

## Supporting information

# Fluorogenic dimers as bright switchable probes for enhanced super-resolution imaging of cell membranes

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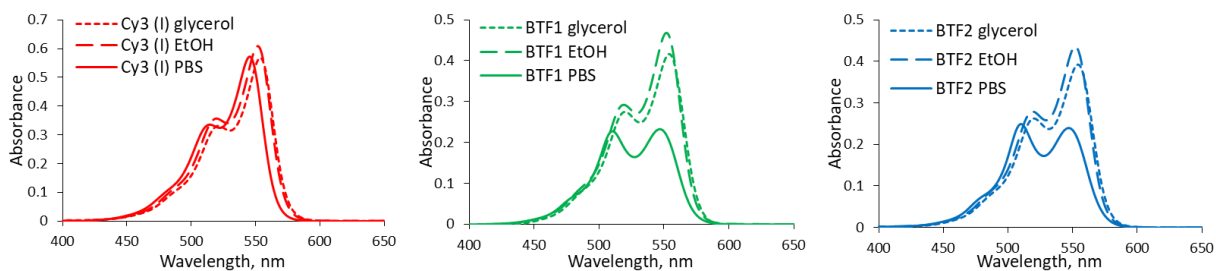
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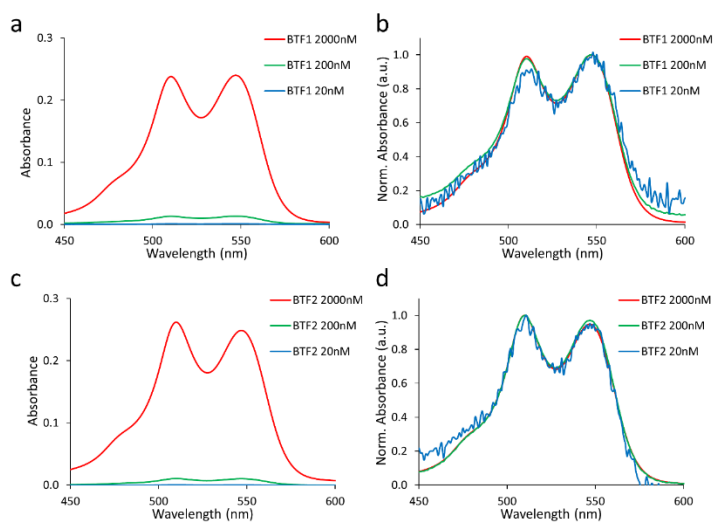
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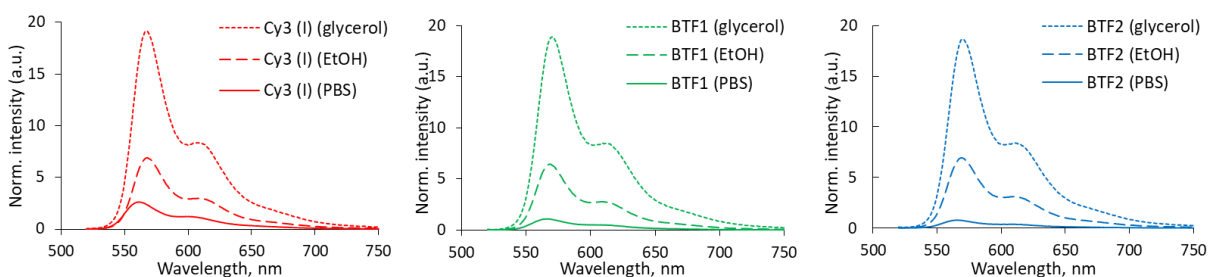
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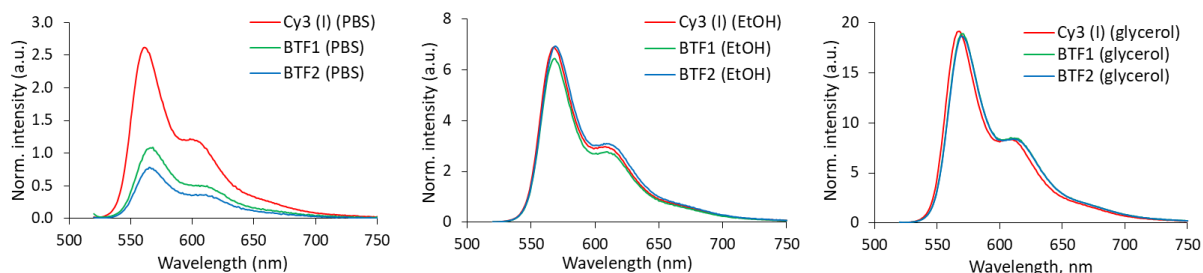
**Figure S1.** Absorption spectra of Cy3 (I), BTF1 and BTF2 in different media measured at 4, 2 and 2  $\mu\text{M}$  concentrations, respectively.



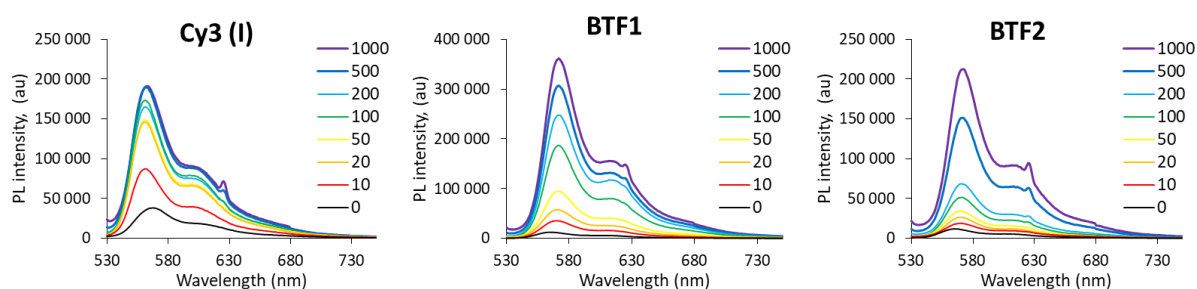
**Figure S2.** Absorption spectra of BTF1 (a,b) and BTF2 (c,d) in PBS at different concentrations. Raw (a,c) and (b,d) normalized spectra are presented.



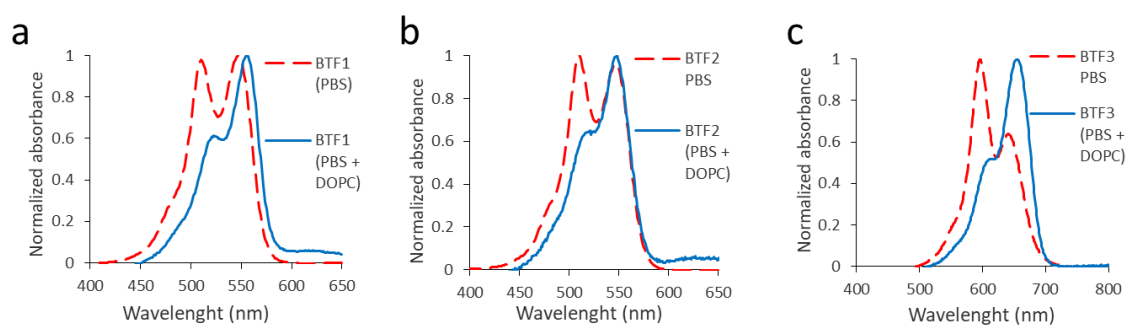
**Figure S3.** Fluorescence spectra of Cy3 (I), BTF1 and BTF2 in different media measured at 0.4, 0.2 and 0.2  $\mu\text{M}$  concentrations, respectively. Excitation wavelength: 510 nm. Fluorescence spectra are normalized with respect to absorptivity at 510 nm.



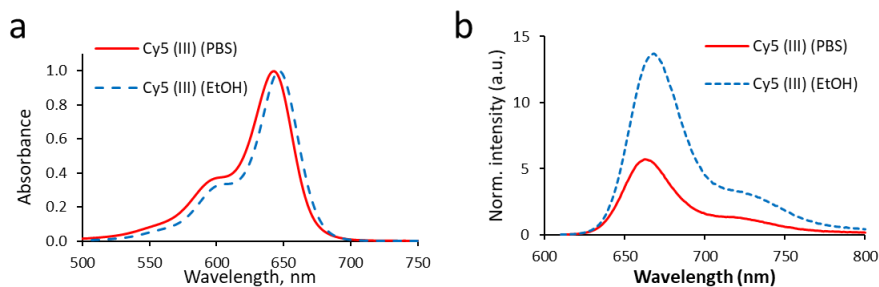
**Figure S4.** Comparison of fluorescence spectra of Cy3 (I), BTF1 and BTF2 in different media measured at 0.4, 0.2 and 0.2  $\mu\text{M}$  concentrations, respectively. Excitation wavelength: 510 nm. Fluorescence spectra are normalized with respect to absorptivity at 510 nm.



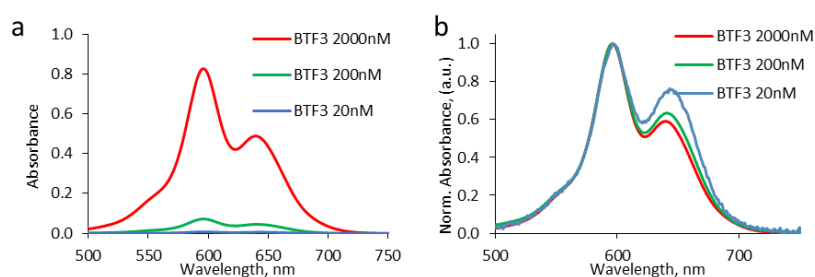
**Figure S5.** Fluorescence spectra of Cy3 (I), BTF1, and BTF2 in presence of liposomes with concentration of DOPC from 0  $\mu\text{M}$  to 1000  $\mu\text{M}$  in phosphate buffer (pH 7.4), measured at 0.2, 0.1 and 0.1  $\mu\text{M}$  the dye concentrations, respectively. Excitation wavelength: 510 nm.



**Figure S6.** Normalized absorption spectra of BTF1 (a), BTF2 (b) and BTF3 (c) in PBS and lipid vesicles. The concentrations of dyes was 100 nM; concentration of DOPC was 1 mM.



**Figure S7.** Absorption and fluorescence spectra of Cy5 (III) in different media measured at 0.4  $\mu\text{M}$  concentrations. Absorption spectra were normalized. Excitation wavelength: 600 nm. Fluorescence spectra are normalized with respect to absorptivity at 600 nm.

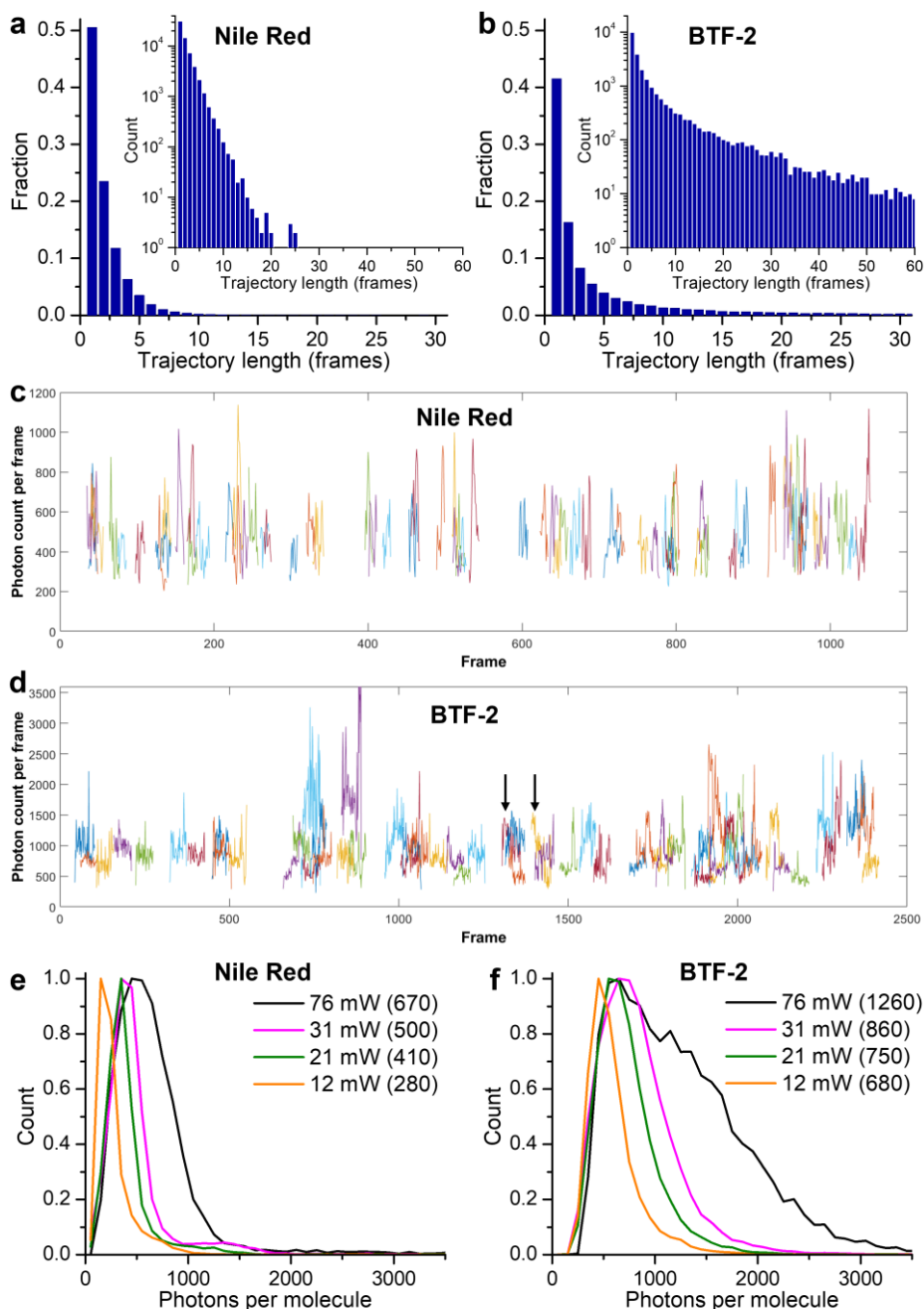


**Figure S8.** Raw (a) and normalized (b) absorption spectra of **BTF3** in PBS at different concentrations.

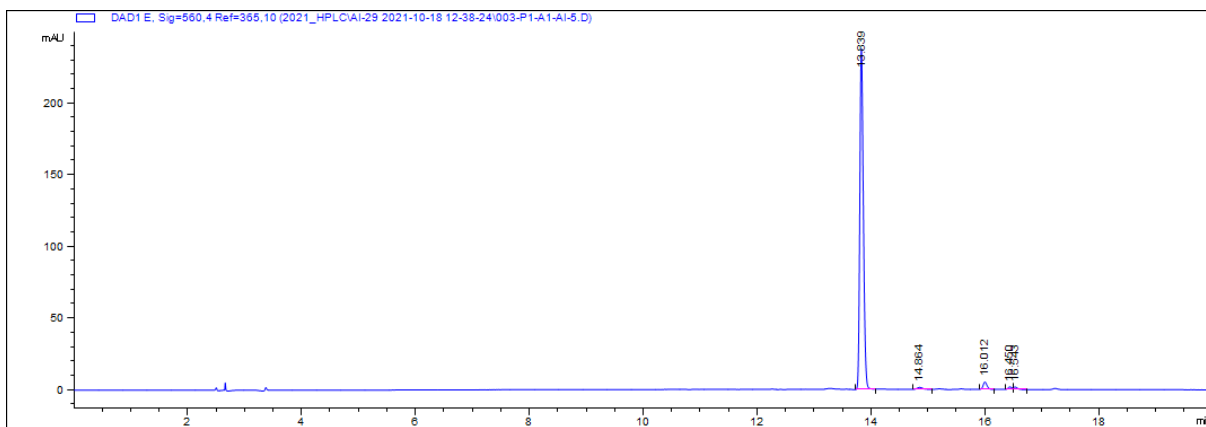
**Table S1.** Photophysical properties of **BTF3** probe and the monomer dye - Cy5 (III).

Probe	Solvent	$\lambda_{\text{abs}}/\lambda_{\text{emis}}$ , (nm) <sup>a</sup>	$I_s/I_L$ <sup>b</sup>	SBR <sup>c</sup>	QY, (%) <sup>d</sup>
Cy5(III)	PBS	643/665	0.33	-/-	21.4 $\pm$ 1.7
	Ethanol	647/668	0.33	2.62	55.2 $\pm$ 1.0
	Glycerol	654/673	0.33	2.5	52.6 $\pm$ 6.9
BTF3	PBS	596/667	1.76	-/-	1.1 $\pm$ 0.1
	Ethanol	647/680	0.49	21.8	24.0 $\pm$ 0.6
	Glycerol	650/682	0.72	14.5	16.5 $\pm$ 2.0

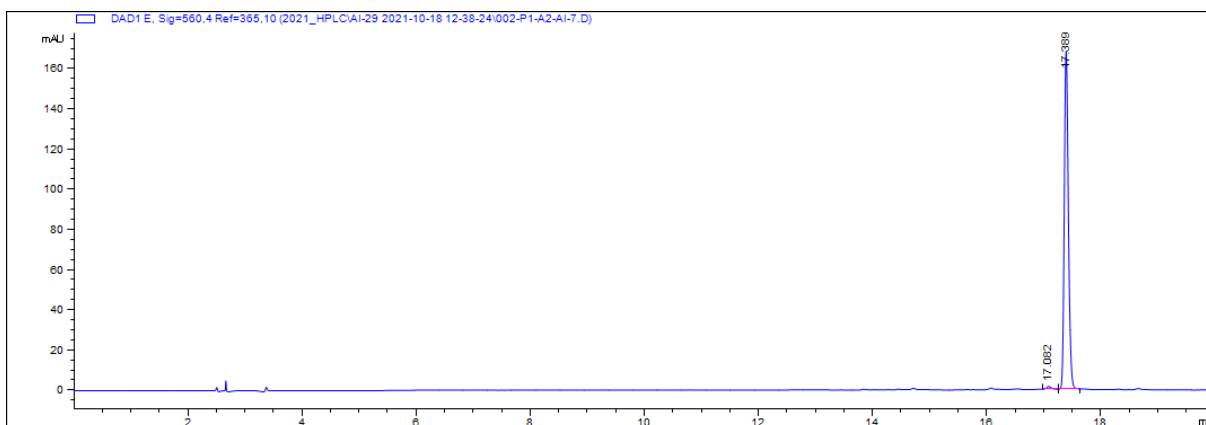
<sup>a</sup> Determined at  $\lambda_{\text{max}}$  for each solvent; <sup>b</sup> determined at  $\lambda_{\text{max}}$  for fluorophore ( $I_L$ ) and its H-aggregated forms ( $I_s$ ); <sup>c</sup> SBR =  $QY_{\text{solv}}/QY_{\text{PBS}}$ ; <sup>d</sup> with respect to DiD in methanol (33%);<sup>1</sup> excitation wavelengths: 600 nm for Cy5.



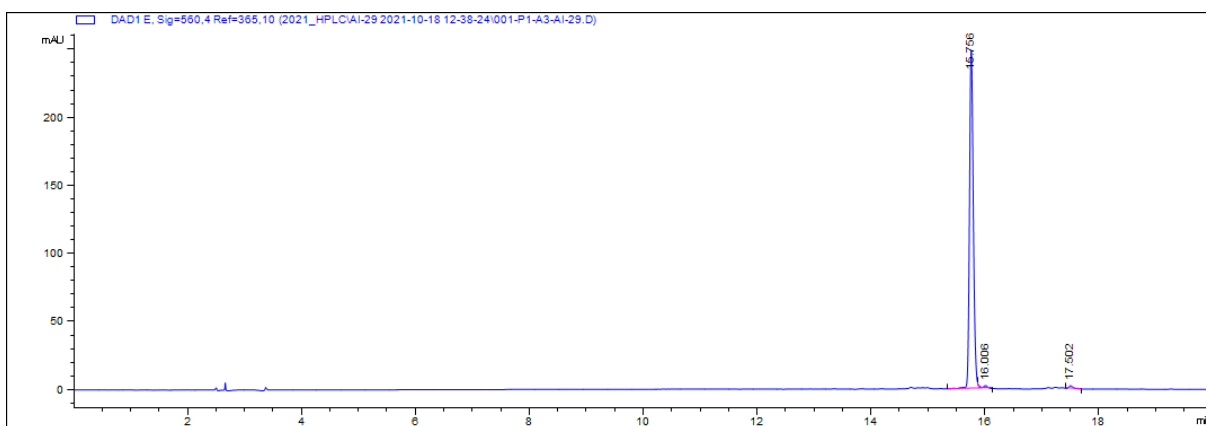
**Figure S9.** Additional characterizations of single-molecule behaviors in SMLM, recorded in cell membranes at 110 frames per second. (a,b) Distribution of the recorded trajectory lengths for single Nile Red (a) and **BTF2** (b) molecules under typical SMLM settings. Insets: Counts on the logarithmic scale for  $\sim 10^4$  trajectories. (c,d) Example intensity (photon count per frame) time traces for the longest ( $>11$  frames for Nile Red and  $>50$  frames for **BTF2**) time traces obtainable for the two dyes. Note the 3x different y-scales. Arrows in (d) indicate rare cases of stepwise photobleaching, consistent with one of the two dye units in the dimer being photobleached. (e,f) Distributions of the single-molecule photon counts recorded in each frame for Nile Red (e) and **BTF2** (f), under illuminations at different total laser powers (12-76 mW; legends). Average photon counts are given in parentheses. Typical SMLM experiments in this work were carried out at  $\sim 50$  mW, corresponding to a power density of  $\sim 1$  kW/cm<sup>2</sup> at the sample.



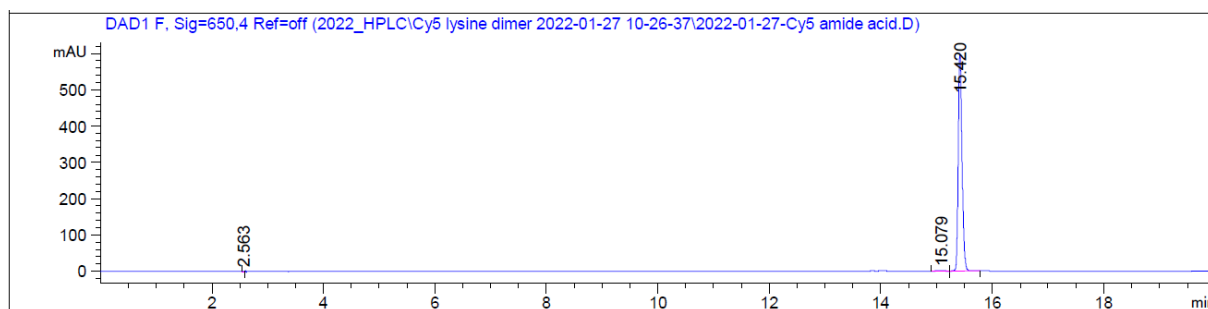
**Figure S10.** RP-HPLC profile for **Cy3 (I)** with absorptivity detection at 560 nm. Retention time for **Cy3 (I)**: 13.84 min.



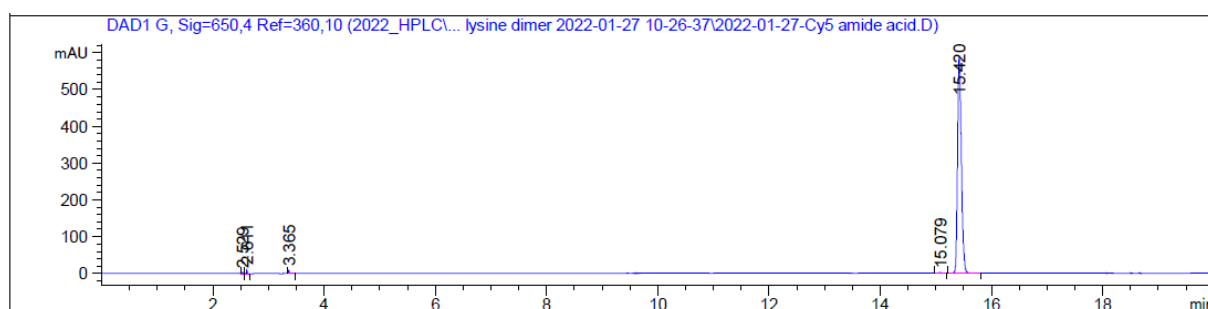
**Figure S11.** RP-HPLC profile for **BTF1** with absorptivity detection at 560 nm. Retention time for **BTF1**: 17.40 min.



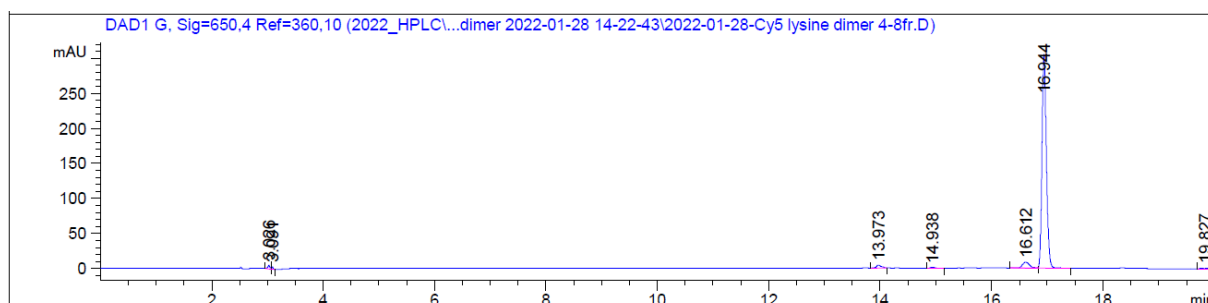
**Figure S12.** RP-HPLC profile for **BTF2** with absorptivity detection at 560 nm. Retention time for **BTF2**: 15.76 min.



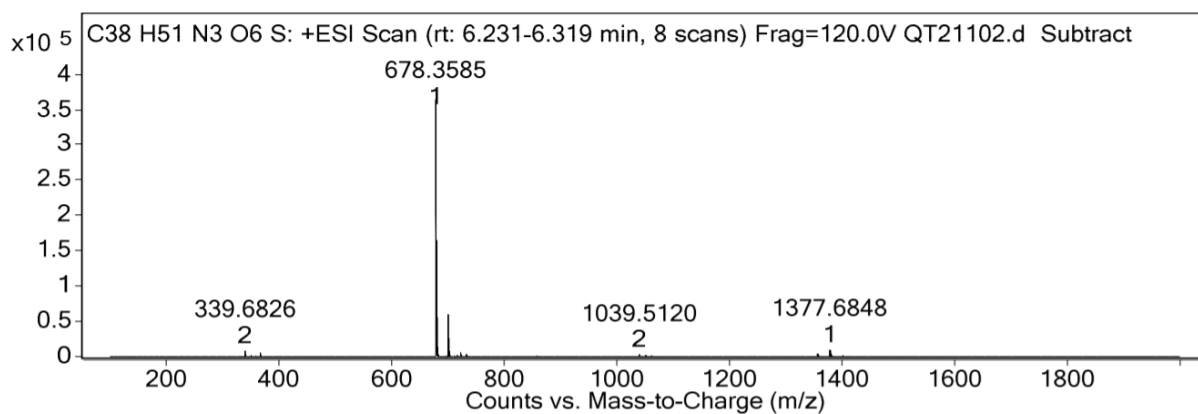
**Figure S13.** RP-HPLC profile for **Cy5 (III)** with absorptivity detection at 650 nm. Retention time for **Cy5 (III)**: 15.42 min.



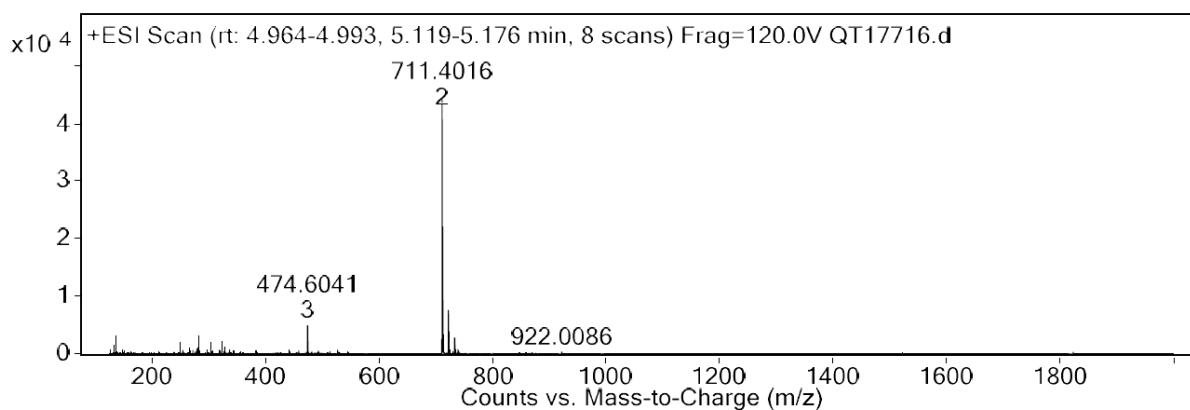
**Figure S14.** RP-HPLC profile for **Cy5 NHS ester (IV)** with absorptivity detection at 650 nm. Retention time for **Cy5 NHS ester (IV)**: 15.42 min.



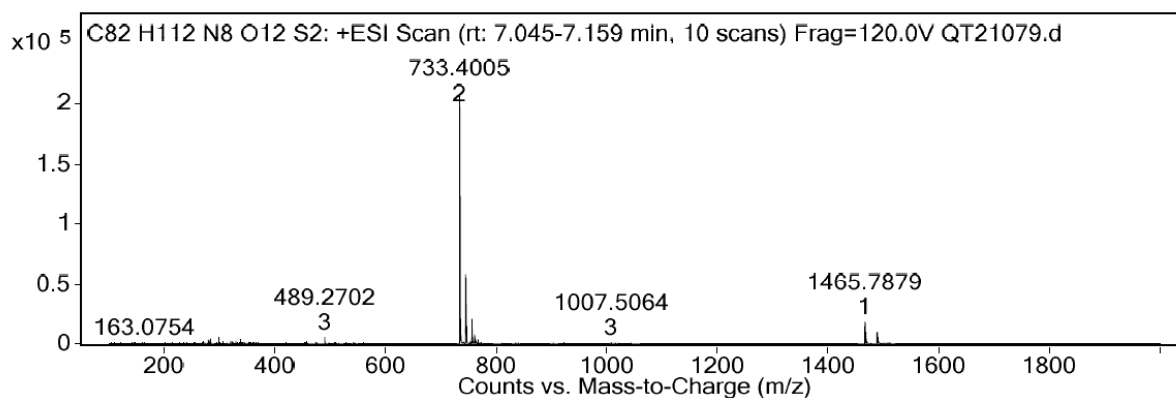
**Figure S15.** RP-HPLC profile for **BTF3** with absorptivity detection at 650 nm. Retention time for **BTF3**: 16.94 min.



**Figure S16.** Positive-mode ESI high-resolution mass spectrum for **Cy3 (I)**.

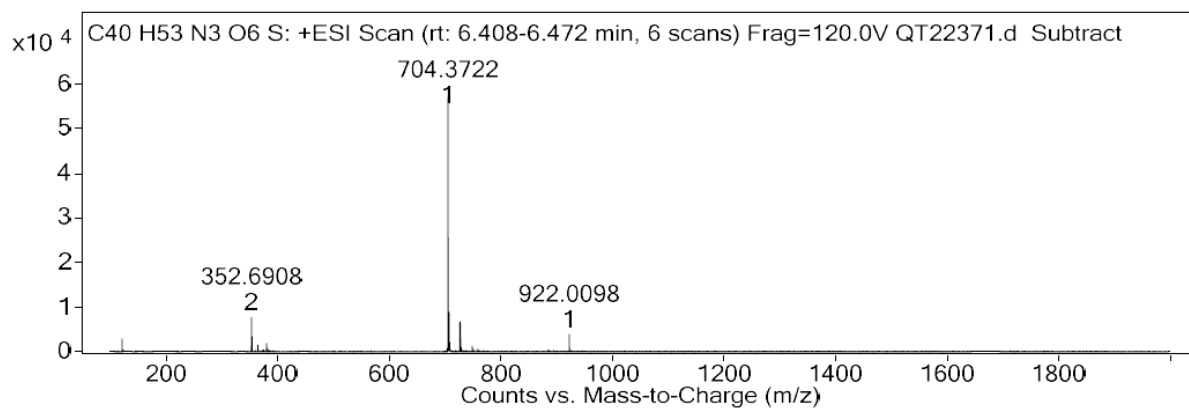


**Figure S17.** Positive-mode ESI high-resolution mass spectrum for **BTF1**.

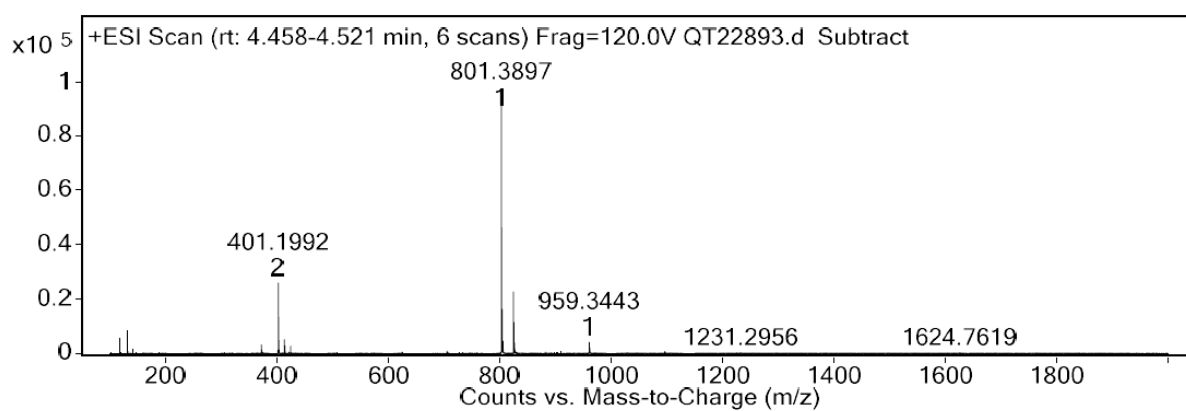


**Figure S18.** Positive-mode ESI high-resolution mass spectrum for **BTF2**.

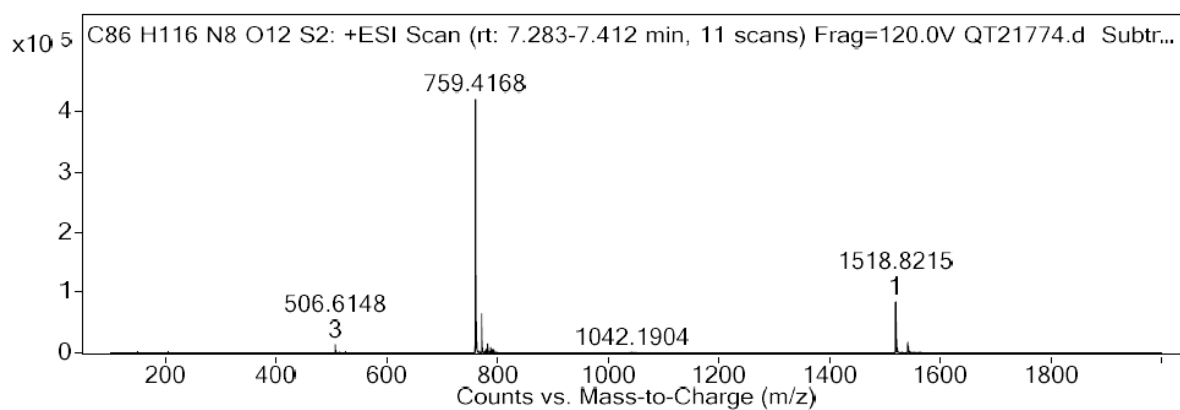




**Figure S19.** Positive-mode ESI high-resolution mass spectrum for **Cy5 (III)**.



**Figure S20.** Positive-mode ESI high-resolution mass spectrum for **Cy5 NHS ester (IV)**.



**Figure S21.** Positive-mode ESI high-resolution mass spectrum for **BTF3**.

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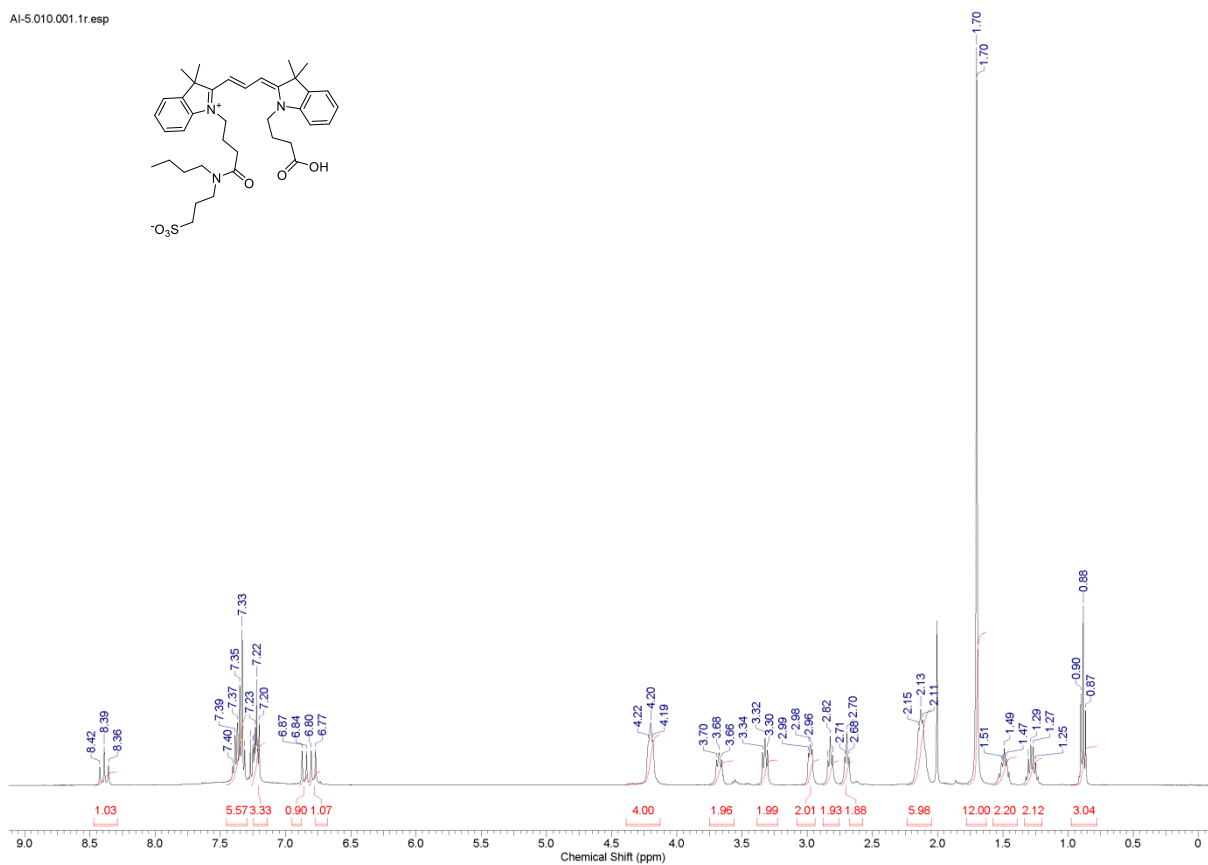


Figure S22. <sup>1</sup>H NMR spectrum for Cy3 (I).

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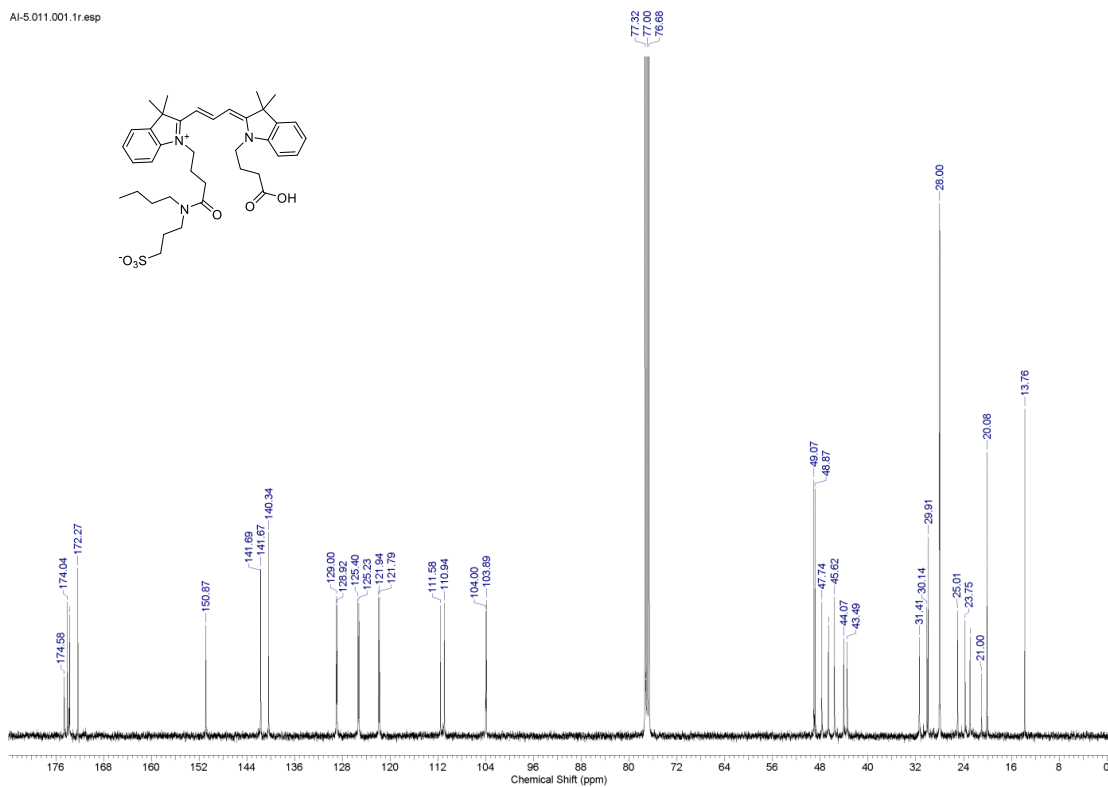
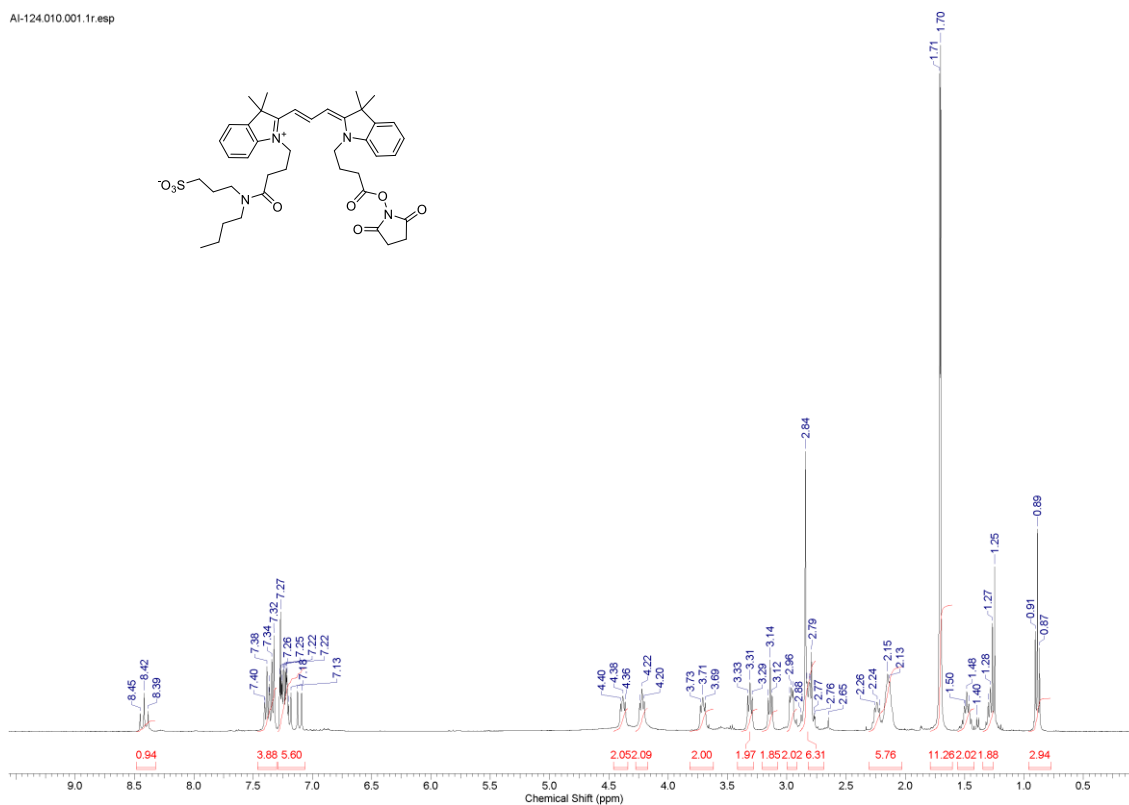
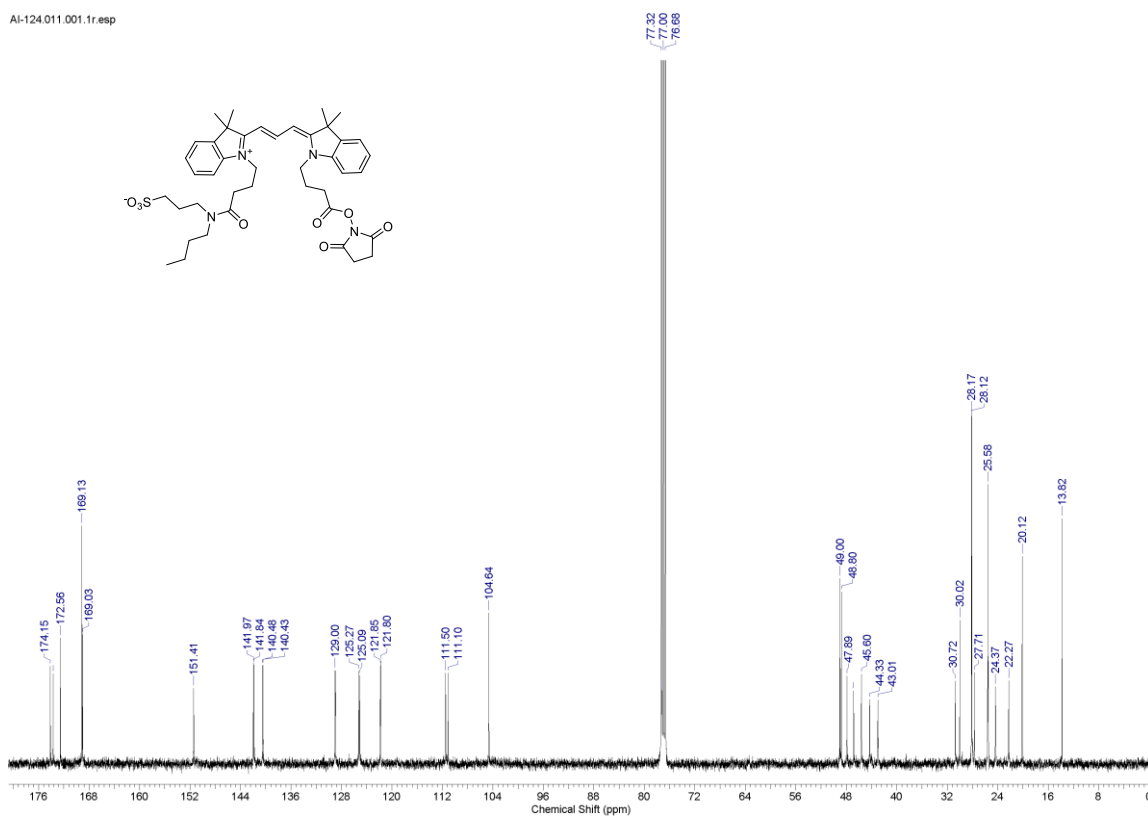


Figure S23. <sup>13</sup>C NMR spectrum for Cy3 (I).

Figure S24. <sup>1</sup>H NMR spectrum for Cy3 (II).Figure S25. <sup>13</sup>C NMR spectrum for Cy3 (II).

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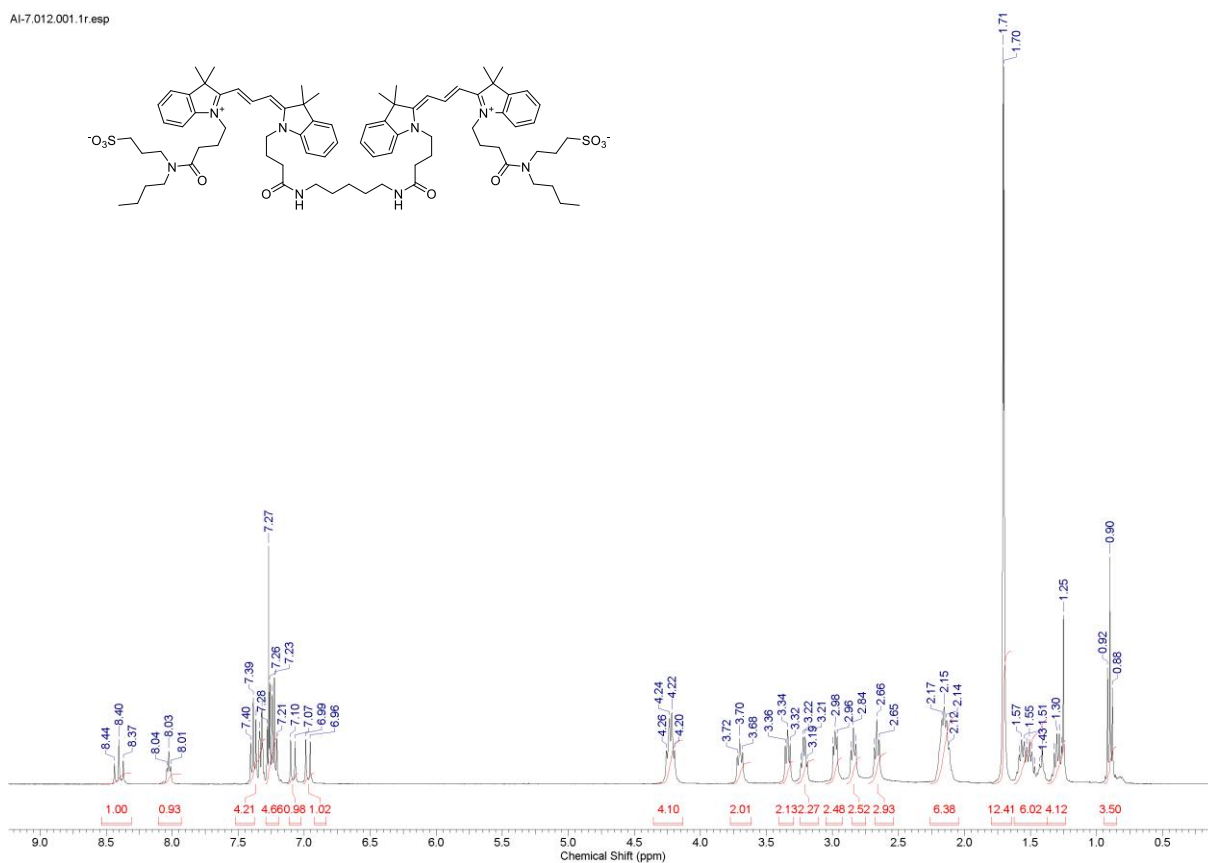


Figure S26. <sup>1</sup>H NMR spectrum for BTF1.

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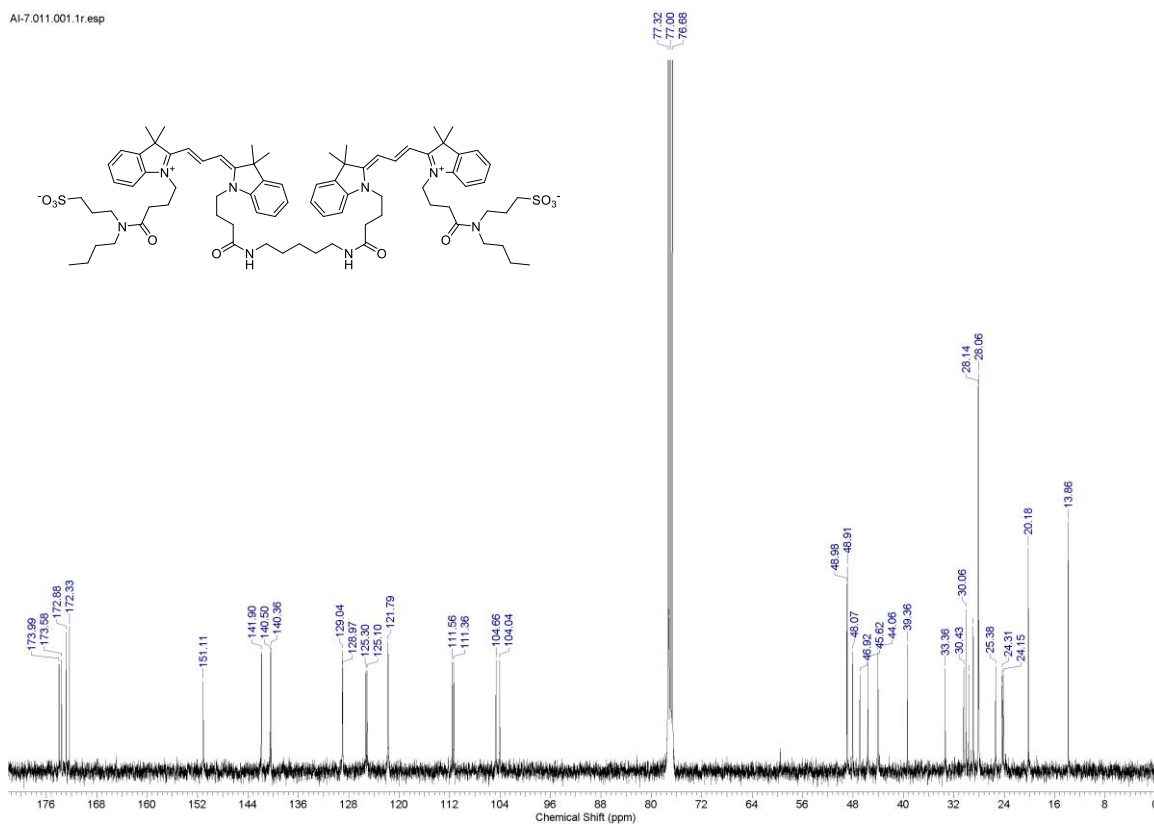


Figure S27. <sup>13</sup>C NMR spectrum for BTF1.

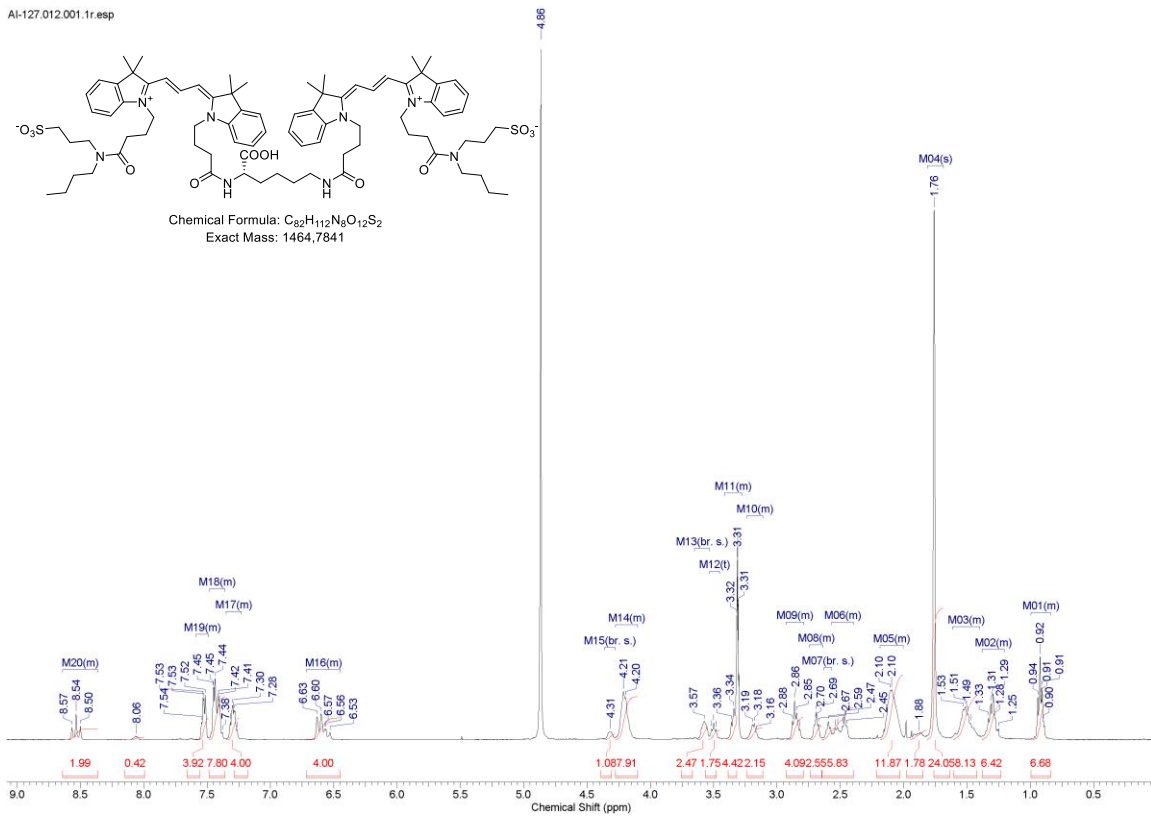


Figure S28.  $^1H$  NMR spectrum for BTF2.

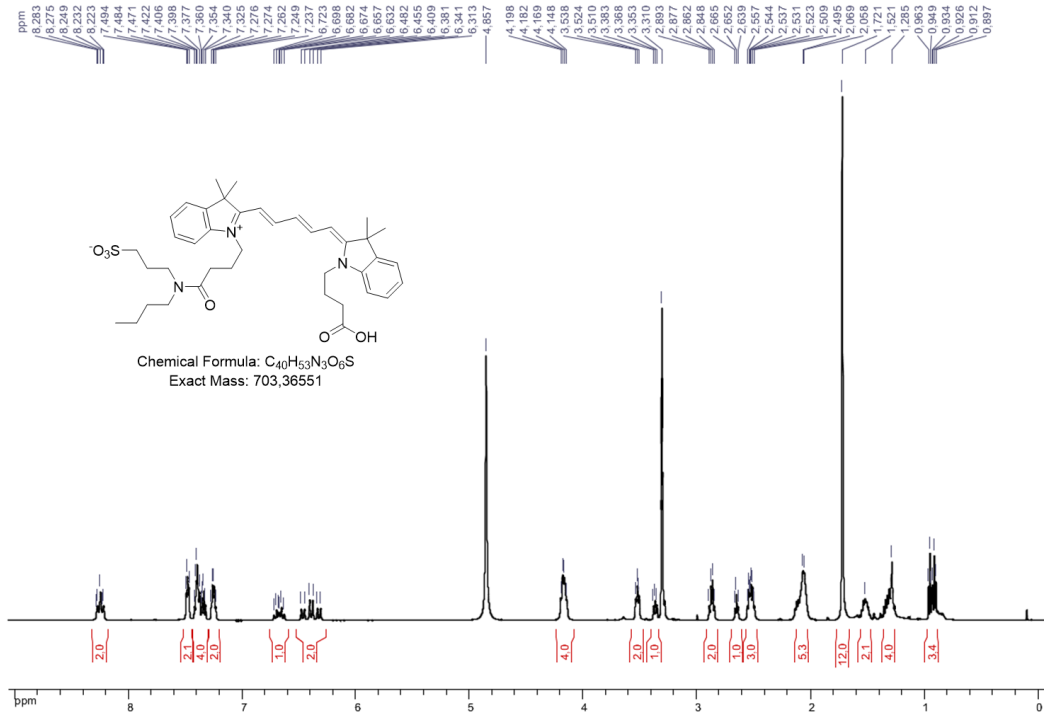


Figure S29.  $^1H$  NMR spectrum for Cy5 (III).

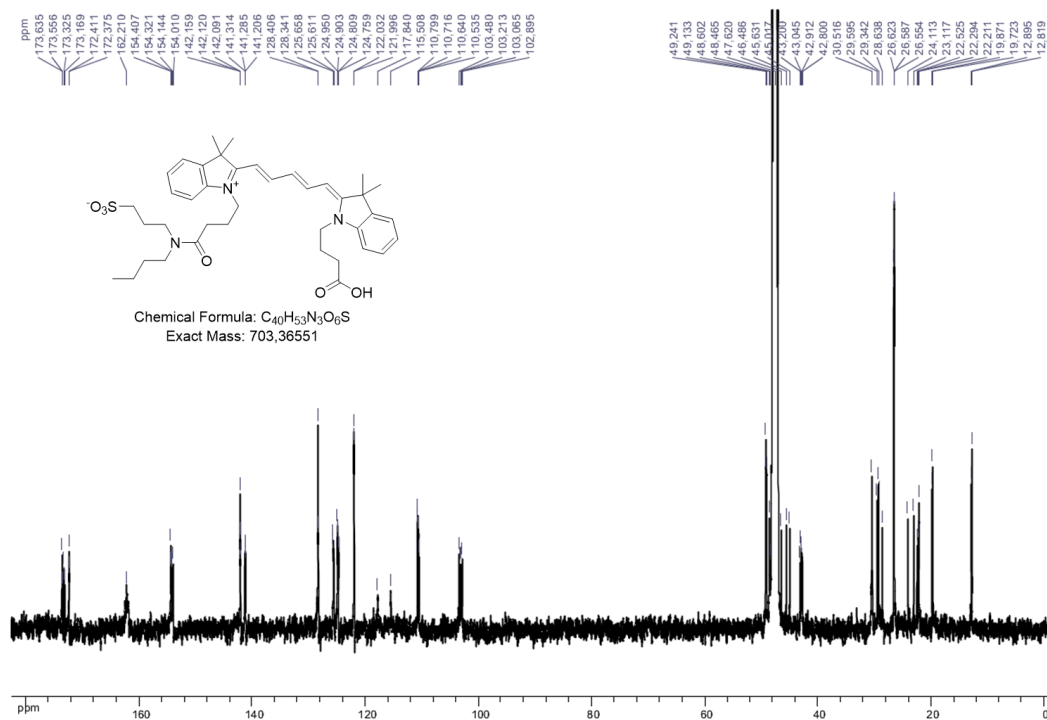


Figure S30.  $^{13}C$  NMR spectrum for Cy5 (III).

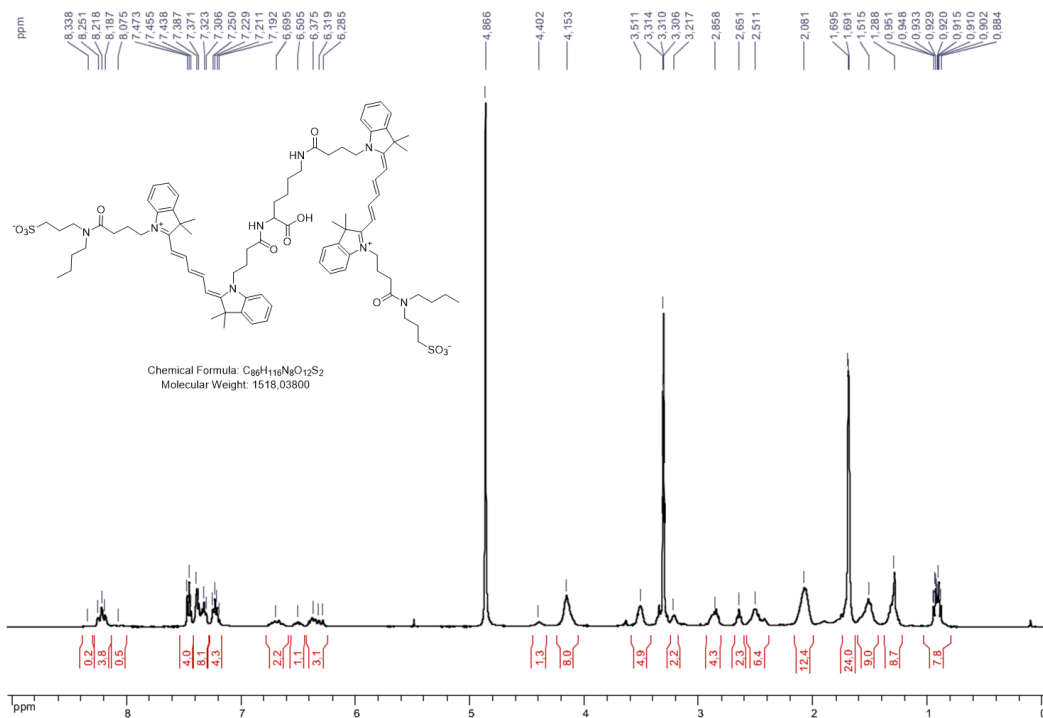


Figure S31.  $^1H$  NMR spectrum for BTF3.

## Supplementary video captions

**Movie S1.** Comparison of single-molecule images of Cy3, **BTF1**, and **BTF2** during 3D-PAINT imaging at the plasma membrane of live COS-7 cells. Single-molecule images were recorded continuously at 110 frames per second (fps) and played back at the standard 24 fps, thus slowed down by 4.6x compared to the real time. Scale bars: 2  $\mu\text{m}$ .

**Movie S2.** Sequential 3D-PAINT images of the **BTF2** labeled plasma membrane of the COS-7 cell shown in Figure 5. Blinking **BTF2** single-molecule images were continuously recorded for 320 s, and the super-resolved 3D positions of single molecules were grouped every 40 seconds to form a 3D-PAINT image. Color presents depth (Z) according to the color scale in Figure 5b. Scale bar: 2  $\mu\text{m}$ .

**Movie S3.** Comparison of single-molecule images of DiD and **BTF3** during 3D-PAINT imaging at the plasma membrane of live COS-7 cells. Single-molecule images were recorded continuously at 110 frames per second (fps) and played back at the standard 24 fps, thus slowed down by 4.6x compared to the real time. Scale bars: 2  $\mu\text{m}$ .

## References

(1) Texier, I.; Goutayer, M.; Da Silva, A.; Guyon, L.; Djaker, N.; Josserand, V.; Neumann, E.; Bibette, J.; Vinet, F. Cyanine-Loaded Lipid Nanoparticles for Improved in Vivo Fluorescence Imaging. *Journal of Biomedical Optics* **2009**, *14*, 054005.