

SUPPORTING INFORMATION FOR

Spectral Fine Tuning of Cyanine Dyes: Electron Donor-Acceptor Substituted Analogues of Thiazole Orange

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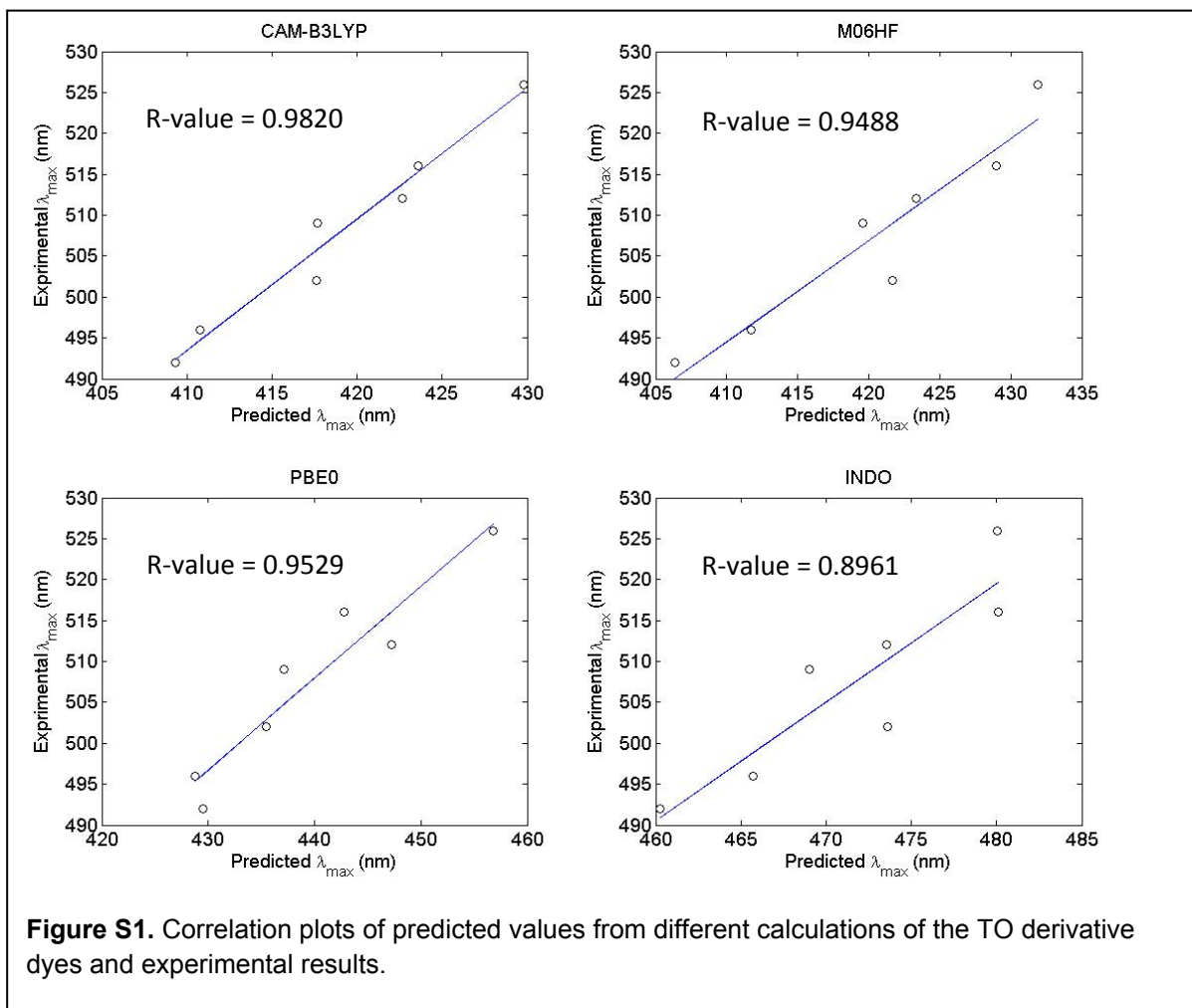
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1. Computations

In the computations reported in the main manuscript, the molecular geometries of the ground electronic state were optimized using DFT with the B3LYP functional¹ and 6-31G** basis set. Vertical excitations were then obtained from single-point TDDFT calculations on these geometries, using the CAM-B3LYP functional and 6-31G** basis². Here, we compare these results to those obtained using the M06HF and PBE0 functionals in single-point TDDFT computations on the same geometries within the same 6-31G** basis set³⁻⁶. Comparisons are also made with INDO theory. All DFT and TDDFT calculations were performed using GAUSSIAN09⁷ and include the effects of the methanol solvent through the Polarization Continuum Model (PCM)⁸. INDO calculations were performed using a direct singles configuration interaction method that includes excitations between all molecular orbitals⁹. Solvent was not included in the INDO computations.

The predicted absorption wavelengths (Table 1) are plotted against the experimental observations in Figure S1. The correlations are significantly better for CAM-B3LYP than for the other methods.

Method (nm)	TO	TO-1F	TO-p2F	TO-4F	TO-CF ₃	TO-OMe	MeO-TO-CF ₃
CAM-B3LYP	417.6	417.66	410.8	409.3	423.6	422.6	429.8
PBE0	435.5	437.2	428.8	429.5	442.8	447.2	456.8
M06HF	421.7	419.6	411.7	406.4	429	423.4	431.9
INDO	473.6	469	465.7	460.3	480.1	473.6	480
Experiment	502	516	512	509	492	496	526



2. Fluorescence Quantum Yield Data

Quantum yields were determined as described in the Materials and Methods section based on the raw data shown below in Figures S2-S4. All samples were excited at 470nm.

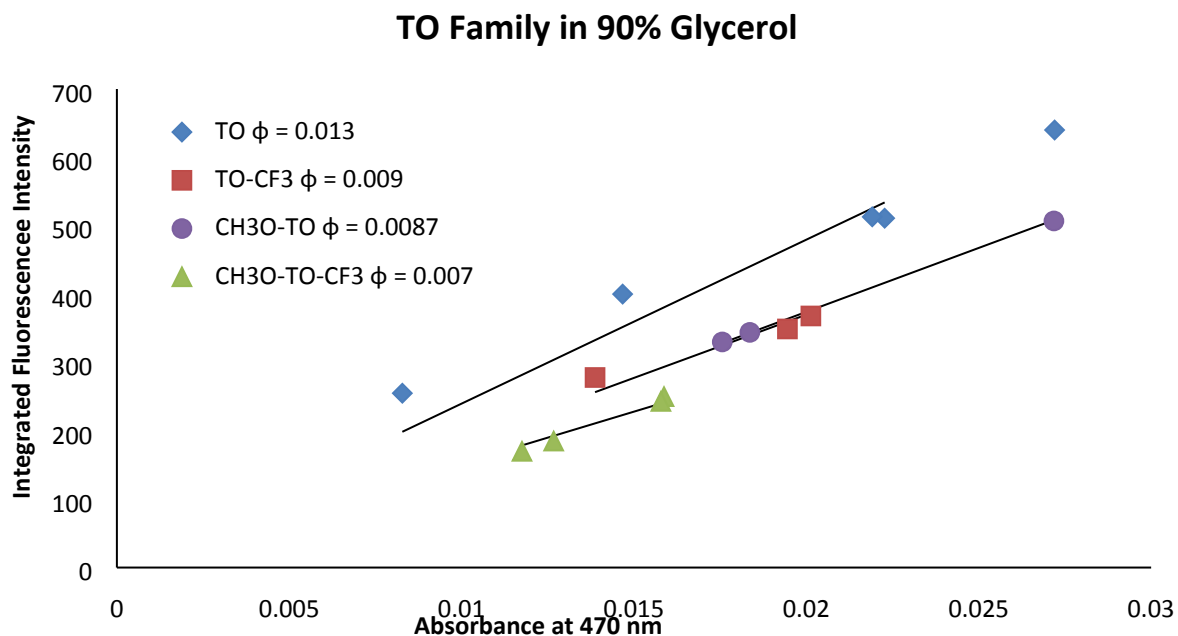


Figure S2. Linear plots for TO dye family in 90% glycerol. The gradient for each sample was used to calculate the quantum yield using equation [eq.1]

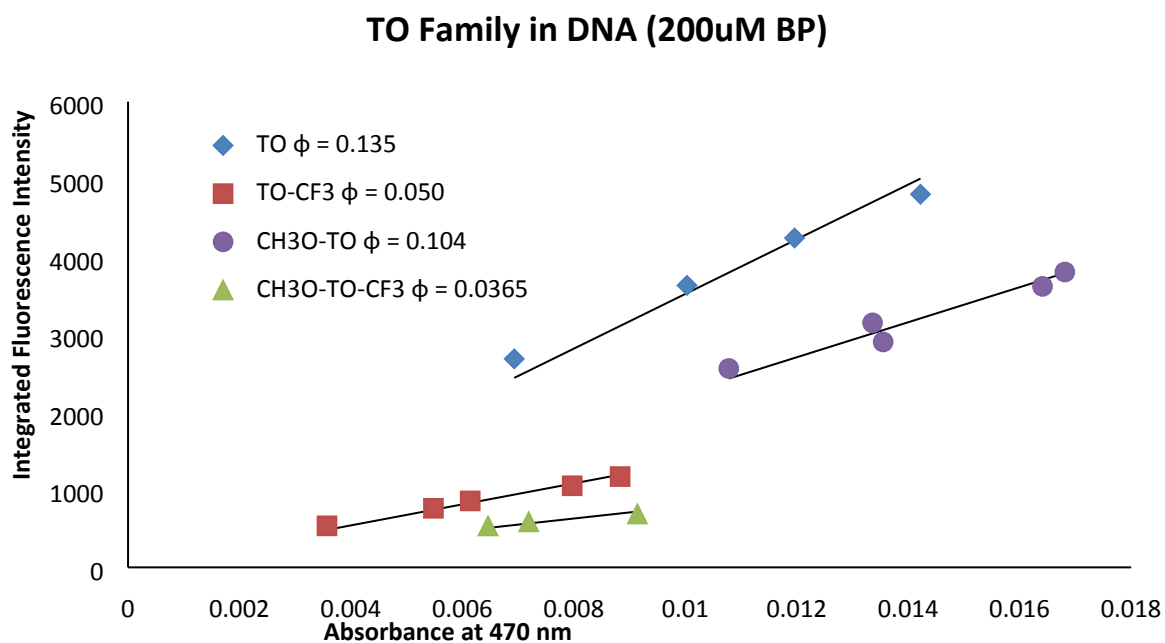


Figure S3. Linear plots for TO Dye family in CT DNA (200 μ M base pairs).

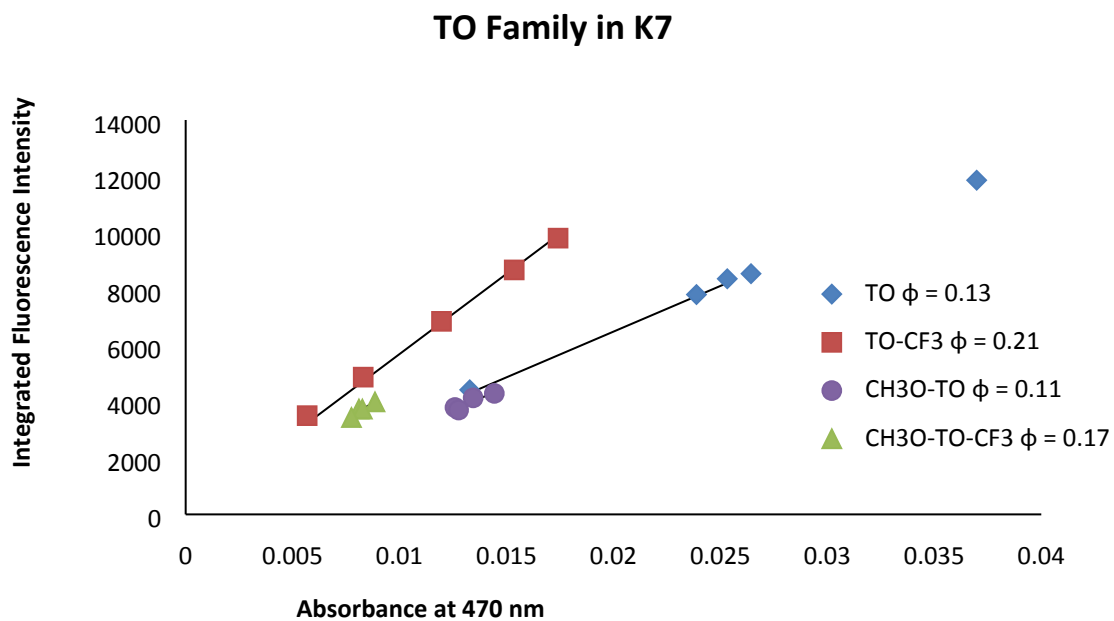


Figure S4. Linear plots for TO dye family in excess soluble protein K7. Concentrations of soluble protein K7 (TO, TO-CF₃) = 2 μ M; CH₃O-TO = 3 μ M; CH₃O-TO-CF₃ = 3.5 μ M.

3. Dye binding affinity to soluble FAPs

In order to determine the fluorogen binding affinity to the protein K7, an initial range finding assay was performed to estimate the concentration at which half of the dye or protein is bound. This concentration was used in the subsequent K_D assay as described in detail in the Materials and Methods section.

3.1 Range Finding Assay

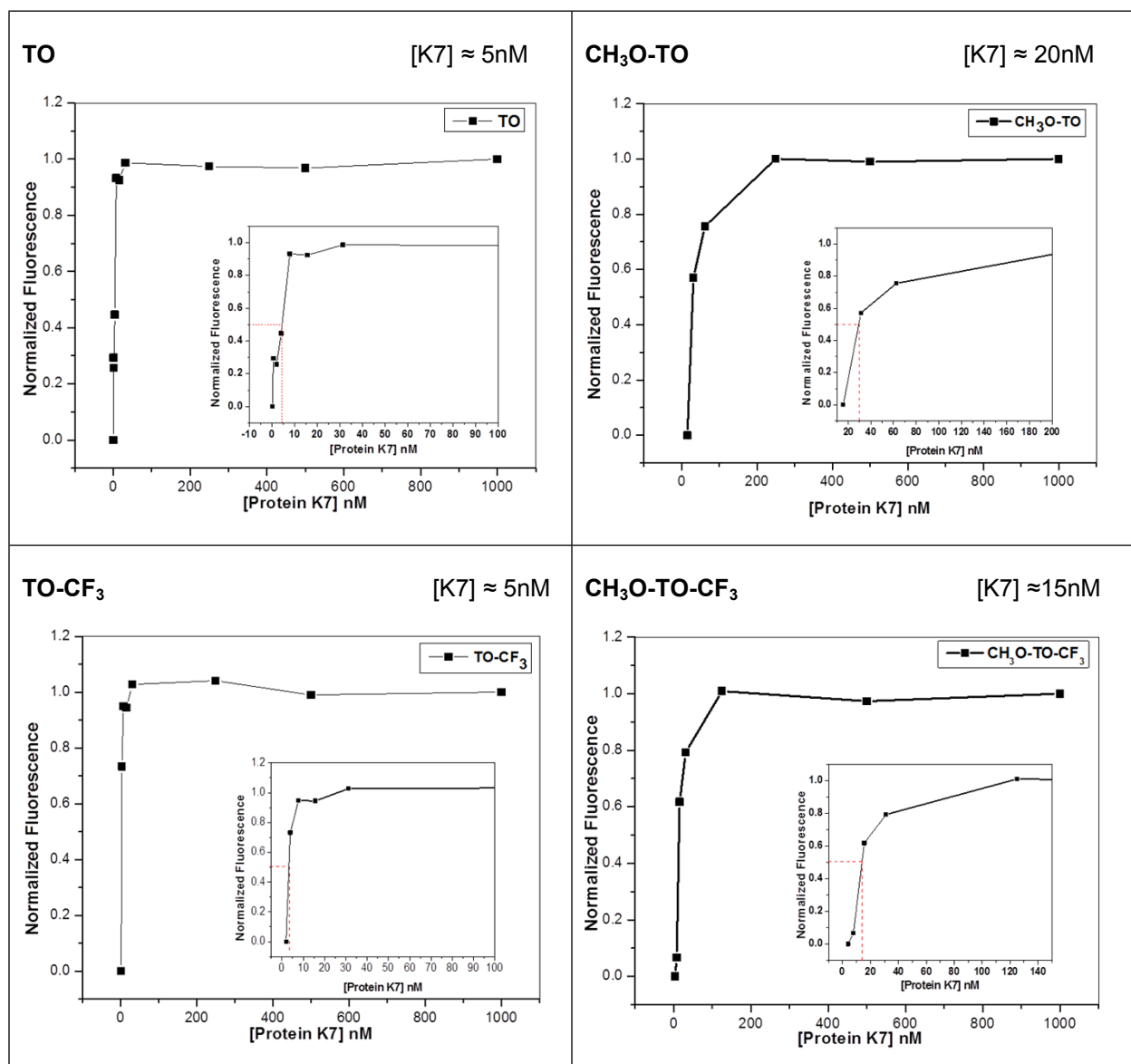


Figure S5. Range-finding data for TO dye family and protein K7. Inset shows estimated protein concentrations when approximately 50% is bound. Samples were excited at 490 nm and emission was monitored at the λ_{max} for each dye. (TO = 520 nm; CH₃O-TO = 536 nm; TO-CF₃ = 536 nm; CH₃O-TO-CF₃ = 552 nm).

3.2 K_D Determination for Dye and Soluble Protein K7

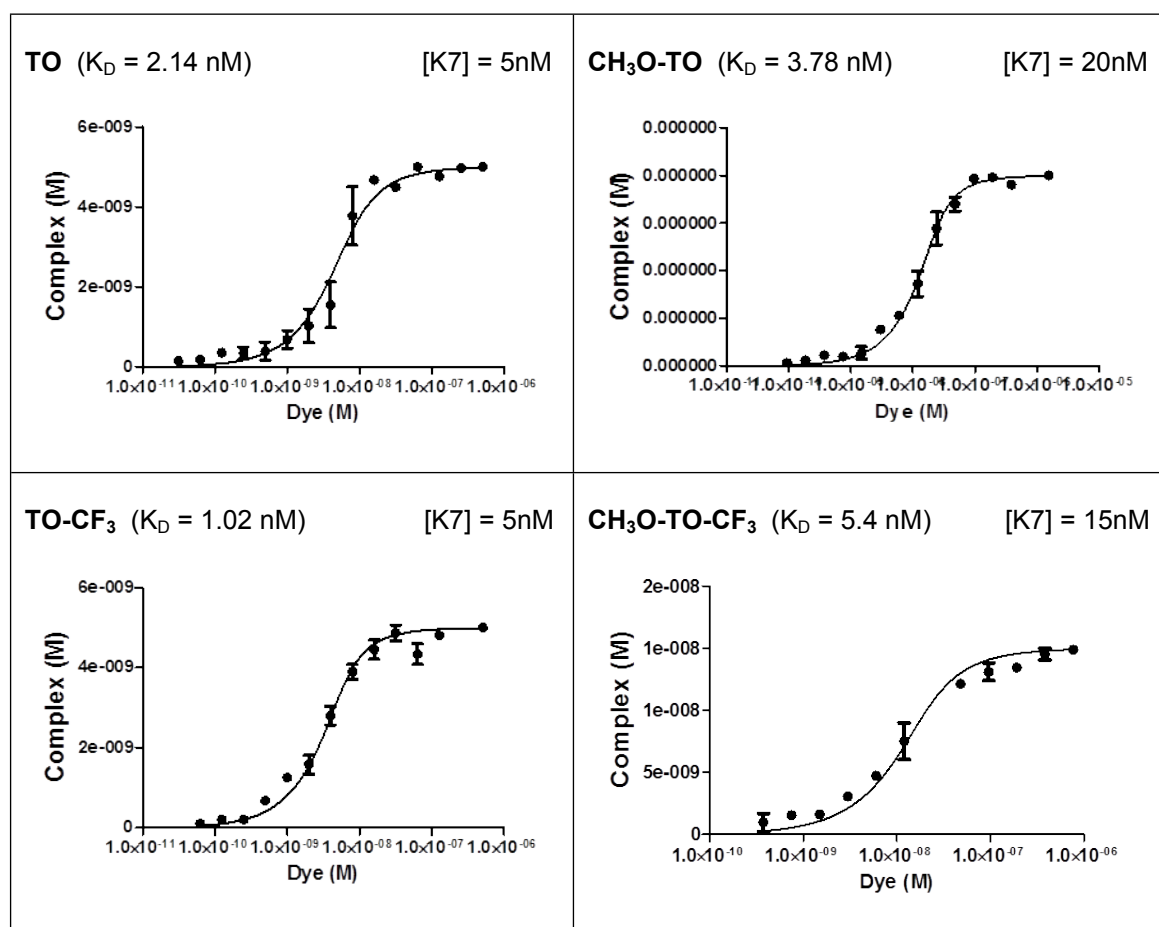


Figure S6. Fluorescence titrations of TO dye into soluble K7 (protein concentrations were determined by range finding assay see **Figure S5**). Samples were excited at 490 nm and emission was monitored at λ_{max} for each dye. (TO = 520 nm; CH₃O-TO = 536 nm; TO-CF₃ = 536 nm; CH₃O-TO-CF₃ = 552 nm)

4. NMR Spectra:

4.1 CH₃O-TO-CF₃

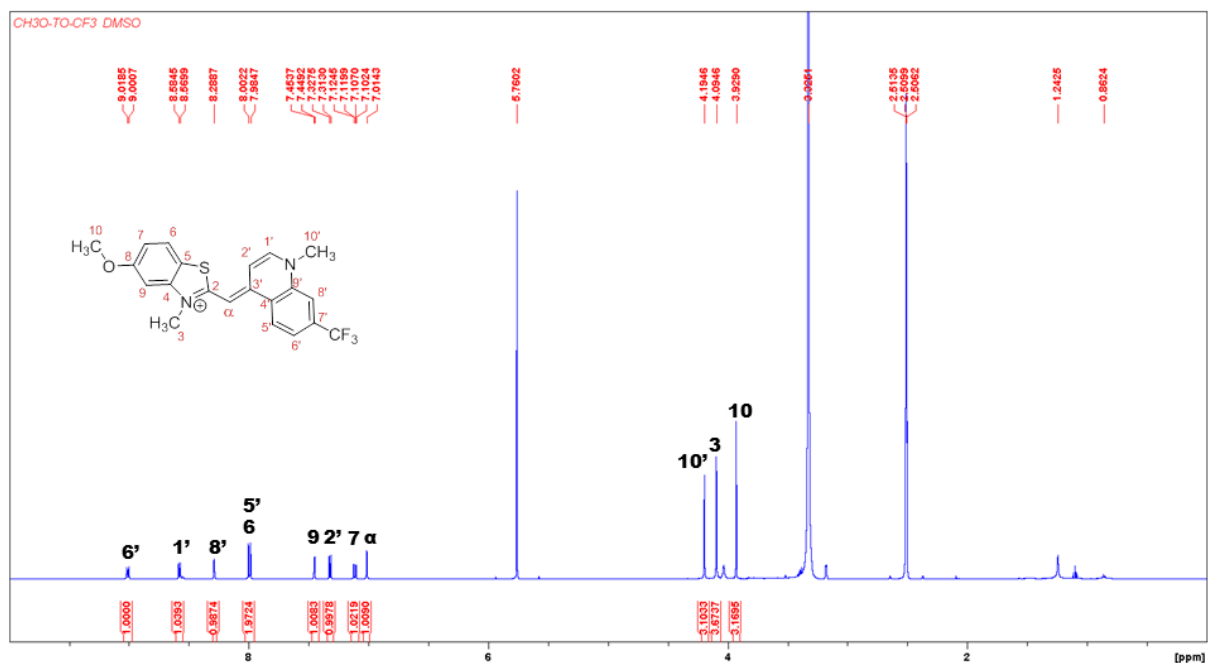


Figure S7. ¹H NMR spectrum of dye CH₃O-TO-CF₃ (500 MHz, (CD₃)₂SO)

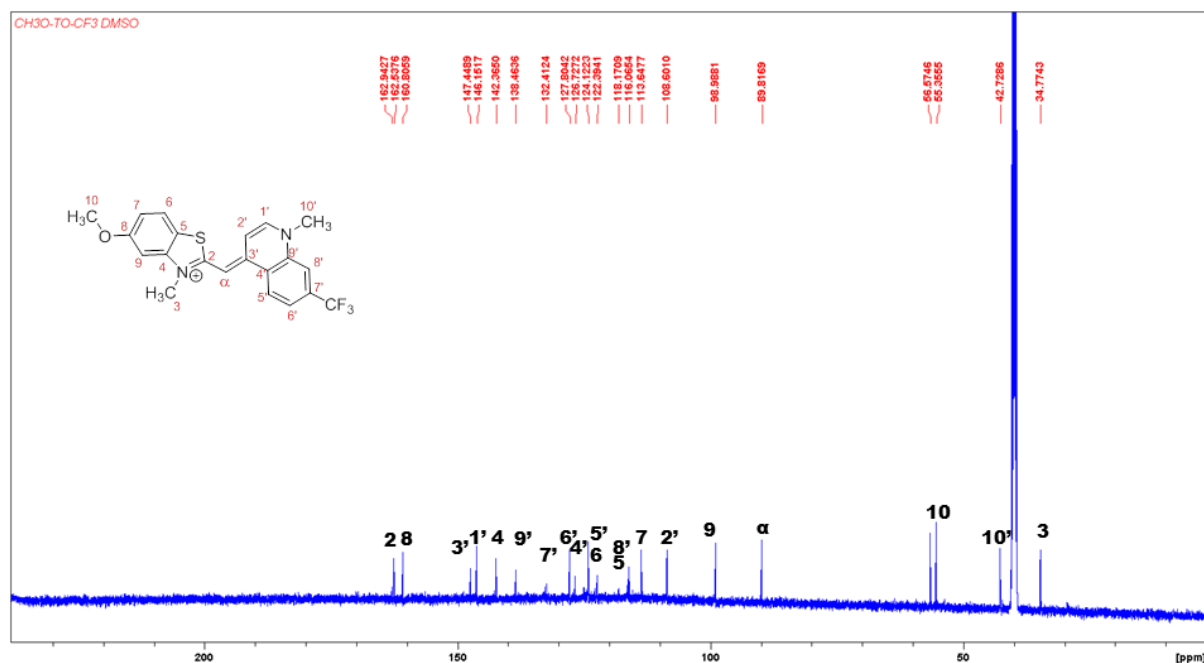


Figure S8. ¹³C NMR spectrum of dye CH₃O-TO-CF₃ (75 MHz, (CD₃)₂SO)

4.2 CH₃O-TO

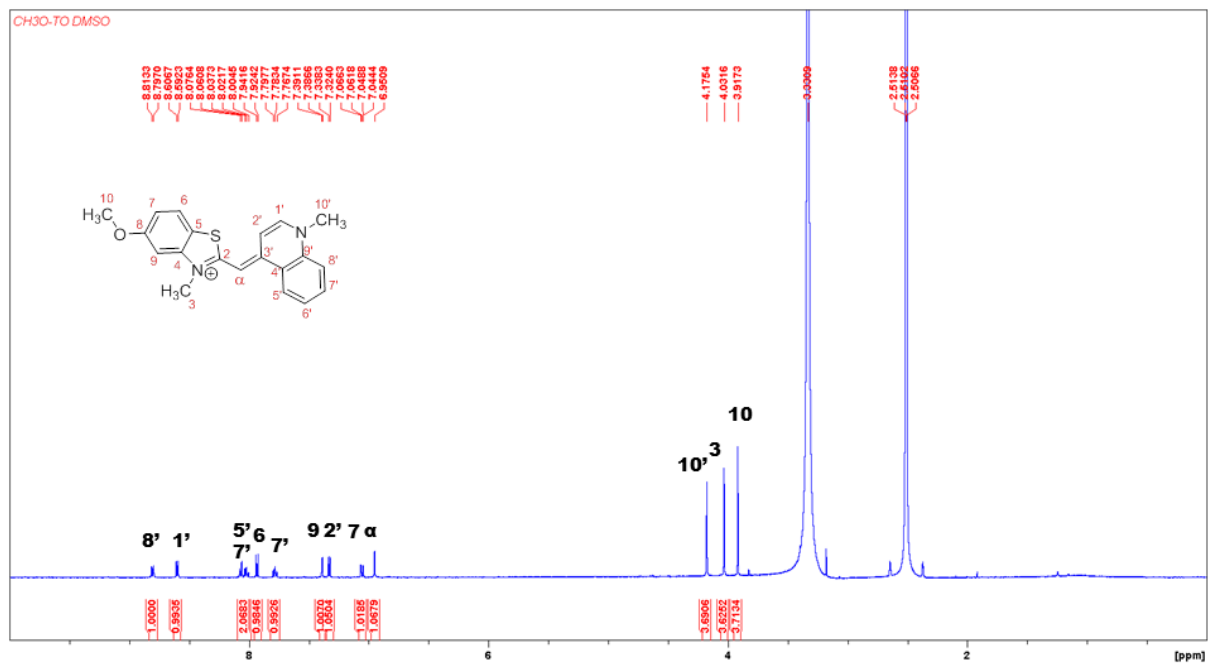


Figure S9. ¹H NMR spectrum of dye CH₃O-TO (500 MHz, (CD₃)₂SO)

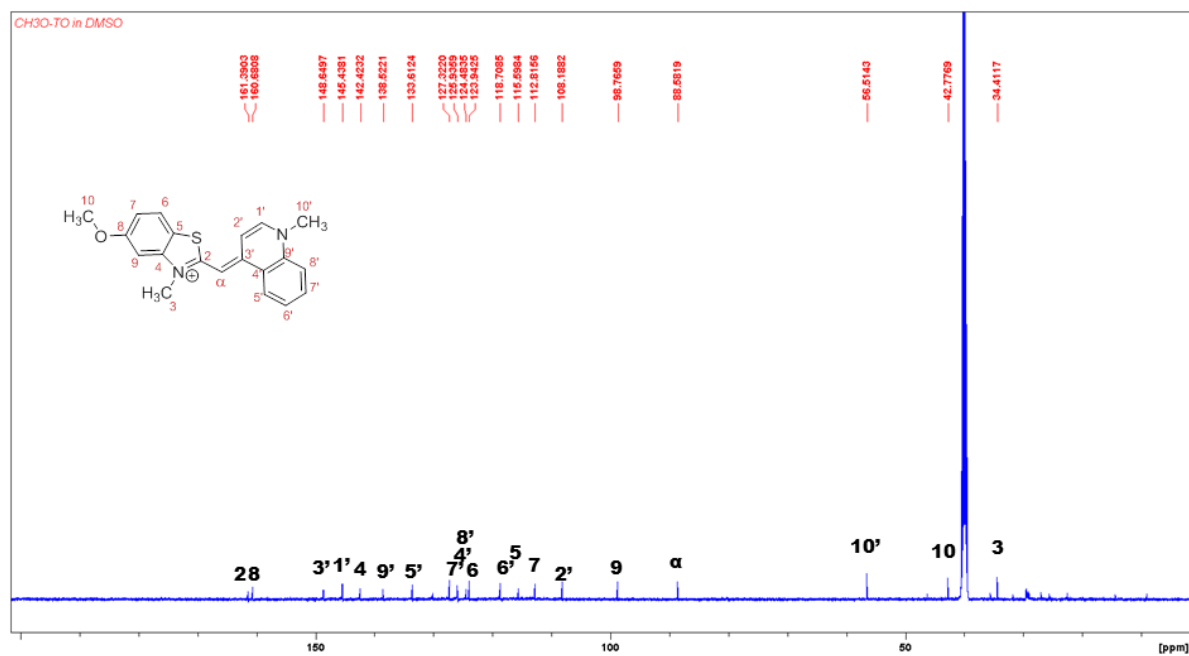


Figure S10. ¹³C NMR spectrum of dye CH₃O-TO (75 MHz, (CD₃)₂SO)

4.3 Q-CF₃

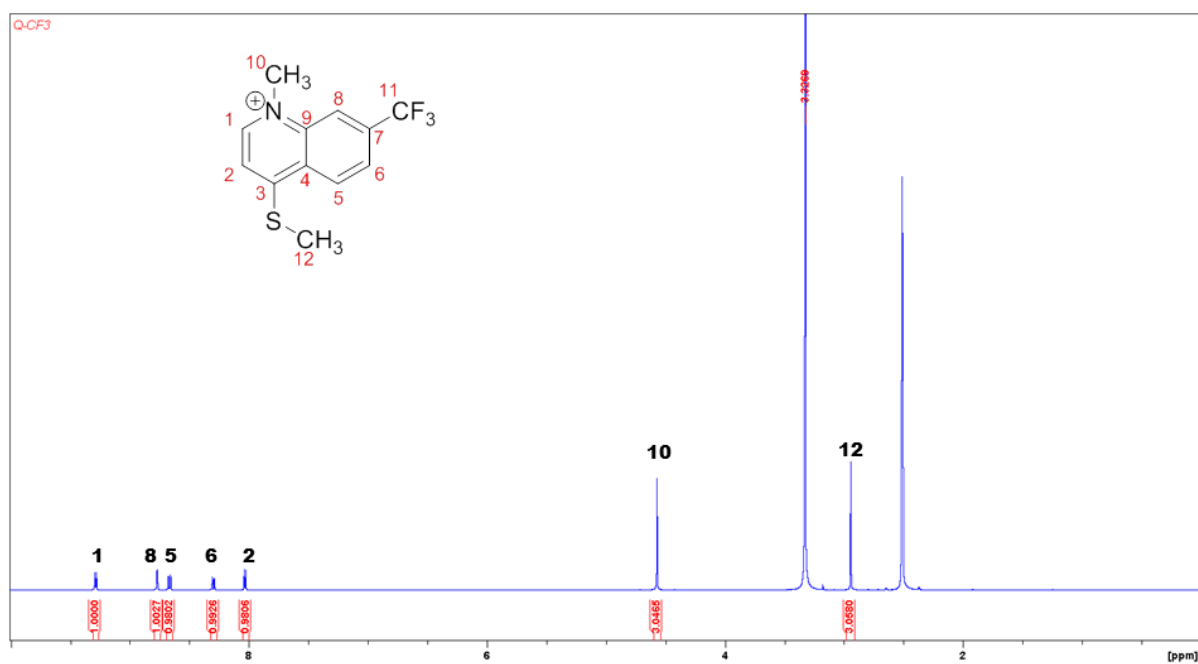


Figure S11. ¹H NMR spectrum of hemi-dye Q-CF₃ (500 MHz, (CD₃)₂SO)

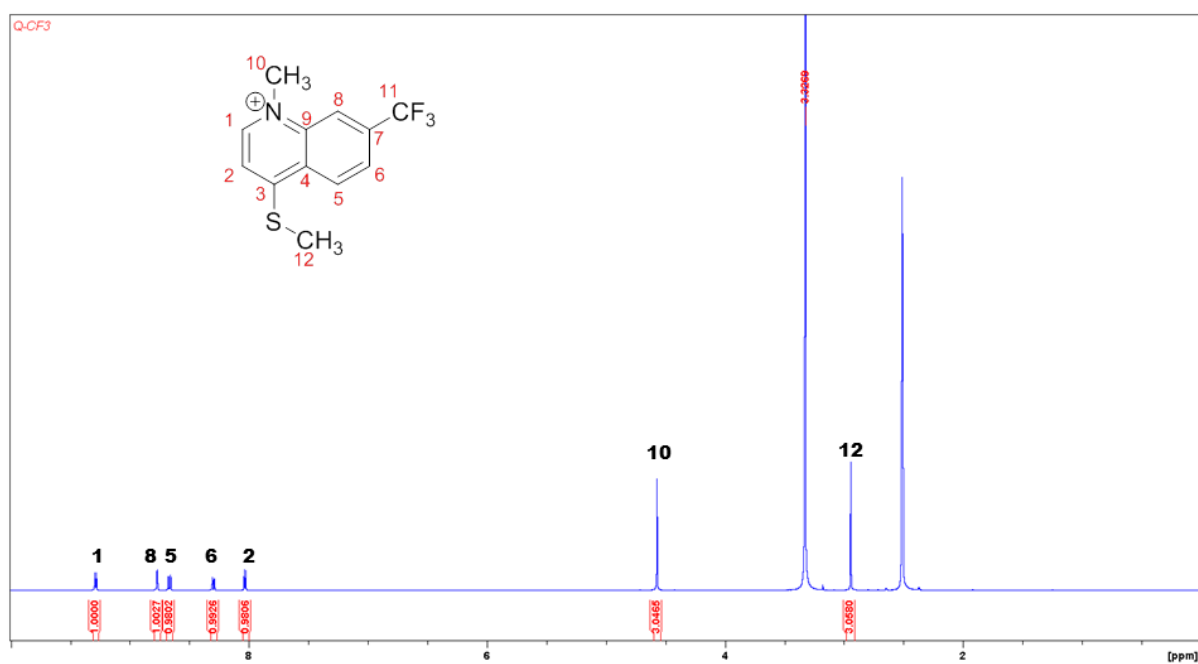


Figure S12. ¹³C NMR spectrum of hemi-dye Q-CF₃ (75 MHz, (CD₃)₂SO)

4.4 CH₃O-BT

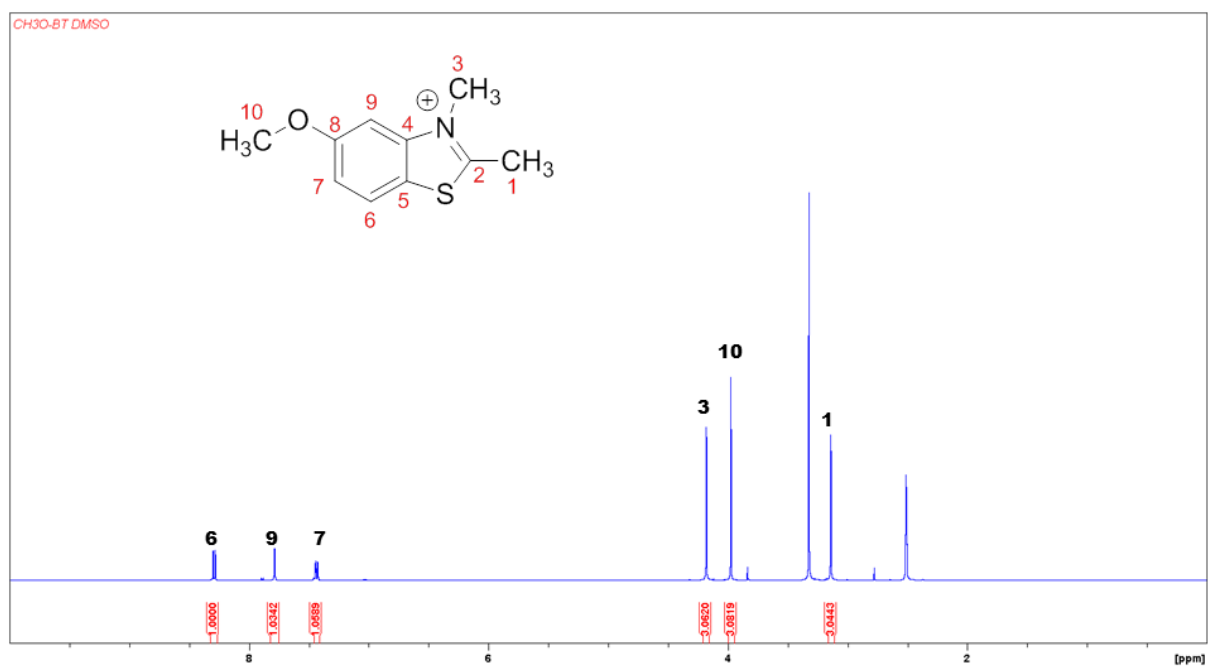


Figure S13. ¹H NMR spectrum of hemi-dye CH₃O-BT (500 MHz, (CD₃)₂SO)

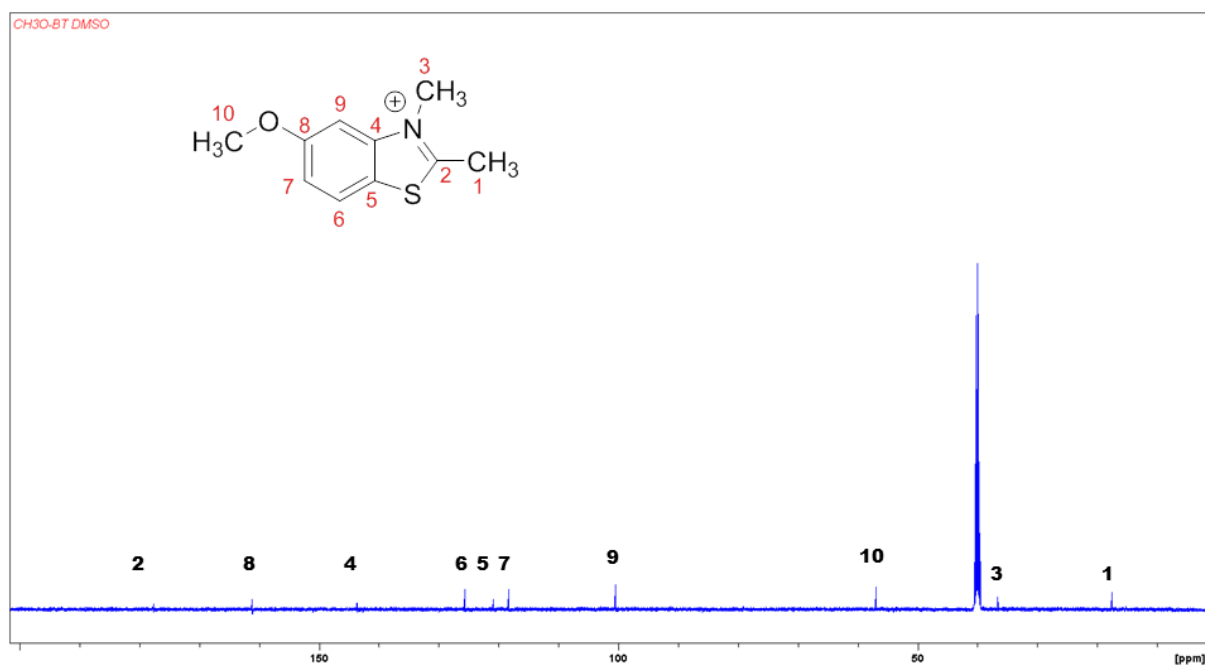


Figure S14. ¹³C NMR spectrum of hemi-dye CH₃O-BT (75 MHz, (CD₃)₂SO)

5 LCQ- ESI –MS Spectra

5.1 CH₃O-BT

LR001 #10-50 RT: 0.30-1.57 AV: 41 NL: 4.49E7
T: + p Full ms [150.00-2000.00]

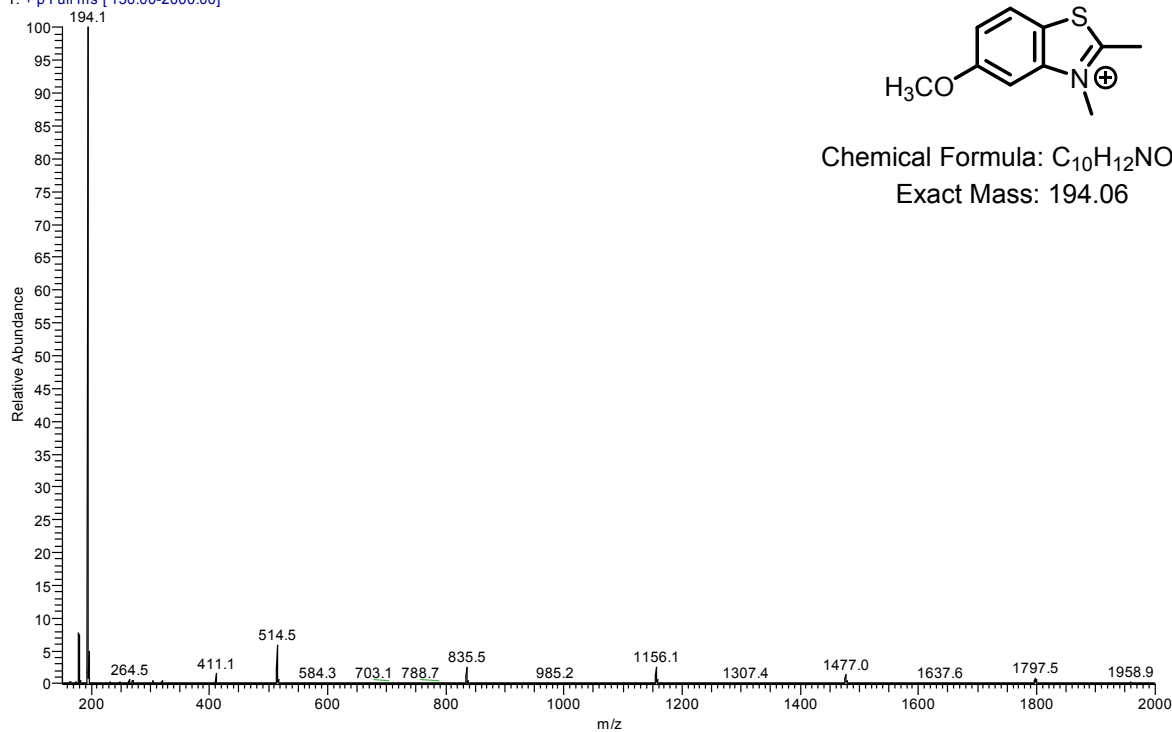


Figure S15. ESI- MS of hemi-dye CH₃O-BT

5.2 Q-CF₃

LR10cry_101208124228 #8 RT: 1.27 AV: 1 NL: 3.16E5
T: + p ms [150.00-2000.00]

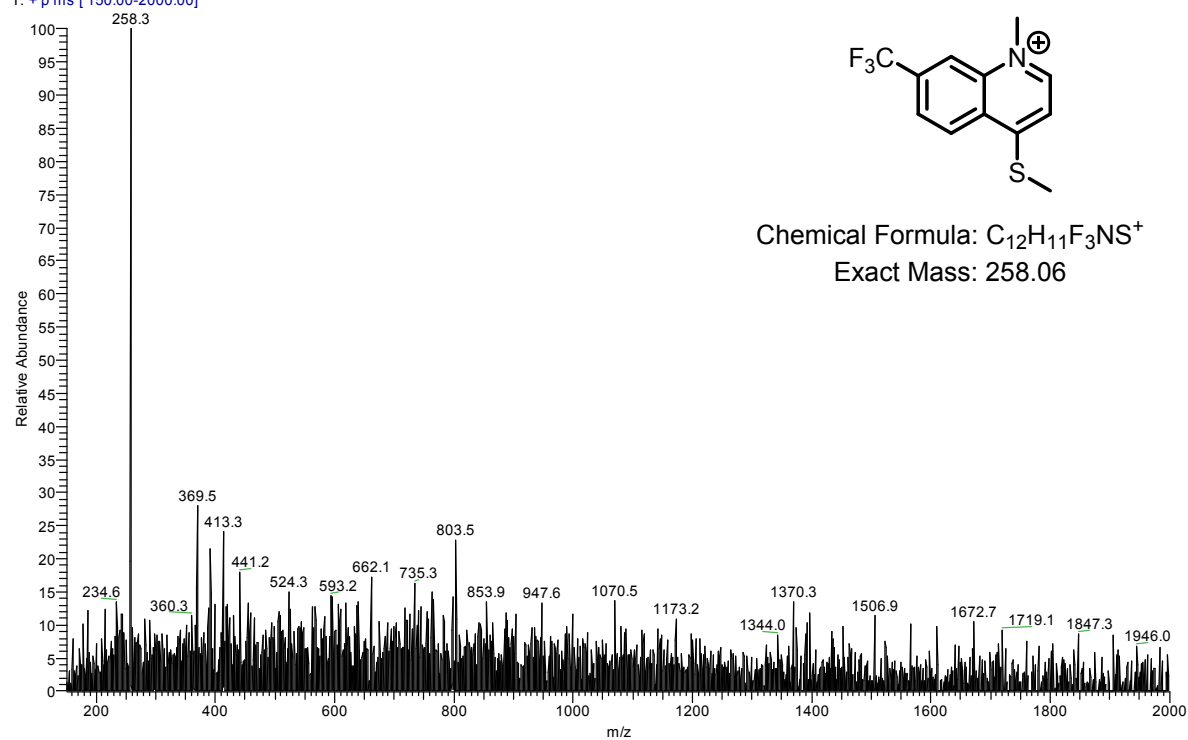


Figure S16. ESI- MS of hemi-dye Q-CF₃

5.3 CH₃O-TO

LR16 #41-78 RT: 1.30-2.47 AV: 38 NL: 5.27E7
T: + p Full ms [150.00-2000.00]

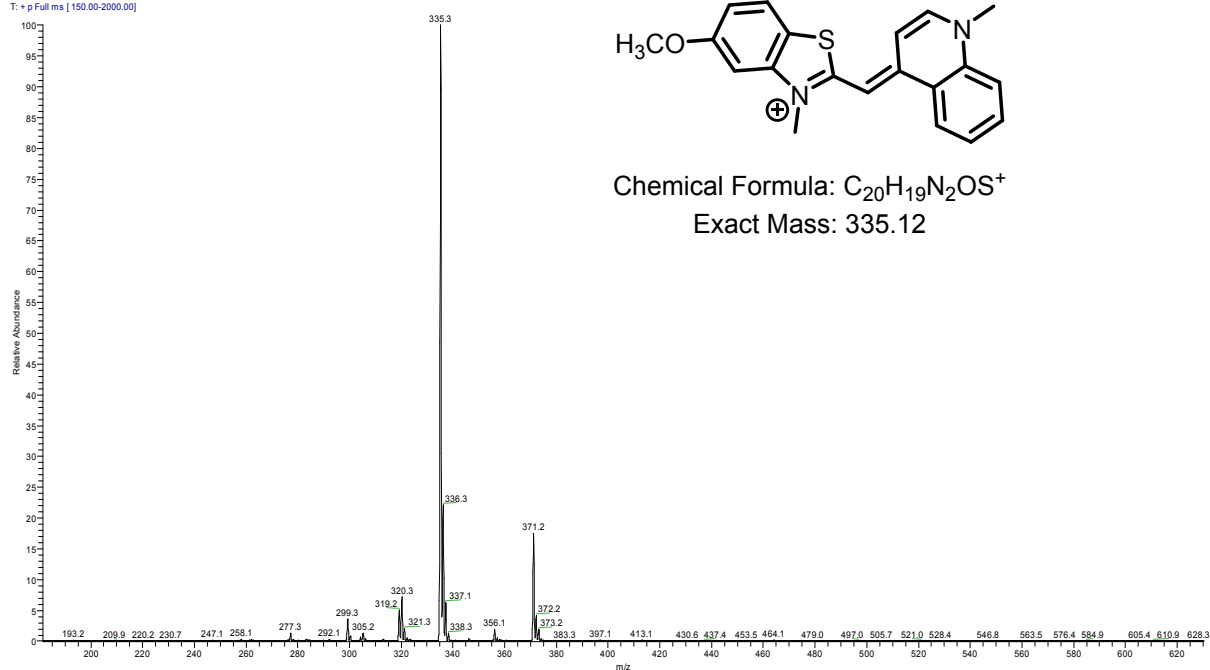


Figure S17. ESI- MS of dye CH₃O-TO

5.4 CH₃O-TO-CF₃

LR13 #36-87 RT: 1.12-2.73 Av: 52 NL: 1.05E7
T: + p Full ms [150.00-2000.00]

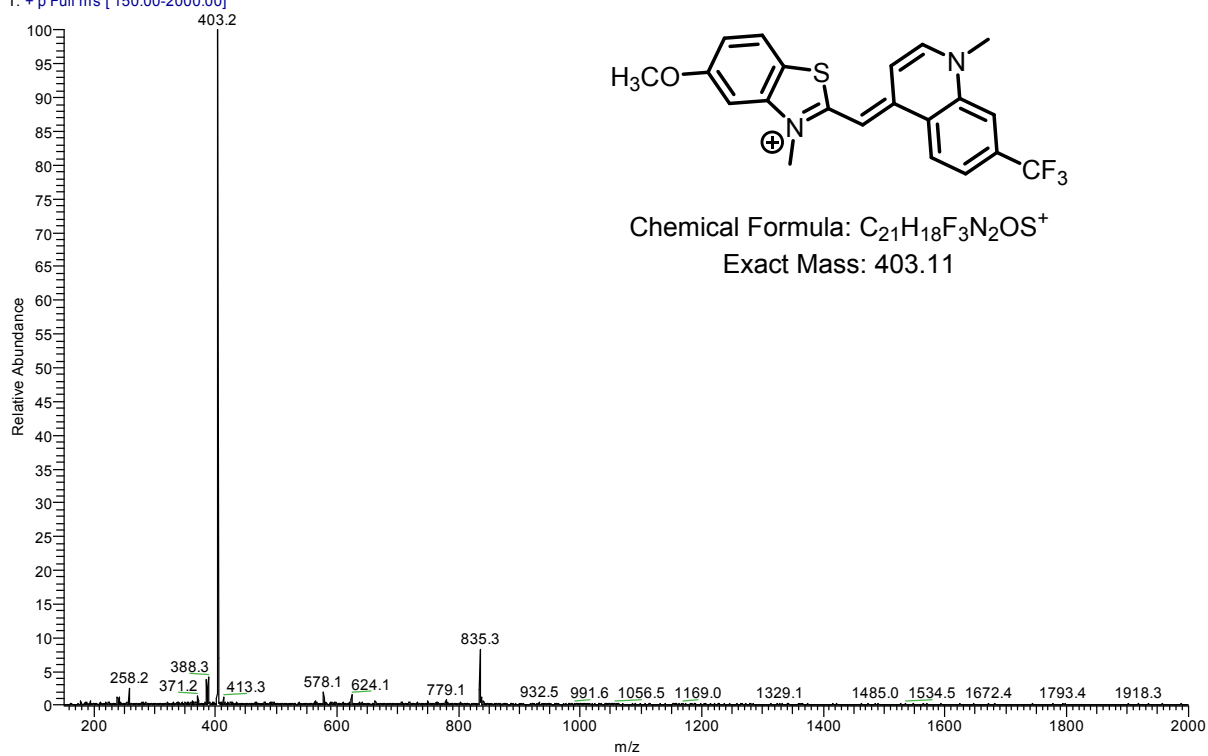


Figure S18. ESI- MS of dye CH₃O-TO-CF₃

References

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