Literature Report

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Fluorescence Imaging

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Targetable Conformationally Restricted Cyanines Enable Photon-Count-Limited Applications**

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Reporter: Kai An

Date: 2021-11-24

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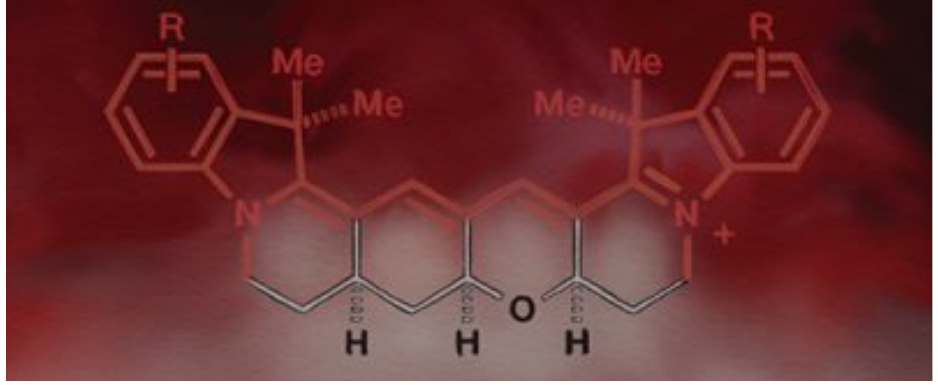

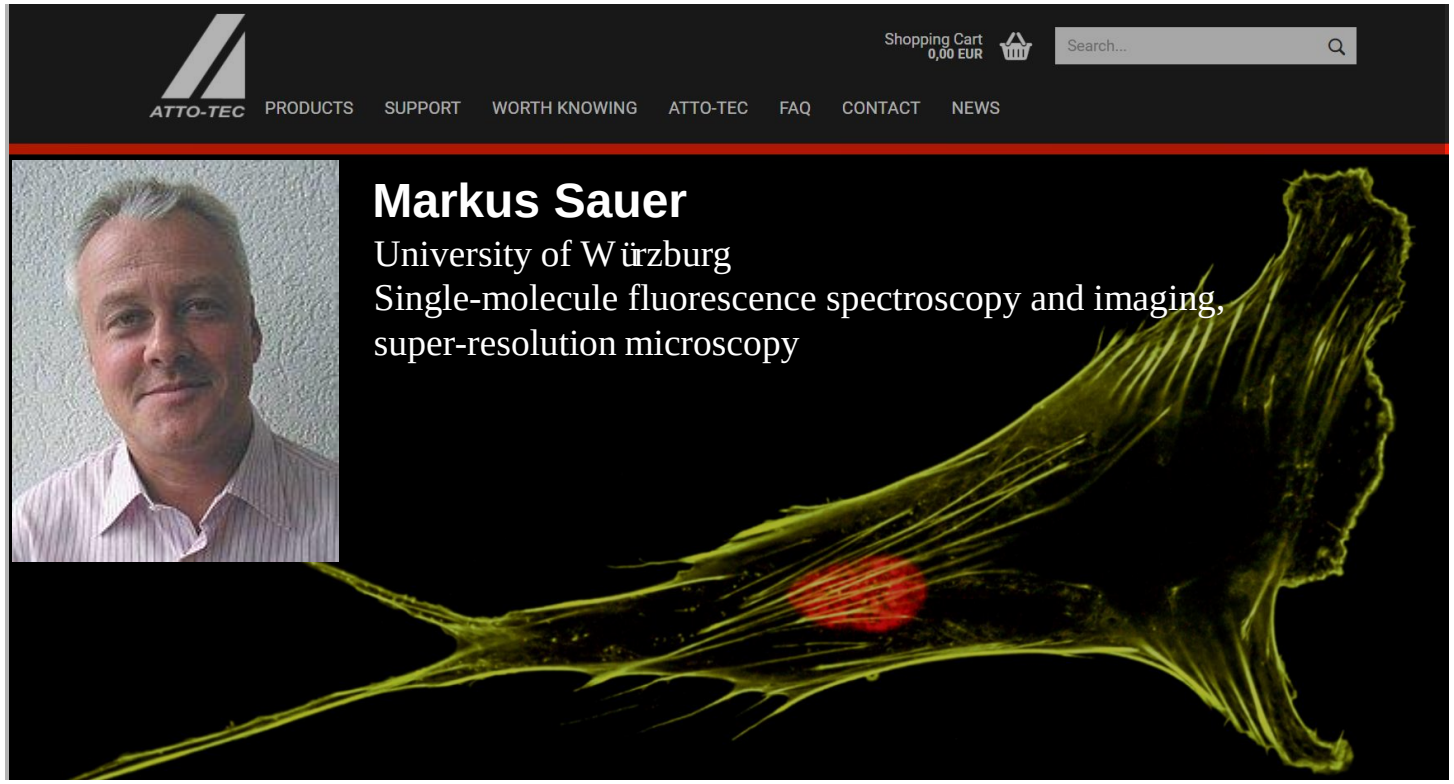

Markus Sauer

University of Würzburg
Single-molecule fluorescence spectroscopy and imaging, super-resolution microscopy

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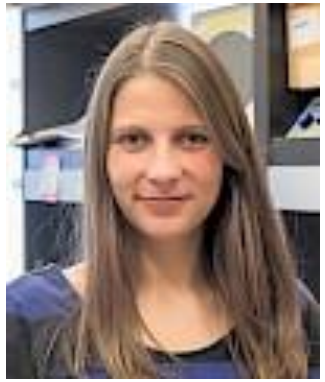
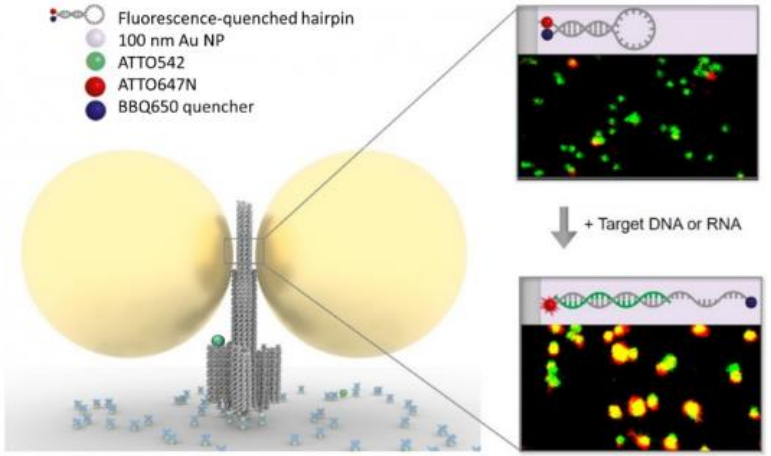
Dr. Viktorija Glembockyté



Single-Molecule Fluorescence,
Superresolution Microscopy,
DNA Nanotechnology, BioSensing

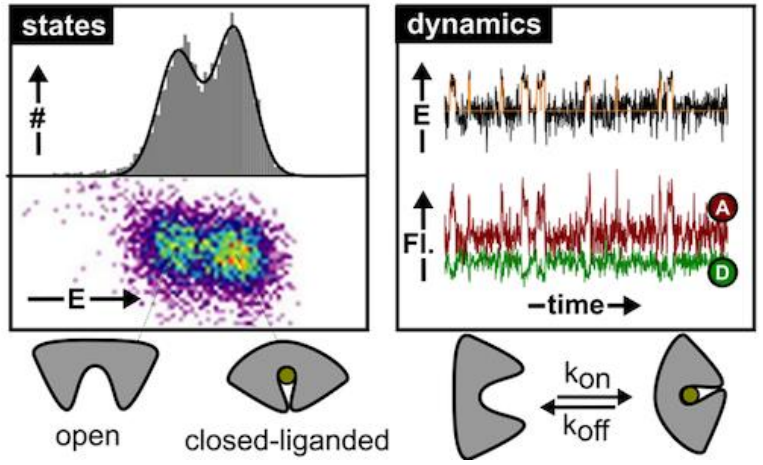


Prof. Dr. Thorben Cordes

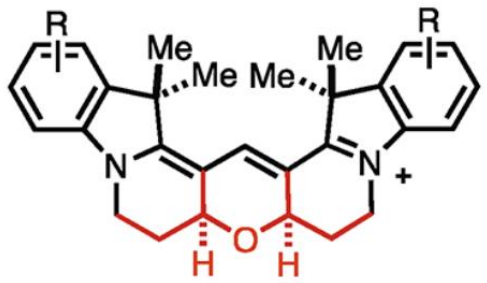
Molecular mechanisms of membrane transport;

Novel approaches to unravel fundamental principles in chemistry and catalysis;

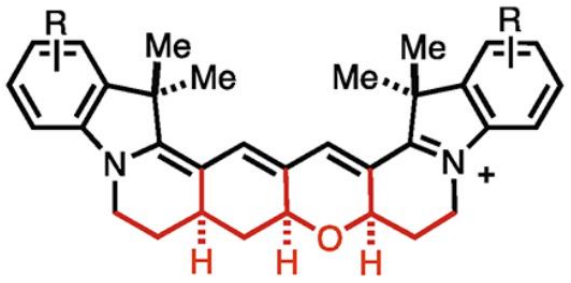
Development of new spectroscopy and microscopy methods



Photoswitch of Cyanine Dyes

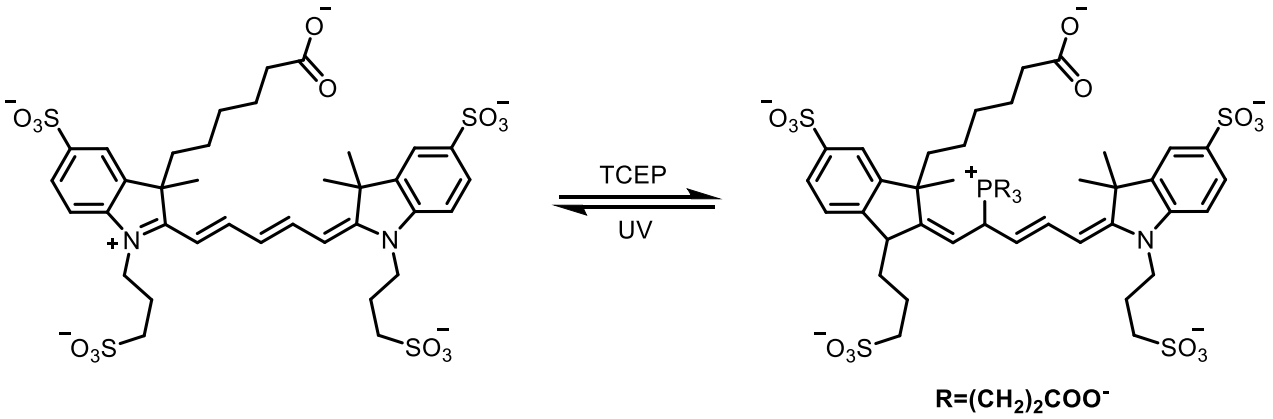


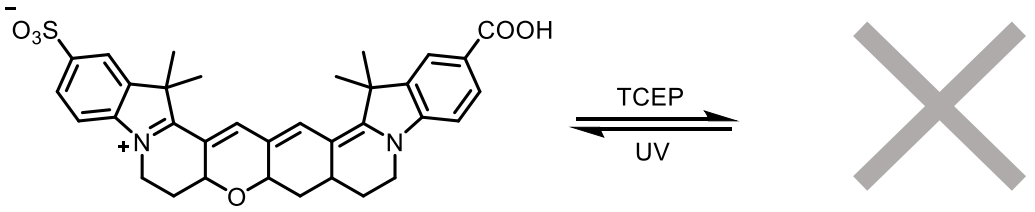
Trimethine Cyanine (black) e.g Cy3	Restrained Variant (black + red) e.g. Cy3B
$\Phi_F = 0.09^a$	$\Phi_F = 0.85^a$

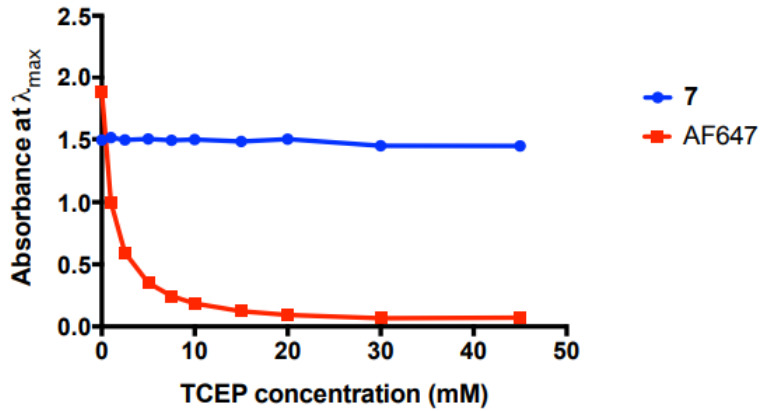


Pentamethine Cyanine (black) e.g. Cy5	Restrained Variant (black + red)
$\Phi_F = 0.15^b$	$\Phi_F = 0.69^b$

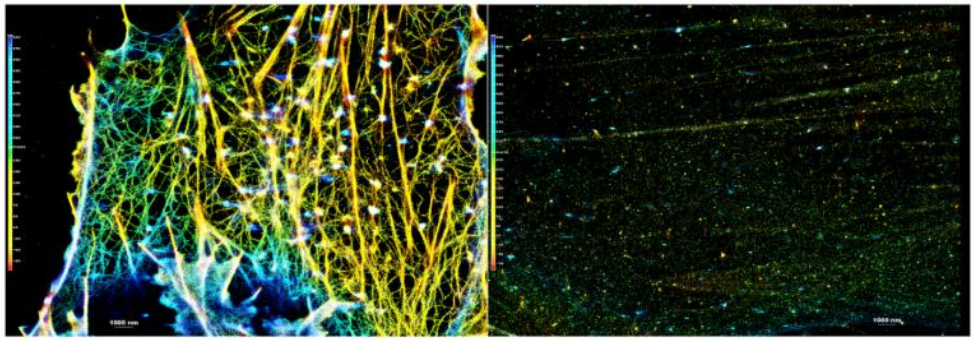
Photoswitch of Cyanine Dyes



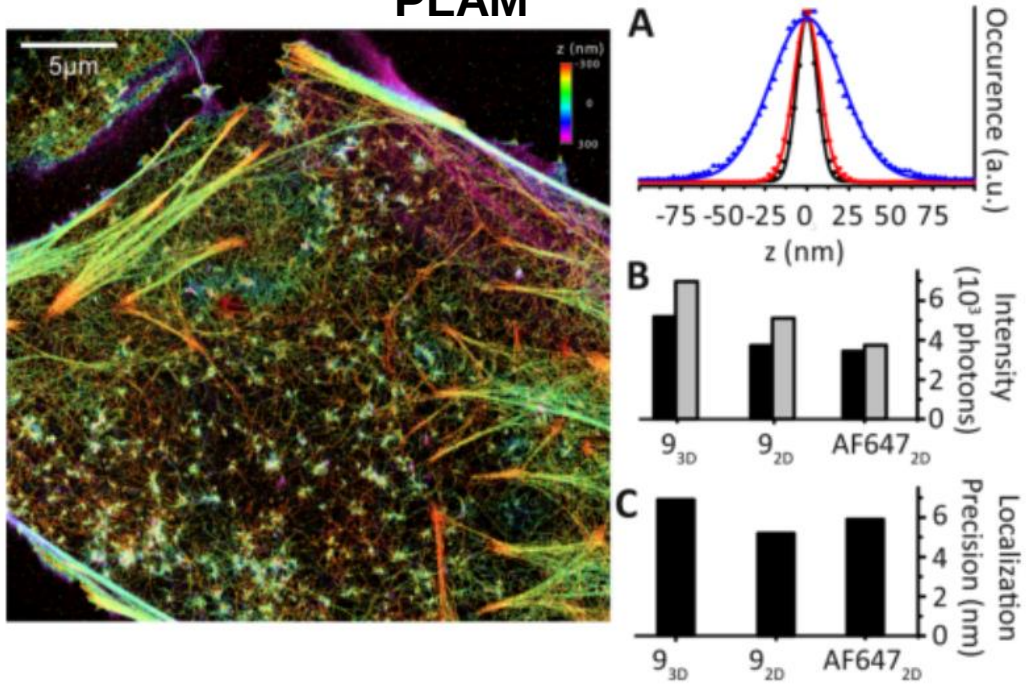




AF647 ***d*STORM** 9

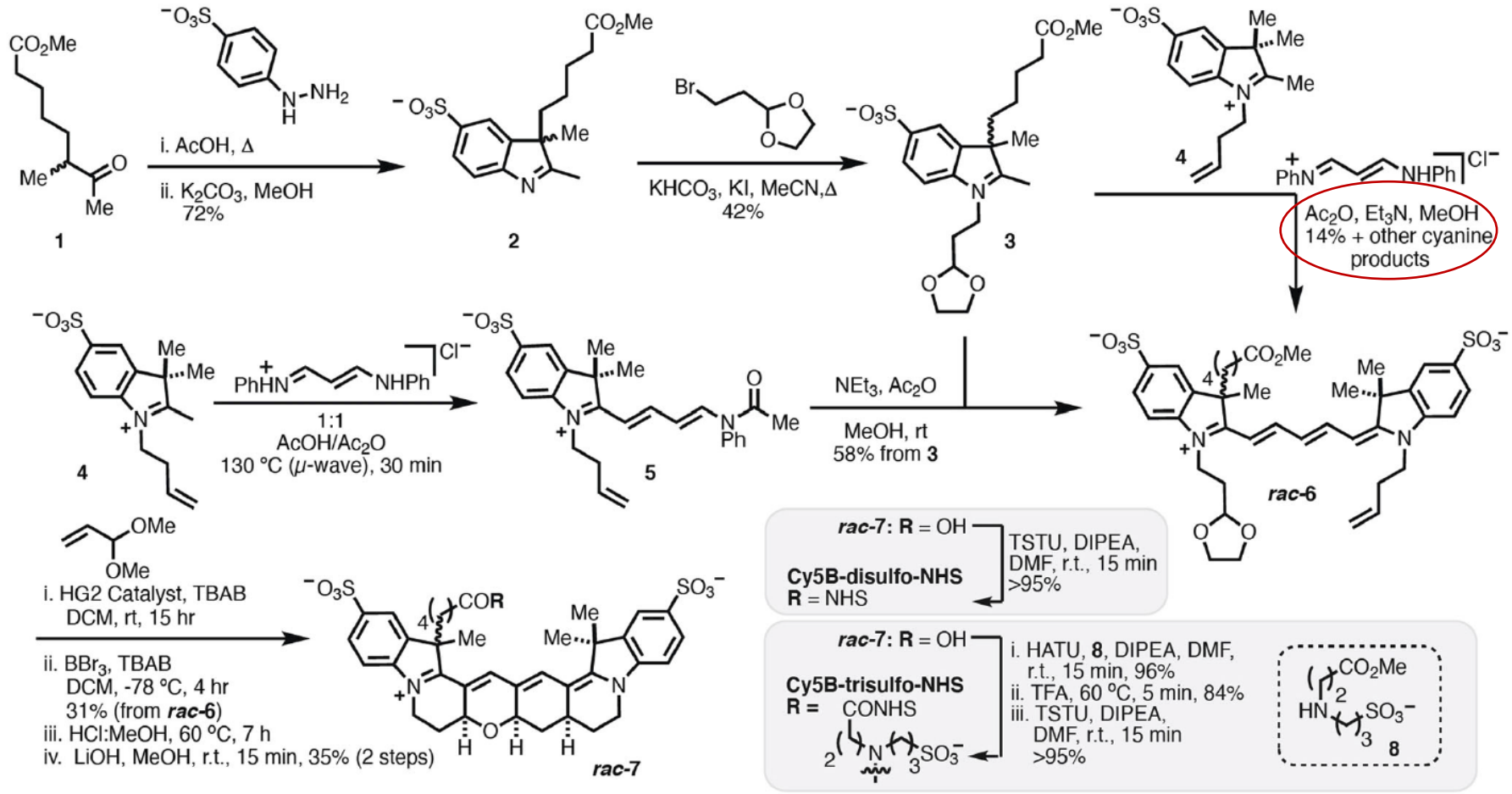


PLAM

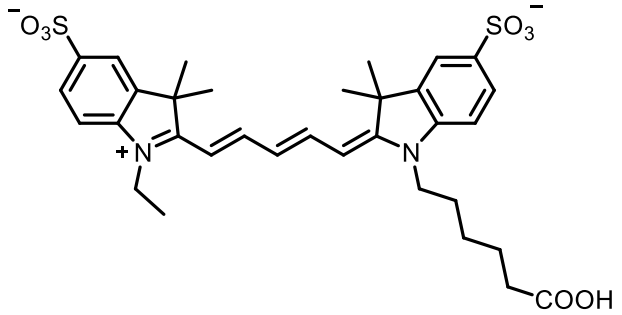


J. Am. Chem. Soc. 2009, 131, 51, 18192–18193

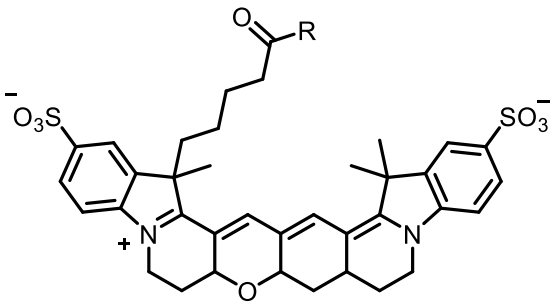
Synthesis

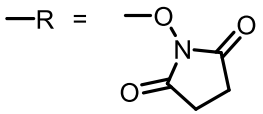

i. AcOH, Δ
ii. K2CO3, MeOH
72%
KHCO3, KI, MeCN,Δ
42%
Ac2O, Et3N, MeOH
14% + other cyanine products
1:1
AcOH/Ac2O
130 °C (μ-wave), 30 min
NEt3, Ac2O
MeOH, rt
58% from 3
i. HG2 Catalyst, TBAB
DCM, rt, 15 hr
ii. BBr3, TBAB
DCM, -78 °C, 4 hr
31% (from rac-6)
iii. HCl:MeOH, 60 °C, 7 h
iv. LiOH, MeOH, r.t., 15 min, 35% (2 steps)
rac-7: R = OH
Cy5B-disulfo-NHS
R = NHS
TSTU, DIPEA,
DMF, r.t., 15 min
>95%
rac-7: R = OH
Cy5B-trisulfo-NHS
R = CONHS
i. HATU, 8, DIPEA, DMF,
r.t., 15 min, 96%
ii. TFA, 60 °C, 5 min, 84%
iii. TSTU, DIPEA,
DMF, r.t., 15 min
>95%
1
2
3
4
5
rac-6
rac-7
8

Spectroscopic Characteristics

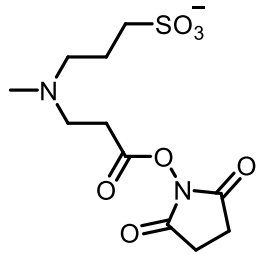


Cy5

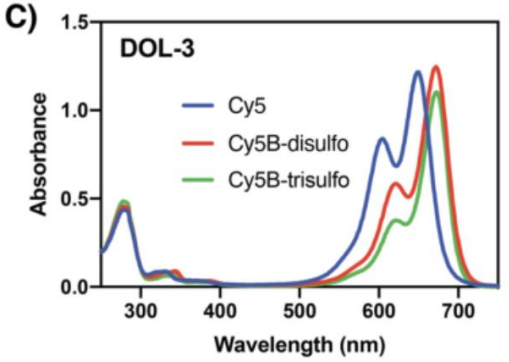




Cy5B-disulfo



Cy5B-trisulfo

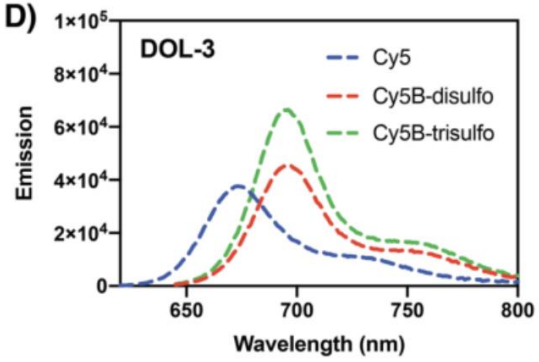


A

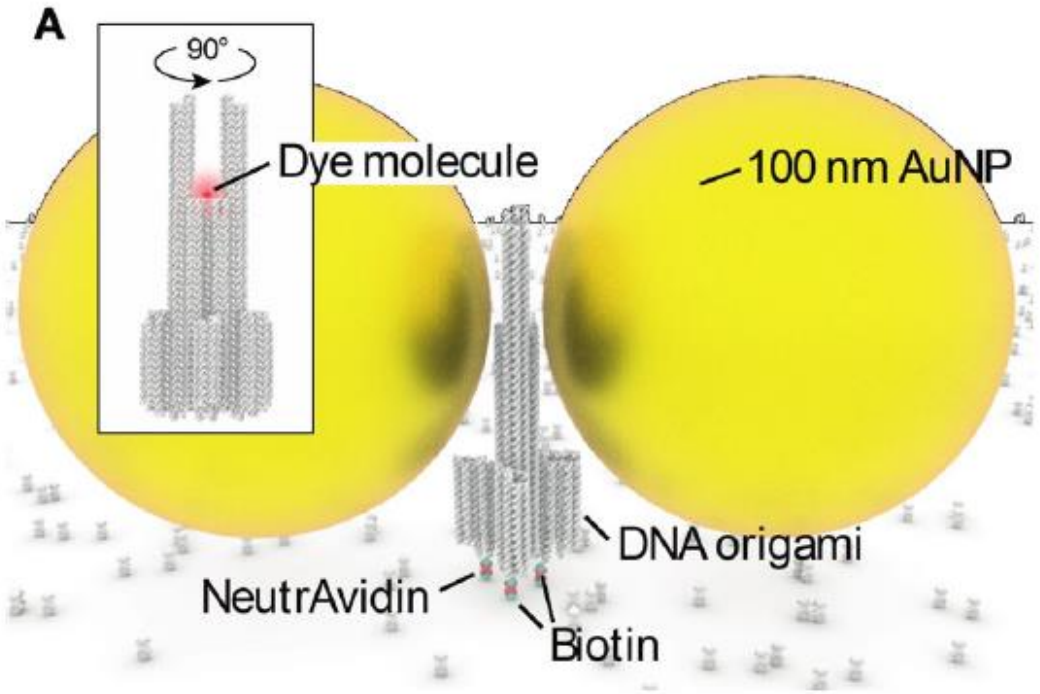
λ_{max} (nm)	ε ($\times 10^4$ $M^{-1}cm^{-1}$)	λ_{em} (nm)	Φ_F	τ_F (ns)	Brightness ($\varepsilon \times \Phi_F$)
Cy5					
647	27.03 ± 0.8	665	0.26	1.09	70,200
Cy5B-disulfo					
669	19.3 ± 2.4	684	0.45	1.89	86,850
Cy5B-trisulfo					
669	24.1 ± 1.9	685	0.40	1.85	96,400

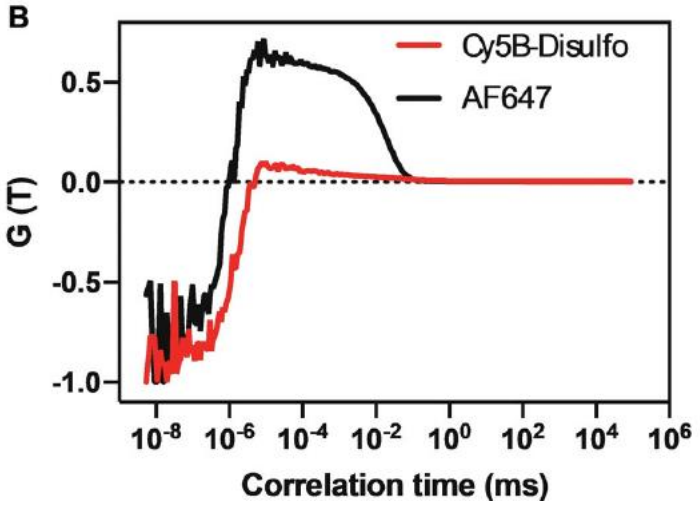
B

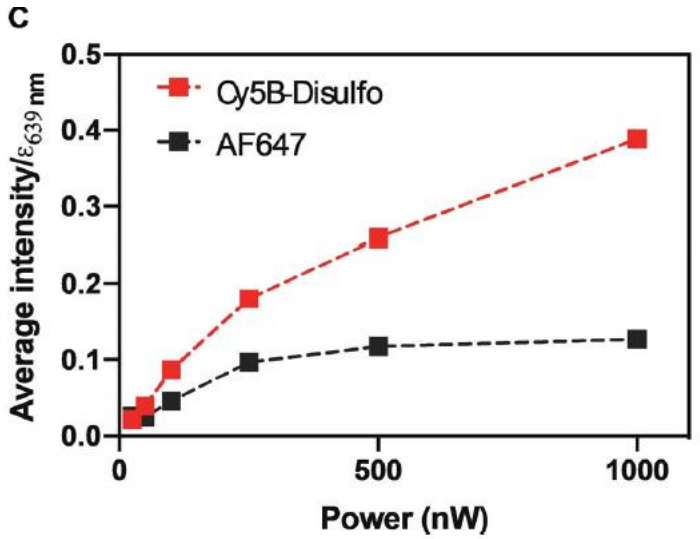
Construct	Φ_F	$\frac{\Phi_F \text{ - labeled}}{\Phi_F \text{ - free dye}}$	τ_F (ns)	$\frac{\tau_F \text{ - labeled}}{\tau_F \text{ - free dye}}$
DOL-1				
Cy5-mAb	0.21	0.81	1.53	1.41
Cy5B-disulfo- mAb	0.38	0.84	2.08	1.10
Cy5B-trisulfo- mAb	0.41	1.03	2.13	1.15
DOL-3				
Cy5-mAb	0.11	0.42	1.22	1.12
Cy5B-disulfo- mAb	0.31	0.69	1.79	0.95
Cy5B-trisulfo- mAb	0.39	0.98	2.21	1.19



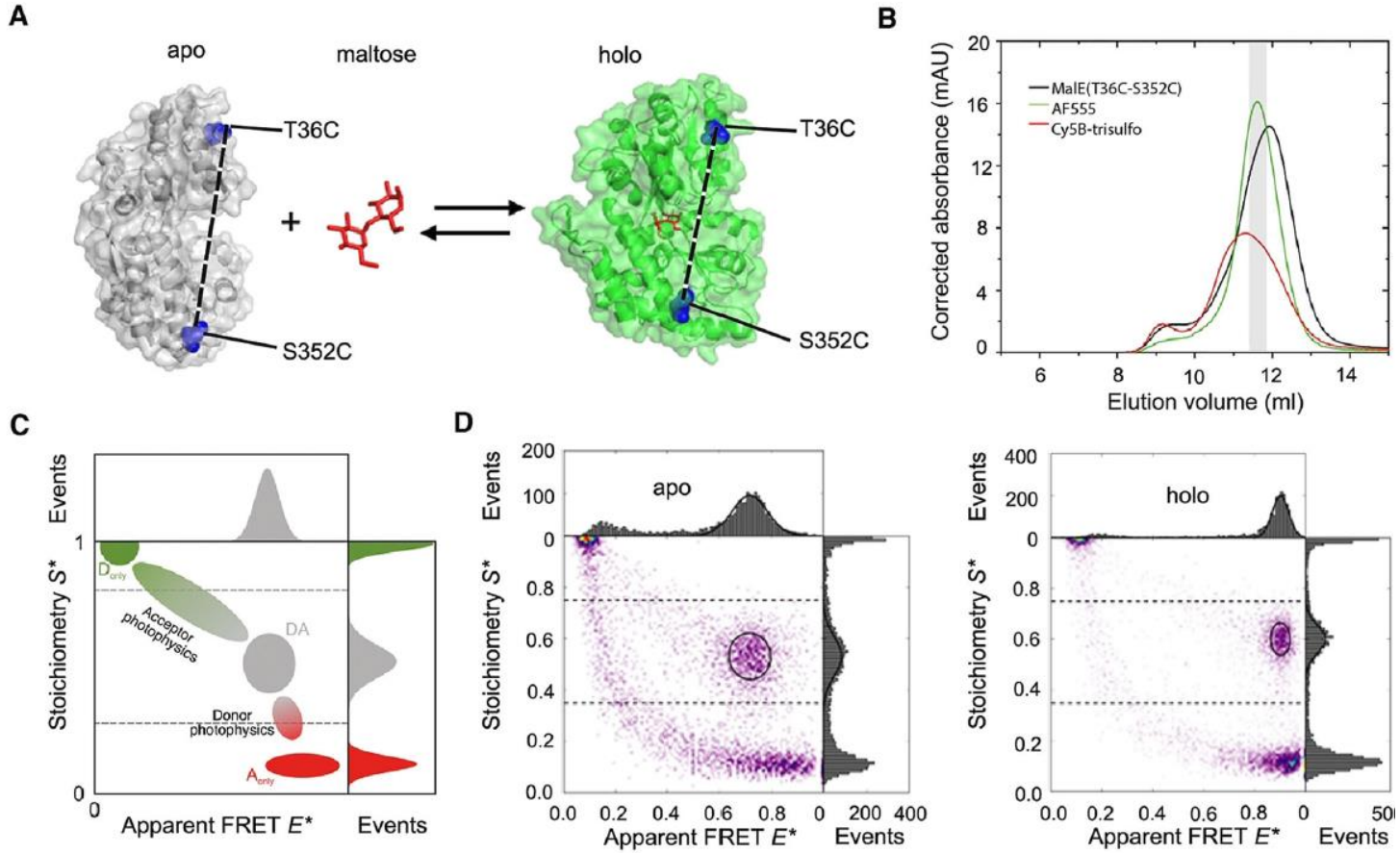
Photophysical Properties of DNA-Conjugates

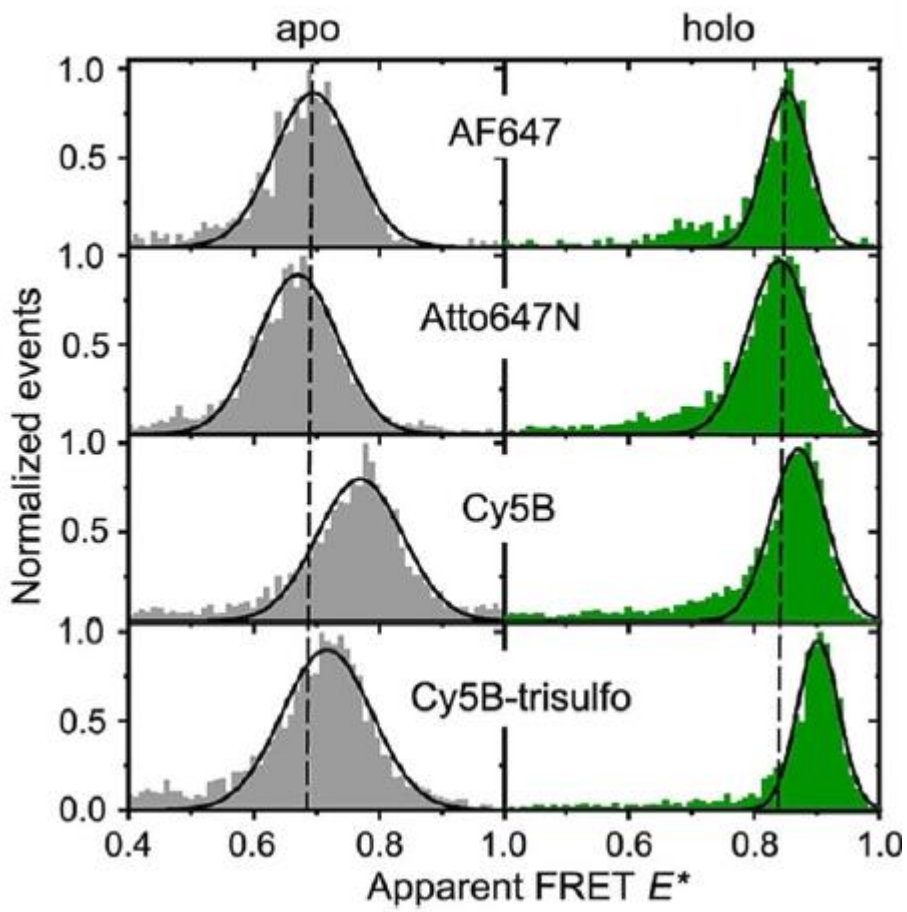






Single-Molecule FRET





FLIM and SMLM