyo 113-0033, Japan, ²Division of Molecular Science, Faculty of Science and Technology, Gunma University, Kiryu 376-8515, Japan, ³Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan, ⁴JST, PRESTO, Saitama 332-0012, Japan, ⁵Center for International Research on Micronano Mechatronics, Institute of Industrial Science, The University of Tokyo, Tokyo 153-8505, Japan, ⁶Laboratory for Cell Polarity Regulation, Quantitative Biology Center, RIKEN, Suita, 565-0874, Japan, ⁷Basic Research Program, Japan Science and Technology Agency, Tokyo 102-0075, Japan. *e-mail: mkamiya@m.u-tokyo.ac.jp; uranokun@m.u-tokyo.ac.jp

Super-resolution Imaging

- Imaging of subcell level: macromolecule
- dSTORM: Direct stochastic optical reconstruction microscopy

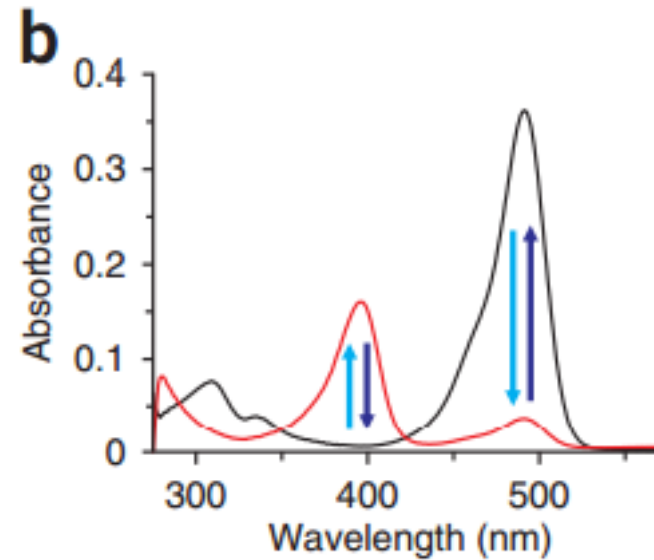


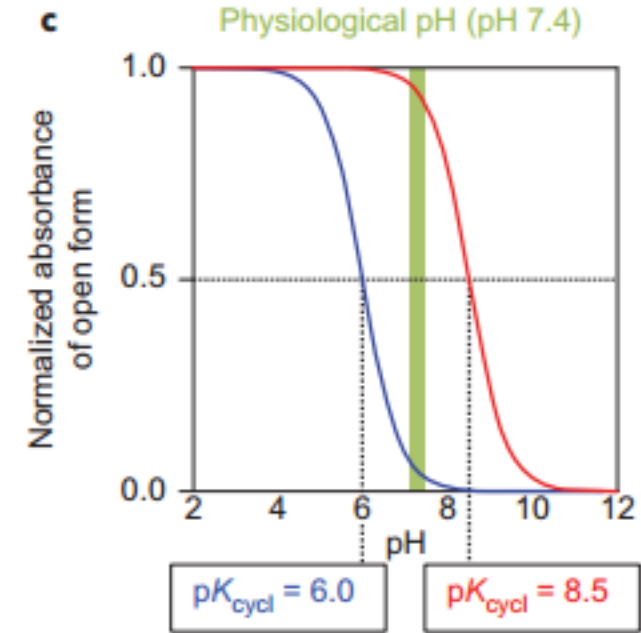
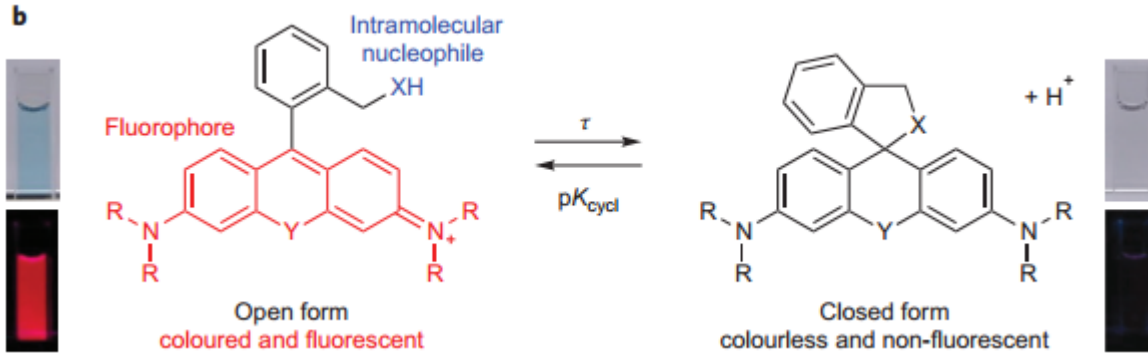
- On-off: blinking
- Reversible blinking: light controlling photoswitch



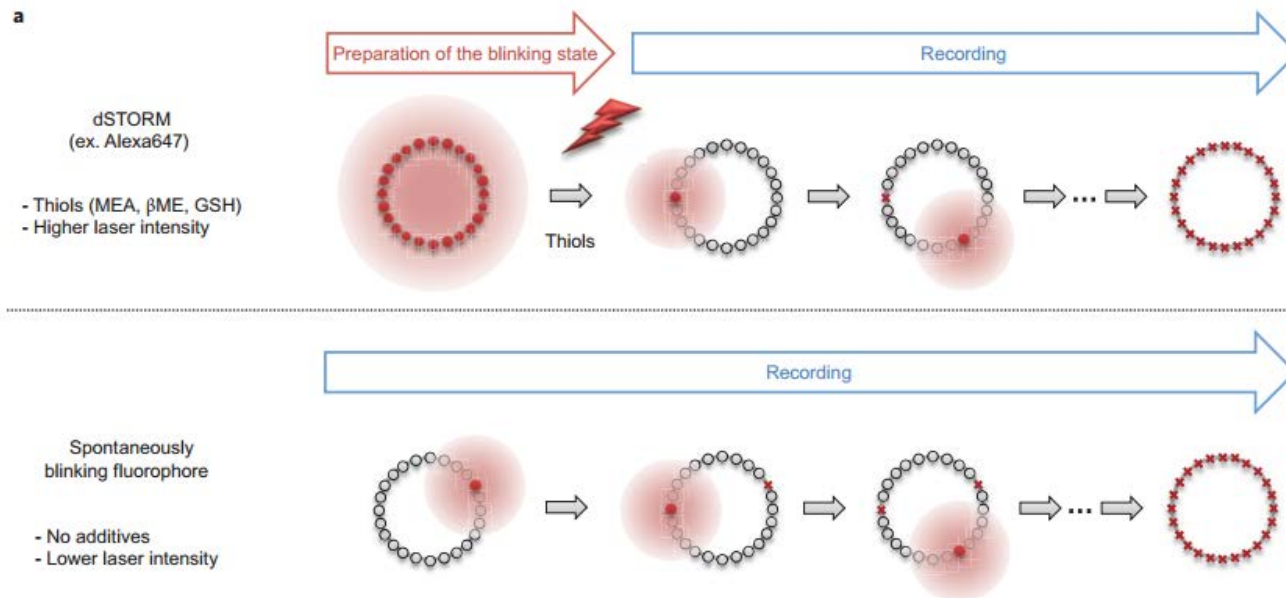
bright

Ex 480

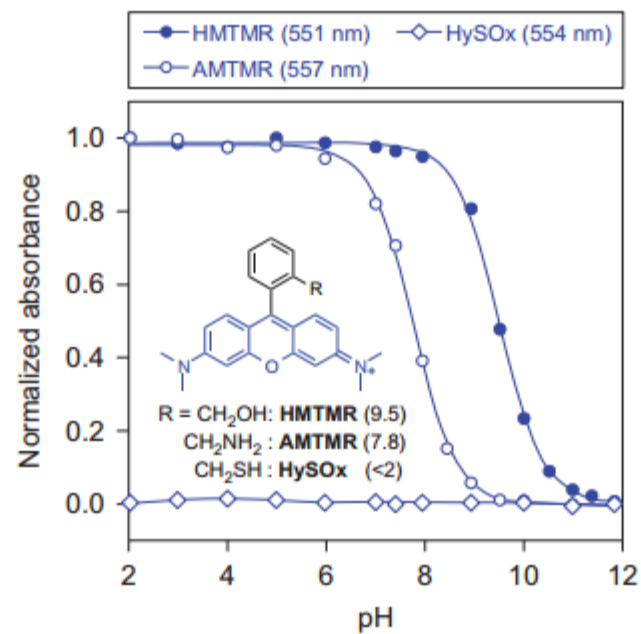
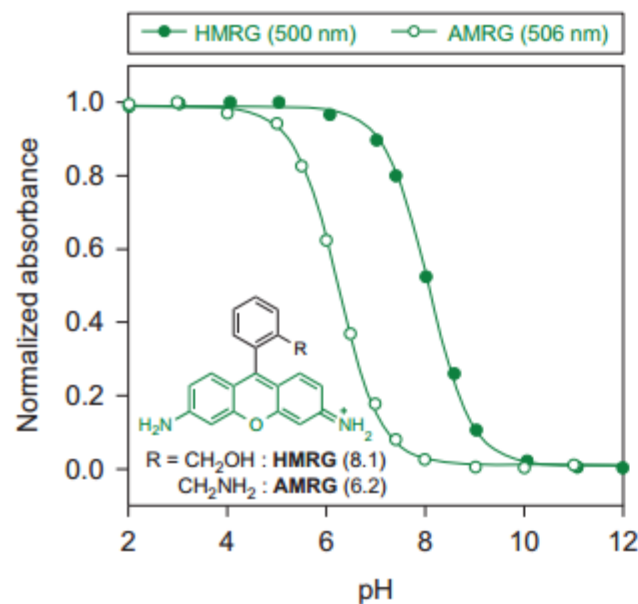
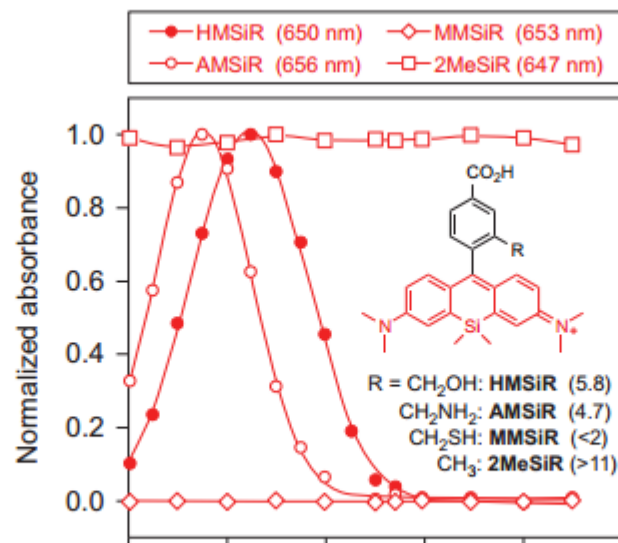
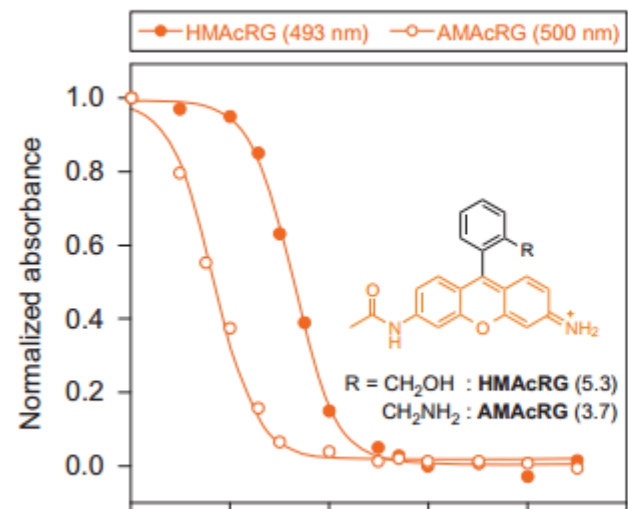
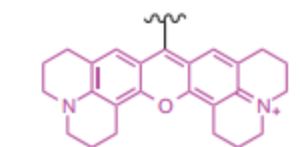
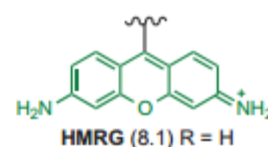
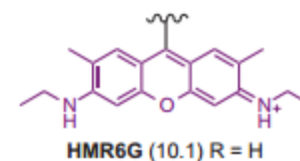
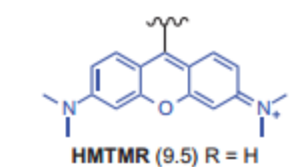
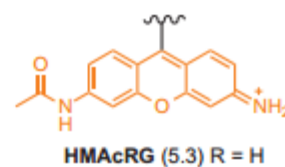
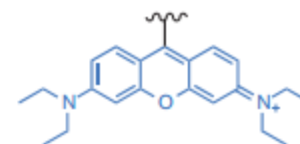
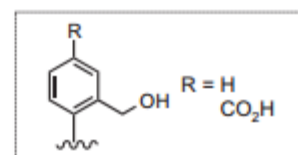
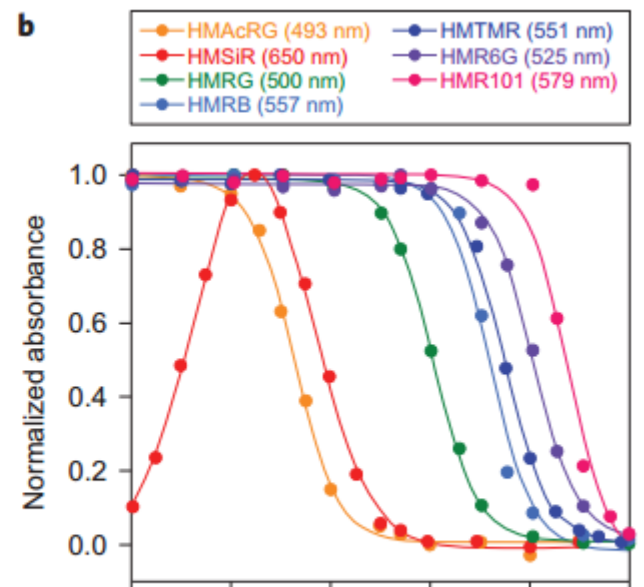




>8.5, most bright at 7.4
 <6, most dark at 7.4: prefer



Without external chemical
 Without switching excitation light beam

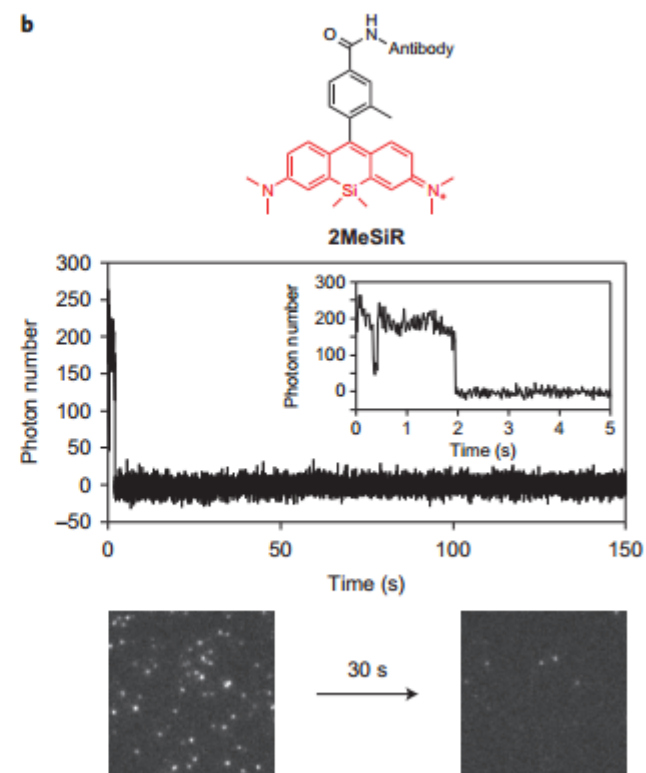
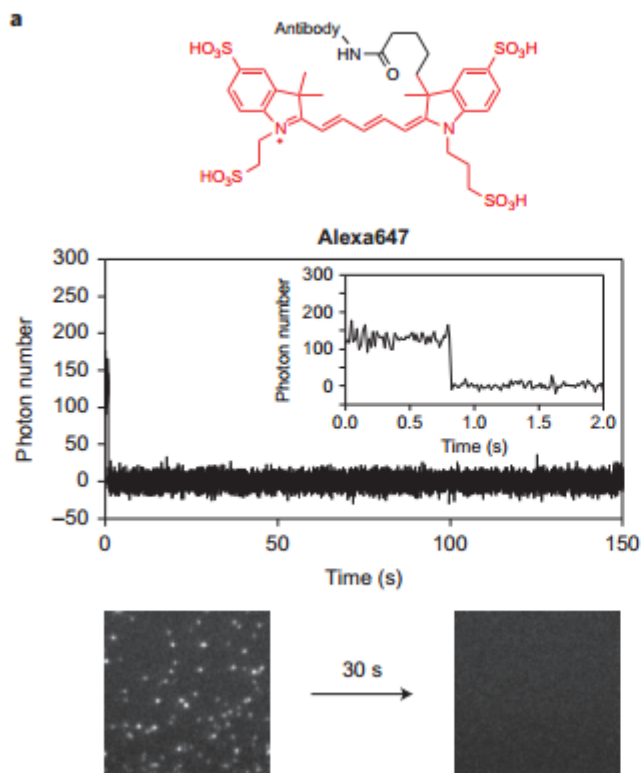
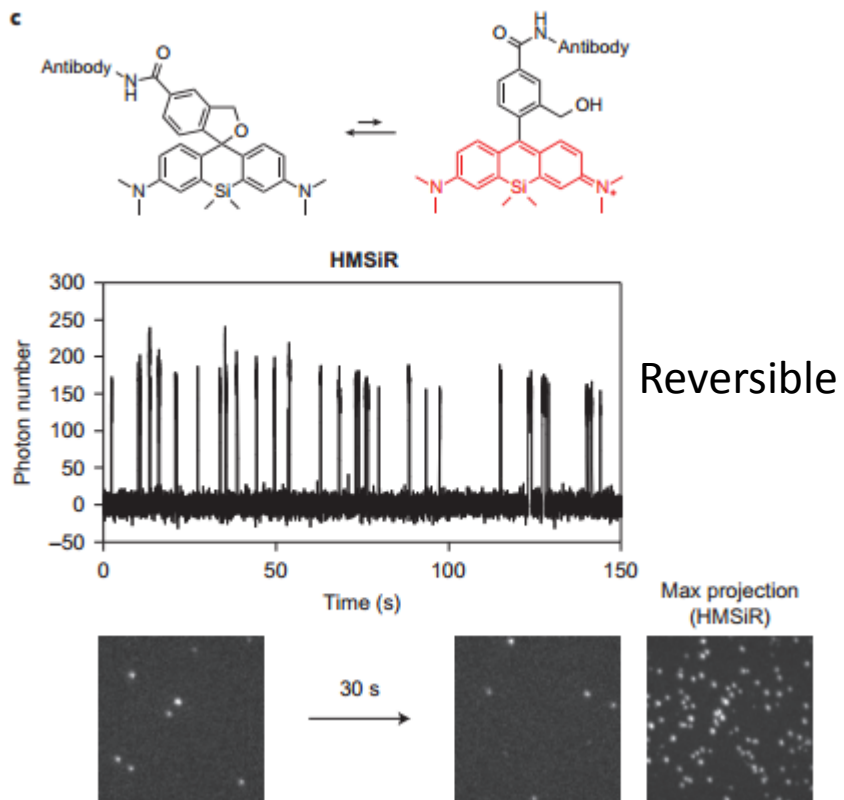
a**b**

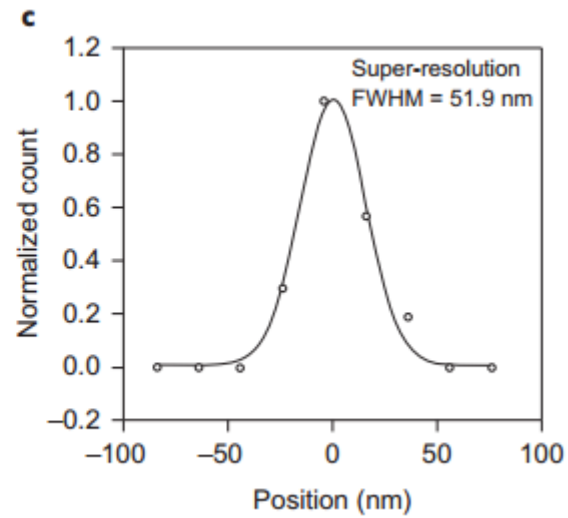
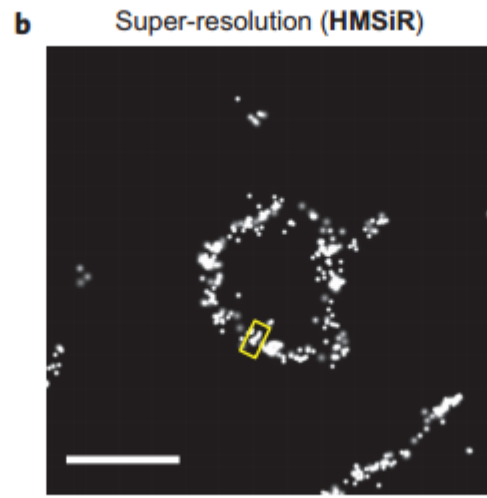
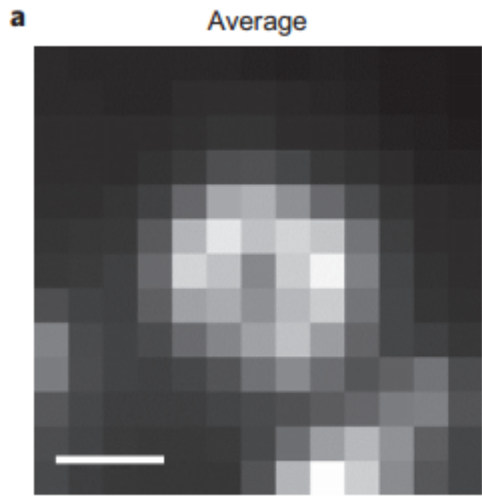
2

Table 1 | Switching properties of rhodamine derivatives determined by laser flash photolysis.

Compounds	pK_{cycl}	τ (s)
HMSiR	5.8	2.45×10^{-1}
HMAcRG	5.3	2.18×10^{-2}
AMSiR	4.7	1.07×10^{-5}
AMAcRG	3.7	3.09×10^{-6}
MMSiR	<2.0	$<5.00 \times 10^{-8}$
HySOx	<2.0	$<5.00 \times 10^{-8}$

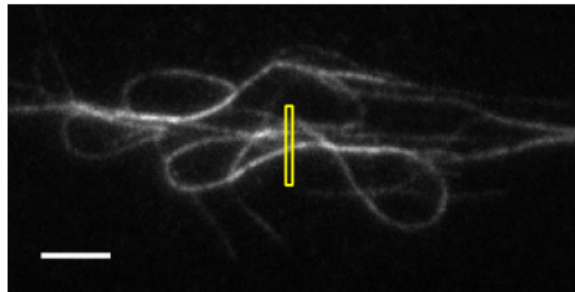
S>N>O



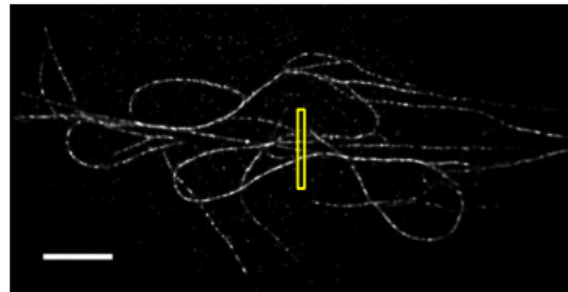


RecA-DNA complex

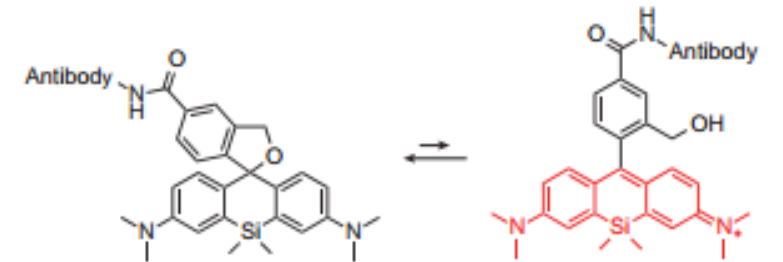
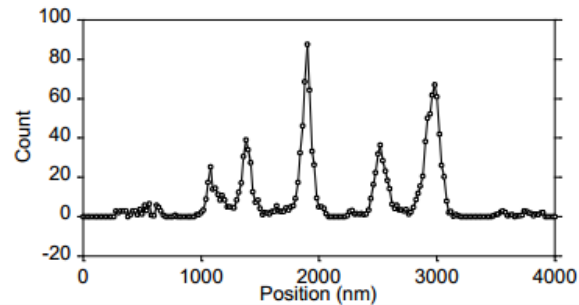
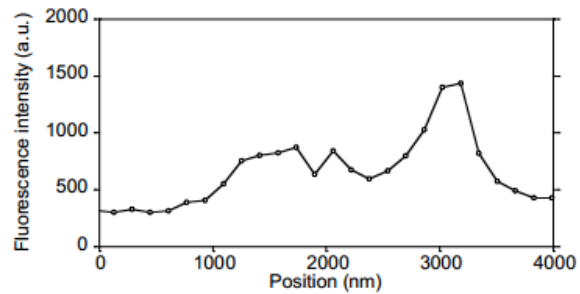
a Conventional (TAMRA)

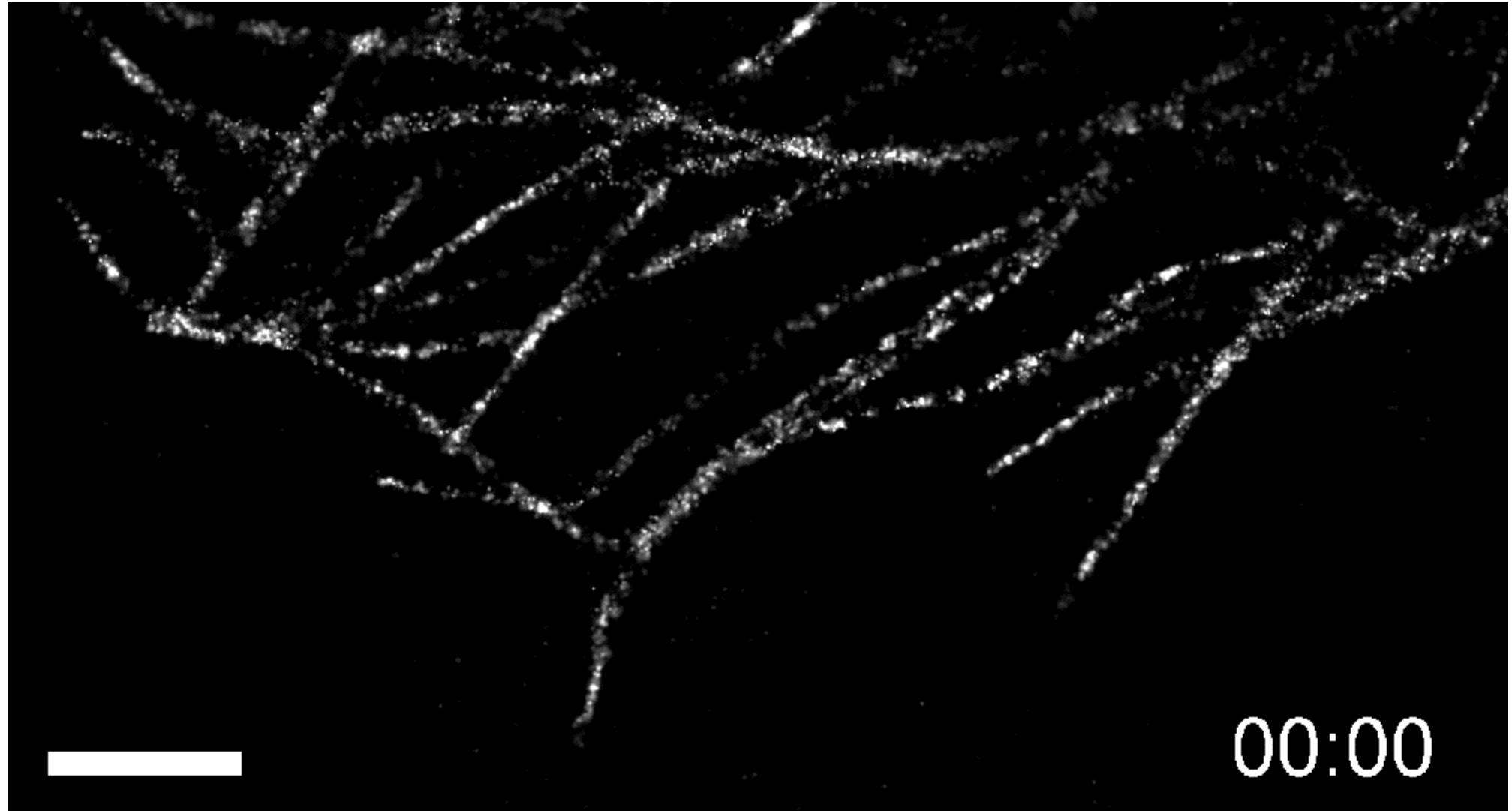


b Super-resolution (HMSiR)



tubulin





Summary

- Fine tuning of the dye
- Dark state without external chemical inducer and strong illumination:
no interruption to data and low cytotoxicity
- Cell permeable dye
- Stable: up to 1h