Chemical Science

ROYAL SOCIETY OF CHEMISTRY

EDGE ARTICLE

View Article Online
View Journal

Check for updates

Cite this: DOI: 10.1039/d0sc05431c

All publication charges for this article have been paid for by the Royal Society of Chemistry

Universal quenching of common fluorescent probes by water and alcohols†

Jimmy Maillard,‡[ab] Kathrin Klehs,‡[c] Christopher Rumble,[a] Eric Vauthey,[a] Mike Heilemann*[c] and Alexandre Fürstenberg*[ab]

Multi-Color, Bleaching-Resistant Super-Resolution Optical Fluctuation Imaging with Oligonucleotide-based Exchangeable Fluorophores

Marius Glogger[1,*], Christoph Spahn[1], Jörg Enderlein[2,3] and Mike Heilemann[1,*]

DOI: 10.1002/ange.202013166

Reporter: Chen Jie
2020-12-24

Authors

Alexandre Fürstenberg

- ***Quantitative fluorescent sensors for single-molecule and super-resolution imaging***
- ***Protein dynamics and G protein-coupled receptors***
- ***Novel fluorescent probes***
- ***Luminescent open-shell coordination complexes***

Mike Heilemann

- ***Super-resolution microscopy***
  - *Binding constants of ligand-receptor-interactions on single cells determined by single-molecule imaging*
  - *New concepts for fluorescence fluctuation in SOFI imaging*
- ***Membrane receptors***
- ***Bacteria***
- ***Photochemistry of organic dyes & labeling***

https://www.unige.ch/sciences/chimie/Fuerstenberg/en/home/
https://www.uni-frankfurt.de/43272324/Welcome_to_the_homepage_of_the_Heilemann_Group

DOI: 10.1002/anie.201302341

Improved Super-Resolution Microscopy with Oxazine Fluorophores in Heavy Water**

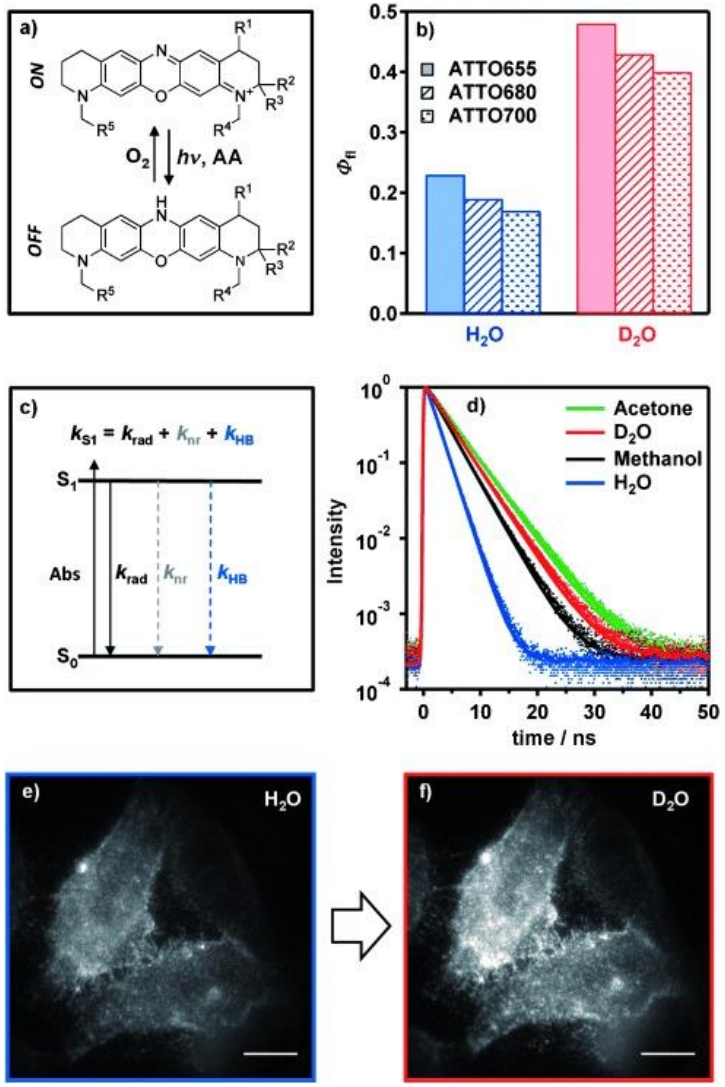


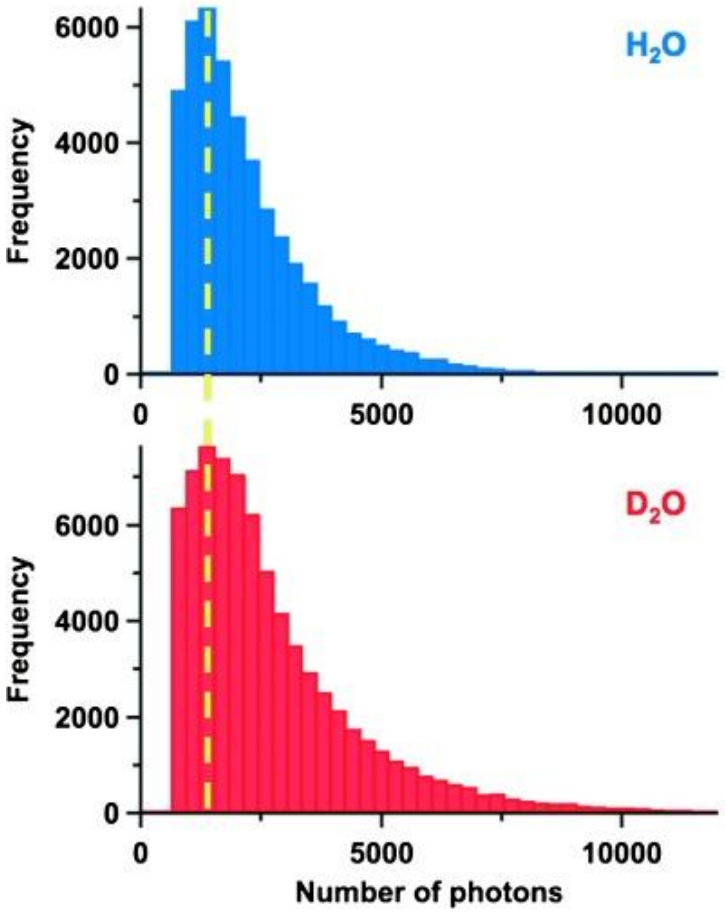
Table 1: Photophysical properties of ATTO655 in various solvents: fluorescence quantum yield (Φ_{fl}), excited-state lifetime (τ_{S1}), radiative rate constant (k_{rad}), and sum of rate constants for nonradiative (k_{nr}) and hydrogen-bond-assisted nonradiative deactivation (k_{HB}).

Solvent	Φ_{fl}	τ_{S1} [ns]	k_{rad} [$\times 10^8$ s^{-1}]	$k_{nr} + k_{HB}$ [$\times 10^8$ s^{-1}]
H_2O	0.23	1.9	1.2	4.1
PBS in H_2O	0.23	1.9	1.2	4.1
D_2O	0.48	3.9	1.2	1.4
PBS in D_2O	0.48	3.9	1.2	1.4
methanol	0.39	3.3	1.2	1.8
ethanol	0.46	3.6	1.3	1.5
2-propanol	0.49	3.8	1.3	1.3
acetone	0.55	4.5	1.2	1.0
acetonitrile	0.57	4.4	1.3	1.0
DMF	0.56	4.1	1.4	1.1
DMSO	0.56	3.9	1.4	1.1

DOI: 10.1002/cphc.201300874

Increasing the Brightness of Cyanine Fluorophores for Single-Molecule and Superresolution Imaging

- ***Alexa Fluor 647***



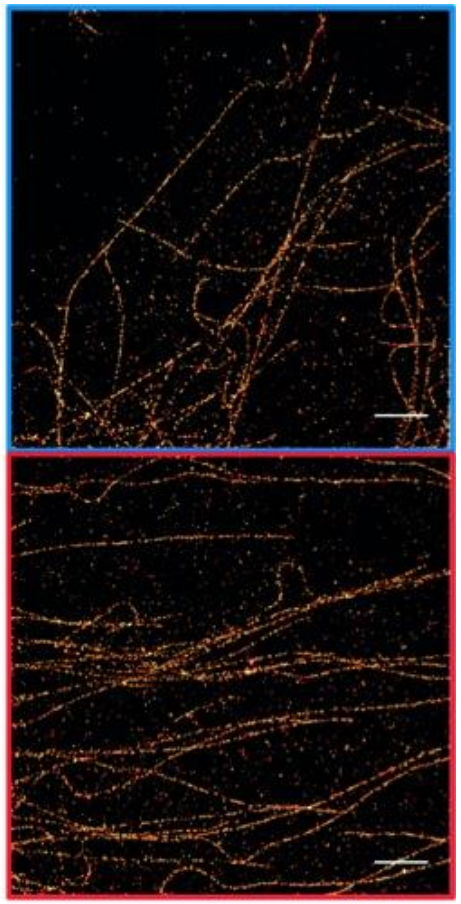
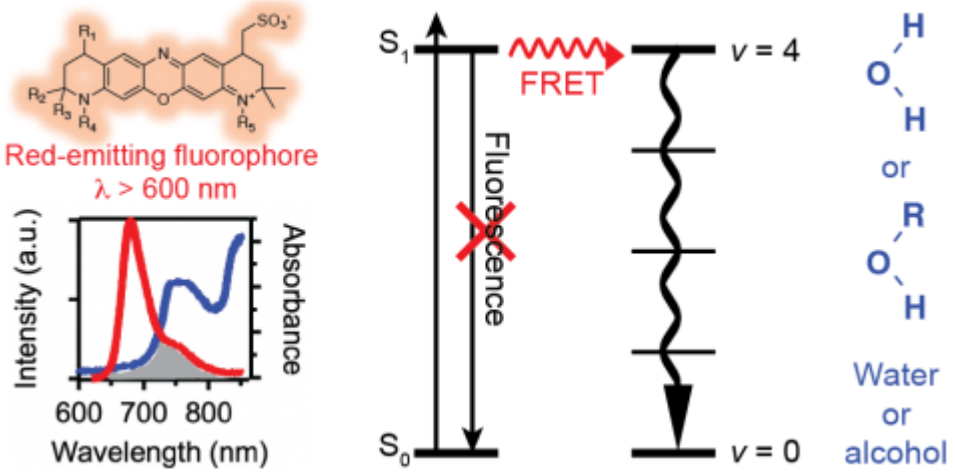


Table 1. Ratios of fluorescence quantum yields (Φ_F) and fluorescence lifetimes (τ_F) measured both in H_2O and in D_2O.

Dye	$\Phi_F(D_2O)/\Phi_F(H_2O)$	$\tau_F(D_2O)/\tau_F(H_2O)$
Cy3	1.1	~1.0[a]
Cy3B	1.1	1.1
Cy3.5	1.2	1.1
Cy5	1.3	1.2
Alexa Fluor 647	1.3	1.2
Cy5.5	1.6	1.4
Alexa Fluor 700[b]	1.7	1.7
Cy7	2.6	2.4

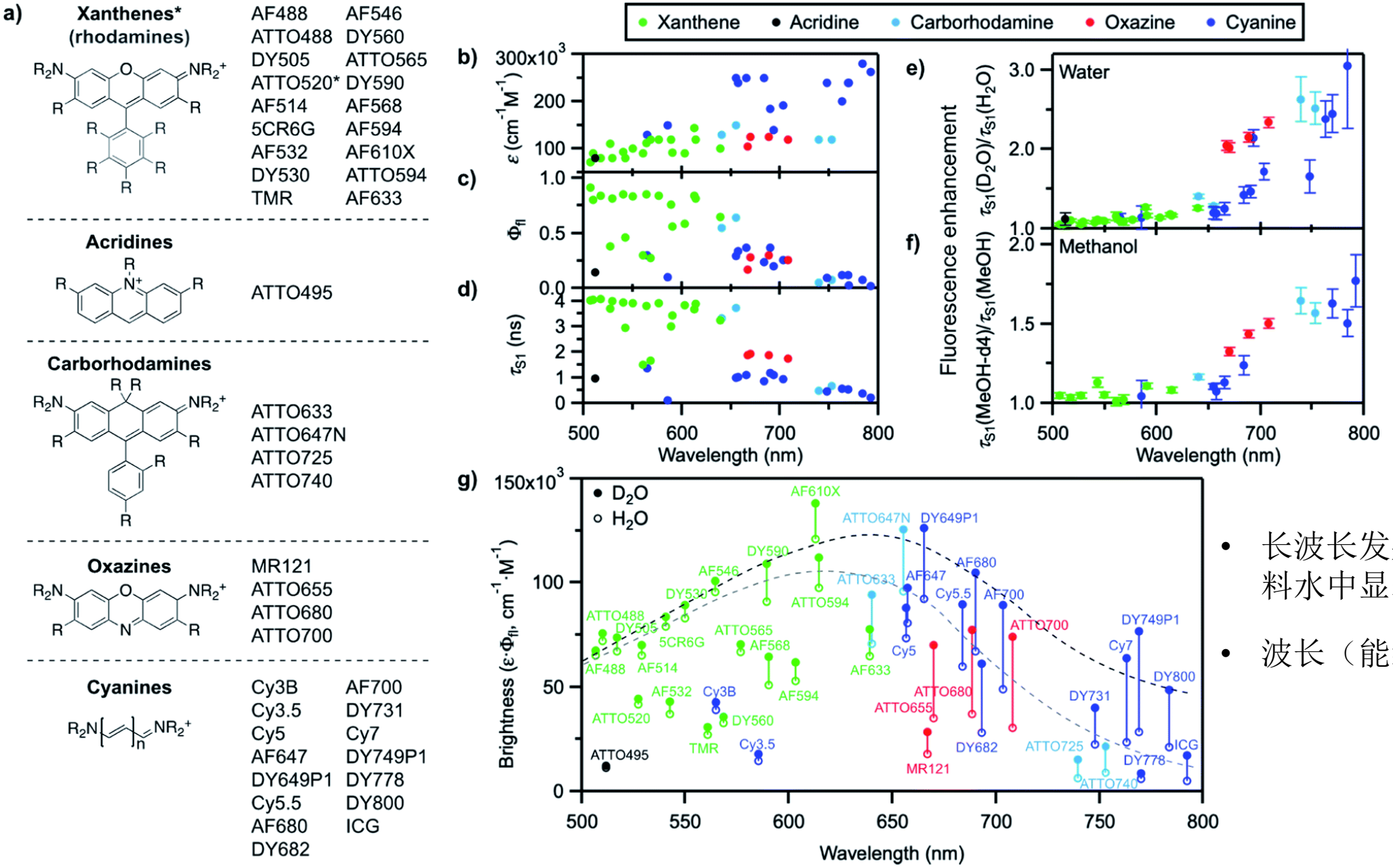
[a] The measured fluorescence lifetime was on the order of the instrument response function in both solvents. [b] Quantum yields and lifetimes were measured in PBS to avoid pH-dependent aggregation of the dye in pure H_2O.

Conclusion



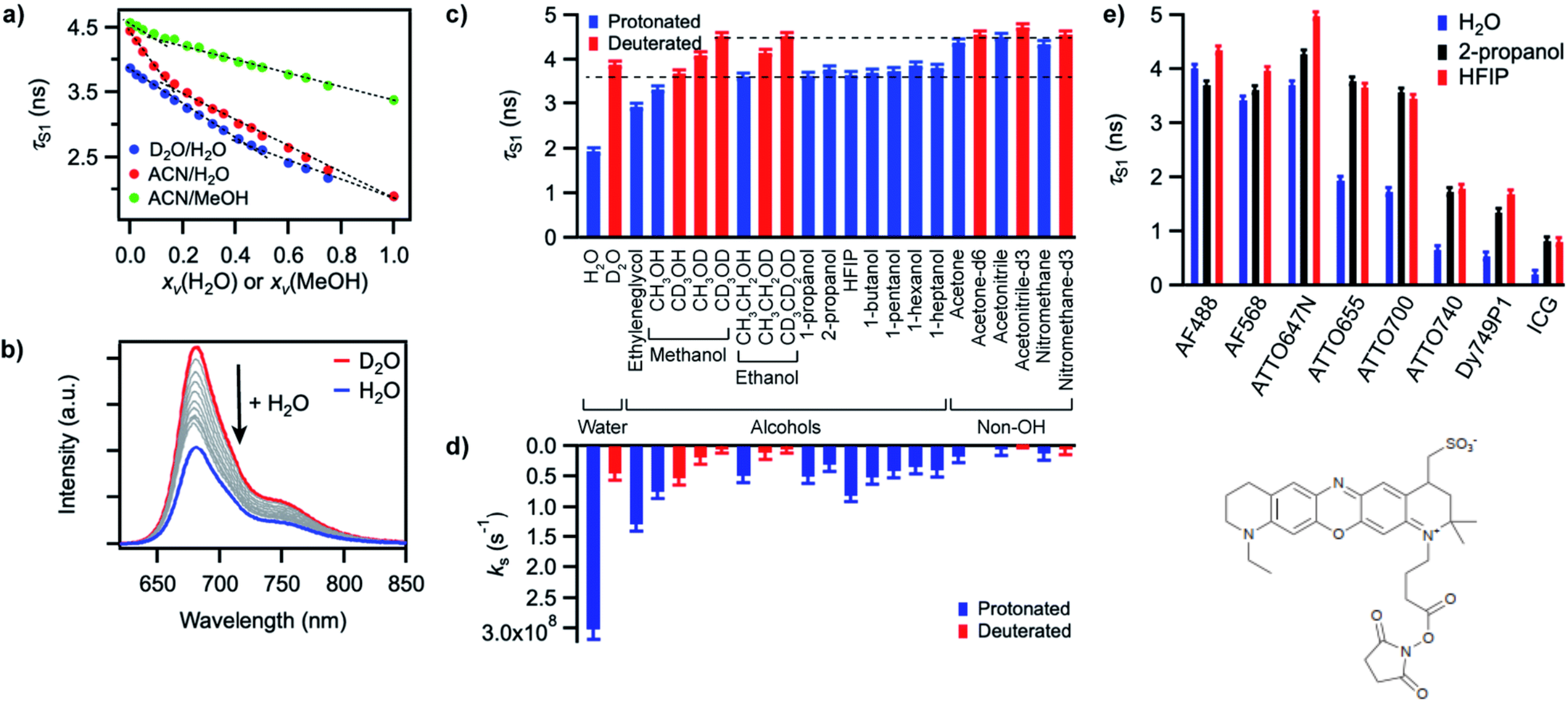
Although biological imaging is mostly performed in aqueous media, it is hardly ever considered that water acts as a classic fluorescence quencher for organic fluorophores. By investigating the fluorescence properties of 42 common organic fluorophores recommended for biological labelling, we demonstrate that H_2O reduces their fluorescence quantum yield and lifetime by up to threefold and uncover the underlying fluorescence quenching mechanism. We show that the quenching efficiency is significantly larger for red-emitting probes and follows an energy gap law. The fluorescence quenching finds its origin in high-energy vibrations of the solvent (OH groups), as methanol and other linear alcohols are also found to quench the emission, whereas it is restored in deuterated solvents. Our observations are consistent with a mechanism by which the electronic excitation of the fluorophore is resonantly transferred to overtones and combination transitions of high-frequency vibrational stretching modes of the solvent through space and not through hydrogen bonds. Insight into this solvent-assisted quenching mechanism opens the door to the rational design of brighter fluorescent probes by offering a justification for protecting organic fluorophores from the solvent *via* encapsulation.

Generality of the fluorescence quenching by water and methanol

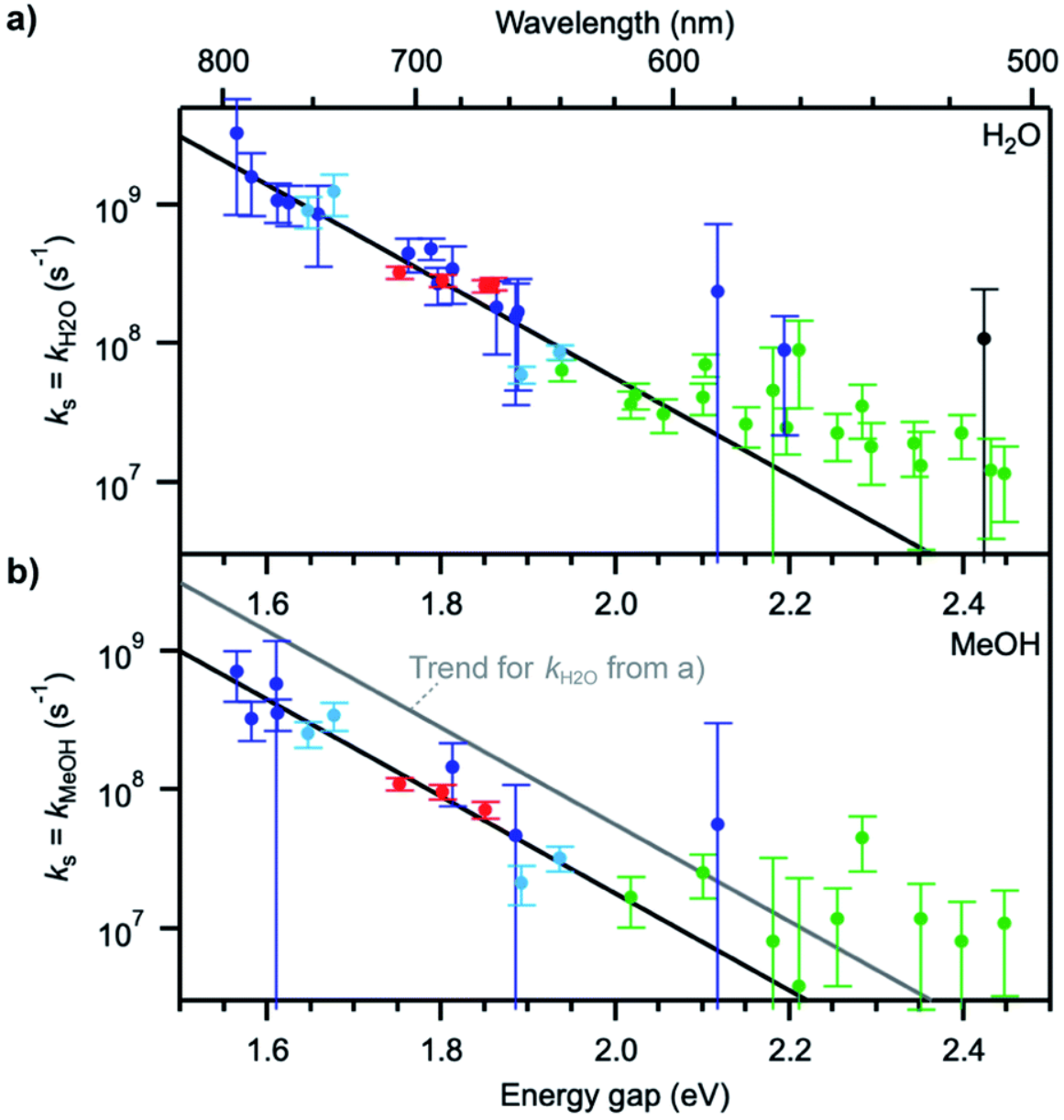


- 长波长发射的染料水中显著淬灭
- 波长（能级）依赖

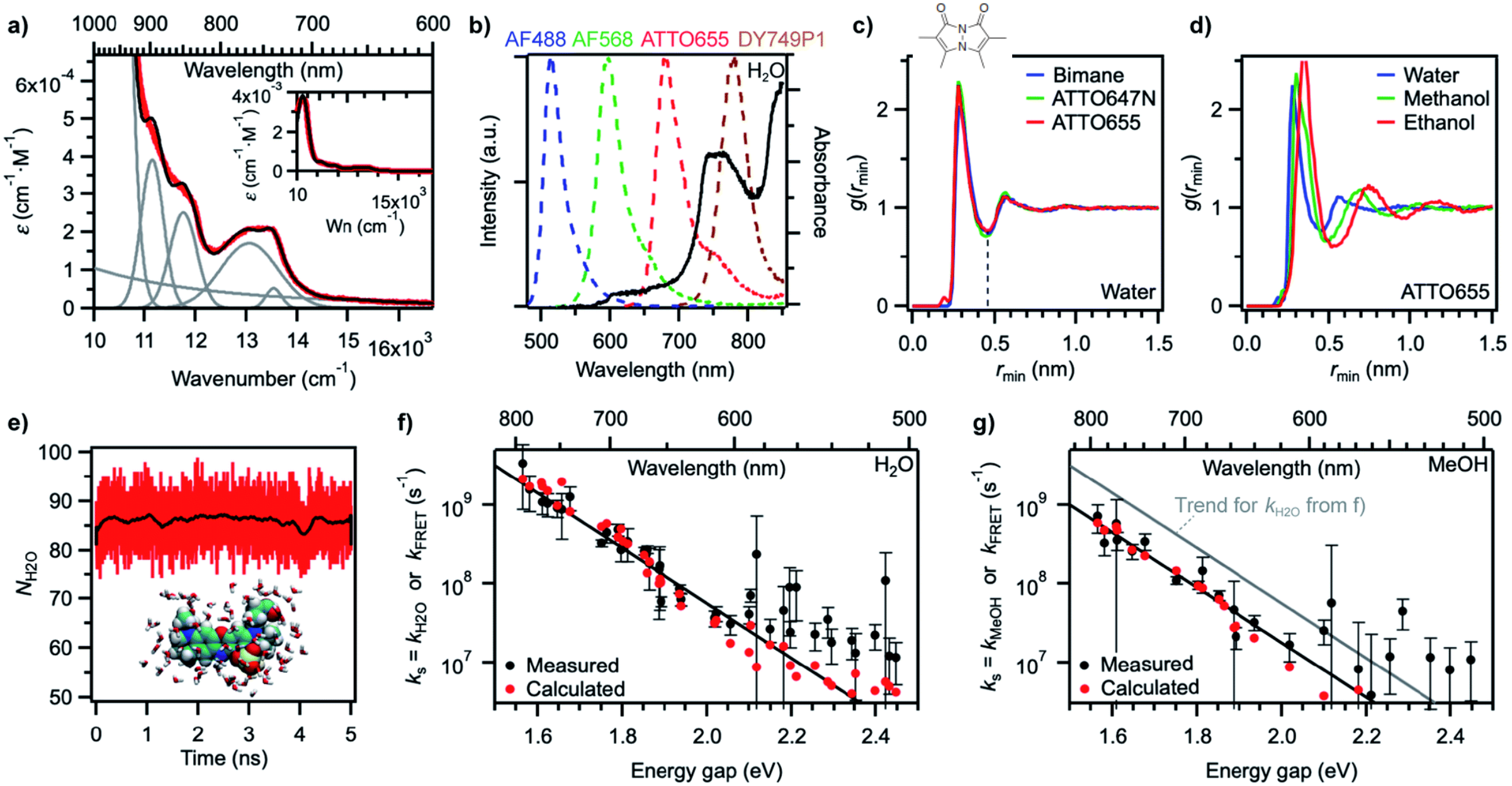
Solvent-assisted quenching through solvent OH moieties



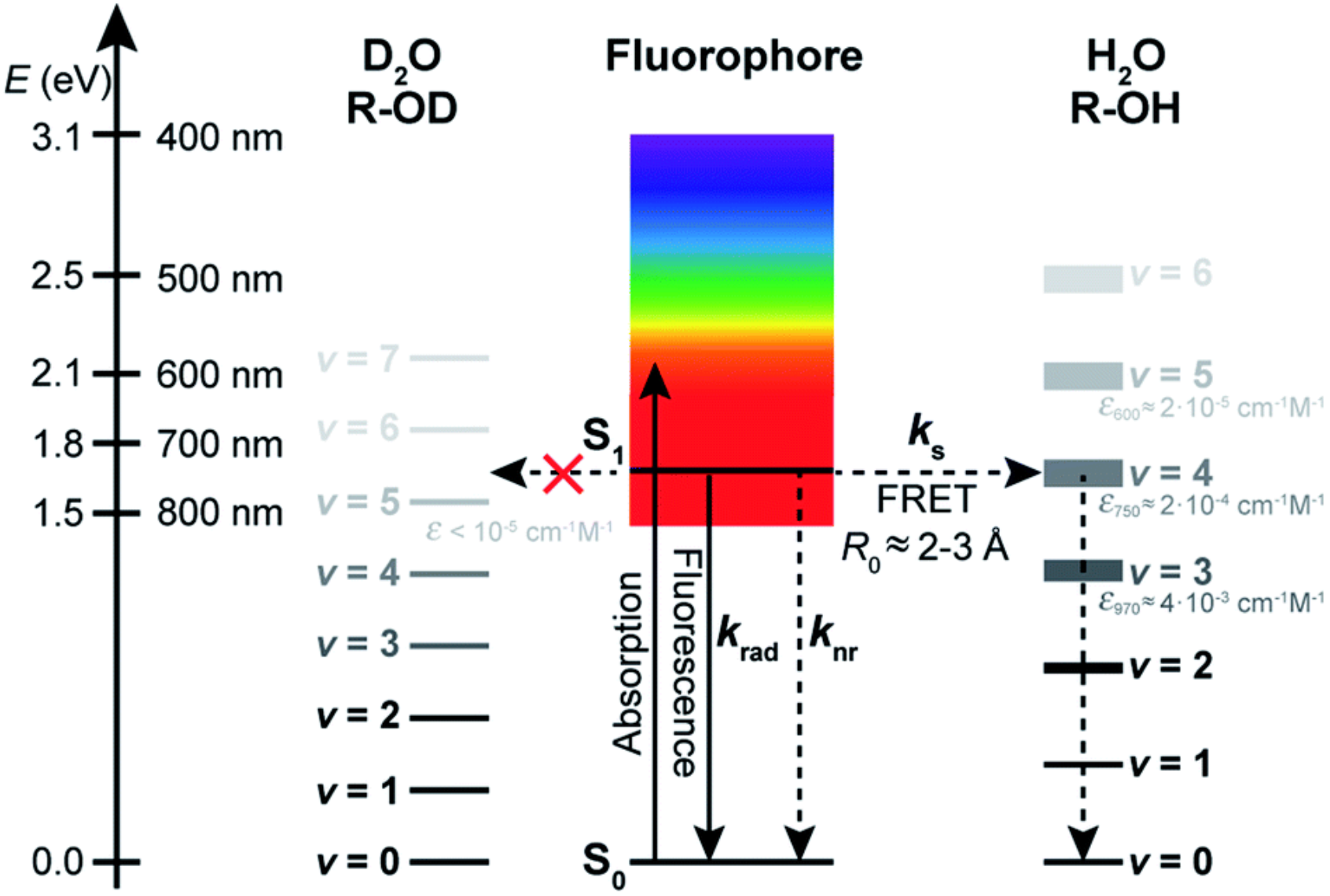
Quenching by the solvent follows an energy gap law



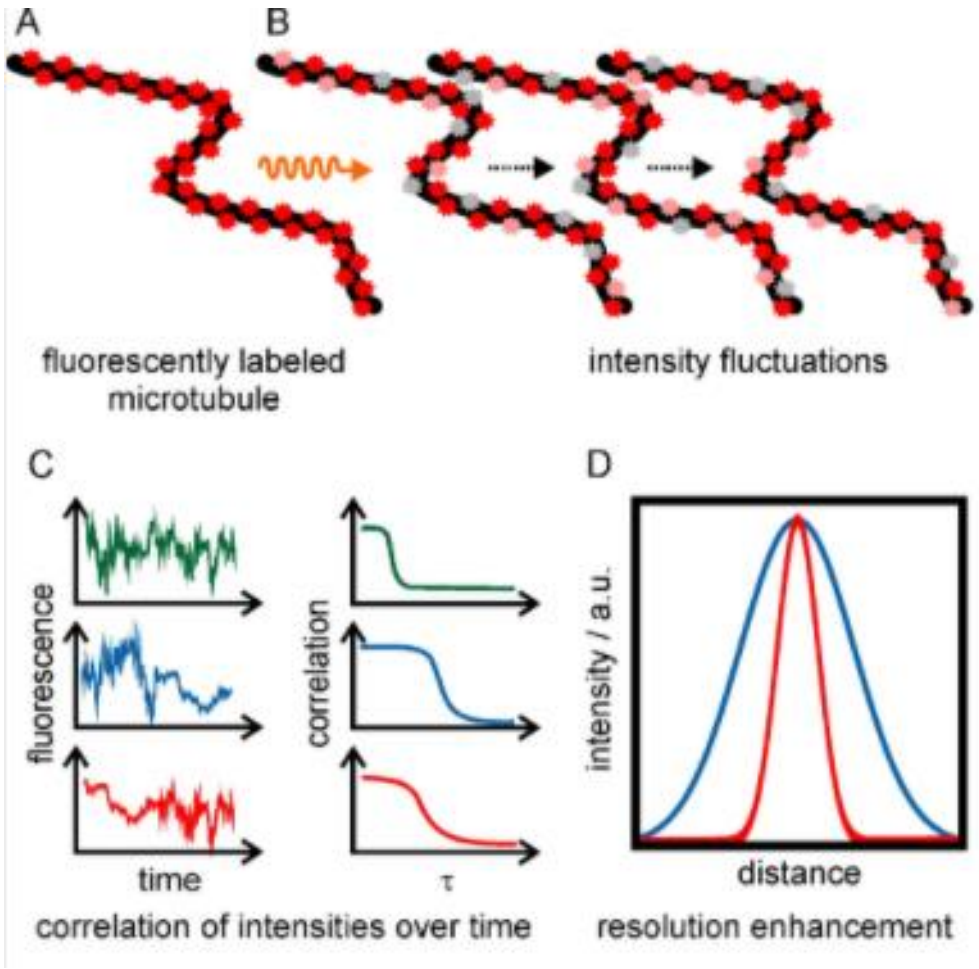
Quenching fluorophores by resonance electronic-to-vibrational energy transfer *via* dipolar coupling



solvent-assisted quenching of red-emitting fluorophores



Multi-Color, Bleaching-Resistant Super-Resolution Optical Fluctuation Imaging with Oligonucleotide-based Exchangeable Fluorophores



- Super-resolution optical fluctuation imaging (SOFI)

利用CCD拍摄获得一系列随时间波动的图像序列，由于同一个单一的荧光粒子具有时间自相关性，所以可以对图像的时间序列自相关计算直接产生高分辨图像。通过计算图像中每个像素的n阶时间累积量，在不进行后续去卷积算法的情况下，SOFI技术的分辨率比传统显微成像高$\sqrt{n}$倍。随着 n 值的增加，噪声和相邻像素去相关的效果得到了提升，便于得到高信噪比的超分辨图像。

- Photostability

SOFI with oligonucleotide-based exchangeable fluorophore labels

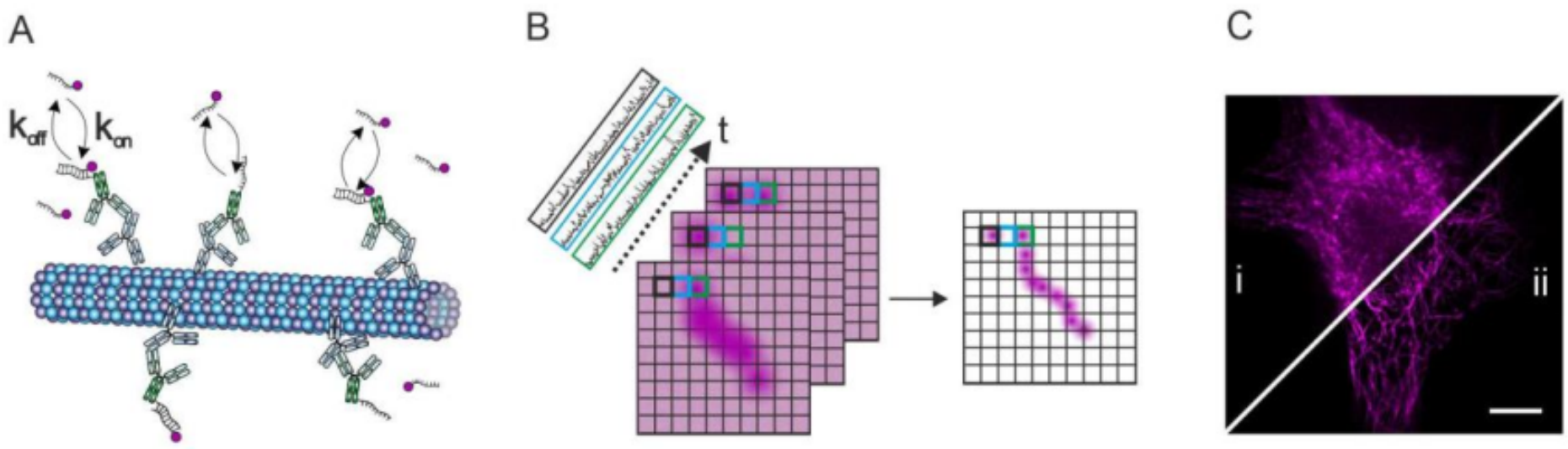


Figure 1. Principle of SOFI with oligonucleotide-based exchangeable fluorophore labels. A) Labeling of cellular structures using oligonucleotide-conjugated (docking strands) secondary antibodies and sequence-complementary, fluorophore-labeled oligonucleotides (imager strands). Transient and reversible binding of imager strands causes target-specific intensity fluctuations. B) Image processing in SOFI via time-trace analysis of stochastically fluctuating emitters. SOFI images are resolution and contrast enhanced and exhibit reduced unspecific background signals. C) Diffraction limited (i) and resolution-enhanced 2nd order SOFI image (ii) of a microtubule labeled U-2 OS cell using P1-AbberiorStar635P as exchangeable imager strand (scale bar 10 µm).

Multi-Color & Bleaching-Resistant SOFI