Supplementary information

for

Defining the Basis of Cyanine Phototruncation Enables A New Approach to Single Molecule Localization Microscopy

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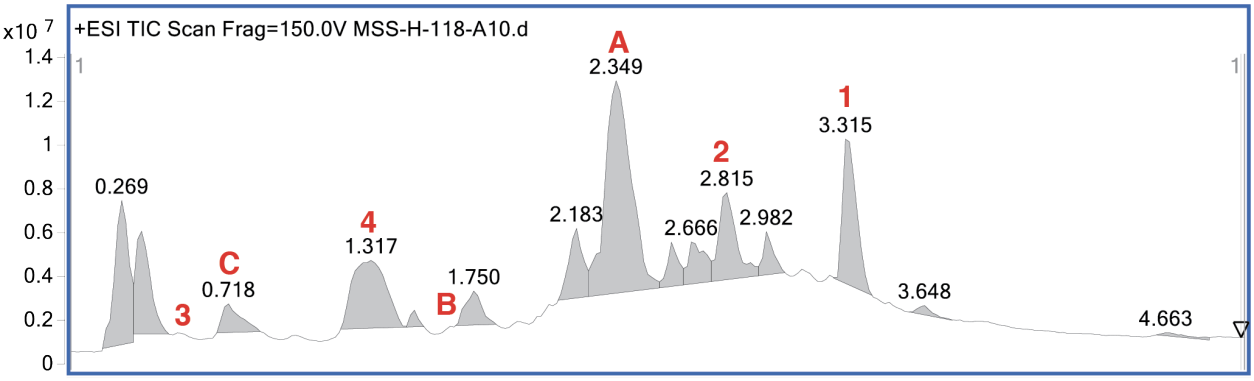
Supplemental Tables

Table S1. Calculated and Observed HRMS (ESI) masses for photo-irradiated products



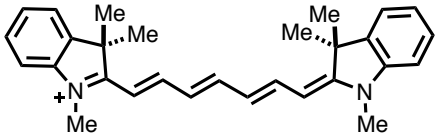
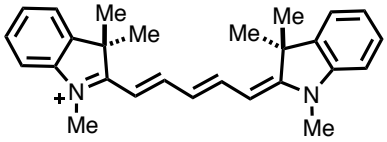
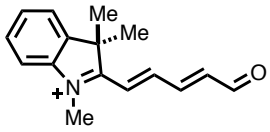
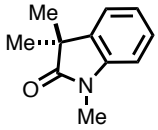
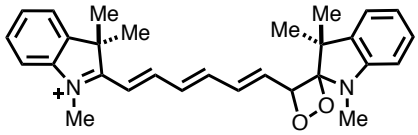
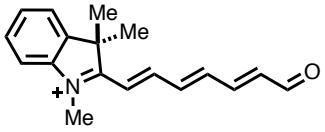
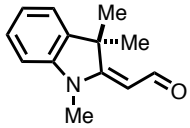
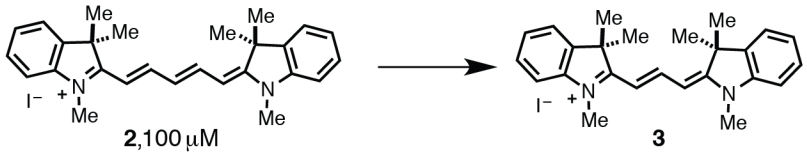
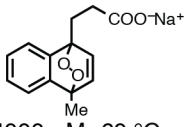
Entry	Compound	Empirical Formula	Calculated m/z	Observed m/z
1		$C_{29}H_{33}N_2^+$	409.2638	409.25553 $[M]^+$
2		$C_{27}H_{31}N_2^+$	383.2482	383.2482 $[M]^+$
3		$C_{16}H_{18}NO^+$	240.1383	240.1387 $[M]^+$
4		$C_{11}H_{13}NO$	175.0997	176.1068 $[M+H]^+$
A		$C_{29}H_{33}N_2O_2^+$	441.2537	441.2539 $[M]^+$
B		$C_{18}H_{20}NO^+$	266.1539	266.1539 $[M[^+$
C		$C_{13}H_{15}NO$	201.1154	202.1224 $[M+H]^+$

Table S2. Evaluating the truncation of **2** to **3** with independently generated ROS



2,100 μM **3**

Entry	Conditions	Yield 2[a]	Yield 3[a]
1	dark PBS (10 mM)	99.5 (± 1.6)	~ 0.30
2	740 nm hν 0.5 W/cm^2 PBS (10 mM)	0.14 (± 0.06)	1.42 (± 0.10)
3	740 nm hν 0.5 W/cm^2 Ethanol	0.8 (± 0.02)	~0.01
4	Zn(TPP) 5 mol% 420 nm hν 2 mW/cm^2 50% MeCN/H_2O	83.2 (± 1.6)	~1.2 (± 0.01)
5	H_2O_2 100 μM PBS (10 mM) pH = 7.4 (H_2O_2)	94.9 (± 1.9)	~0.1 (± 0.16)
6	KO_2 100 μM PBS (10 mM) pH = 7.4 ($O_2^{\bullet -}$)	86.3 (± 3.1)	~0.1 (± 0.02)
7	$FeCl_2$ 200 μM H_2O_2 1 mM $NaHPO_4$ (50 mM) pH = 6 ($HO^{\bullet}$)	3.4 (± 1.2)	~0.1 (± 0.03)
8	 4000 μM, 60 °C PBS (10 mM)	86.9 (± 1.4)	~1.1 (± 0.23)

[a] Determined by UV-vis and reported in percent. Values are average of experimental triplicate. Error expressed as standard deviation in parentheses.

Table S3. Evaluating the role of irradiation on **1** to **2** phototruncation keeping the total photons constant.

#	Power (mW cm^{-2})	Time (min)	% 2 formed[a]
1	8	128 min	1.96 ± 0.15
2	16	64 min	1.81 ± 0.13
3	31	32 min	1.85 ± 0.16
4	62	16 min	1.80 ± 0.10
5	125	8 min	1.93 ± 0.14
6	250	4 min	1.71 ± 0.12
7	500	2 min	1.82 ± 0.10

[a]The yield of **2** formed was calculated using Beer-lambert law (from a starting **1** concentration of 100 μM). The absorbance at 640 nm was measured after photo-irradiating the sample in a glass autosampler vial (containing a fused insert). The absorption coefficient (ε) of **2** in water was calculated to be 230,400 $M^{-1}cm^{-1}$. Experiments conducted in triplicate with the error expressed in standard deviation of mean.

Table S4. Evaluating the role of irradiation power on **1** to **2** phototruncation keeping the irradiation time constant.

#	Power (mW cm^{-2})	Time (min)	% 2 formed[a]
1	8	2 min	–
2	16	2 min	0. 03 ± 0. 01
3	31	2 min	0.06 ± 0.05
4	62	2 min	0.29 ± 0.07
5	125	2 min	0.64 ± 0.15
6	250	2 min	1.13 ± 0. 22
7	500	2 min	1.82 ± 0.10

[a]The yield of **2** formed was calculated using Beer-lambert law (from a starting **1** concentration of 100 μM). The absorbance at 640 nm was measured after photo-irradiating the sample in a glass autosampler vial (containing a fused insert). The absorption coefficient (ε) of **2** in water was calculated to be 230,400 $M^{-1}cm^{-1}$. Experiments conducted in triplicate with the error expressed in standard deviation of mean.

Table S5. Top-20 conditions from Comprehensive screen.

Buffer #	Solute	Concentration	pH	Buffer Name	Buffer Conc.	Solvent	% conversion (384-well plate method)	% conversion (using larger volumes in a glass vial)			Average (Method - II)	std dev (Method - II)
								Attempt-1	Attempt-2	Attempt-3		
1			9.5	CAPSO	1000 mM	water	9.5	14.7	15.5	15.4	15.2	0.4
2			9.2	CAPSO with saline	1000 mM	water	8.5	13.3	13.5	11.6	12.8	1.0
3	1,3-diaminopropanol	100 mM	9			water	7.4	10.6	11.1	11.4	11.0	0.4
4	Arginine	100 mM	9	CAPSO	1000 mM	water	6.6	10.4	10.1	10.8	10.4	0.4
5	Arginine	100 mM	9			water	6.6	10.2	10.4	11.2	10.6	0.5
6	Arginine	100 mM	9.8			water	6.4	9.7	10.3	10.4	10.1	0.4
7	Arginine	100 mM	10			water	6.3	10.2	9.5	9.7	9.8	0.4
8	Arginine	100 mM	9.2	CAPSO with saline	1000 mM	water	5.7	8.6	8.3	9.8	8.9	0.8
9	1,3-diaminopropanol	100 mM	9.25	CAPSO	1000 mM	water	5.3	9.5	9.9	9.8	9.7	0.2
10	1,3-diaminopropanol + lysine	100 mM	8.9	CAPSO	1000 mM	water	5.2	9.6	9.2	8.8	9.2	0.4
11	Histidine	10 mM	7.4	Phosphate Buffer	10 mM	water	5.1	9.3	8.5	9.3	9.0	0.5
12	Arginine	100 mM	9.2	BIS-TRIS	1000 mM	water	5.0	8.7	8.1	7.9	8.2	0.4
13	Histidine	100 mM	10			water	4.9	9.3	8.5	8.4	8.7	0.5
14	Lysine	100 mM	10			water	4.9	8.7	8.1	9.4	8.7	0.7
15	Alanine	100 mM	10			water	4.8	8.8	8.2	7.8	8.3	0.5
16	Lysine	10 mM	8.5	TRIS	100 mM	water	4.8	8.3	8.1	7.3	7.9	0.5
17	Arginine	10 mM	7.4	Phosphate Buffer	10 mM	water	4.7	8.2	7.6	8.1	8.0	0.3
18	Histidine	100 mM	7.4			water	4.6	8.2	8.5	7.8	8.2	0.4
19	1,3-diaminopropanol	100 mM	9.5	CAPSO with saline	1000 mM	water	4.6	8.2	8.5	8.1	8.3	0.2
20	Lysine	100 mM	9.2	BIS-TRIS	1000 mM	water	4.6	7.4	7.6	7.1	7.4	0.3

The yield of **2** formed was calculated using Beer-lambert law (from a starting **1** concentration of 50 μM). The absorbance at 640 nm (**2**) was measured after photo-irradiating the sample in a glass autosampler vial (containing a fused insert). The absorption coefficients (ε) of **2** in PBS were calculated to be 230,400 $M^{-1}cm^{-1}$. Experiments conducted in triplicate with the error expressed in standard deviation of mean.

Supplemental Figures

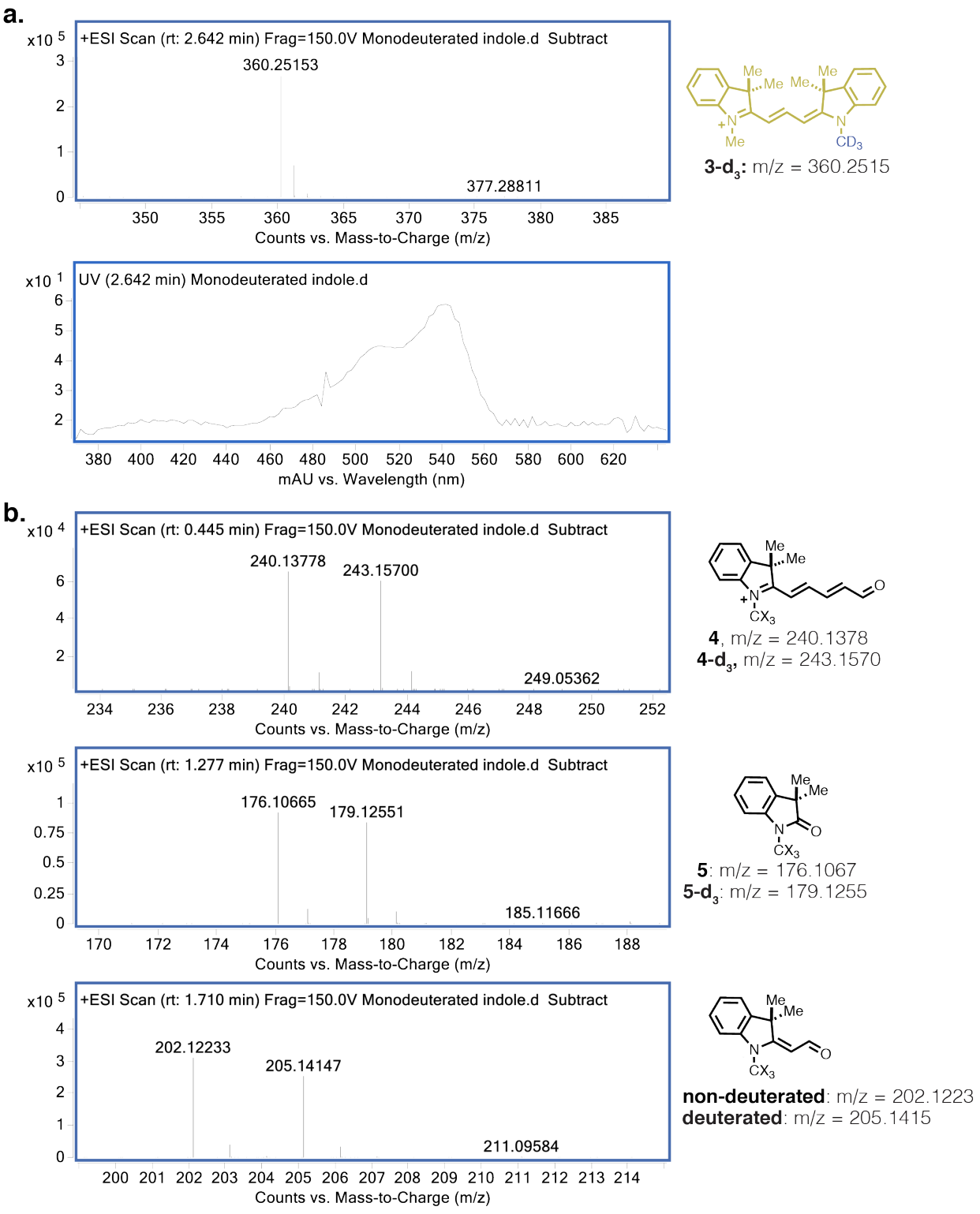


Figure S1 (continued on next page). HRESIMS analysis of the product mixture obtained from the irradiation of **2-d3** with 630 LED (0.2 mW cm^{-2}) in PBS (pH = 7.4) for 60 min. (a) m/z and extracted UV-vis spectra of **3-d3** (b) other products observed.

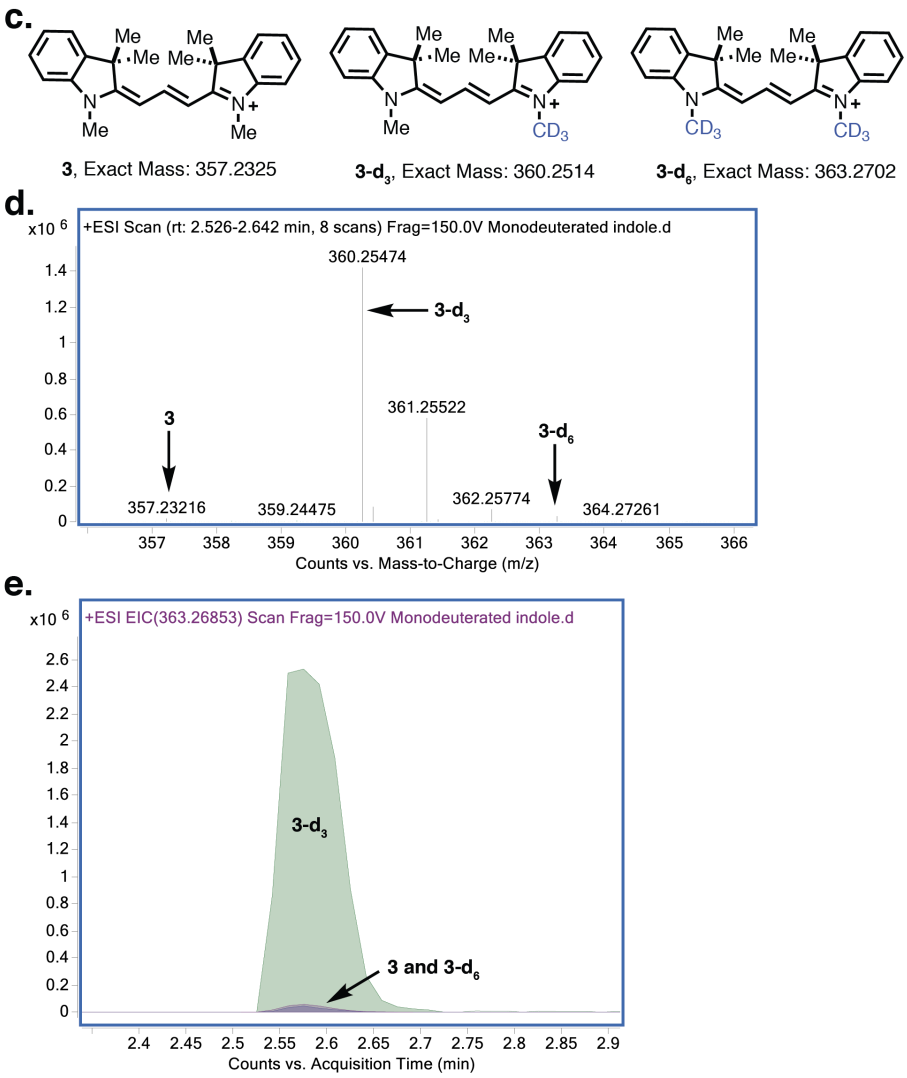


Figure S1 (continued). HRESIMS analysis of the product mixture obtained from the irradiation of **2-d$_3$** with 630 LED (0.2 mW cm^{-2}) in PBS (pH = 7.4) for 60 min. (c) Compounds analyzed. (d) m/z for the designated compounds and their (e) extracted ion counts at 357.2321, 360.2547 and 363.2685 within ± 10 ppm. The relative ratio of the extracted ion counts for **3**, **3-d$_3$**, and **3-d$_6$** is measured to be 97.8: 0.6: 1.6.

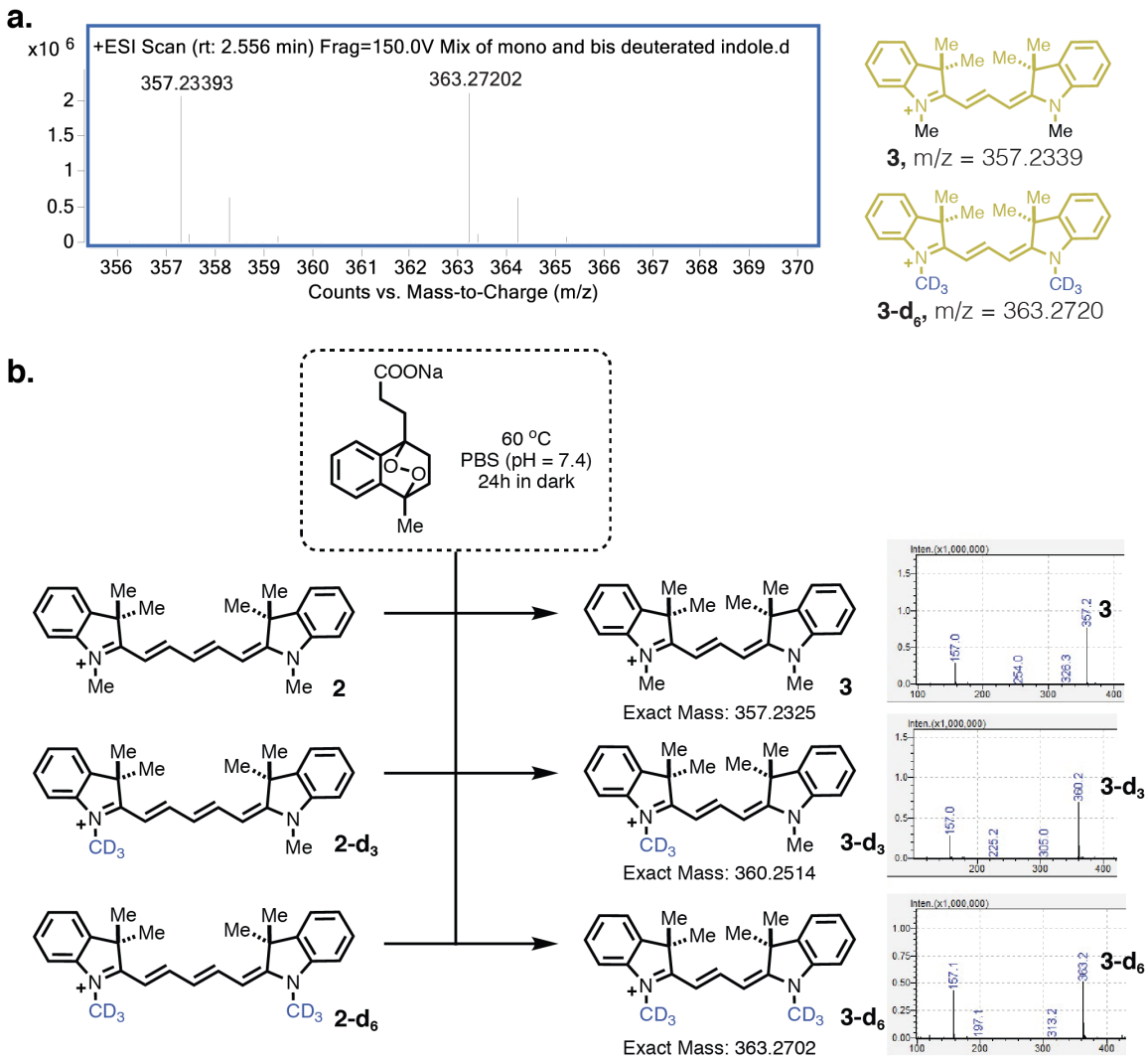
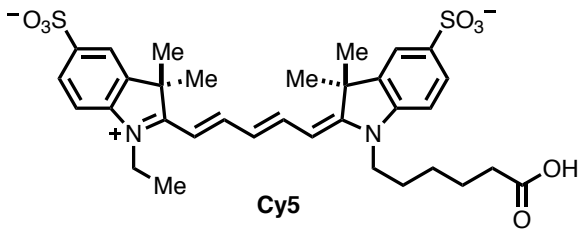
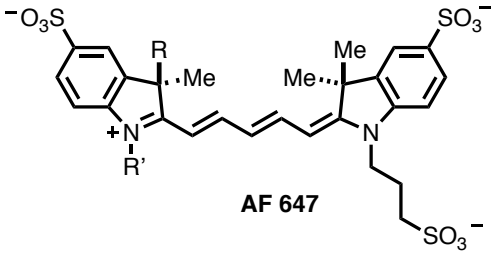


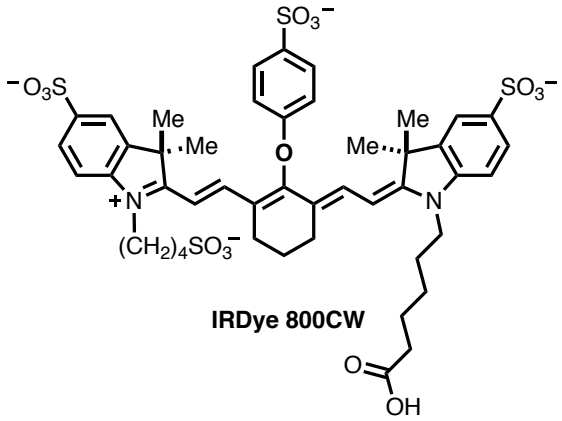
Figure S2: (a) HRESIMS analysis of the product mixture obtained from the irradiation of equimolar ratio of **2** and **2-d$_6$** with 630 LED (0.2 mW cm^{-2}) in PBS (pH = 7.4) for 60 min. (b) LC-MS analysis of independently generated singlet oxygen mediated truncation of **2**, **2-d$_3$**, **2-d$_6$** to the corresponding trimethine cyanines (upon reacting with 4000 μM of **8** at 60 °C (in dark) for 24 h in PBS (pH = 7.4).

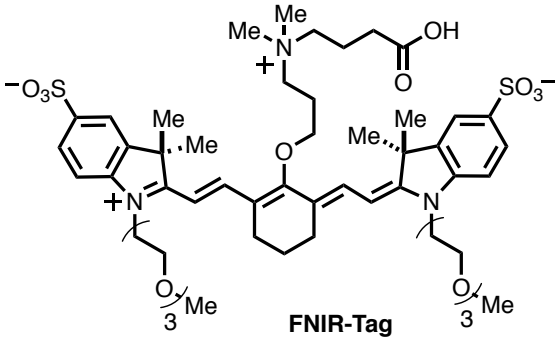
Pentamethine cyanines analyzed:

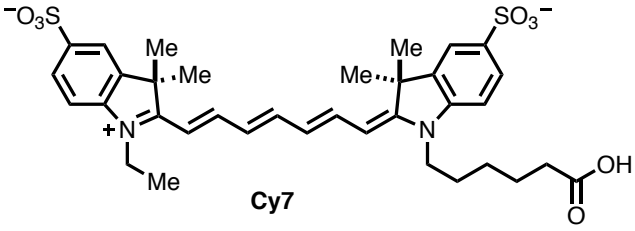


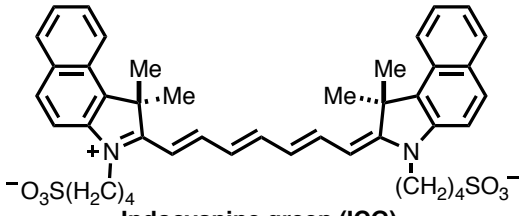


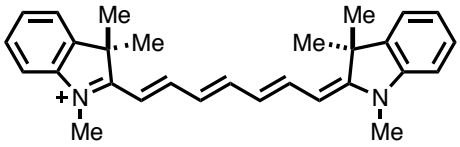
Heptamethine cyanines analyzed:











a.

b.

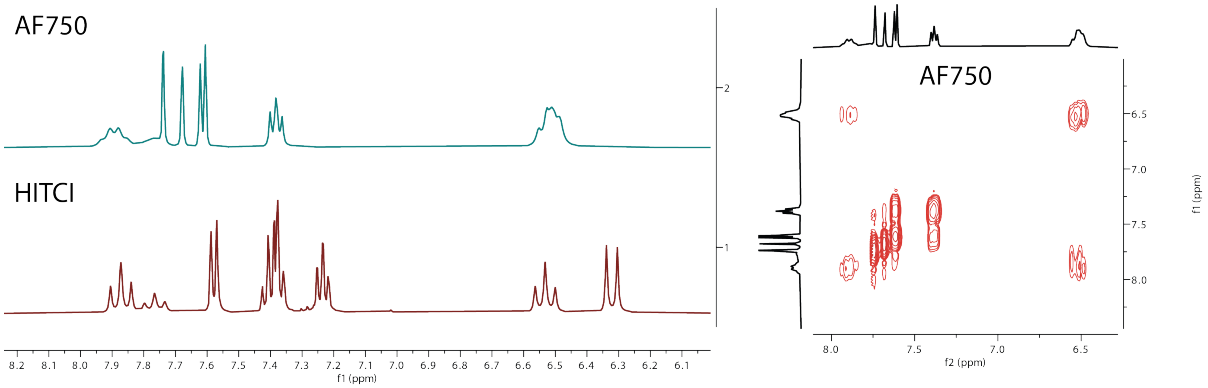


Figure S3. (a) Chemical structures of the penta- and heptamethine cyanines analyzed. The exact structure of AF 647 has not been reported. The chemical structure for Alexa Fluor 750 (AF 750) is not yet reported. However, examination of the ^{1}H NMR (DMSO-d_6) in (b), suggests an unmodified polymethine chain.

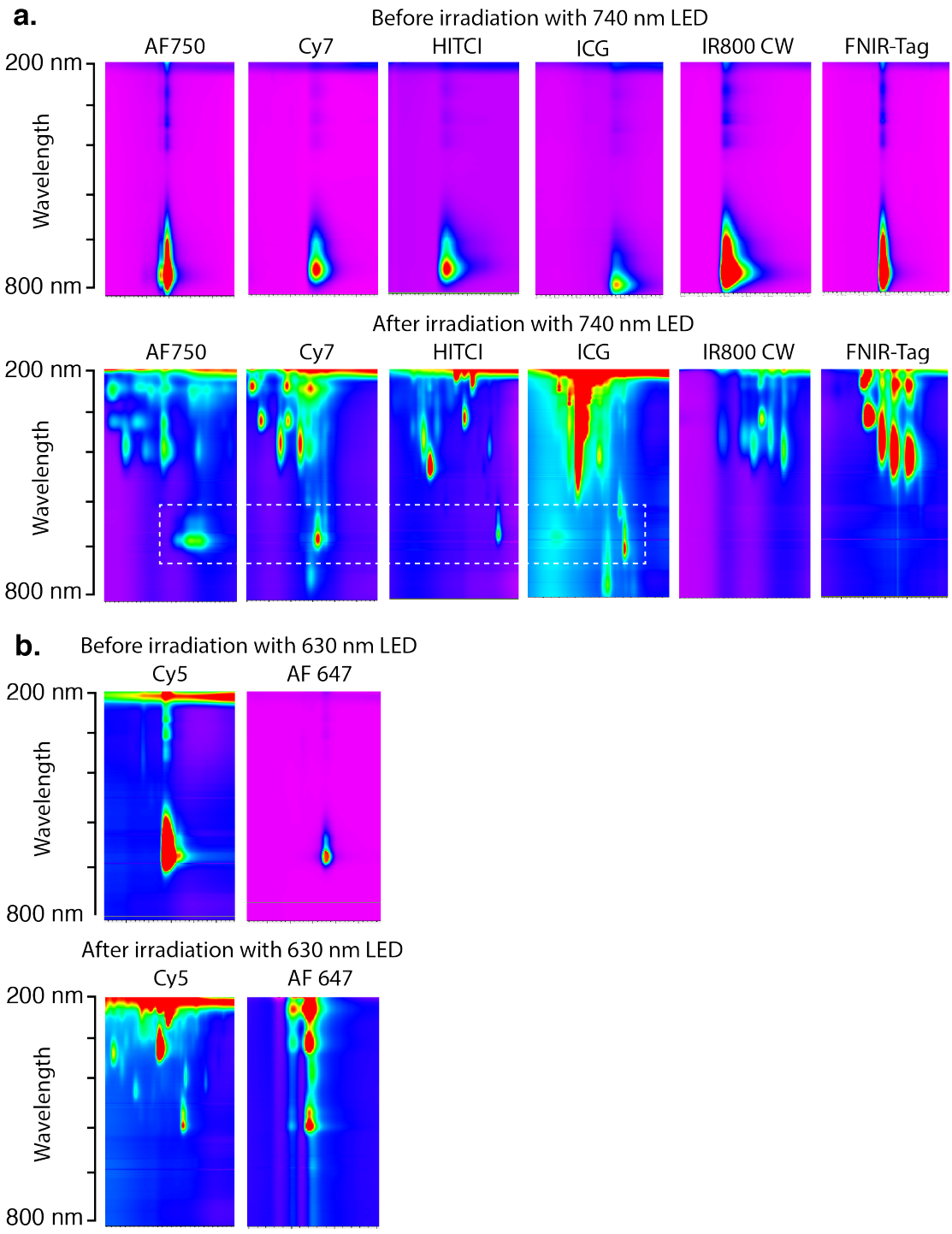


Figure S4. Analysis of product distribution of the pre- and post-irradiated (a) heptamethine cyanines and (b) pentamethine cyanines by LC-MS-coupled to photo-diode array detector. Irradiated was conducted on a 50 μM sample in PBS with 740 nm LED at 0.5 W cm^{-2} or 630 nm LED at 0.2 mW cm^{-2} for 1h. Absorbance around 650 nm (dotted box) in the case of AF750, Cy7, HITCI, and ICG suggests photoblueing to the corresponding pentamethine cyanine. Mass ions (not shown) of the pentamethine cyanine-like peaks in the case of Cy7, HITCI and ICG matched their corresponding pentamethine cyanine variants.

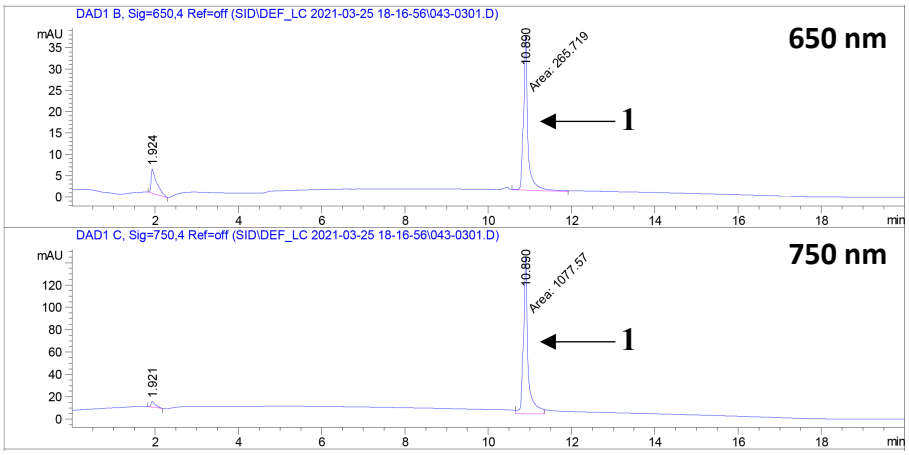


Figure 5. Reference HPLC traces of 50 μM **1** (R_t = 10.9 min) at 650 nm (top) and 750 nm (bottom).

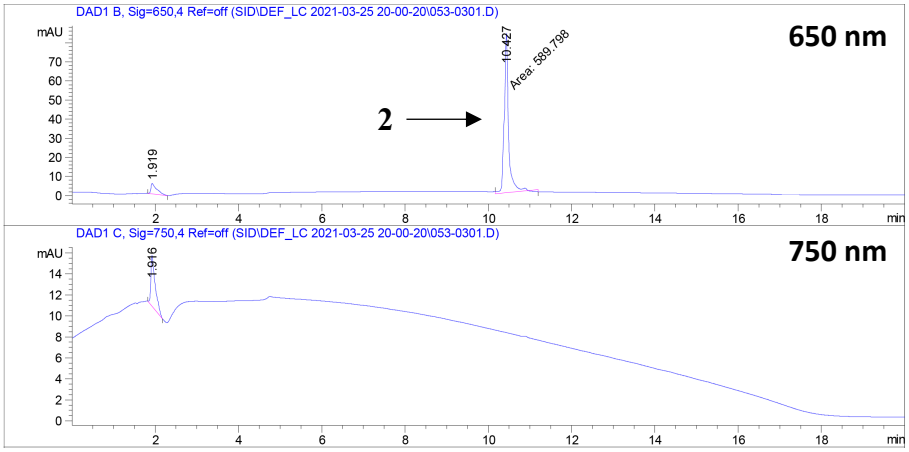


Figure S6. Reference HPLC traces of 50 μM **2** (R_t = 10.4 min) at 650 nm (top) and 750 nm (bottom).

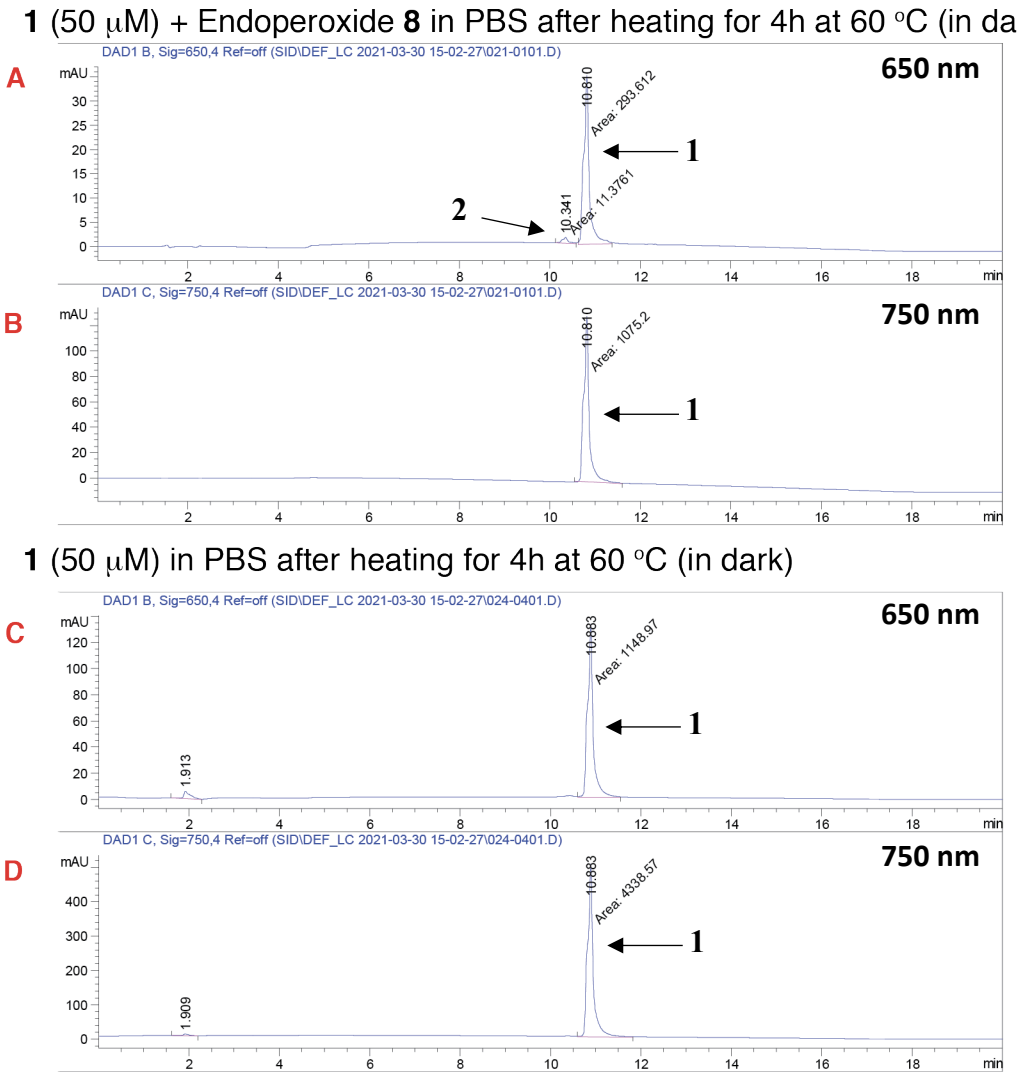
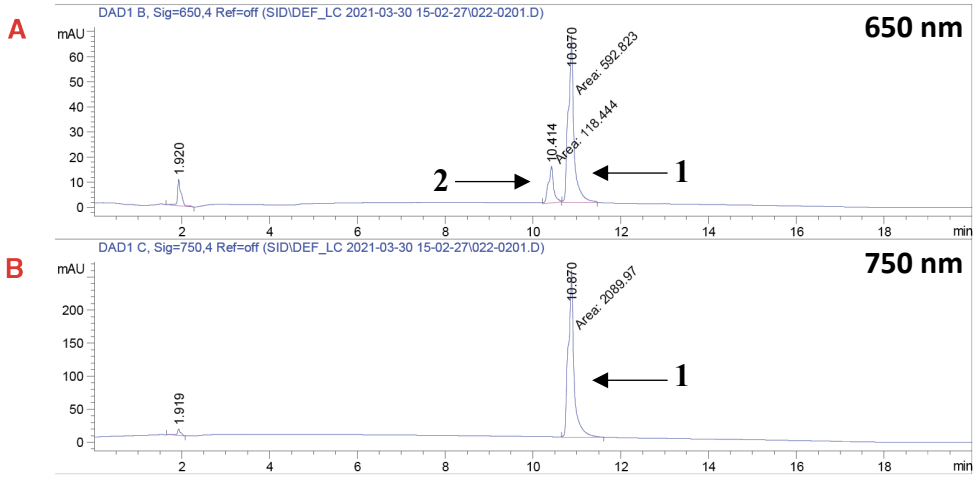


Figure S7. HPLC traces at 650 nm (A/C) and 750 nm (B/D) upon heating **1** with (A/B) and without (C/D) the endoperoxide **8** for 4 h in 10 mM PBS at 60 °C. Amount of **2** formed in A (at 10.3 min) was measured using the standard curve on Figure S11 and found to be 1.3%. The amount of **1** remaining in D (at 10.9 min) was measured using the standard curve on Figure S10 and found to be 99.2%. HPLC peaks in Figure S5 and S6 used as reference for **1** and **2** respectively.

1 (50 μM) + Endoperoxide **8** in 1M CAPSO (pH = 9.5) after heating for 4h at 60 °C (in dark)



1 (50 μM) in 1M CAPSO (pH = 9.5) after heating for 4h at 60 °C (in dark)

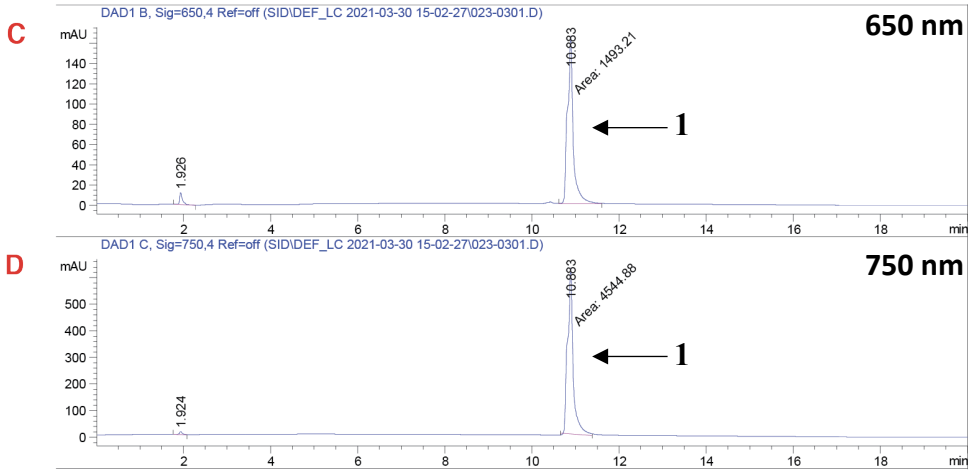


Figure S8. HPLC traces at 650 nm (A/C) and 750 nm (B/D) upon heating **1** with (A/B) and without (C/D) the endoperoxide **8** for 4 h in 1M CAPSO at 60 °C. Amount of **2** formed in A (at 10.4 min) was measured using the standard curve Figure S11 and found to be 3.8%. The amount of **1** remaining in D (at 10.9 min) was measured using the standard curve Figure S10 and found to be ~100%. HPLC peaks in Figure S5 and S6 used as reference for **1** and **2** respectively.

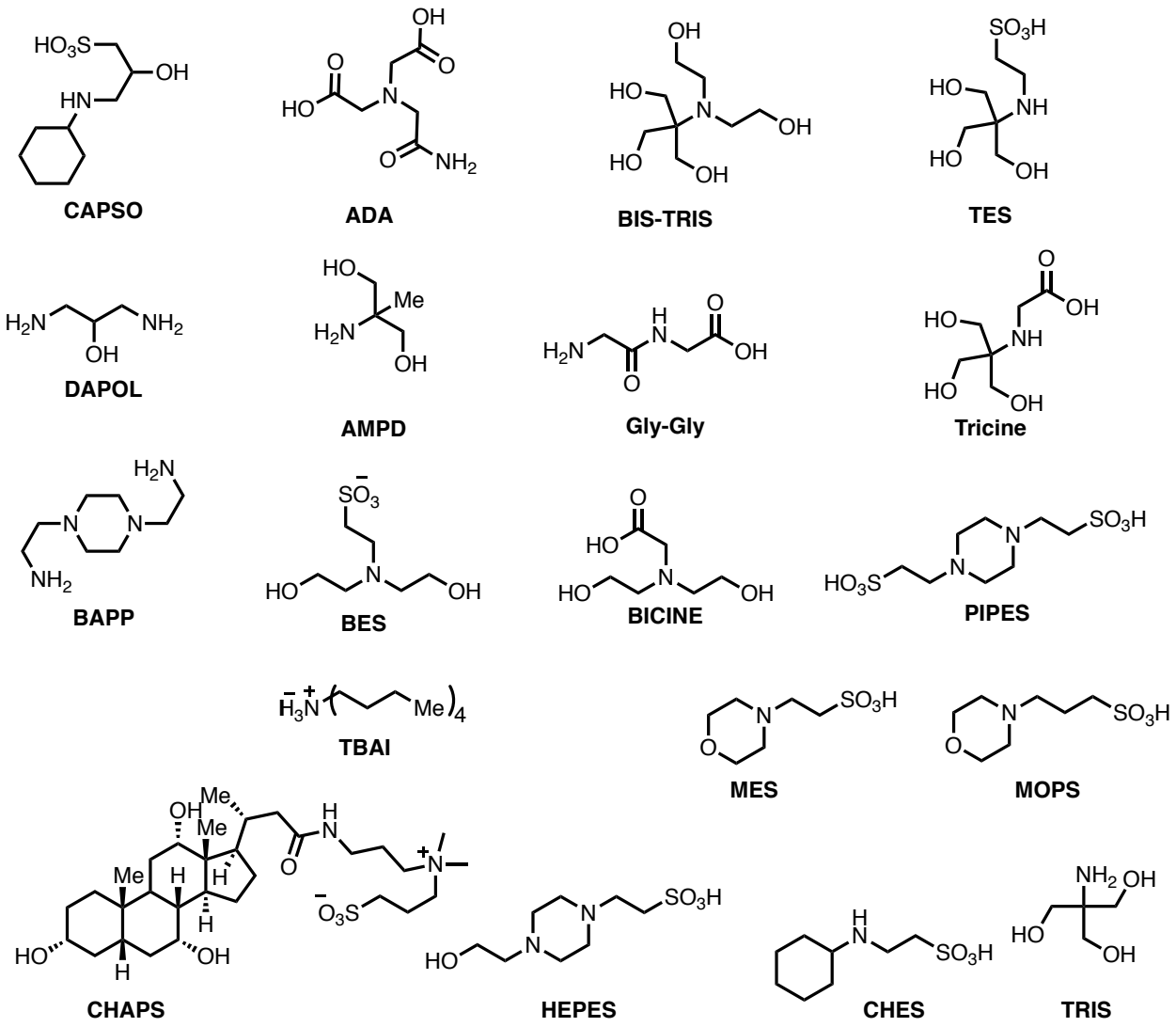


Figure S9. Chemical structures of the additives and buffers screened in Fig. 4.

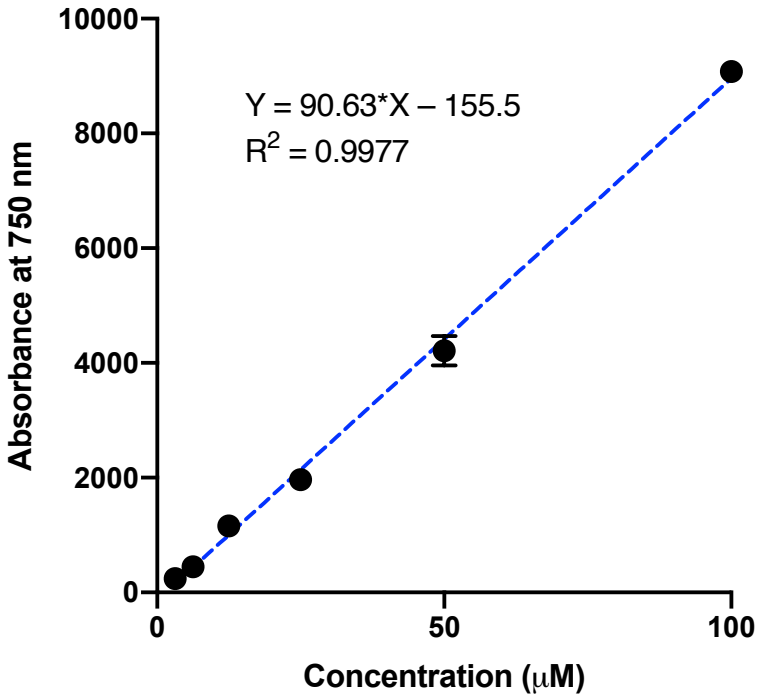


Figure S10. Standard curve of **1** in PBS at 750 nm (R_t =10.9 min). Error bars represent ± SD (n = 3).

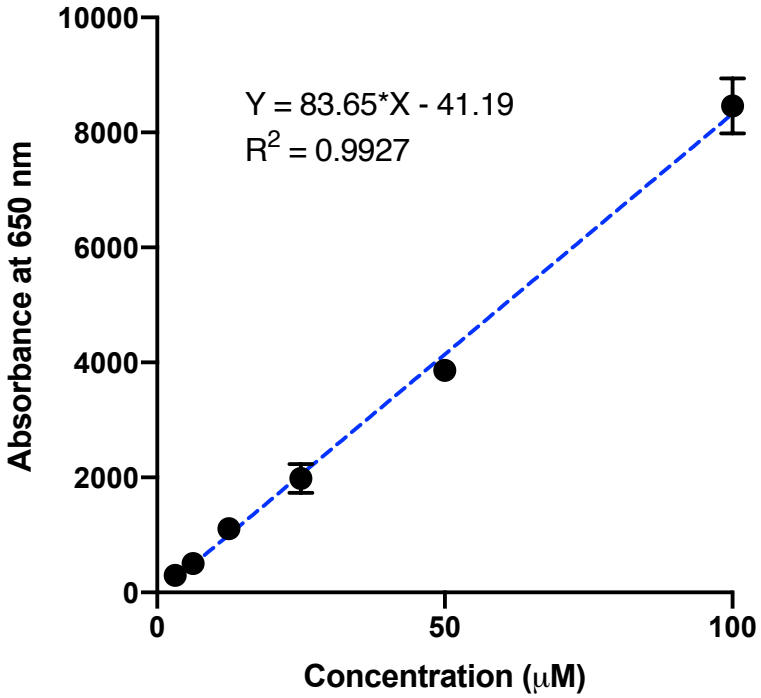
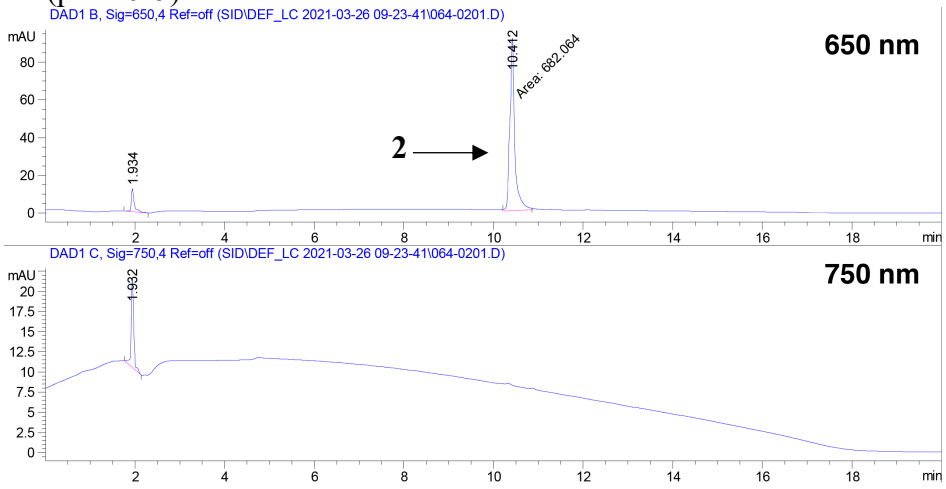
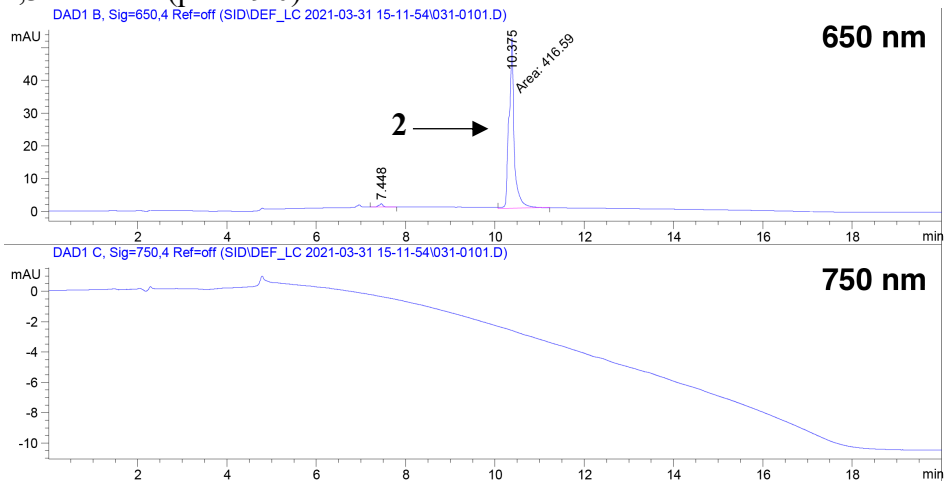


Figure S11. Standard curve of **2** in PBS at 650 nm (R_t = 10.4 min). Error bars represent ± SD (n = 3).

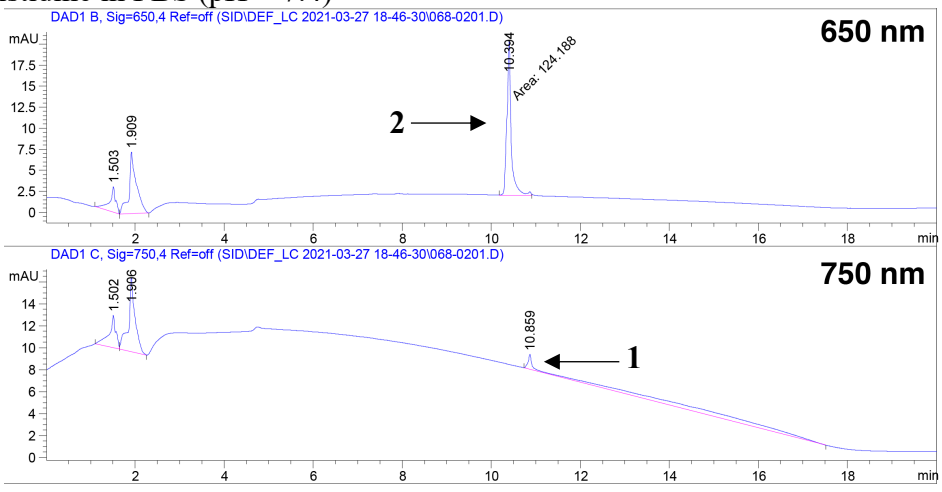
a) 1M CAPSO (pH = 9.5)



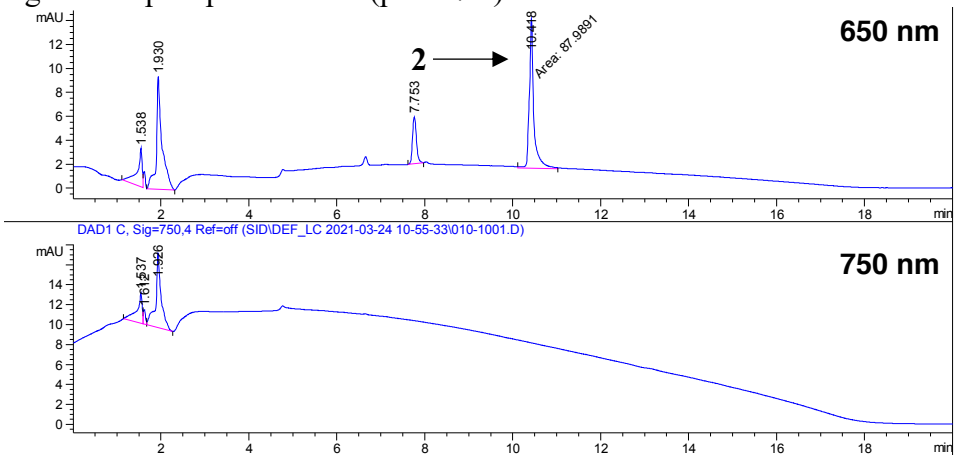
b) 100 mM 1,3-DAPOL (pH = 9.0)



c) 10 mM histidine in PBS (pH = 7.4)



d) 10 mM Arginine in phosphate buffer (pH = 7.4)



e) 100 mM PBS (pH = 7.4)

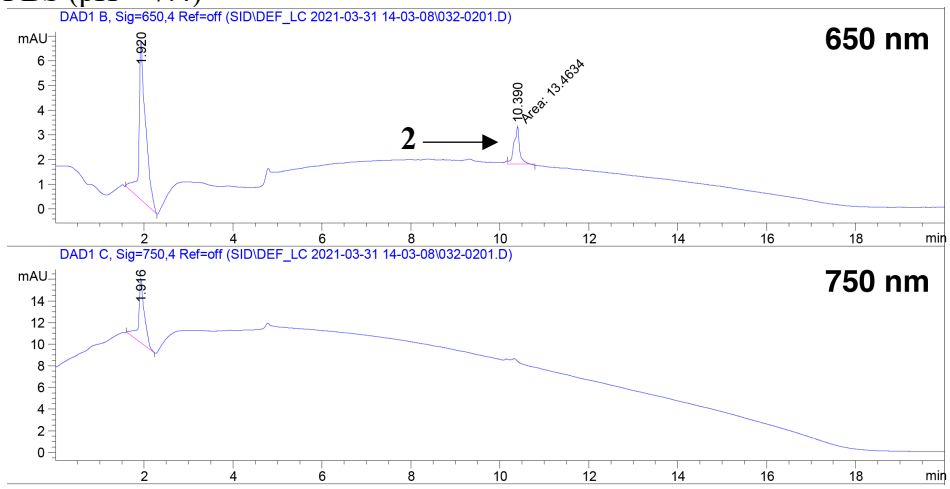


Figure S12. HPLC traces of an irradiated (using 740 nm LED at 0.5 W cm^{-2} for 60 min) sample containing **1** (50 µM) in (a) 1M CAPSO (pH = 9.5), (b) 100 mM DAPOL (pH = 9.0), (c) 10 mM histidine in PBS (pH = 7.4), (d) 10 mM arginine in phosphate buffer (pH = 7.4), and (e) 10 mM PBS (pH = 7.4). HPLC peaks in Figure S5 and S6 used as reference for **1** and **2** respectively.

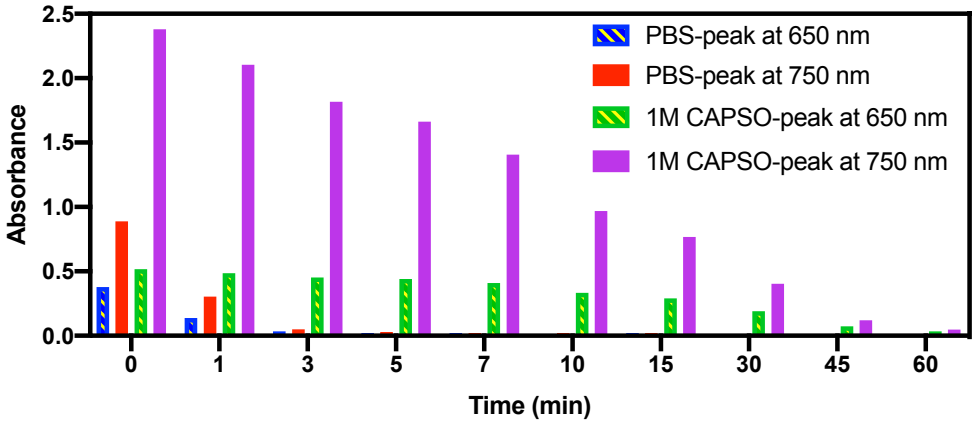


Figure S13. Absorbance at 650 nm and 750 nm in 10 mM PBS and 1M CAPSO buffers over 60 mins upon irradiation of **1** (100 μM) with 640 nm LED (0.2 W cm^{-2}).

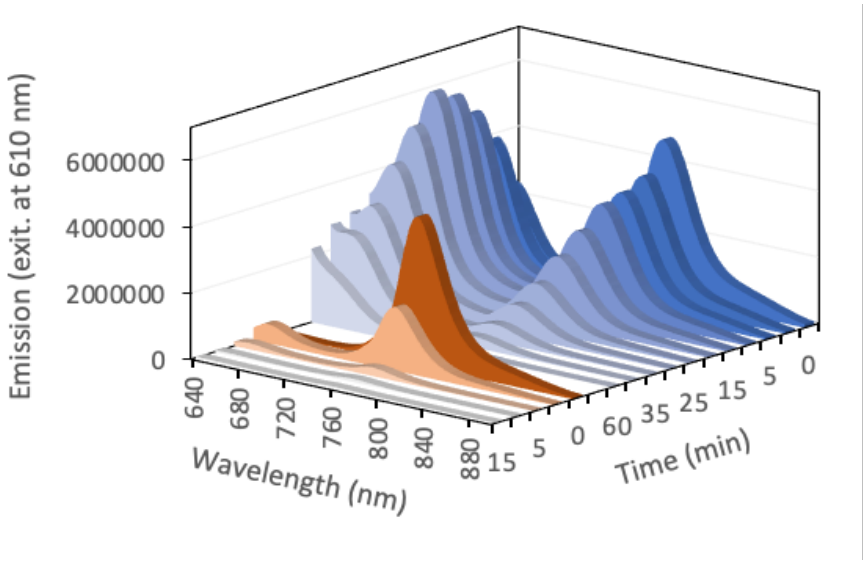


Figure S14. Ensemble fluorescence experiments following irradiating **1** for the indicated time with a 630 nm LED in 10 mM PBS (pH = 7.4, orange), 1M and 0.5 M CAPSO (pH = 9.5, blue) buffer. Fluorescence traces measured following excitation using a 630 nm LED (0.2 W cm^{-2}) for upto 60 mins.

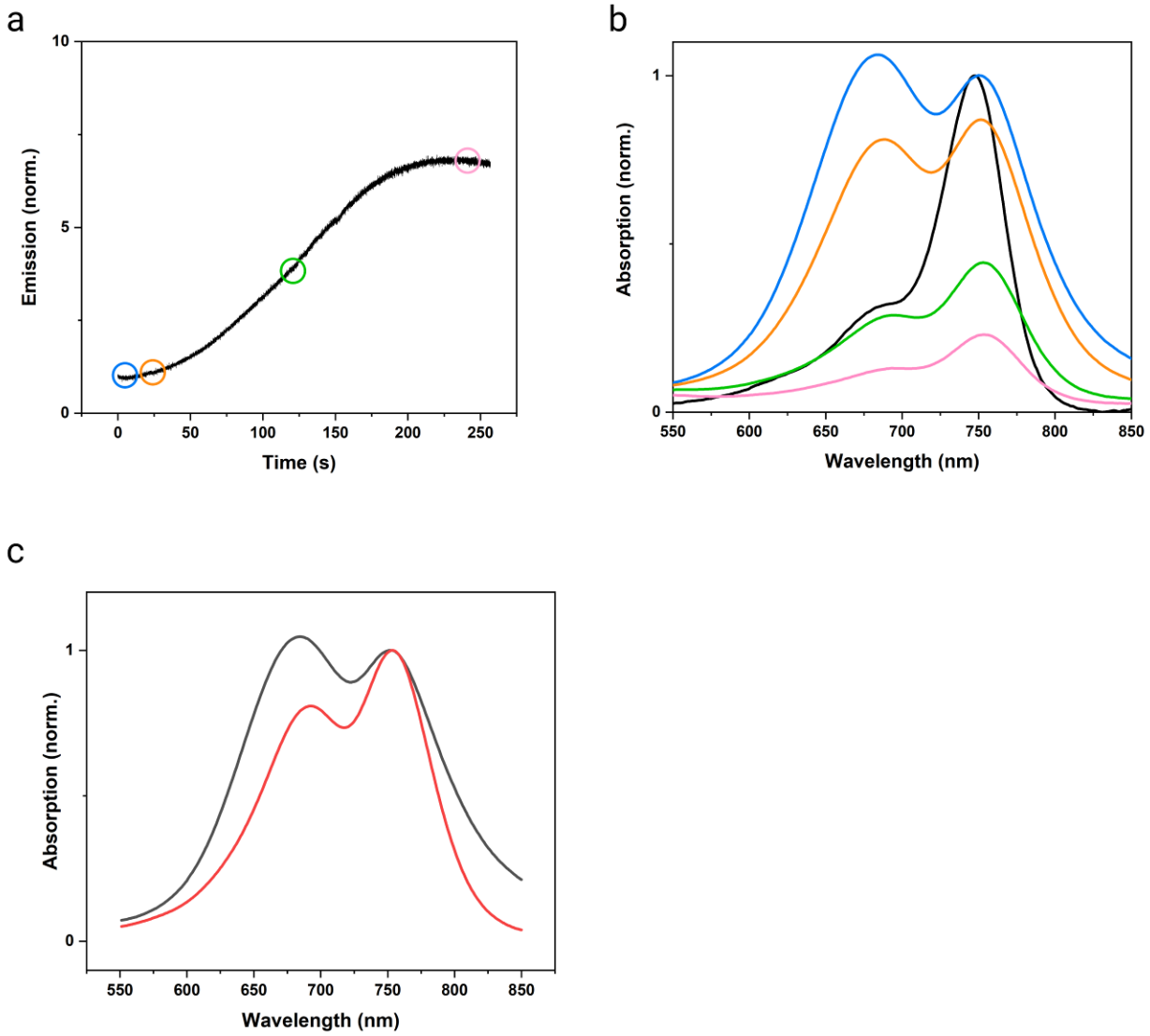


Figure S15. Decrease of H-aggregate prior to Cy7 fluorescence increase. Ensemble cuvette measurements of 1 µM IgG-gar-Cy7 (DOL ~4) illuminated at 640 nm with an irradiation intensity of 0.1 kW cm^{-2}. (a) Fluorescence time course measurement of Cy7 fluorescence in PBS, pH 7.4 beyond a bandpass filter (835 ± 35 nm). (b) Ensemble absorption spectra of the illuminated sample in (a) at different time points (blue: 0 min, orange: 20 min, green: 2 h and rose: 4h) compared to 1 µM free Cy7 fluorophores (black). (c) Absorption spectra of 1 µM IgG-gar-Cy7 (DOL ~4) in PBS, pH 7.4, (black) and 1M CAPSO, pH 9.5, (red), revealing different dimerization efficiencies.

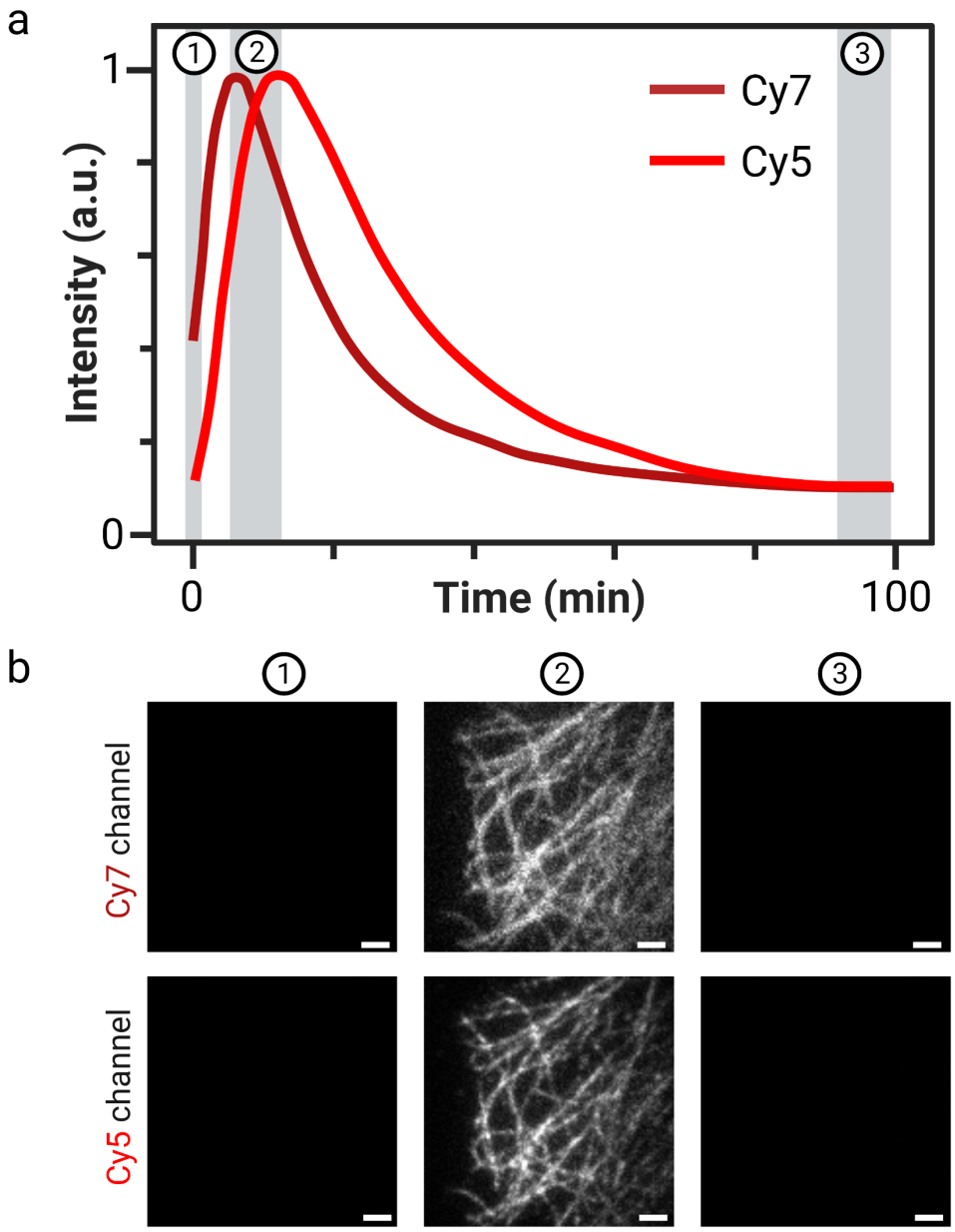
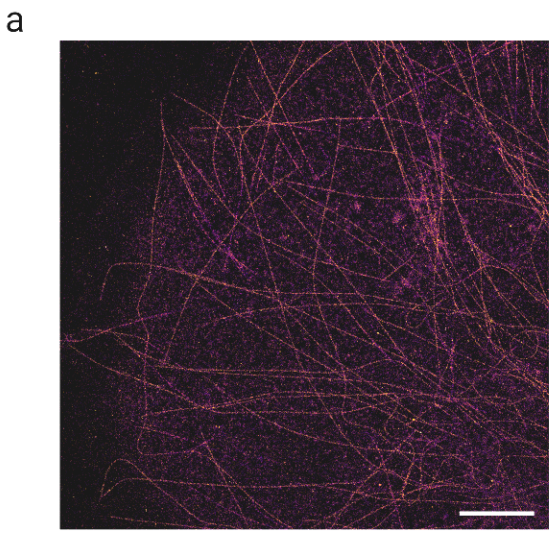
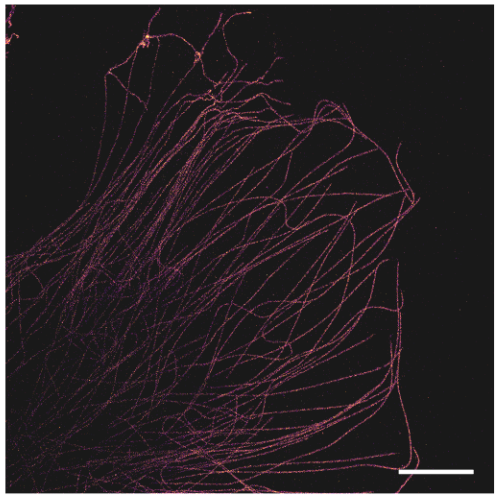
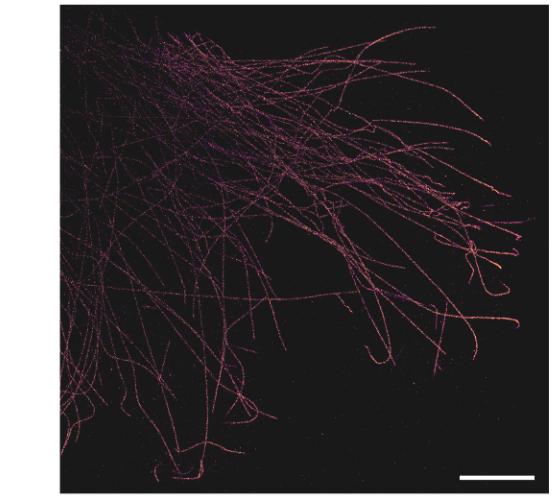


Figure S16. (a) Time course of fluorescence signals recoded on a longer wavelength Cy7 channel and a shorter wavelength Cy5 channel in PBS, pH 7.4. (b) Fluorescence imaging was performed in PBS, pH 7.4 at the designated time points (1-3). Bandpass filters used: Cy5 red-channel (679 nm ± 21 nm) and Cy7 far-red channel (835 ± 35 nm).







d

	Condition	resolution (nm)
Cy7	0.5kW/cm^2 500mM NaCl 10mM Histidine	16.8 +/- 0.5
	1.5kW/cm^2 500mM NaCl 10mM Histidine	26.0 +/- 2.2
Cy5	0.5kW/cm^2 500mM NaCl	26.6 +/- 1.6
	1.5kW/cm^2 500mM NaCl	38.3 +/- 5.6
	1.5kW/cm^2 500mM NaCl 10mM Histidine	27.8 +/- 2.2

Figure S17. Phototruncation DNA-PAINT and conventional DNA-PAINT at high irradiation intensity. (a) Cy7-phototruncation DNA-PAINT image recorded with high irradiation intensity (1.5 kW cm^{-2} at 640 nm, 100 ms frame^{-1}, 50.000 frames, 100 nM Cy7-imager strand in PBS, pH 7.4, 500 mM NaCl containing 10 mM histidine). (b,c). Conventional DNA-PAINT image recorded with an irradiation intensity of 1.5 kW cm^{-2} at 640 nm, 100 ms frame^{-1}, 50.000 frames, 100 pM Cy5-imager strand in PBS, pH 7.4, 500 mM NaCl in absence (b) and presence (c) 10 mM histidine). (d) Fourier Ring Correlation (FRC) estimated spatial resolutions for different imaging conditions and irradiation intensities. Scale bars: 5 µm.

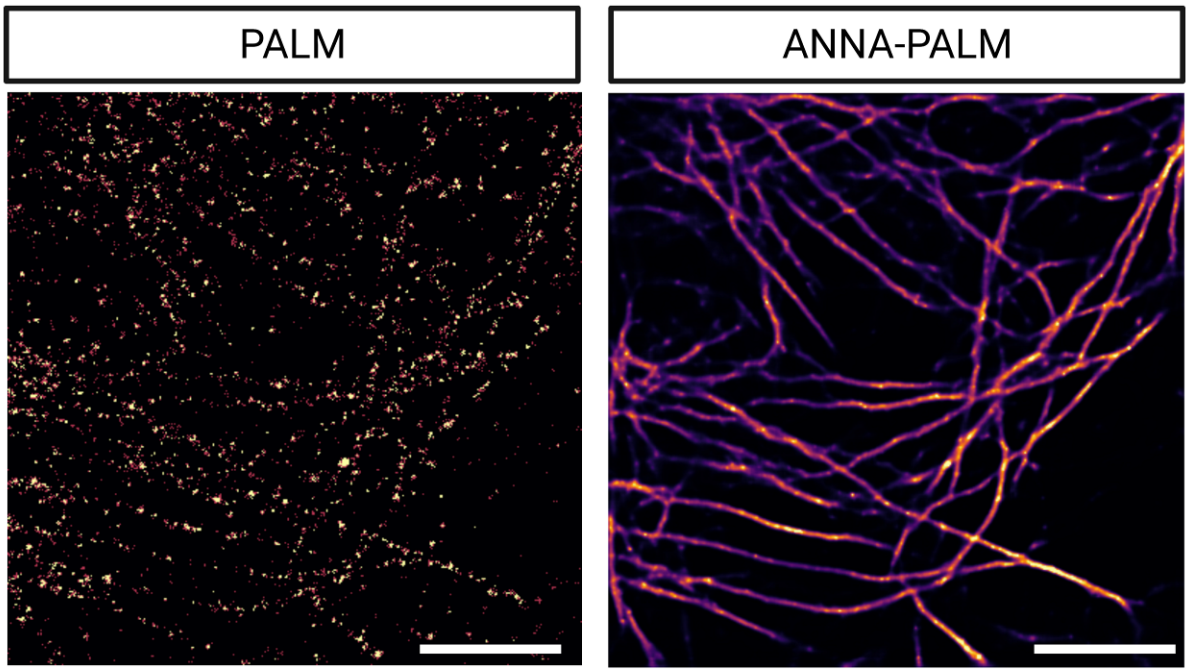


Figure S18. SMLM image of microtubules reconstructed after an acquisition time of 83.3 min at 100 ms exposure time using solely 640 nm irradiation at an intensity of 1 kW cm^{-2}. The left image shows the raw reconstructed PALM-like, i.e., Cy5 is photoactivated via phototruncation Cy7→Cy5 followed by detection, localization, and photobleaching or further photoconversion. SMLM image obtained after applying a trained artificial neural network (ANNA-PALM) algorithm to the reconstructed raw PALM image. Scale bars: 2 µm.

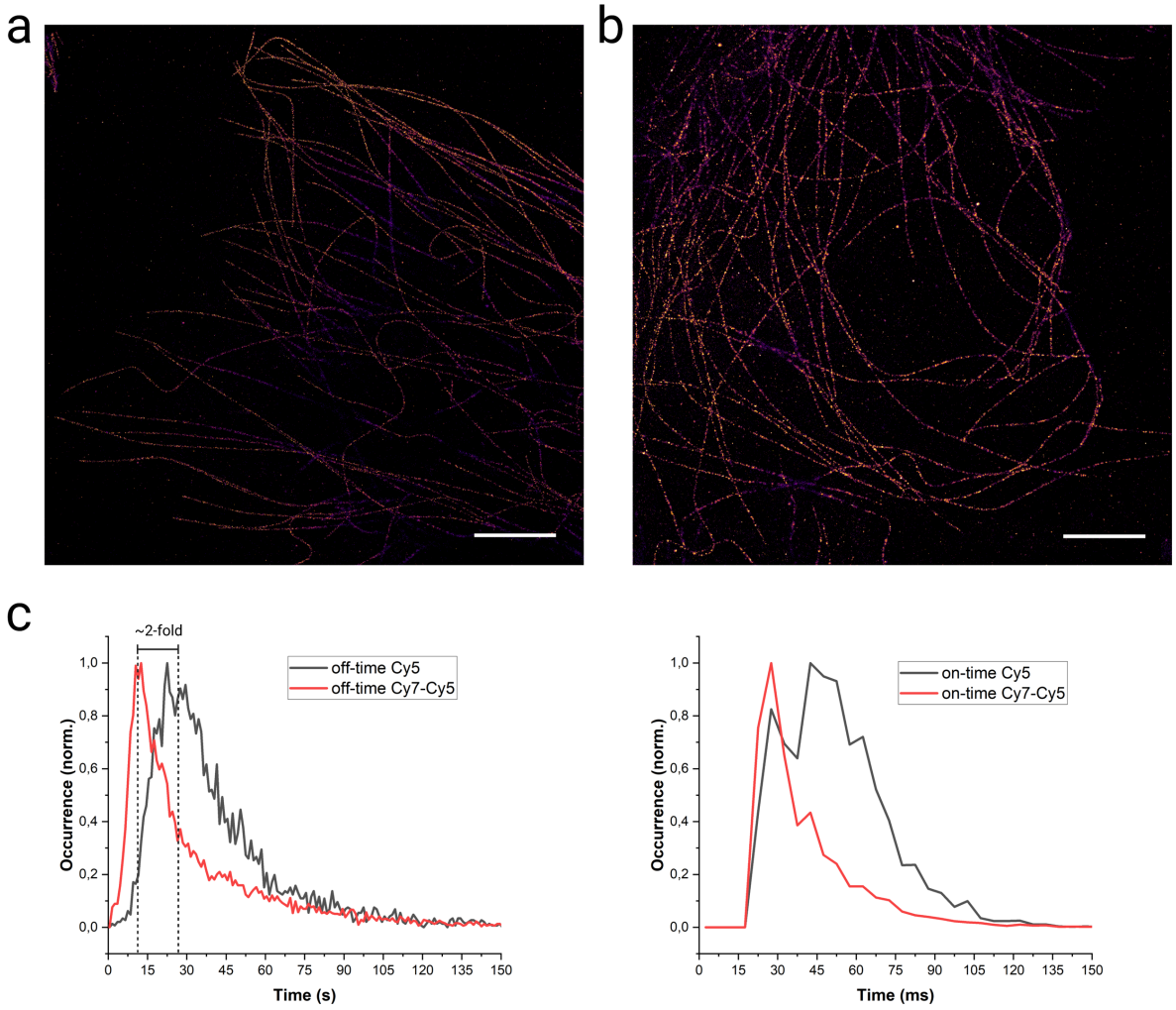


Figure S19. On- and off-times of conventional Cy5 and Cy7-Cy5 phototruncation DNA-PAINT. a,b DNA-PAINT images of immunostained microtubules in COS7 using docking strand labeled secondary α-tubulin antibodies. Irradiation was performed at 640 nm in TIRF-mode using an irradiation intensity of 0.5 kW cm^{-2}. The Cy5 fluorescence signal was detected behind a bandpass filter (679 nm ± 21 nm). (a) Image of Cy5 signals measured with 100 pM Cy5 labeled imager strands in 500 mM NaCl, pH 7.4, measured at 20 ms $frame^{-1}$. (b) Image of Cy5 signals measured with 100 nM Cy7 labeled imager strand in PBS, 500 mM NaCl + 10 mM histidine, pH 7.4, measured at 20 ms $frame^{-1}$. (c) Histograms of off-times (binning 1s) and on-times (signal capturing on-times recorded at a binning of 5 ms) determined from conventional Cy5 and Cy7-Cy5 phototruncation DNA-PAINT. Scale bars: 5 µm. The off-times of 100 nM Cy7-imager strands are ~2.2-fold shorter than the off-times measured for 100 pM Cy5 imager strands reducing the imaging time by the same factor.

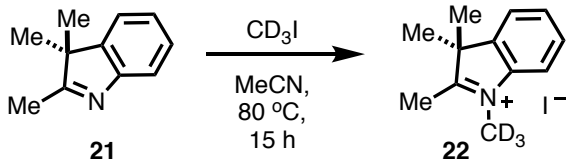
General experimental

Unless stated otherwise, reactions were conducted in oven- dried glassware under an atmosphere of nitrogen or argon using anhydrous solvents (passed through activated alumina columns). All commercially obtained reagents were used as received. Flash column chromatography was performed using reversed phase (100 Å, 20-40-micron particle size) and silica on a CombiFlash® Rf 200i (Teledyne Isco, Inc.). High-Resolution ElectroSpray Ionization Mass Spectrometry High-resolution (HRESIMS) analysis was conducted on the 6530 Series accurate-mass Q-TOF LC/MS (Agilent Technologies, Inc., Santa Clara, CA) with an API-electrospray source using the Zorbax 300SB-C18 Poroshell column 2.1x75 mm (particle size 5 μm) in positive ion mode. Runs employed a gradient of 0→95% MeCN/0.1% in aqueous formic acid over 4 minutes at a flow rate of 1 mL/min. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometers (at 500 MHz or at 125 MHz) and are reported relative to deuterated solvent signals. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz), and integration. Data for ^{13}C NMR spectra are reported in terms of chemical shift. Absorption curves for quantum yield measurements were performed on a BioTek's Synergy™ Mx Microplate Reader operated by Gen5™ 2.05 software. Fluorescence traces were recorded on a PTI QuantaMaster steady state spectrofluorometer operated by FelixGX 4.2.2 software, with 5 nm excitation and emission slit widths, 0.1 s integration rate, and enabled emission correction. Data analysis and curve fitting were performed using MS Excel 2011 and GraphPad Prism 7. Light intensity measurements were performed with a Thorlabs PM200 optical power and energy meter fitted with an S120VC standard Si photodiode power sensor (200 – 1100 nm, 50 nW – 50 mW).

Synthesis and NMR analysis

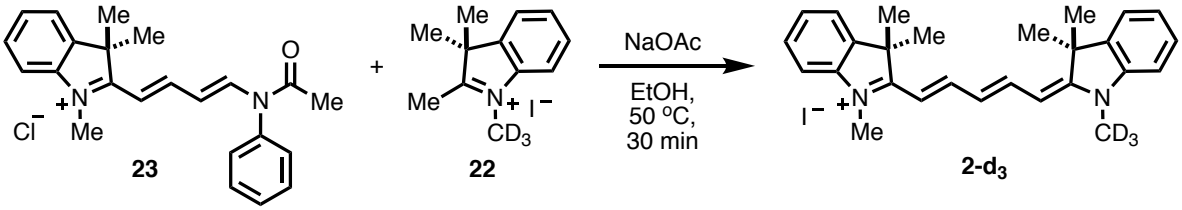
Compound **23** was synthesized according to the previously reported procedure.[1]

Synthesis of 22:



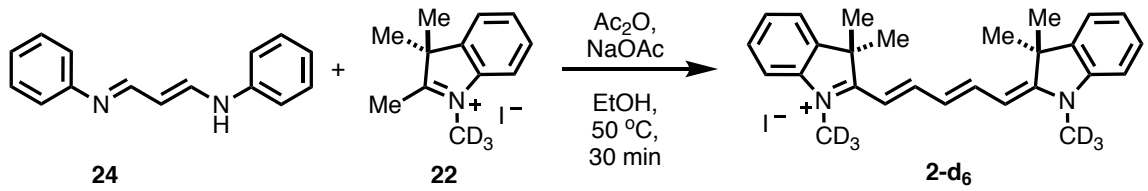
Iodomethane-d_3 (1.36 g, 9.43 mmol, 1.5 eq.) was added to a solution of **21** (1 g, 6.29 mmol, 1 eq.) in dry MeCN (5 mL) under N_2. The reaction mixture was heated to 50 ºC for 15 h and the progress was monitored by LC-MS. Upon completion, the reaction mixture was precipitated in 50 mL diethyl ether. The precipitate was filtrated and washed (3 x 50 mL) and dried in vacuo to result in 1.64 g (86%) of **22** as a pale-red colored amorphous solid. The dried precipitate was taken forwarded without any further purification. HRESIMS calculated for $C_{12}H_{13}D_3N^+$ $(M)^+$ 177.1466, observed 177.14671.

Synthesis of 2-d_3:

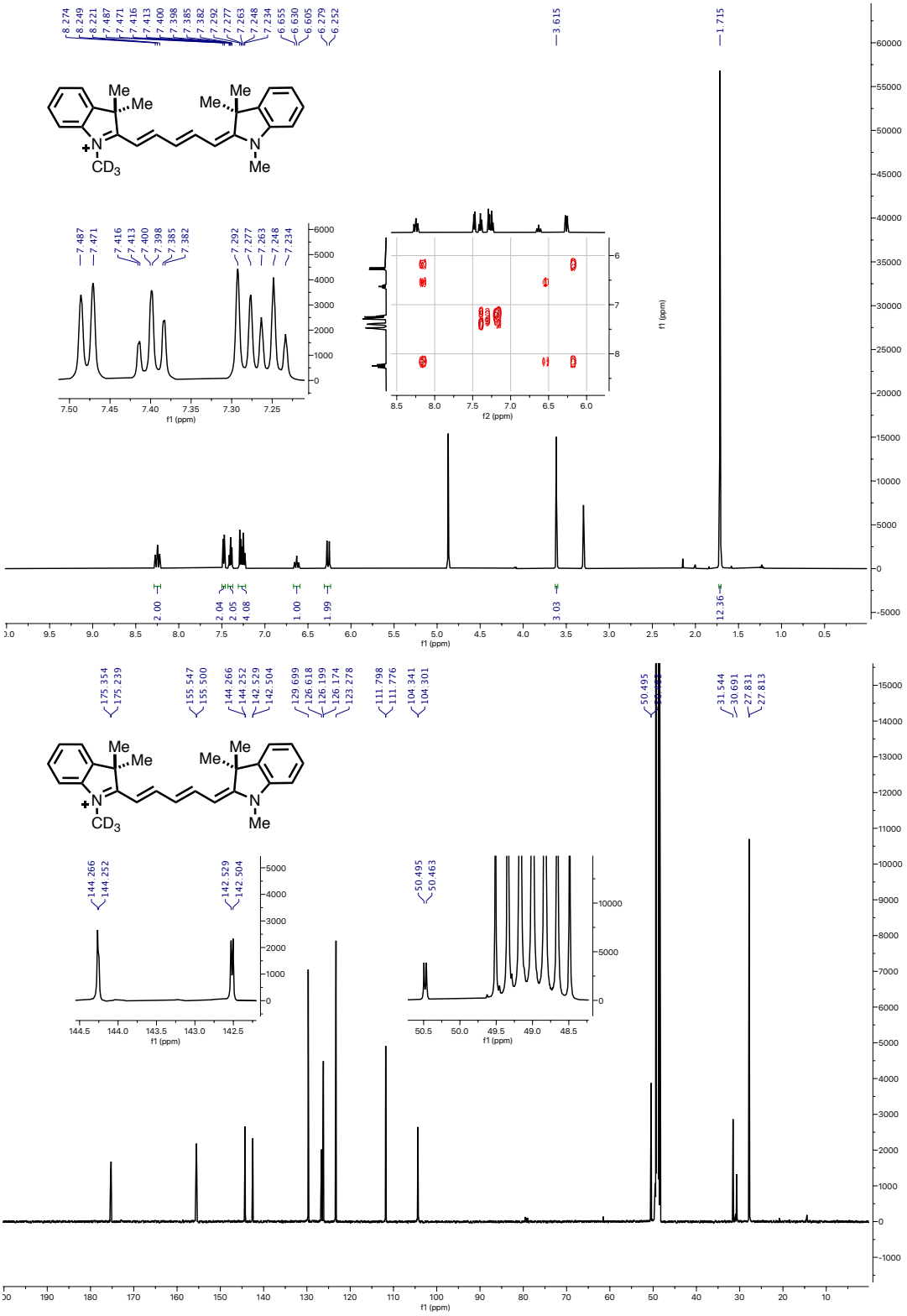


A solution of **23** (0.1 g, 0.26 mmol, 1.0 eq), **22** (64 mg, 0.21 mmol, 0.8 eq), and NaOAc (25.8 mg, 0.32 mmol, 1.2 eq) in EtOH (2.5 mL, 0.11 M) was heated to 50 ºC for 30 min. The reaction progress was monitored by LC-MS and upon completion, cooled to room temperature and filtered through a celite plug. The filtrate was concentrated under reduced pressure and purified by normal-phase column chromatography (24 g silica gel column, 0-10% MeOH/CH_2Cl_2) and concentrated in vacuo to yield **2-d_3** (113 mg, 84%) as a bluish-green amorphous solid. ^{1}H NMR (500 MHz, MeOD) δ 8.29 – 8.21 (m, 2H), 7.48 (d, J = 8.1 Hz, 2H), 7.40 (td, J = 7.7, 1.3 Hz, 2H), 7.31 – 7.22 (m, 4H), 6.63 (t, J = 12.4 Hz, 1H), 6.27 (d, J = 13.7 Hz, 2H), 3.62 (s, 3H), 1.72 (s, 12H). ^{13}C NMR (125 MHz, MeOD) δ 175.35, 175.24, 155.55, 155.50, 144.27, 144.25, 142.53, 142.50, 129.70, 126.62, 126.20, 126.17, 123.28, 111.80, 111.78, 104.34, 104.30, 50.50, 50.46, 31.54, 30.69, 27.83, 27.81. HRESIMS calculated for $C_{27}H_{28}D_3N_2$ $(M)^+$ 386.2670, observed 386.2676.

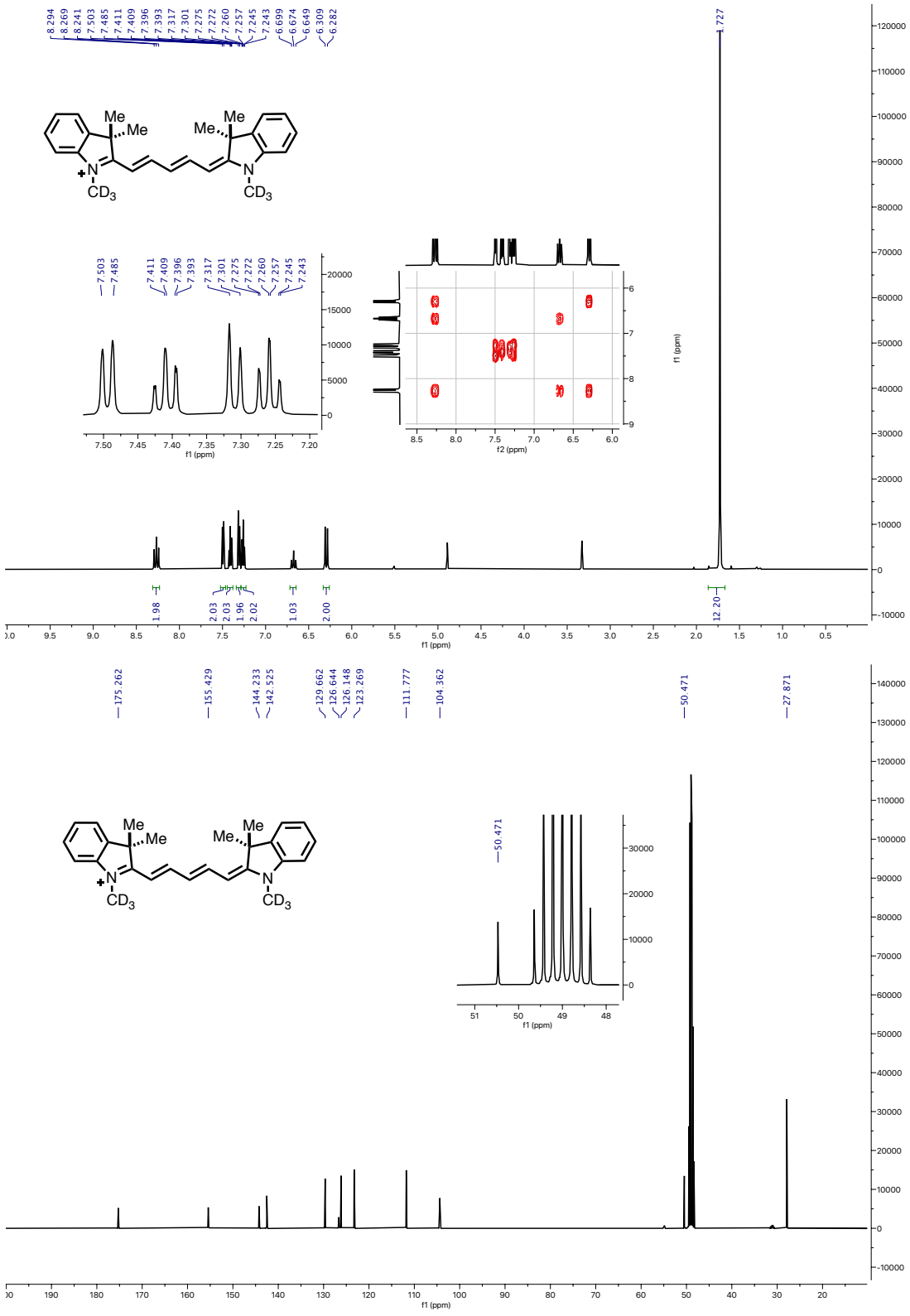
Synthesis of 2-d_6:



To a solution of **22** (0.15 g, 0.49 mmol, 1 eq), **24** (44 mg, 0.20 mmol, 0.4 eq), and NaOAc (45 mg, 0.54 mmol, 1.1 eq) in EtOH (2.5 mL, 0.34 M) was added Ac_2O (55 mg, 0.54 mmol, 1.1 eq). The reaction mixture was heated to 50 ºC for 60 min. The reaction progress was monitored by LC-MS and upon completion, cooled to room temperature and filtered through a celite plug. The filtrate was concentrated under reduced pressure and purified by normal-phase column chromatography (24 g silica gel column, 0-10% MeOH/CH_2Cl_2) and concentrated in vacuo to yield **2-d_6** (232 mg, 91%) as a bluish-green amorphous solid. ^{1}H NMR (400 MHz, MeOD) δ 8.29 – 8.17 (m, 2H), 7.46 (d, J = 7.3 Hz, 2H), 7.38 (td, J = 7.5, 1.2 Hz, 2H), 7.31 – 7.19 (m, 4H), 6.64 (t, J = 12.4 Hz, 1H), 6.27 (d, J = 13.8 Hz, 2H), 5.48 (s, 1H), 1.70 (s, 12H). ^{13}C NMR (100 MHz, MeOD) δ 175.26, 155.43, 144.23, 142.53, 129.66, 126.64, 126.15, 123.27, 111.78, 104.36, 54.84, 50.47, 27.87. HRESIMS calculated for $C_{27}H_{25}D_6N_2$ $(M)^+$ 389.2858, observed 389.2861.



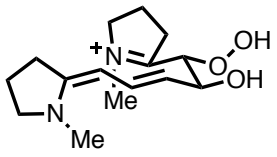
^{1}H NMR (500 MHz) and ^{13}C (125 MHz) of **2-d$_3$**.



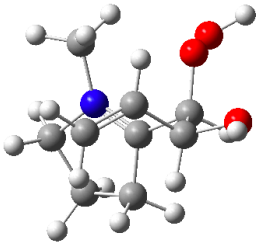
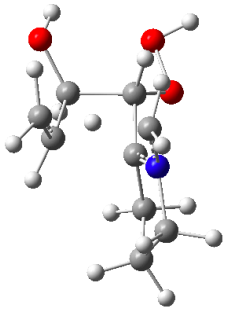
^{1}H NMR (400 MHz) and ^{13}C (100 MHz) NMR of **2-d$_6$**.

Computational analysis

DFT calculations show the peroxy intermediate **9** to exist as a minimum on the potential energy surface. This peroxy intermediate is unlike the reaction of $^1O_2$ with simple alkenes that typically react via a two-step no-intermediate mechanism, in which perepoxides reside on an upper caldera segment of the potential energy surfaces. Dienes and polyenes with no allylic hydrogens can undergo alternate pathways, including formation of peroxy intermediates that are rationalized to be zwitterionic due to their capacity to react with nucleophilic solvents such as methanol.[2-5]



16

Axial view	Lateral view
	

Axial and lateral view of **16** adopting a chair-like structure, which enable the intramolecular attack of the vinylogous enamine

Descriptions of energies and geometries of stationary points

2 (pentamethine cyanine)

Zero-point correction=	0.520957 (Hartree/Particle)
Thermal correction to Energy=	0.548917
Thermal correction to Enthalpy=	0.549862
Thermal correction to Gibbs Free Energy=	0.461639
Sum of electronic and zero-point Energies=	-1155.849879
Sum of electronic and thermal Energies=	-1155.821919
Sum of electronic and thermal Enthalpies=	-1155.820975
Sum of electronic and thermal Free Energies=	-1155.909197

	E (Thermal) KCal/Mol	CV Cal/Mol-Kelvin	S Cal/Mol-Kelvin
Total	344.451	109.040	185.679

+1 1

1	6	0	-1.565446	-4.332102	0.992063
2	6	0	-1.462812	-4.119787	-0.379662
3	6	0	-0.711542	-3.062941	-0.863025
4	6	0	-0.067590	-2.226501	0.052174
5	6	0	-0.181517	-2.454462	1.422460
6	6	0	-0.936487	-3.517125	1.921335
7	6	0	-2.815517	-5.997865	0.049845
8	1	0	-0.621819	-2.883890	-1.930690
9	1	0	0.525948	-1.392833	-0.305447
10	1	0	0.324617	-1.795607	2.119268
11	1	0	-1.014275	-3.679621	2.990032
12	6	0	-2.260454	-5.175889	-1.115406
13	6	0	-1.327189	-6.014859	-2.007072
14	1	0	-1.868372	-6.796631	-2.543843
15	1	0	-0.854154	-5.360563	-2.744087
16	1	0	-0.540015	-6.483943	-1.412371
17	6	0	-3.386203	-4.512801	-1.929765
18	1	0	-2.943600	-3.835452	-2.664860
19	1	0	-3.991031	-5.247301	-2.465267
20	1	0	-4.043348	-3.929434	-1.280769
21	7	0	-2.383784	-5.466031	1.207645
22	6	0	-2.718342	-5.993748	2.518940
23	1	0	-2.245860	-5.380885	3.282497
24	1	0	-3.800879	-5.973107	2.668202
25	1	0	-2.356800	-7.020689	2.615357
26	6	0	-3.636102	-7.123461	0.014247
27	1	0	-3.924432	-7.571909	0.959949
28	6	0	-4.120923	-7.725551	-1.145386
29	1	0	-3.852697	-7.302390	-2.110585
30	6	0	-4.939380	-8.850478	-1.162952
31	1	0	-5.232129	-9.307334	-0.220155
32	6	0	-5.393313	-9.406375	-2.354799
33	1	0	-5.076659	-8.917196	-3.273021
34	6	0	-6.213139	-10.530500	-2.436302
35	1	0	-6.509547	-10.993203	-1.500002
36	6	0	-6.677520	-11.100004	-3.620172
37	6	0	-7.210194	-11.698668	-5.833688
38	6	0	-7.811020	-12.581729	-4.941162
39	7	0	-7.467157	-12.189154	-3.626026
40	6	0	-7.911585	-12.877137	-2.426439
41	1	0	-8.502137	-12.202331	-1.801498
42	1	0	-8.528724	-13.726361	-2.708931
43	1	0	-7.050308	-13.238956	-1.859162
44	6	0	-6.422040	-10.660212	-5.063431

45	6	0	-4.929564	-10.754244	-5.429117
46	1	0	-4.810924	-10.558643	-6.498090
47	1	0	-4.327648	-10.026413	-4.881350
48	1	0	-4.540649	-11.753439	-5.220008
49	6	0	-6.991103	-9.256470	-5.338084
50	1	0	-8.047825	-9.205616	-5.065851
51	1	0	-6.453633	-8.482397	-4.786702
52	1	0	-6.902714	-9.037811	-6.405521
53	6	0	-7.389218	-11.861616	-7.196156
54	1	0	-6.926884	-11.181003	-7.905427
55	6	0	-8.599814	-13.642794	-5.359937
56	1	0	-9.069555	-14.331862	-4.667755
57	6	0	-8.773349	-13.797454	-6.736163
58	6	0	-8.179290	-12.923058	-7.644600
59	1	0	-8.332358	-13.068637	-8.707878
60	1	0	-9.383743	-14.616047	-7.101055

3 (trimethine cyanine)

Zero-point correction=	0.487034 (Hartree/Particle)
Thermal correction to Energy=	0.512886
Thermal correction to Enthalpy=	0.513830
Thermal correction to Gibbs Free Energy=	0.430867
Sum of electronic and zero-point Energies=	-1078.512163
Sum of electronic and thermal Energies=	-1078.486312
Sum of electronic and thermal Enthalpies=	-1078.485368
Sum of electronic and thermal Free Energies=	-1078.568331

	E (Thermal) KCal/Mol	CV Cal/Mol-Kelvin	S Cal/Mol-Kelvin
Total	321.841	101.107	174.610

+1 1

1	6	0	-1.924390	-5.205840	0.224475
2	6	0	-1.869236	-4.990150	-1.148891
3	6	0	-1.095480	-3.962119	-1.658593
4	6	0	-0.381121	-3.158723	-0.766542
5	6	0	-0.448244	-3.390003	0.606383
6	6	0	-1.225341	-4.423772	1.131301
7	6	0	-3.277371	-6.815241	-0.671423
8	1	0	-1.041378	-3.780334	-2.728165
9	1	0	0.231354	-2.348004	-1.144380
10	1	0	0.112975	-2.756873	1.284508
11	1	0	-1.265418	-4.589688	2.201526
12	6	0	-2.737228	-6.009923	-1.855539
13	6	0	-1.874412	-6.881572	-2.785558

14	1	0	-2.469194	-7.636684	-3.304489
15	1	0	-1.403958	-6.244141	-3.538613
16	1	0	-1.085322	-7.387646	-2.224814
17	6	0	-3.865968	-5.299246	-2.623738
18	1	0	-3.425637	-4.629432	-3.366841
19	1	0	-4.512021	-6.006884	-3.148253
20	1	0	-4.482279	-4.701898	-1.948088
21	7	0	-2.780044	-6.308267	0.468049
22	6	0	-3.081973	-6.828075	1.791279
23	1	0	-2.745980	-7.864684	1.874865
24	1	0	-2.565422	-6.228988	2.536898
25	1	0	-4.157215	-6.775784	1.979037
26	6	0	-4.142090	-7.910707	-0.677656
27	1	0	-4.404073	-8.356119	0.277095
28	6	0	-4.697690	-8.480433	-1.820033
29	1	0	-4.450261	-8.047812	-2.781912
30	6	0	-5.554781	-9.577154	-1.790908
31	1	0	-5.788460	-9.999208	-0.818362
32	6	0	-6.132822	-10.168626	-2.915121
33	6	0	-6.870446	-10.812534	-5.055940
34	6	0	-7.416533	-11.650865	-4.089111
35	7	0	-6.947694	-11.231470	-2.819846
36	6	0	-7.305856	-11.874035	-1.566419
37	1	0	-7.833639	-11.169659	-0.918539
38	1	0	-7.956762	-12.720086	-1.771915
39	1	0	-6.407502	-12.234671	-1.059485
40	6	0	-5.990837	-9.776839	-4.387824
41	6	0	-4.539274	-9.922782	-4.878674
42	1	0	-4.510898	-9.768677	-5.960428
43	1	0	-3.875385	-9.189138	-4.415740
44	1	0	-4.153735	-10.922400	-4.665811
45	6	0	-6.544515	-8.365476	-4.653661
46	1	0	-7.570983	-8.274583	-4.291296
47	1	0	-5.938786	-7.592659	-4.174945
48	1	0	-6.544171	-8.178019	-5.730518
49	6	0	-7.171126	-11.007893	-6.392632
50	1	0	-6.753230	-10.362506	-7.159962
51	6	0	-8.269207	-12.697764	-4.404676
52	1	0	-8.697567	-13.351491	-3.653902
53	6	0	-8.565703	-12.885552	-5.755535
54	6	0	-8.027375	-12.056234	-6.738078
55	1	0	-8.276310	-12.227006	-7.779222
56	1	0	-9.229162	-13.694149	-6.041309

9

Zero-point correction= 0.527803 (Hartree/Particle)

Thermal correction to Energy=	0.557735
Thermal correction to Enthalpy=	0.558679
Thermal correction to Gibbs Free Energy=	0.465551
Sum of electronic and zero-point Energies=	-1306.030039
Sum of electronic and thermal Energies=	-1306.000107
Sum of electronic and thermal Enthalpies=	-1305.999163
Sum of electronic and thermal Free Energies=	-1306.092292

	E (Thermal) KCal/Mol	CV Cal/Mol-Kelvin	S Cal/Mol-Kelvin
Total	349.984	115.922	196.005

+1 1

1	6	0	-1.494150	-4.383126	1.454640
2	6	0	-1.690473	-3.786523	0.215302
3	6	0	-1.311960	-2.467957	0.023211
4	6	0	-0.745240	-1.781389	1.099685
5	6	0	-0.562791	-2.401901	2.336986
6	6	0	-0.938949	-3.729621	2.541549
7	6	0	-2.437311	-5.998990	0.198724
8	1	0	-1.452177	-1.974973	-0.934094
9	1	0	-0.443180	-0.747550	0.974370
10	1	0	-0.122870	-1.843617	3.155572
11	1	0	-0.804745	-4.214923	3.501540
12	6	0	-2.319870	-4.798078	-0.714818
13	6	0	-1.397898	-5.083169	-1.916095
14	1	0	-1.830953	-5.818679	-2.596427
15	1	0	-1.255752	-4.154167	-2.473856
16	1	0	-0.417762	-5.436542	-1.587720
17	6	0	-3.726289	-4.362369	-1.169263
18	1	0	-3.640095	-3.445602	-1.758013
19	1	0	-4.194501	-5.130086	-1.790310
20	1	0	-4.372551	-4.165456	-0.311077
21	7	0	-1.970109	-5.727181	1.383881
22	6	0	-1.942530	-6.618544	2.555984
23	1	0	-0.923767	-6.598915	2.948004
24	1	0	-2.631236	-6.202903	3.294602
25	1	0	-2.282822	-7.633955	2.303180
26	6	0	-3.633765	-7.727518	-1.365482
27	1	0	-3.280152	-7.297057	-2.301584
28	6	0	-4.572262	-8.695848	-1.346639
29	1	0	-4.815934	-9.125117	-0.370839
30	6	0	-5.164907	-9.214380	-2.540336
31	1	0	-4.863319	-8.775788	-3.488865
32	6	0	-6.070821	-10.236220	-2.502143
33	1	0	-6.327620	-10.628141	-1.522918

34	6	0	-6.697205	-10.823575	-3.632198
35	6	0	-7.496324	-11.446467	-5.746793
36	6	0	-8.069152	-12.244661	-4.762531
37	7	0	-7.549252	-11.832837	-3.504649
38	6	0	-7.920740	-12.454126	-2.235697
39	1	0	-8.474744	-11.743457	-1.619131
40	1	0	-8.546324	-13.319429	-2.437168
41	1	0	-7.023419	-12.781813	-1.708671
42	6	0	-6.551561	-10.456519	-5.104092
43	6	0	-5.112023	-10.693580	-5.601585
44	1	0	-5.084681	-10.543400	-6.683670
45	1	0	-4.404090	-10.001037	-5.143019
46	1	0	-4.787898	-11.715083	-5.390169
47	6	0	-7.021172	-9.014115	-5.373320
48	1	0	-8.037815	-8.858134	-5.005391
49	1	0	-6.363966	-8.279830	-4.904096
50	1	0	-7.016908	-8.837128	-6.451695
51	6	0	-7.832644	-11.642265	-7.075757
52	1	0	-7.396563	-11.031171	-7.860414
53	6	0	-8.984771	-13.247921	-5.042063
54	1	0	-9.435293	-13.864976	-4.273645
55	6	0	-9.316273	-13.435233	-6.383568
56	6	0	-8.750564	-12.647259	-7.386740
57	1	0	-9.029241	-12.818237	-8.420385
58	1	0	-10.028544	-14.208681	-6.647640
59	8	0	-3.960856	-7.560930	0.975569
60	8	0	-3.669731	-8.892618	1.491273
61	6	0	-3.035931	-7.362233	-0.031433
62	1	0	-2.208023	-8.081451	0.138275

15

Zero-point correction=	0.486703 (Hartree/Particle)
Thermal correction to Energy=	0.511943
Thermal correction to Enthalpy=	0.512887
Thermal correction to Gibbs Free Energy=	0.432614
Sum of electronic and zero-point Energies=	-1078.473152
Sum of electronic and thermal Energies=	-1078.447912
Sum of electronic and thermal Enthalpies=	-1078.446967
Sum of electronic and thermal Free Energies=	-1078.527240

	E (Thermal) KCal/Mol	CV Cal/Mol-Kelvin	S Cal/Mol-Kelvin
Total	321.249	101.069	168.949

+1 1

1	6	0	-4.807876	-7.773333	2.817738

2	6	0	-4.350522	-6.752774	1.996824
3	6	0	-3.508658	-5.783966	2.519094
4	6	0	-3.164962	-5.868168	3.870014
5	6	0	-3.641820	-6.904624	4.675560
6	6	0	-4.481644	-7.890859	4.158973
7	6	0	-5.777236	-8.181245	0.808818
8	1	0	-3.128831	-4.975848	1.901519
9	1	0	-2.516578	-5.114936	4.303438
10	1	0	-3.358496	-6.944183	5.721111
11	1	0	-4.857583	-8.691876	4.785595
12	6	0	-4.868435	-6.978642	0.595627
13	6	0	-3.662049	-7.339414	-0.309171
14	1	0	-3.953636	-7.481324	-1.348451
15	1	0	-2.950024	-6.512135	-0.260951
16	1	0	-3.158993	-8.242430	0.044770
17	6	0	-5.658809	-5.786492	0.035519
18	1	0	-4.991000	-4.926811	-0.057552
19	1	0	-6.064462	-6.017476	-0.952477
20	1	0	-6.483253	-5.513190	0.697779
21	7	0	-5.658383	-8.619462	2.038802
22	6	0	-6.239087	-9.821923	2.635775
23	1	0	-5.439860	-10.353964	3.154058
24	1	0	-7.012229	-9.538076	3.353067
25	1	0	-6.666188	-10.446034	1.855072
26	6	0	-6.737084	-8.725002	-0.165800
27	6	0	-6.317061	-8.861344	-1.559975
28	6	0	-6.719721	-9.805457	-2.452163
29	6	0	-7.164620	-10.923850	-4.482994
30	6	0	-7.612666	-11.686993	-3.406967
31	7	0	-7.312049	-11.017107	-2.198232
32	6	0	-7.293204	-11.690972	-0.923777
33	1	0	-8.148659	-11.425660	-0.288828
34	1	0	-7.297461	-12.767794	-1.096106
35	1	0	-6.372121	-11.425302	-0.394047
36	6	0	-6.525711	-9.653017	-3.966915
37	6	0	-5.031288	-9.598366	-4.320712
38	1	0	-4.909738	-9.573405	-5.407123
39	1	0	-4.565872	-8.697239	-3.908178
40	1	0	-4.504260	-10.474595	-3.935846
41	6	0	-7.250797	-8.405257	-4.487899
42	1	0	-8.312539	-8.433566	-4.231629
43	1	0	-6.812999	-7.496898	-4.062739
44	1	0	-7.161238	-8.350361	-5.576331
45	6	0	-7.334345	-11.383403	-5.776656
46	1	0	-6.984364	-10.799838	-6.623695
47	6	0	-8.249201	-12.906404	-3.579984

48	1	0	-8.620799	-13.487556	-2.743041
49	6	0	-8.416037	-13.360208	-4.891031
50	6	0	-7.966359	-12.614395	-5.977305
51	1	0	-8.111903	-12.988387	-6.984332
52	1	0	-8.911727	-14.309879	-5.060676
53	6	0	-8.002166	-8.927975	0.266197
54	1	0	-8.299039	-8.788788	1. 300130
55	1	0	-8.781021	-9.177326	-0.446478
56	1	0	-5.733886	-8.050764	-1.980167

16

Zero-point correction=	0.397408 (Hartree/Particle)
Thermal correction to Energy=	0.418901
Thermal correction to Enthalpy=	0.419845
Thermal correction to Gibbs Free Energy=	0.346596
Sum of electronic and zero-point Energies=	-920.703882
Sum of electronic and thermal Energies=	-920.682388
Sum of electronic and thermal Enthalpies=	-920.681444
Sum of electronic and thermal Free Energies=	-920.754693

	E (Thermal) Kcal/mol	CV cal/mol-Kelvin	S cal/mol-Kelvin
Total	262.864	80.378	154.165

0 1

1	6	0	-2.996353	-0.439537	-0.172917
2	6	0	-2.195019	-1.666910	-0.701767
3	1	0	-3.813357	-0.787019	0.471996
4	1	0	-2.894883	-2.203170	-1.355071
5	6	0	-2.038623	0.461513	0.542237
6	6	0	-1.402009	1.659059	-0.092470
7	1	0	-1.164870	1.467126	-1.138949
8	1	0	-2.136111	2.475390	-0.060265
9	7	0	-1.614798	0.285171	1.746460
10	6	0	-0.178853	1.904340	0.804145
11	1	0	0.074938	2.959300	0.899154
12	1	0	0.679322	1.368992	0.387369
13	6	0	-0.589052	1.284870	2.141943
14	1	0	0.215788	0.767891	2.668614
15	1	0	-1.062987	1.992177	2.830507
16	8	0	-1.906772	-2.410433	0.465746
17	1	0	-1.393022	-3.184021	0.199584
18	8	0	-3.497797	0.347450	-1.231070
19	8	0	-4.455981	-0.464681	-1.901308
20	1	0	-5.222326	0.129423	-1.911136

21	6	0	1.456668	-0.725185	-1.546736
22	1	0	1.375565	-0.381514	-2.573538
23	6	0	2.677000	-0.742004	-0.937690
24	6	0	5.013807	-0.681904	-0.710284
25	1	0	5.786971	0.085999	-0.795993
26	1	0	5.436370	-1.636405	-1.056334
27	6	0	4.433294	-0.798637	0.699923
28	1	0	4.416500	0.189013	1.167849
29	1	0	5.005628	-1.472923	1.337293
30	6	0	2.998914	-1.285223	0.441960
31	1	0	2.954222	-2.379807	0.408851
32	1	0	2.290551	-0.951416	1.204618
33	7	0	3.840661	-0.313157	-1.496249
34	6	0	3.944280	0.037261	-2.893408
35	1	0	3.728710	-0.820152	-3.544377
36	1	0	3.241466	0.840935	-3.132829
37	1	0	4.954282	0.393510	-3.098130
38	6	0	-2.044332	-0.699287	2.739349
39	1	0	-1.217782	-1.386410	2.924864
40	1	0	-2.295305	-0.159637	3.654743
41	1	0	-2.892068	-1.270131	2.373269
42	6	0	-0.968508	-1.245164	-1.461085
43	6	0	0.257066	-1.191251	-0.901621
44	1	0	-1.122775	-0.934077	-2.491483
45	1	0	0.351186	-1.529770	0.132966

17

Zero-point correction=	0.394401 (Hartree/Particle)
Thermal correction to Energy=	0.414145
Thermal correction to Enthalpy=	0.415090
Thermal correction to Gibbs Free Energy=	0.346165
Sum of electronic and zero-point Energies=	-920.657548
Sum of electronic and thermal Energies=	-920.637803
Sum of electronic and thermal Enthalpies=	-920.636859
Sum of electronic and thermal Free Energies=	-920.705783

	E (Thermal) Kcal/mol	CV cal/mol-Kelvin	S cal/mol-Kelvin
Total	259.880	76.579	145.065

0 1

1	6	0	-0.976275	-0.481124	-0.677430
2	6	0	-3.042247	-0.465772	0.076211
3	6	0	-2.094475	-1.537554	-0.509155

4	1	0	-0.862922	-0.152840	-1.712852
5	1	0	-3.475420	-0.717447	1.054599
6	1	0	-2.461146	-1.973260	-1.442878
7	6	0	-1.908788	0.588360	0.086525
8	6	0	-2.162454	1.954969	-0.541958
9	1	0	-2.169544	1.909739	-1.631214
10	1	0	-3.142980	2.317965	-0.218513
11	7	0	-1.348748	0.899994	1.384212
12	6	0	-0.820848	-0.097297	2.092693
13	1	0	-1.365899	-1.039980	2.132949
14	1	0	0.139320	-0.663307	1.155038
15	1	0	-0.203068	0.170018	2.946491
16	6	0	-1.039751	2.824596	0.036641
17	1	0	-1.274710	3.889053	0.012300
18	1	0	-0.111035	2.670024	-0.518958
19	6	0	-0.887207	2.303209	1.467671
20	1	0	0.136935	2.341807	1.845859
21	1	0	-1.541789	2.836611	2.163667
22	8	0	-1.705790	-2.514594	0.426331
23	1	0	-2.379544	-3.204835	0.452656
24	8	0	-4.019168	-0.028015	-0.829425
25	8	0	-4.858430	-1.164019	-1.049679
26	1	0	-5.730871	-0.779352	-0.875347
27	6	0	0.362418	-0.795806	-0.083597
28	1	0	0.562050	-1.869860	-0.048958
29	6	0	1.467636	0.068757	-0.447454
30	1	0	1.245715	1.012790	-0.933174
31	6	0	2.771030	-0.290209	-0.223205
32	6	0	5.112920	-0.274071	-0.363802
33	1	0	5.891150	0.404777	-0.006623
34	1	0	5.442223	-0.706149	-1.318334
35	6	0	4.733971	-1.351073	0.655437
36	1	0	4.859573	-0.957450	1.666771
37	1	0	5.341306	-2.250799	0.559356
38	6	0	3.244064	-1.599545	0.370348
39	1	0	3.105514	-2.390791	-0.376128
40	1	0	2.677608	-1.884362	1.259948
41	7	0	3.852537	0.449323	-0.538509
42	6	0	3.772124	1.686364	-1.281681
43	1	0	3.404195	1.519255	-2.301330
44	1	0	3.098606	2.384876	-0.775056
45	1	0	4.762598	2.138505	-1.331462

18

Zero-point correction=	0.397503 (Hartree/Particle)
Thermal correction to Energy=	0.418572
Thermal correction to Enthalpy=	0.419517
Thermal correction to Gibbs Free Energy=	0.346942
Sum of electronic and zero-point Energies=	-920.686945
Sum of electronic and thermal Energies=	-920.665875
Sum of electronic and thermal Enthalpies=	-920.664931
Sum of electronic and thermal Free Energies=	-920.737505

	E (Thermal) Kcal/mol	CV cal/mol-Kelvin	S cal/mol-Kelvin
Total	262.658	79.221	152.745

0 1

1	6	0	-1.463683	-0.824802	-0.665588
2	6	0	-3.387974	-0.524662	0.375109
3	6	0	-2.660247	-1.718782	-0.274697
4	1	0	-1.534754	-0.461573	-1.693758
5	1	0	-3.790544	-0.701821	1.380773
6	1	0	-3.202112	-2.158532	-1.118047
7	6	0	-2.127767	0.363013	0.296629
8	6	0	-2.231361	1.715152	-0.406934
9	1	0	-2.417350	1.631431	-1.477830
10	1	0	-3.064862	2.265276	0.038780
11	7	0	-1.521512	0.703777	1.533667
12	6	0	-0.889481	2.364666	-0.049692
13	1	0	-0.930021	3.454305	-0.068020
14	1	0	-0.122181	2.051835	-0.766078
15	6	0	-0.569011	1.801680	1.346414
16	1	0	0.468694	1.437098	1.405235
17	1	0	-0.701325	2.541218	2.143496
18	8	0	-2.232430	-2.683306	0.655204
19	1	0	-2.996678	-3.206253	0.926316
20	8	0	-4.357445	0.035077	-0.476019
21	8	0	-5.387437	-0.949865	-0.582874
22	1	0	-6.152355	-0.446217	-0.266101
23	6	0	1.020067	-0.819048	-0.909342
24	1	0	0.972565	-0.035933	-1.660329
25	6	0	2.293974	-1.306378	-0.501958
26	6	0	4.618097	-1.467974	-0.348791
27	1	0	5.060009	-0.738343	0.338588
28	1	0	5.352454	-1.711185	-1.119236
29	6	0	4.038189	-2.695002	0.360440
30	1	0	4.550697	-2.909608	1.297213
31	1	0	4.125695	-3.571012	-0.284988
32	6	0	2.555070	-2.339944	0.562376

33	1	0	1.889999	-3.199786	0.472927
34	1	0	2.364717	-1.874968	1.537981
35	7	0	3.430202	-0.858692	-0.977809
36	6	0	3.561716	0.213170	-1.960764
37	1	0	3.068576	-0.070883	-2.892820
38	1	0	3.119795	1.135911	-1.576929
39	1	0	4.620146	0.378376	-2.154436
40	6	0	-1.127114	-0.305087	2.482455
41	1	0	-0.969221	0.158627	3.459742
42	1	0	-1.893350	-1.075251	2.577588
43	1	0	-0.183136	-0.806712	2.192977
44	6	0	-0.124135	-1.312594	-0.361821
45	1	0	-0.066839	-2.116991	0.369476

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Zero-point correction=	0.394657 (Hartree/Particle)
Thermal correction to Energy=	0.415882
Thermal correction to Enthalpy=	0.416827
Thermal correction to Gibbs Free Energy=	0.343976
Sum of electronic and zero-point Energies=	-920.631702
Sum of electronic and thermal Energies=	-920.610476
Sum of electronic and thermal Enthalpies=	-920.609532
Sum of electronic and thermal Free Energies=	-920.682382

	E (Thermal) Kcal/mol	CV cal/mol-Kelvin	S cal/mol-Kelvin
Total	260.970	79.495	153.327

0 1

1	6	0	-0.654018	-0.919914	-0.697587
2	6	0	-3.014362	-0.385945	0.145850
3	6	0	-2.665366	-1.634468	0.775436
4	1	0	-1.188616	-1.694682	-1.242171
5	1	0	-3.486829	0.334186	0.824518
6	1	0	-2.743768	-2.560789	0.207040
7	6	0	-1.505801	0.281139	-0.364315
8	6	0	-1.829422	1.212617	-1.544448
9	1	0	-0.865625	1.403116	-2.027653
10	1	0	-2.497651	0.765665	-2.278712
11	7	0	-1.028419	1.173038	0.607435
12	6	0	-0.587482	0.769652	1.923859
13	1	0	-0.049849	-0.178309	1.862597
14	1	0	0.095036	1.529682	2.316085
15	1	0	-1.425432	0.673164	2.631564

16	6	0	-2.384941	2.467551	-0.864270
17	1	0	-3.471709	2.395935	-0.767154
18	1	0	-2.167555	3.375845	-1.426605
19	6	0	-1.715152	2.462697	0.524673
20	1	0	-0.985125	3.268395	0.655500
21	1	0	-2.454928	2.562682	1.334872
22	8	0	-2.298847	-1.672387	2.005735
23	1	0	-2.028729	-2.557440	2.300978
24	8	0	-3.757660	-0.648215	-1.002130
25	8	0	-4.957753	-1.296927	-0.534063
26	1	0	-5.591012	-1.014290	-1.212803
27	6	0	0.702180	-1.004875	-0.654912
28	1	0	1.104106	-1.951678	-1.015312
29	6	0	1.670106	-0.044509	-0.232845
30	1	0	1.339685	0.952647	0.030257
31	6	0	3.012405	-0.335674	-0.228775
32	6	0	5.332494	0.028344	-0.127582
33	1	0	6.014743	0.352464	0.662405
34	1	0	5.708627	0.410290	-1.086609
35	6	0	5.116971	-1.485854	-0.157361
36	1	0	5.194044	-1.884985	0.856817
37	1	0	5.843216	-2.001007	-0.785967
38	6	0	3.674390	-1.626312	-0.667561
39	1	0	3.643365	-1.680216	-1.761985
40	1	0	3.169682	-2.511338	-0.277109
41	7	0	3.983746	0.529611	0.130646
42	6	0	3.727761	1.915600	0.447187
43	1	0	3.346082	2.462907	-0.423517
44	1	0	2.992115	1.986636	1.254625
45	1	0	4.654344	2.382503	0.781345

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Zero-point correction=	0.394548 (Hartree/Particle)
Thermal correction to Energy=	0.417122
Thermal correction to Enthalpy=	0.418066
Thermal correction to Gibbs Free Energy=	0.339456
Sum of electronic and zero-point Energies=	-920.708673
Sum of electronic and thermal Energies=	-920.686099
Sum of electronic and thermal Enthalpies=	-920.685155
Sum of electronic and thermal Free Energies=	-920.763765

	E (Thermal) Kcal/mol	CV cal/mol-Kelvin	S cal/mol-Kelvin
Total	261.748	81.283	165.448

0 1

1	6	0	-1.884760	-0.048123	-1.651792
2	6	0	1.443124	-0.308267	0.638809
3	6	0	-2.083316	0.350032	-0.399181
4	1	0	-2.342822	0.466404	-2.493612
5	1	0	1.517107	-0.206798	-0.442814
6	1	0	-1.659242	-0.135739	0.472477
7	6	0	0.851012	0.731857	1.335344
8	1	0	0.693637	0.634083	2.404657
9	6	0	0.363679	1.868550	0.671530
10	6	0	0.627505	2.250754	-0.764880
11	6	0	-0.394144	3.366379	-1.047457
12	1	0	0.516785	1.398826	-1.438368
13	1	0	1.661993	2.604116	-0.837570
14	6	0	-0.812060	3.871052	0.339867
15	1	0	0.022424	4.162754	-1.663685
16	1	0	-1.263858	2.952349	-1.559420
17	1	0	-0.316448	4.803998	0.630613
18	1	0	-1.893523	3.997407	0.433815
19	8	0	-0.990054	-1.052054	-1.914638
20	1	0	-1.269429	-1.525024	-2.706655
21	8	0	-2.735734	1.543087	-0.180038
22	8	0	-3.392662	1.469066	1.082054
23	1	0	-4.301256	1.244727	0.823866
24	7	0	-0.382892	2.795811	1.241895
25	6	0	-0.824531	2.760221	2.626770
26	1	0	-1.326699	3.699239	2.855757
27	1	0	-1.530798	1.937734	2.771189
28	1	0	0.030674	2.644853	3.296950
29	6	0	1.925334	-1.490699	1.199884
30	1	0	1.906058	-1.613536	2.277810
31	6	0	2.422262	-2.515435	0.400552
32	6	0	2.509173	-2.519921	-1.109133
33	1	0	3.346524	-1.880674	-1.412551
34	1	0	1.599261	-2.128427	-1.567851
35	6	0	2.774229	-3.996733	-1.445091
36	1	0	3.387229	-4.127117	-2.336559
37	1	0	1.826458	-4.517302	-1.600059
38	6	0	3.101992	-3.948754	2.275546
39	1	0	3.783608	-3.227937	2.737942
40	1	0	2.139355	-3.913463	2.793106
41	1	0	3.518828	-4.949695	2.379286
42	7	0	2.924779	-3.657119	0.864559
43	6	0	3.453571	-4.536681	-0.184298
44	1	0	3.198659	-5.575979	0.035711

45	1	0	4.546377	-4.448321	-0.22333

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