SUPPORTING INFORMATION

Environment Sensing Merocyanine Dyes for Live Cell Imaging Applications

Christopher J. MacNevin†‡[1], Dmitriy Gremyachinskiy†[1], Chia-Wen Hsu†, Li Li†, Marie Rougie†, Tamara T. Davis†, and Klaus M. Hahn†*

†*Department of Pharmacology, 120 Mason Farm Road, University of North Carolina, Chapel Hill, 27599.* ‡*Center for Integrative Chemical Biology and Drug Discovery, 120 Mason Farm Road, University of North Carolina, Chapel Hill, 27599.*

[1]These authors contributed equally to this work.

Protein expression: The Cdc42-binding domain (CBD), derived from Wiskott-Aldrich syndrome protein (WASP, residues 201-320), was mutated to include a single cysteine (F271C) for dye attachment and fused at its C-terminus with maltose-binding protein (MBP) through a GSGSGS linker. The CBD-MBP fragment was subcloned into a pET23b plasmid to generate a C-terminal 6xHis fusion protein. The CBD-Cerulean fusion protein was prepared with C49S mutation in Cerulean to eliminate off-target dye labeling and subcloned into a pET23b plasmid to generate a C-terminal 6xHis fusion protein. The constitutively active mutant, Cdc42 Q61L, was subcloned into the pQE-80L plasmid to generate an N-terminal 6xHis fusion protein. The resulting constructs were transformed into the BL21(DE3) strain from *E. Coli* (Stratagene) and the bacteria was cultured at 37 °C, 245 rpm (New Brunswick Scientific, Innova 4000) in Leuria-Bertani medium in the presence of 100 μg/mL carbenicillin until OD600 reached 0.8. Protein expression was conducted at 32 °C for 2 hours after addition of 40 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). The bacteria pellet was collected by centrifugation (Beckman, model J-68) at 4000 rpm for 20 min, 4 °C, and stored at -80 °C prior to purification.

Protein purification: For lysis, the bacterial pellets were resuspended in 30 mL of the lysis buffer (50 mM NaH_2PO_4, pH 7.0, 300 mM NaCl) and disrupted by sonication (Branson Digital Sonifier). The supernatant was collected by centrifugation (Sorvall, model T21) at 6000 rpm for 15 min, 4 °C, followed by incubation with Talon Metal Affinity Resin (Clontech) on a nutator mixer (BD Clay Adams, model 1105) for 1 hour at 4 °C. The protein-bound resin was collected after centrifugation (Eppendorf, model 5810) at 1600 rpm for 2 min, 4 °C, washed with the lysis buffer, and loaded onto a polypropylene column (BioRad). The immobilized proteins were washed with 20 mL of the washing buffer (50 mM NaH_2PO_4, pH 7.5) and eluted with 1~2 mL elution buffer (300 mM imidazole, pH 7.0). To fully reduce the F271C residue of CBD-MBP, 1μl of β-mercaptoethanol (BioRad) was added to the eluate and the resulting protein solution was concentrated to 200 μl by centrifuge (VivaSpin 20 MWCO 10K; Eppendorf, model 5810). The excess imidazole and β-mercaptoethanol were removed by buffer exchange on a dextran desalting column (Thermo Scientific Pierce, 5K MWCO) using the protein labeling buffer (50 mM NaH_2PO_4, pH 7.5). The protein aliquots were stored at -80 °C.

Cell culture and microinjection: NIH 3T3 mouse embryonic fibroblasts (MEF) were maintained in 10% CO_2 at 37^{o}C in Dulbecco's modified Eagle's medium (DMEM, Cellgro) with 10% fetal bovine serum (HyClone, Thermo Scientific) and 2 mM GlutaMax (Gibco, Life

Technologies). The cells were plated on coverslips coated with fibronectin (Sigma-Aldrich) overnight. Micropipettes were made fresh with a flaming/brown micropipette puller (Sutter Instrument Co., Model P-97) and thin-walled glass capillary tubes (World Precision Instruments, 1.2 O.D./ 0.9 I.D., TW120F-4). Cells were microinjected on an inverted microscope (Ziess Axiovert S100 TV) with a 20X objective (LD Achrostigmat, 0.30 N.A., Ph1), followed by recovery at 37 °C in the cell incubator in Ham's F-12K medium without phenol red (Gibco) with 5% fetal bovine serum, 15 mM HEPES, and 2mM GlutaMax for at least 30 min prior to imaging.

Image acquisition: Live cell imaging was carried out on an Olympus IX81 microscope with UPLFLN 40X oil objective (NA 1.3) and mercury lamp excitation (103W HBO bulb). Filters used for Cerulean were 430/24 excitation and 470/24 emission. Excitation was through a ND1.5 (3% transmission) neutral density filter, with 200 ms exposure. For **mero61**, filters were 545/50 excitation and 630/45 emission. Excitation was through a ND2.0 (1%T) filter with 200 ms exposure. Imaging of **mero62** and **mero87** was carried out using 590/30 excitation, 630/40 emission, ND2.0 (1%T), and 200 ms exposure. Images were acquired with a Coolsnap ES2 camera (Photometrics) with a Sony 6.45 x 6.45 μM/pixel chip using 2 x 2 binning. All image acquisition, processing, and analysis was carried out with Metamorph software.

Table S1: Polarity characteristics of solvents selected for brightness screening.

solvent	solubility parameter dispersion	polar	H-bonding
MeOH	14.7	12.3	22.3
BuOH	16.0	5.7	15.8
DMSO	18.4	16.4	10.2
1,4-dioxane	17.5	1.8	9.0

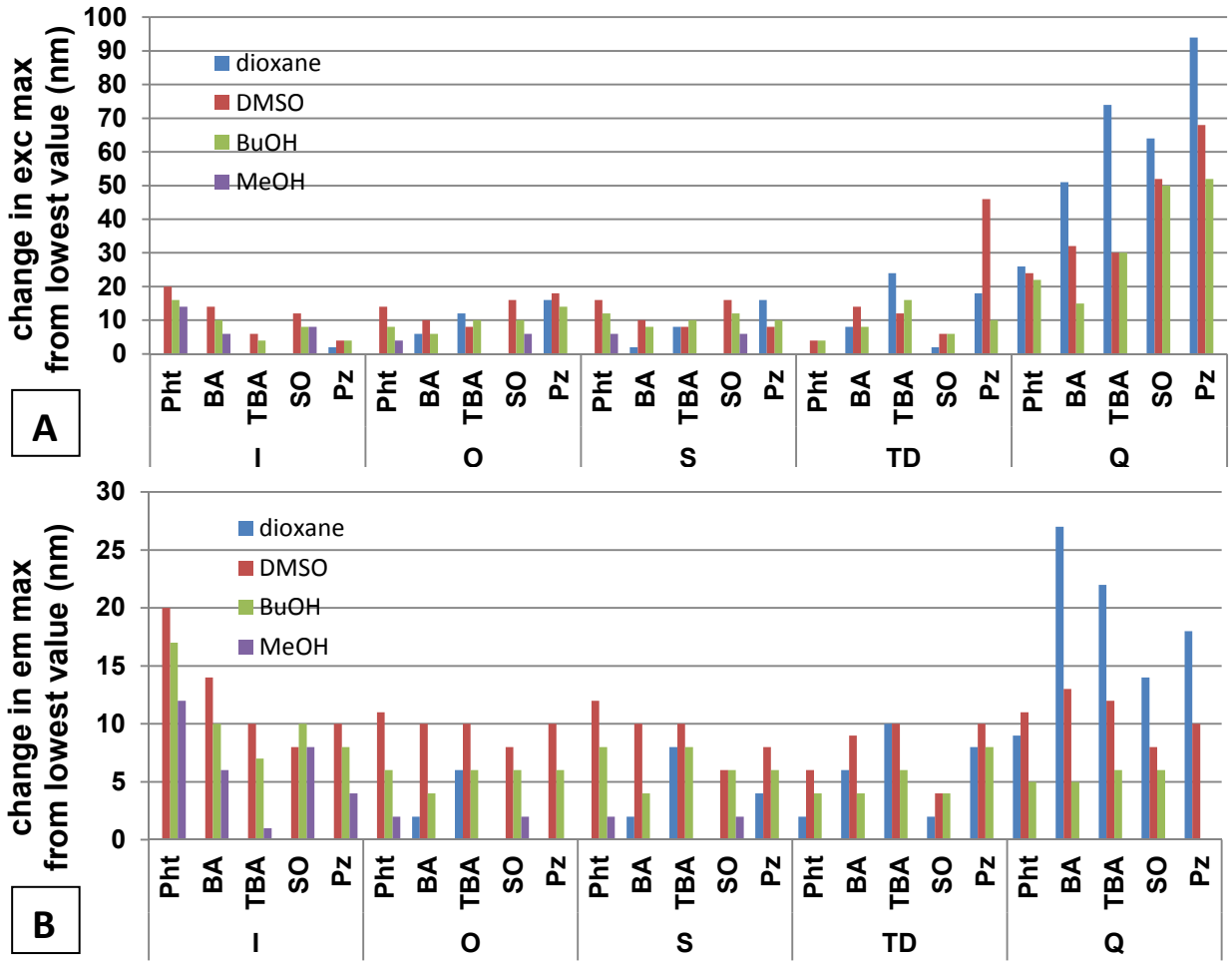


Figure S1: Change in excitation maximum (A) and emission maximum (B) from smallest value for set of four solvents.

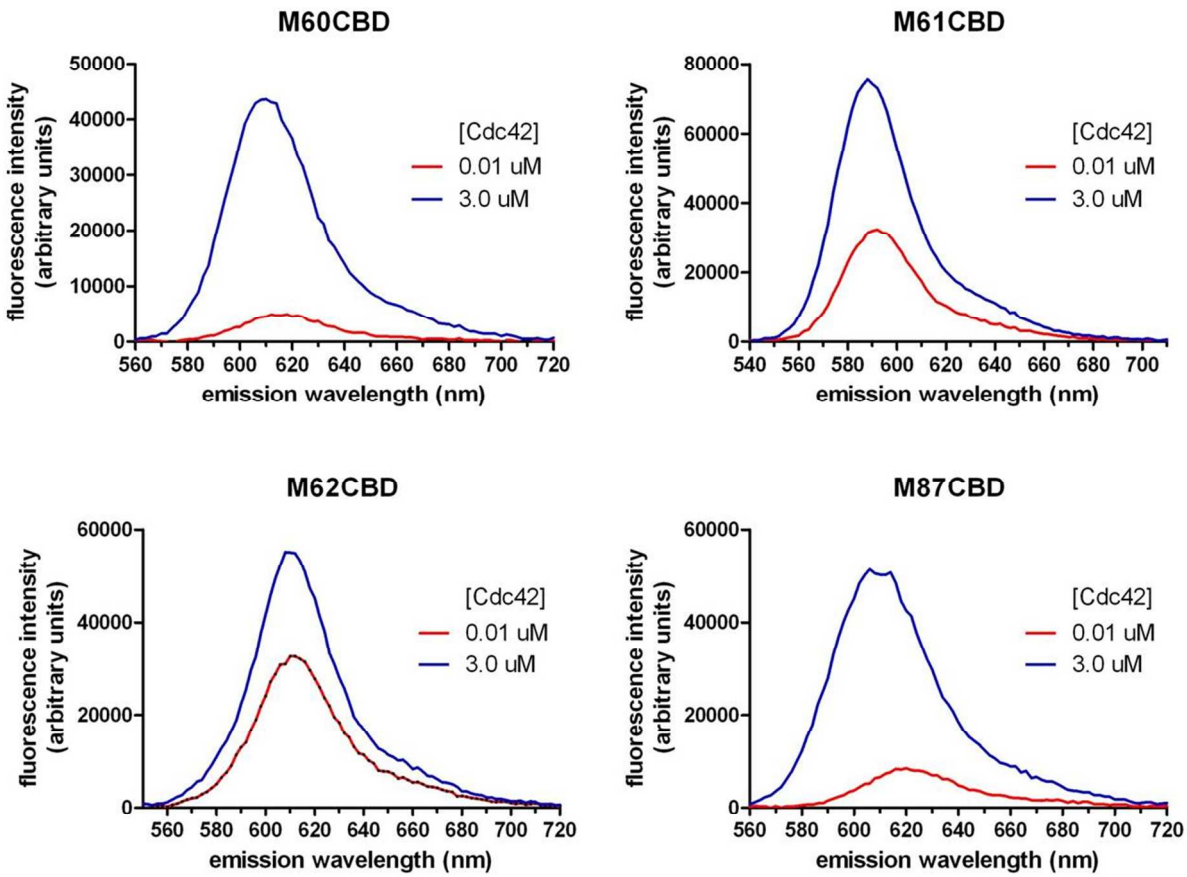
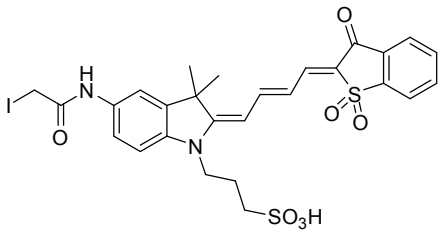


Figure S2: Complete emission spectra for binding of merocyanine-labeled CBD-MBP fragment at low (0.01 uM) and high (3.0 uM) concentrations of Cdc42.



Solvent	X max	M max	ε	QY	ε x QY
Water	599	630	143000	0.004	600
MeOH	601	634	138000	0.01	1400
BuOH	607	639	150000	0.06	9000

Figure S3: Structure and photophysical properties of **mero221**.

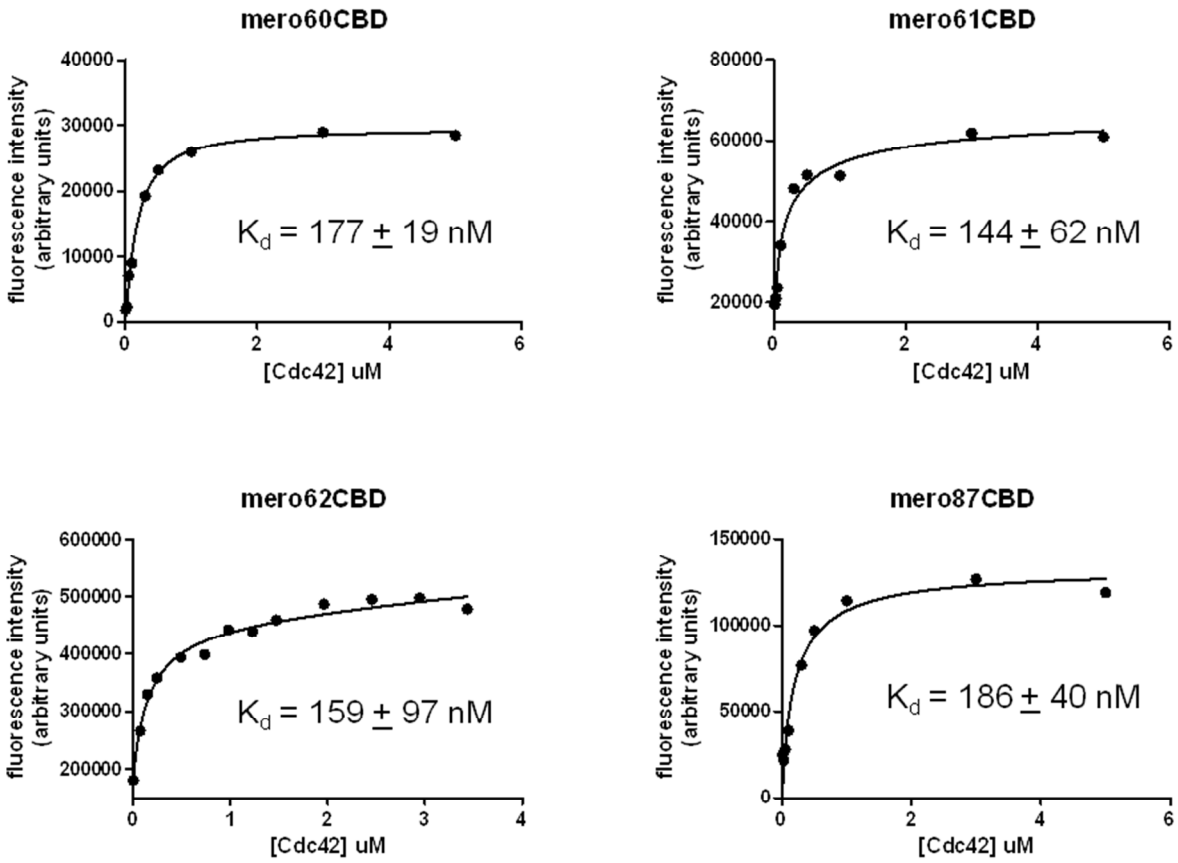


Figure S4: Dissociation constant (K_d) values calculated for meroCBD binding with Cdc42 in 50 mM NaH_2PO_4, 150 mM NaCl buffer at pH 7.5.

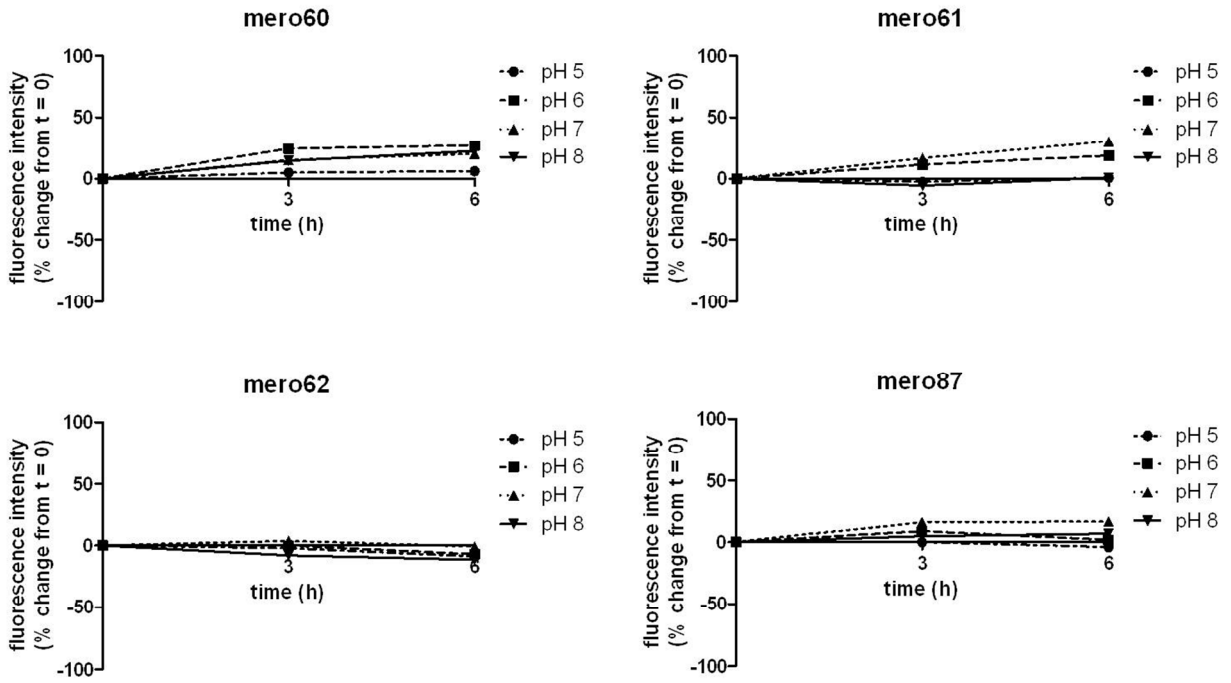


Figure S5: Fluorescence emission intensity of the four conjugatable merocyanines over time at different pH. Each dye was prepared as a 1.0 μM solution in aqueous sodium phosphate/citric acid buffer.

Compound characterization data:

Quaternized donor heterocycles:

O: Yield = 51%. ^{1}H NMR (400 MHz, DMSO) δ 8.17 – 8.08 (m, 2H), 7.83 – 7.74 (m, 2H), 4.08 (s, 3H), 3.07 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 169.0, 147.2, 130.3, 128.6, 127.6, 114.4, 112.8, 32.6, 13.4.

S: Yield = 93%. ^{1}H NMR (400 MHz, DMSO) δ 8.48 – 8.42 (m, 1H), 8.29 (d, *J* = 8.4 Hz, 1H), 7.89 (ddd, *J* = 8.5, 7.3, 1.3 Hz, 1H), 7.80 (ddd, *J* = 8.2, 7.3, 1.0 Hz, 1H), 4.21 (s, 3H), 3.18 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 177.2, 141.5, 129.2, 128.6, 128.0, 124.5, 116.7, 36.3, 17.2.

TD: Yield = 98%. ^{1}H NMR (400 MHz, DMSO) δ 4.17 (s, 3H), 3.02 (s, 3H), 2.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 174.4, 166.7, 41.4, 39.5, 15.9, 14.9.

Q: Yield = 95%. ^{1}H NMR (400 MHz, DMSO) δ 9.37 (d, *J* = 6.1 Hz, 1H), 8.58 – 8.43 (m, 2H), 8.27 (ddd, *J* = 8.7, 7.0, 1.4 Hz, 1H), 8.12 – 8.00 (m, 2H), 4.58 (s, 3H), 3.01 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 158.1, 148.9, 137.6, 134.9, 129.6, 128.4, 126.7, 122.4, 119.5, 45.0, 39.5, 19.6.

Acceptor enol ethers:

BA: Yield = 61%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 – 7.97 (m, 1H), 7.58 – 7.38 (m, 2H), 3.98 (m, 4H), 3.92 (s, 3H), 1.22 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 162.4, 162.2, 157.5, 151.0, 110.9, 105.8, 58.7, 37.2, 36.6, 13.6, 13.5.

SO: Yield = 73%. Spectra matched those previously reported.[S1] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.6 Hz, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.86 (t, *J* = 7.6 Hz, 1H), 7.78 (t, *J* = 7.6 Hz, 1H), 7.67 (d, *J* = 12.8 Hz, 1H), 7.45 (d, *J* = 12.0 Hz, 1H), 6.45 (t, *J* = 12.4 Hz, 1H), 3.96 (s, 1H).

TBA: Yield = 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 11.8 Hz, 1H), 7.60 – 7.45 (m, 2H), 4.51 (q, *J* = 7.0 Hz, 4H), 3.96 (s, 3H), 1.28 (dt, *J* = 12.8, 6.5 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.3, 172.6, 161.0, 160.4, 159.2, 111.5, 106.7, 77.2, 59.1, 43.8, 43.2, 12.7, 12.6.

Pz: Yield = 70%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.67 (m, 1H), 7.50 (d, *J* = 12.3 Hz, 1H), 7.44 – 7.27 (m, 9H), 7.20 – 7.08 (m, 2H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 164.4 (2C), 152.1, 137.2 (2C), 129.1, 129.0 (2C), 126.3, 126.2, 122.4, 122.3, 111.0, 104.1, 58.7.

Library dyes:

I-BA: Yield = 89%. ^{1}H NMR (300 MHz, DMSO) δ 8.12 (dt, *J* = 13.1, 6.5 Hz, 2H), 7.71 (t, *J* = 13.0 Hz, 1H), 7.50 (d, *J* = 6.8 Hz, 1H), 7.40 – 7.21 (m, 2H), 7.21 – 7.06 (m, 1H), 6.13 (d, *J* = 13.5 Hz, 1H), 3.84 (q, *J* = 6.8 Hz, 3H), 3.51 (s, 3H), 1.62 (s, 6H), 1.10 (td, *J* = 6.8, 1.6 Hz, 6H). MS-ESI *m/z* 394.1 ($[M + H]^+$ requires 394.2).

I-SO: Yield = 97%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.4 Hz, 1H), 7.94 (d, *J* = 7.4 Hz, 1H), 7.83 – 7.67 (m, 4H), 7.34 – 7.26 (m, 2H), 7.11 (t, *J* = 7.5 Hz, 1H), 6.92 (d, *J* = 7.9 Hz, 1H), 6.86 (t, *J* = 13.2 Hz, 1H), 5.89 (d, *J* = 12.8 Hz, 1H), 3.46 – 3.31 (s, 3H), 1.62 (s, 6H). MS-ESI *m/z* 392.0 ($[M + H]^+$ requires 392.1).

I-TBA: Yield = 93%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 – 8.04 (m, 1H), 7.95 – 7.81 (m, 2H), 7.36 – 7.28 (m, 2H), 7.16 (dt, *J* = 7.5, 0.7 Hz, 1H), 6.97 (d, *J* = 7.9 Hz, 1H), 6.05 – 5.90 (m, 1H), 4.58 (q, *J* = 6.9 Hz, 4H), 3.38 (s, 3H), 1.62 (s, 6H), 1.38 – 1.26 (m, 6H). MS-ESI *m/z* 410.1 ($[M + H]^+$ requires 410.2).

I-Pz: Yield = 87%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (dd, *J* = 25.0, 13.0 Hz, 2H), 7.57 (t, *J* = 13.1 Hz, 1H), 7.48 – 7.40 (m, 4H), 7.33 – 7.26 (m, 6H), 7.14 – 7.07 (m, 3H), 6.91 (d, *J* = 7.8 Hz, 1H), 5.87 (d, *J* = 13.0 Hz, 1H), 3.36 (s, 3H), 1.65 (s, 6H). MS-ESI *m/z* 462.1 ($[M + H]^+$ requires 462.2).

O-Pht: Yield = 59%. ^{1}H NMR (400 MHz, DMSO) δ 7.91 (t, *J* = 12.5 Hz, 1H), 7.70 – 7.56 (m, 6H), 7.55 – 7.32 (m, 4H), 6.10 (d, *J* = 13.5 Hz, 1H), 3.72 (s, 3H). MS-ESI *m/z* 330.1 ($[M + H]^+$ requires 330.1).

O-BA: Yield = 29%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, *J* = 12.6 Hz, 1H), 7.83 (td, *J* = 25.3, 13.1 Hz, 2H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.35 – 7.24 (m, 2H), 7.13 – 7.06 (m, 1H), 5.45 (d, *J* = 11.9 Hz, 1H), 4.02 (dq, *J* = 7.0, 1.3 Hz, 4H), 3.53 (s, 3H), 1.25 (dt, *J* = 7.0, 3.0 Hz, 6H). MS-ESI *m/z* 368.1 ($[M + H]^+$ requires 368.2).

O-SO: Yield = 29%. ^{1}H NMR (400 MHz, DMSO) δ 8.01 (t, *J* = 13.0 Hz, 1H), 7.92 (d, *J* = 6.5 Hz, 1H), 7.85 – 7.76 (m, 3H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.69 – 7.57 (m, 2H), 7.48 (t, *J* = 7.2 Hz, 1H), 7.41 (t, *J* = 7.2 Hz, 1H), 6.55 (t, *J* = 13.1 Hz, 1H), 6.26 (d, *J* = 13.5 Hz, 1H), 3.75 (s, 3H). MS-ESI *m/z* 366.1 ($[M + H]^+$ requires 366.1).

O-TBA: Yield = 85%. ^{1}H NMR (400 MHz, DMSO) δ 8.04 – 7.96 (m, 1H), 7.83 – 7.72 (m, 4H), 7.53 (dt, *J* = 7.7, 1.3 Hz, 1H), 7.48 (dt, *J* = 7.6, 1.2 Hz, 1H), 6.42 (d, *J* = 13.8 Hz, 1H), 4.43 (q, *J* = 6.9 Hz, 4H), 3.83 (s, 3H), 1.17 (t, *J* = 6.9 Hz, 6H). MS-ESI *m/z* 384.1 ($[M + H]^+$ requires 384.1).

O-Pz: Yield =78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (t, *J* = 12.8 Hz, 1H), 7.66 (d, *J* = 13.2 Hz, 1H), 7.51 (t, *J* = 13.2 Hz, 1H), 7.46 – 7.36 (m, 5H), 7.31 – 7.21 (m, 6H), 7.12 – 7.05 (m, 3H), 5.42 (d, *J* = 12.4 Hz, 1H), 3.51 (s, 3H). MS-ESI *m/z* 437.0 ($[M + H]^+$ requires 436.2).

S-Pht: Yield = 72%. ^{1}H NMR (400 MHz, DMSO, 90 °C) δ 7.87 (d, *J* = 7.9 Hz, 1H), 7.74 – 7.45 (m, 9H), 7.33 (t, *J* = 7.1 Hz, 1H), 6.43 (d, *J* = 12.4 Hz, 1H), 3.76 (s, 3H). MS-ESI *m/z* 346.0 ($[M + H]^+$ requires 346.1).

S-BA: Yield = 70%. ^{1}H NMR (300 MHz, DMSO) δ 7.95 – 7.81 (m, 2H), 7.72 – 7.60 (m, 3H), 7.51 (t, *J* = 7.7 Hz, 1H), 7.34 (t, *J* = 7.5 Hz, 1H), 6.50 (d, *J* = 11.5 Hz, 1H), 3.83 (q, *J* = 6.9 Hz, 4H), 3.80 (s, 3H), 1.10 (t, *J* = 6.8 Hz, 6H). MS-ESI *m/z* 384.0 ($[M + H]^+$ requires 384.1).

S-SO: Yield = 78%. ^{1}H NMR (400 MHz, DMSO) δ 7.93 (d, *J* = 7.3 Hz, 1H), 7.91 – 7.72 (m, 5H), 7.63 (t, *J* = 10.7 Hz, 2H), 7.58 – 7.50 (m, 1H), 7.44 – 7.30 (m, 1H), 6.98 – 6.68 (m, 1H), 6.55 (d, *J* = 13.0 Hz, 1H), 3.82 (s, 3H). MS-ESI *m/z* 382.0 ($[M + H]^+$ requires 382.1).

S-TBA: Yield = 93%. ^{1}H NMR (400 MHz, DMSO) δ 8.10 (d, *J* = 8.0 Hz, 1H), 7.94 – 7.83 (m, 4H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 1H), 6.82 (d, *J* = 13.5 Hz, 1H), 4.43 (q, *J* = 6.8 Hz, 4H), 3.94 (s, 3H), 1.17 (t, *J* = 7.0 Hz, 6H). MS-ESI *m/z* 400.1 ($[M + H]^+$ requires 400.1).

S-Pz: Yield = 70%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 12.9 Hz, 1H), 7.55 (t, *J* = 11.3 Hz, 2H), 7.46 – 7.37 (m, 6H), 7.31 – 7.22 (m, 5H), 7.18 (d, *J* = 8.0 Hz, 1H), 7.13 – 7.04 (m, 2H), 6.02 (d, *J* = 12.3 Hz, 1H), 3.61 (s, 3H). MS-ESI *m/z* 452.0 ($[M + H]^+$ requires 452.1).

TD-Pht: Yield = 82%. 1H NMR (400 MHz, DMSO) δ 7.68 – 7.49 (m, 5H), 7.38 (d, *J* = 5.4 Hz, 2H), 6.32 (d, *J* = 12.8 Hz, 1H), 3.84 (s, 3H), 2.57 (s, 3H). MS-ESI *m/z* 311.0 ($[M + H]^+$ requires 311.1).

TD-BA: Yield = 61%. ^{1}H NMR (400 MHz, DMSO) δ 7.85 – 7.70 (m, 1H), 7.67 – 7.47 (m, 2H), 6.44 – 6.19 (m, 1H), 3.86 (s, 3H), 3.82 (q, *J* = 6.9 Hz, 4H), 2.58 (s, 3H), 1.07 (t, *J* = 6.9 Hz, 6H). MS-ESI *m/z* 349.1 ($[M + H]^+$ requires 349.1).

TD-SO: Yield = 77%. ^{1}H NMR (400 MHz, DMSO) δ 7.88 (d, *J* = 6.2 Hz, 1H), 7.84 – 7.73 (m, 3H), 7.68 (t, *J* = 12.6 Hz, 1H), 7.56 (d, *J* = 13.9 Hz, 1H), 6.45 (t, *J* = 14.0 Hz, 2H), 3.88 (s, 3H), 2.60 (s, 3H). MS-ESI *m/z* 347.0 ($[M + H]^+$ requires 347.0).

TD-TBA: Yield = 70%. ^{1}H NMR (400 MHz, DMSO) δ 7.76 – 7.55 (m, 3H), 6.68 – 6.48 (m, 1H), 4.42 (q, *J* = 6.9 Hz, 4H), 3.96 (s, 3H), 2.64 (s, 3H), 1.16 (t, *J* = 6.9 Hz, 6H). MS-ESI *m/z* 365.0 ($[M + H]^+$ requires 365.1).

TD-Pz: Yield = 53%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 13.0 Hz, 1H), 7.50 – 7.40 (m, 4H), 7.31 – 7.24 (m, 4H), 7.15 (d, *J* = 12.7 Hz, 2H), 7.11 – 7.05 (m, 2H), 5.75 (d, *J* = 12.7 Hz, 1H), 3.69 (s, 3H), 2.49 (s, 3H). MS-ESI *m/z* 417.1 ($[M + H]^+$ requires 417.1).

Q-Pht: Yield = 56%. ^{1}H NMR (400 MHz, DMSO) δ 8.57 (d, *J* = 8.5 Hz, 1H), 8.27 (d, *J* = 7.3 Hz, 1H), 8.14 (t, J = 12.0 Hz, 1H), 7.91 (m, 2H), 7.70 – 7.47 (m, 7H), 7.35 (d, *J* = 13.8 Hz, 1H), 7.06 (d, *J* = 13.6 Hz, 1H), 4.05 (s, 3H). MS-ESI *m/z* 340.1 ($[M + H]^+$ requires 340.1).

Q-BA: Yield = 45%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 8.6 Hz, 1H), 8.04 (d, *J* = 13.2 Hz, 1H), 7.93 (t, *J* = 12.8 Hz, 1H), 7.79 (t, *J* = 12.7 Hz, 1H), 7.69 (ddd, *J* = 8.5, 7.1, 1.4 Hz, 1H), 7.46 (ddd, *J* = 8.3, 7.1, 1.1 Hz, 1H), 7.42 – 7.38 (m, 1H), 7.28 (d, *J* = 7.4 Hz, 1H), 6.95 (d, *J* = 7.5 Hz, 1H), 6.75 (d, *J* = 12.8 Hz, 1H), 4.02 (q, *J* = 7.0 Hz, 4H), 3.82 (s, 3H), 1.24 (dt, *J* = 7.2, 6.8 Hz, 6H). MS-ESI *m/z* 378.1 ($[M + H]^+$ requires 378.2).

Q-SO: Yield = 20%. ^{1}H NMR (400 MHz, DMSO) δ 8.65 (d, *J* = 8.7 Hz, 1H), 8.46 (d, *J* = 7.0 Hz, 1H), 8.25 – 8.15 (m, 1H), 8.04 – 7.94 (m, 2H), 7.86 – 7.82 (m, 1H), 7.79 – 7.67 (m, 5H), 7.54 – 7.34 (m, 1H), 7.19 (d, *J* = 14.0 Hz, 1H), 6.77 – 6.51 (m, 1H), 4.14 (s, 3H). MS-ESI *m/z* 376.0 ($[M + H]^+$ requires 376.1).

Q-TBA: Yield = 69%. ^{1}H NMR (400 MHz, DMSO) δ 8.72 – 8.65 (m, 2H), 8.19 – 8.10 (m, 2H), 8.08 – 8.00 (m, 2H), 7.86 – 7.76 (m, 2H), 7.63 (d, *J* = 14.4 Hz, 1H), 7.26 (d, *J* = 14.4 Hz, 1H), 4.45 (q, *J* = 7.0 Hz, 4H), 4.24 (s, 3H), 1.17 (t, *J* = 6.9 Hz, 6H). MS-ESI *m/z* 394.1 ($[M + H]^+$ requires 394.2).

Q-Pz: Yield = 39%. ^{1}H NMR (400 MHz, DMSO) δ 8.62 (d, *J* = 8.7 Hz, 1H), 8.46 (d, *J* = 7.0 Hz, 1H), 8.32 – 8.11 (dd, *J* = 14.0, 11.2 Hz, 1H), 7.99 (q, *J* = 8.9 Hz, 2H), 7.79 (d, *J* = 7.2 Hz, 1H), 7.71 (t, *J* = 7.3 Hz, 1H), 7.49 – 7.21 (m, 10H), 7.18 – 6.96 (m, 3H), 4.14 (s, 3H). MS-ESI *m/z* 446.2 ($[M + H]^+$ requires 446.2).

Intermediates for conjugatable dye synthesis:

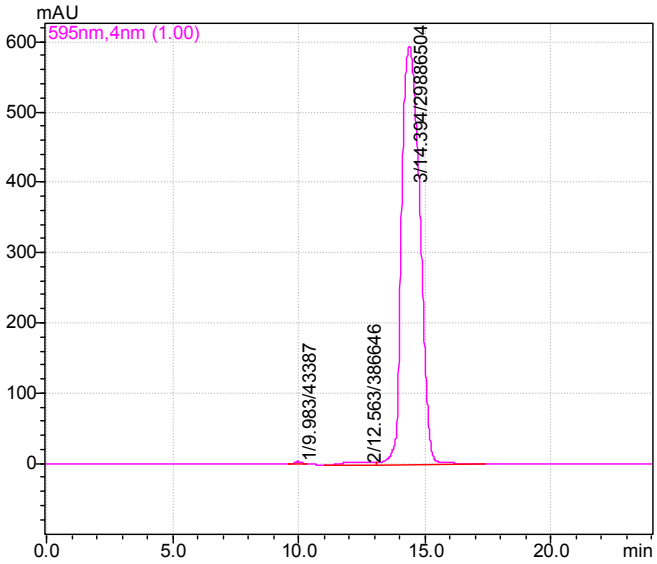
INS: ^{1}H NMR (400 MHz, D_2O) δ 7.86 – 7.75 (m, 2H), 7.73 – 7.65 (m, 2H), 4.80 (s, 3H), 4.67 – 4.57 (m, 2H), 3.40 – 3.32 (m, 2H), 3.32 – 3.25 (m, 2H), 3.05 (t, *J* = 7.3 Hz, 2H), 2.91 – 2.83 (m, 1H), 2.50 – 2.35 (m, 2H), 2.25 – 2.11 (m, 2H), 1.61 (s, 6H); ^{13}C NMR (100 MHz, D_2O) δ 197.7, 141.6, 140.5, 130.0, 129.2, 123.5, 117.7, 114.8, 114.6, 54.6, 47.7, 46.4, 44.6, 44.2, 23.9, 21.6, 21.1.

References:

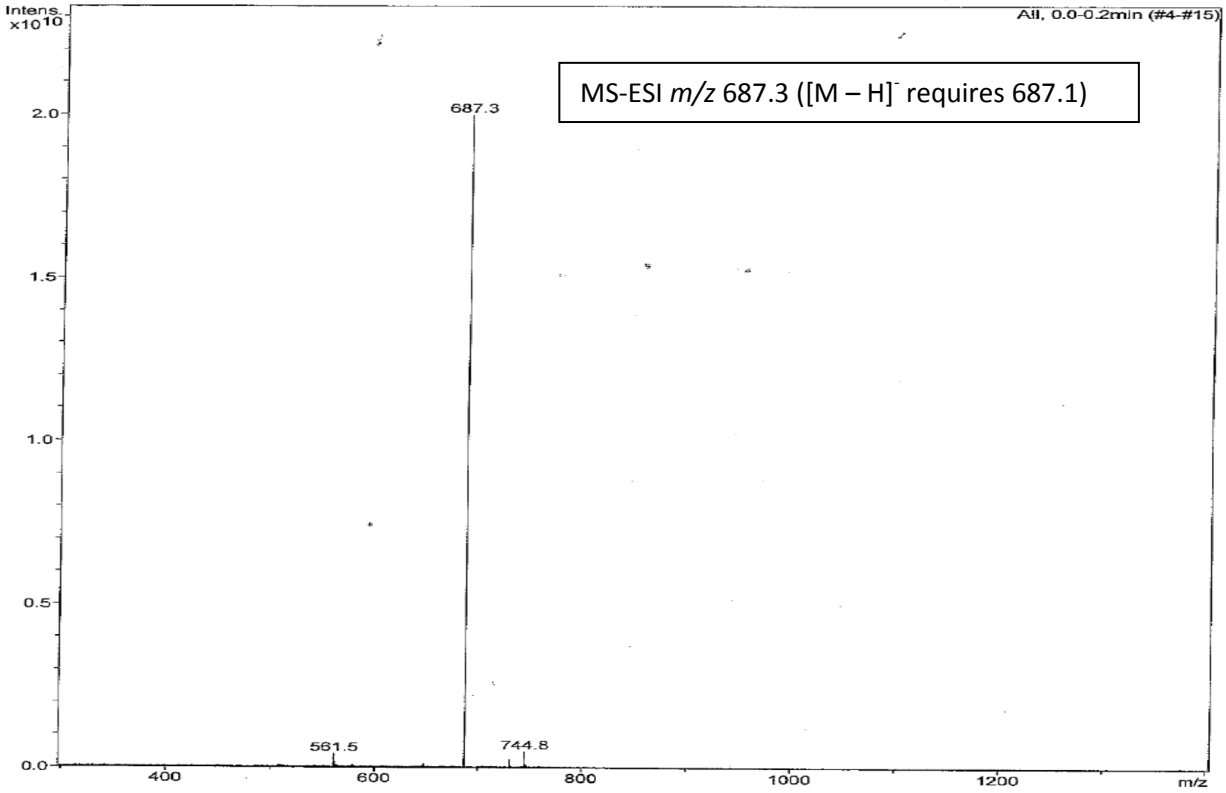
(S1) Toutchkine, A., Kraynov, V., and Hahn, K. (2003) Solvent-sensitive dyes to report protein conformational changes in living cells. *J. Am. Chem. Soc. 125*, 4132-4145.

HPLC and ESI-MS data for conjugatable merocyanine final products:

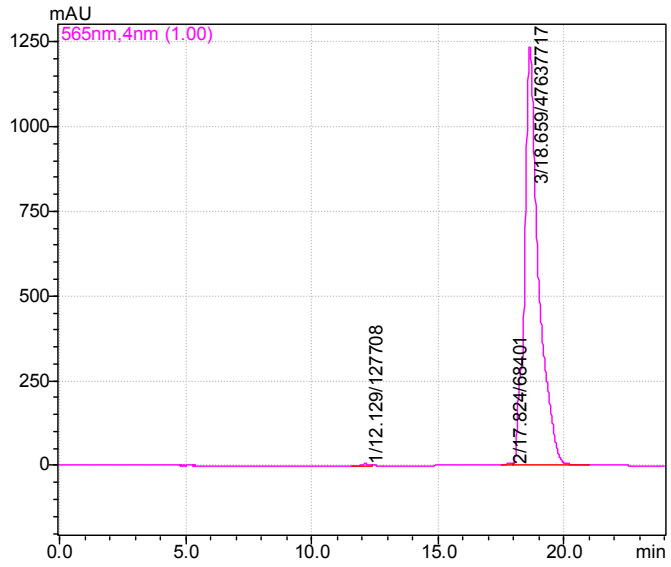
mero60



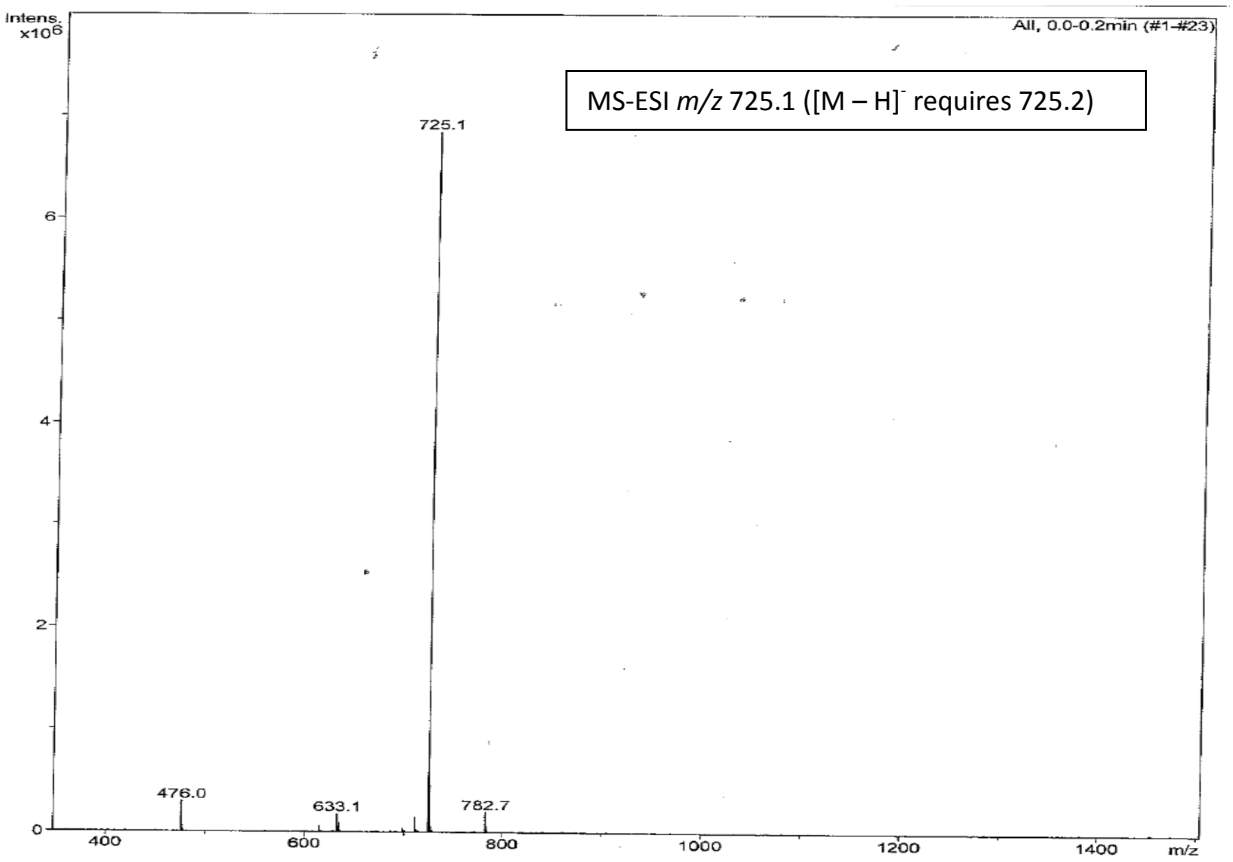
peak #	peak ID	ret. time	area	height	area %
1		9.983	43387	2860	0.1431
2		12.563	386646	4423	1.2754
3	mero60	14.394	29886504	595810	98.5815



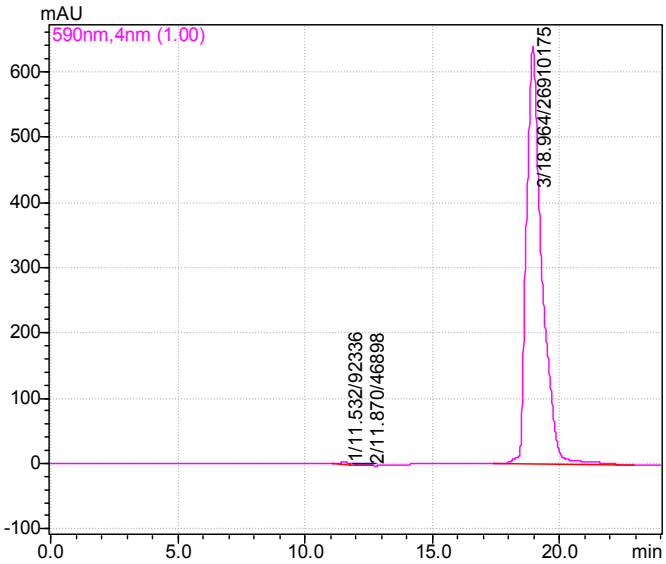
mero61



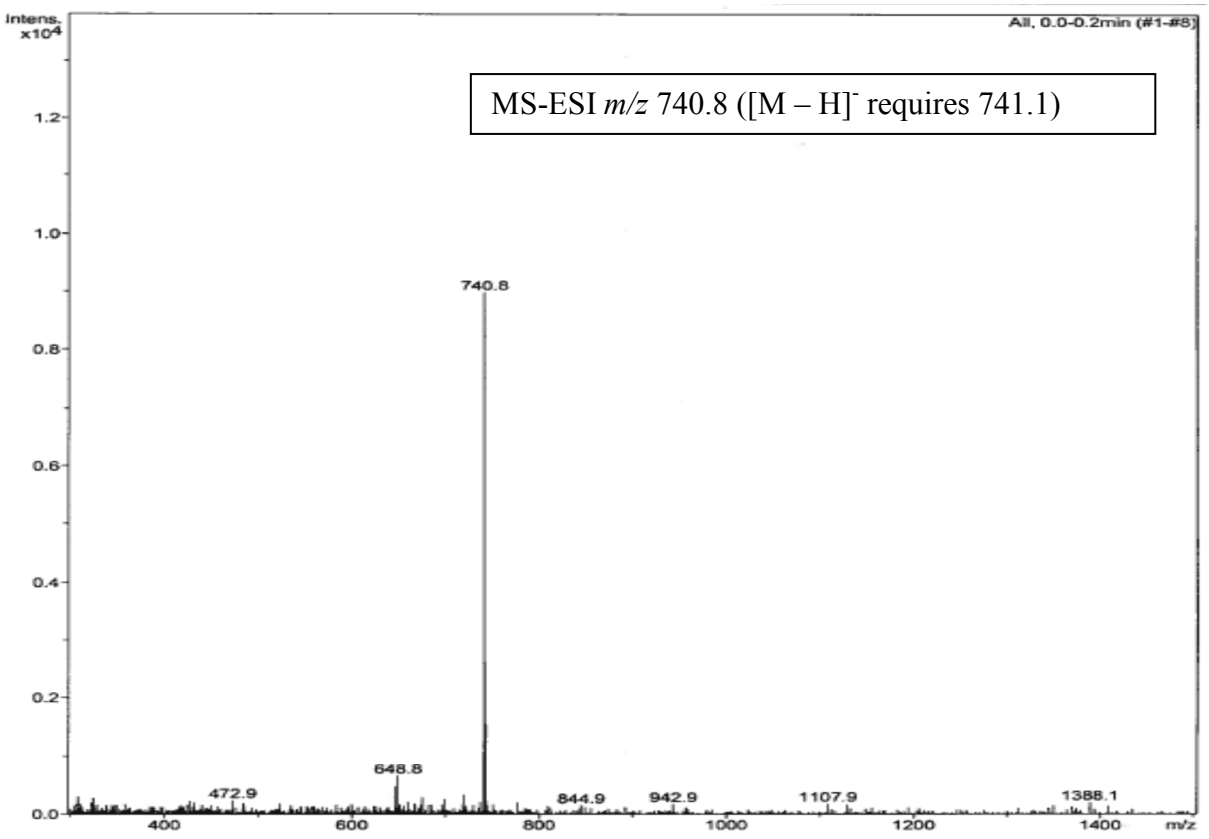
peak #	peak ID	ret. time	area	height	area %
1		12.129	127708	6407	0.2670
2		17.824	68401	3701	0.1430
3	mero61	18.659	47637717	1233625	99.5900



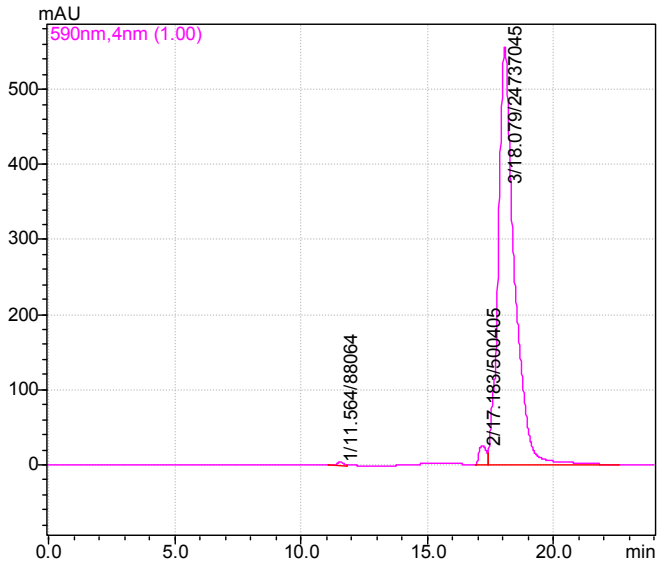
mero62



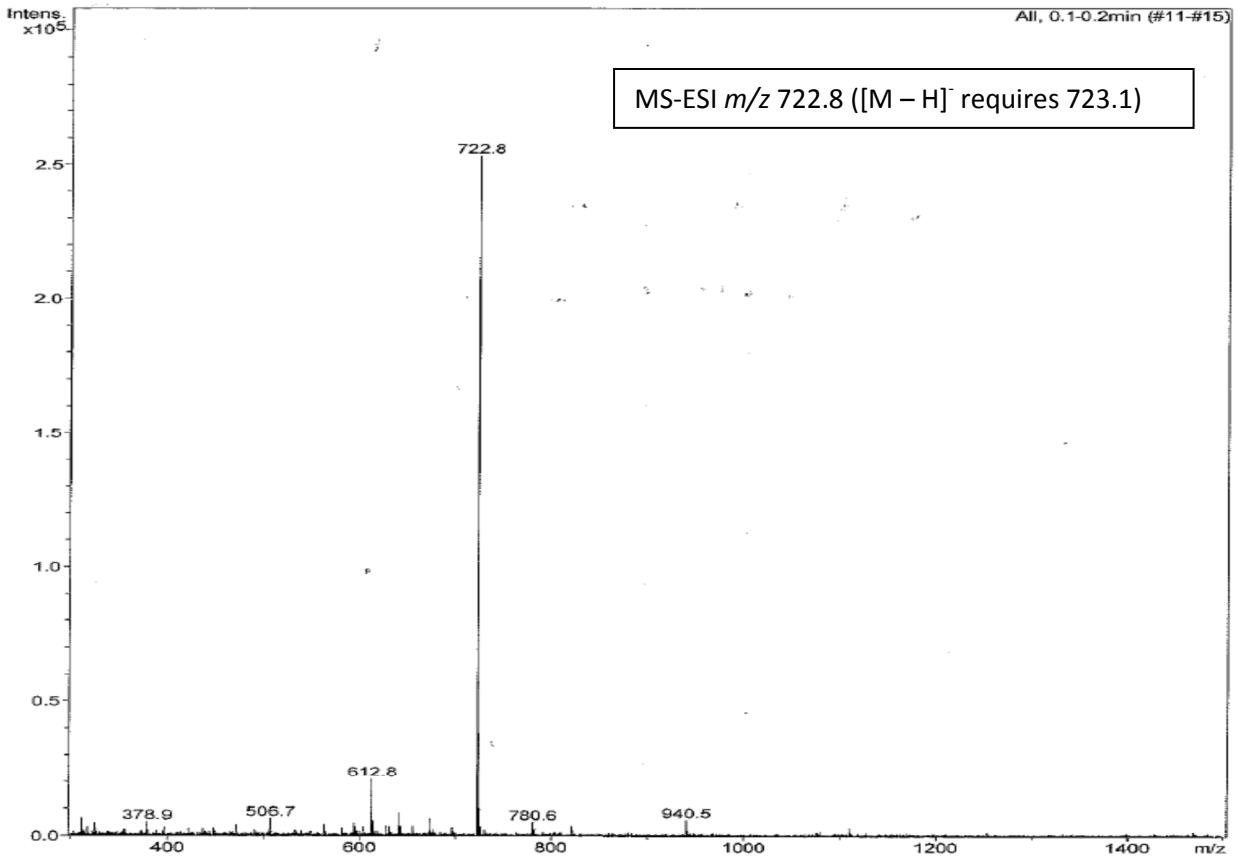
peak #	peak ID	ret. time	area	height	area %
1		11.532	92336	4516	0.3414
2		11.870	46898	2608	0.1734
3	mero62	18.964	26910175	638499	99.4853



mero87

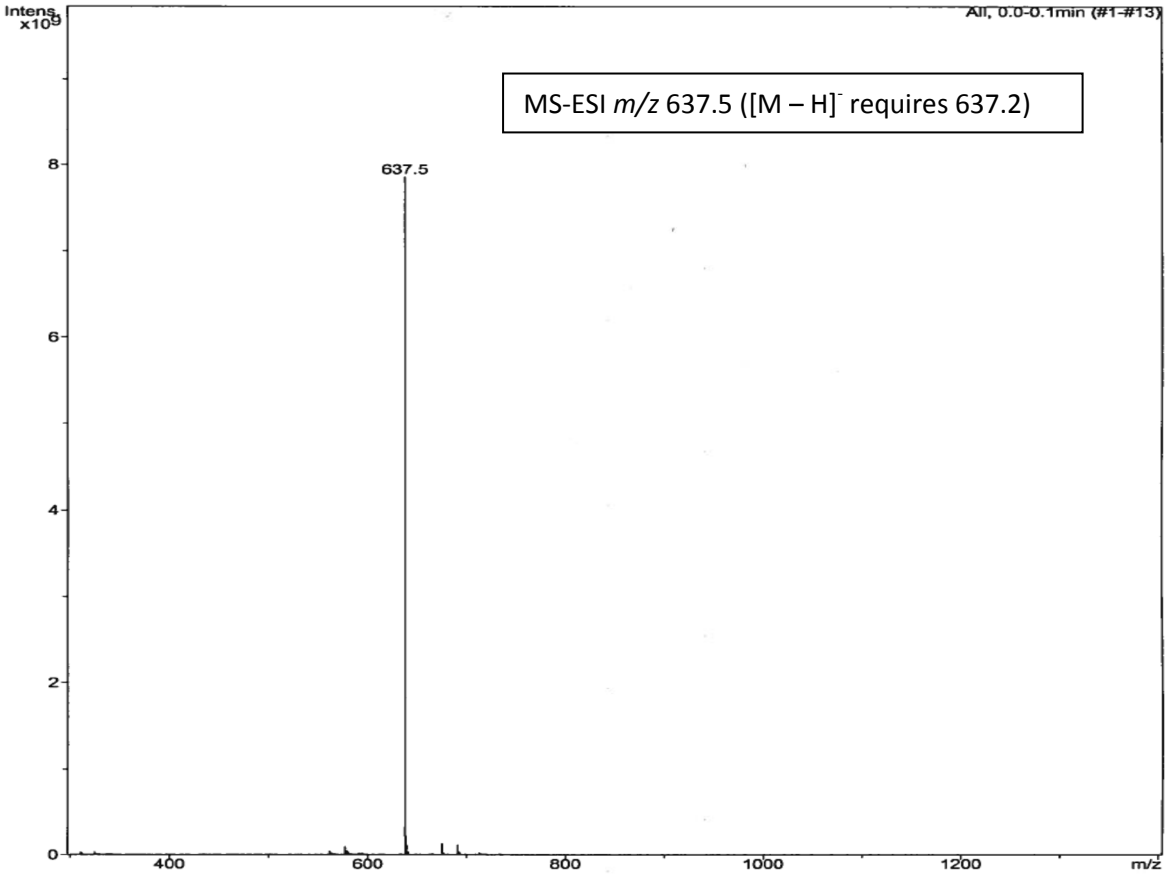


peak #	peak ID	ret. time	area	height	area %
1		11.564	88064	4541	0.3477
2		17.183	500405	25247	1.9759
3	mero87	18.079	24737045	556481	97.6764

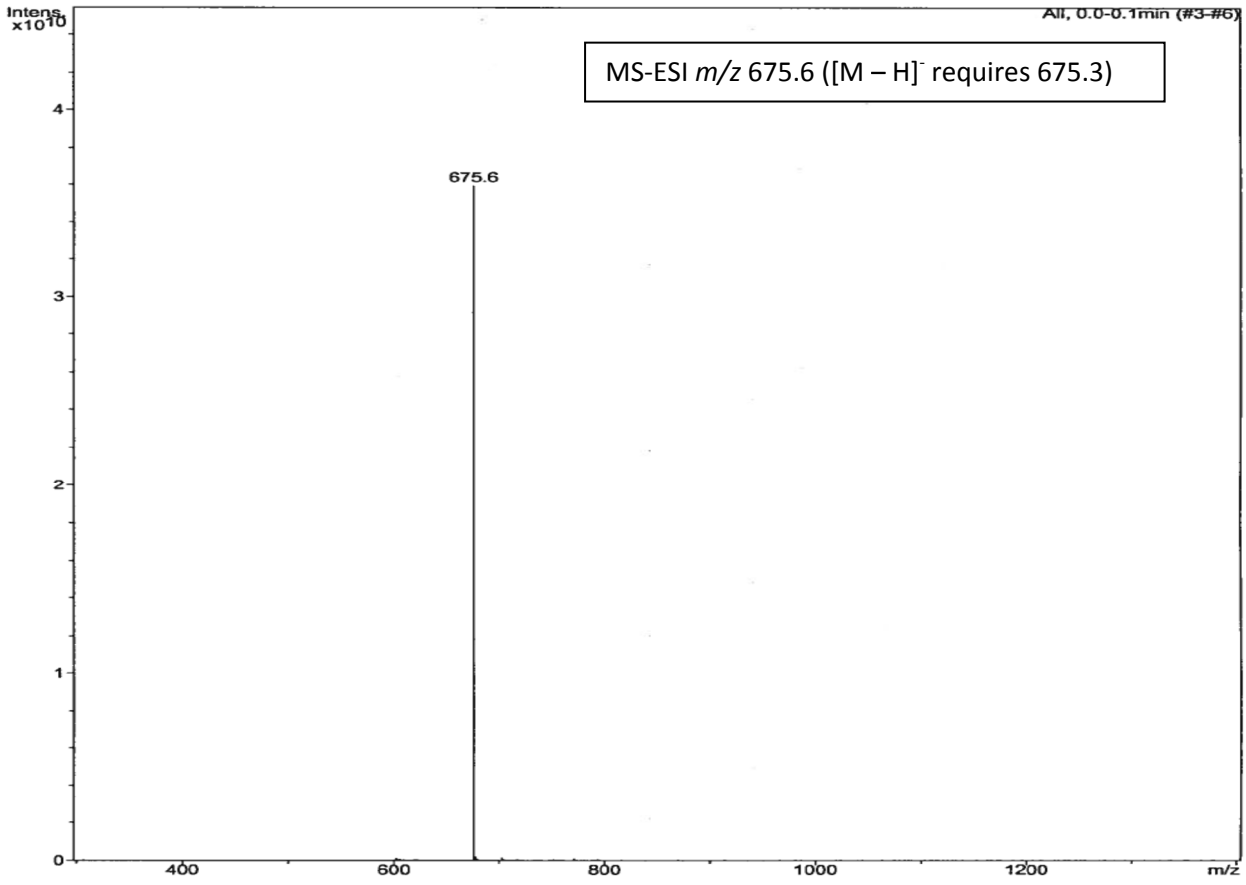


HPLC and ESI-MS data for β-mercaptoethanol derivatives of conjugatable merocyanines:

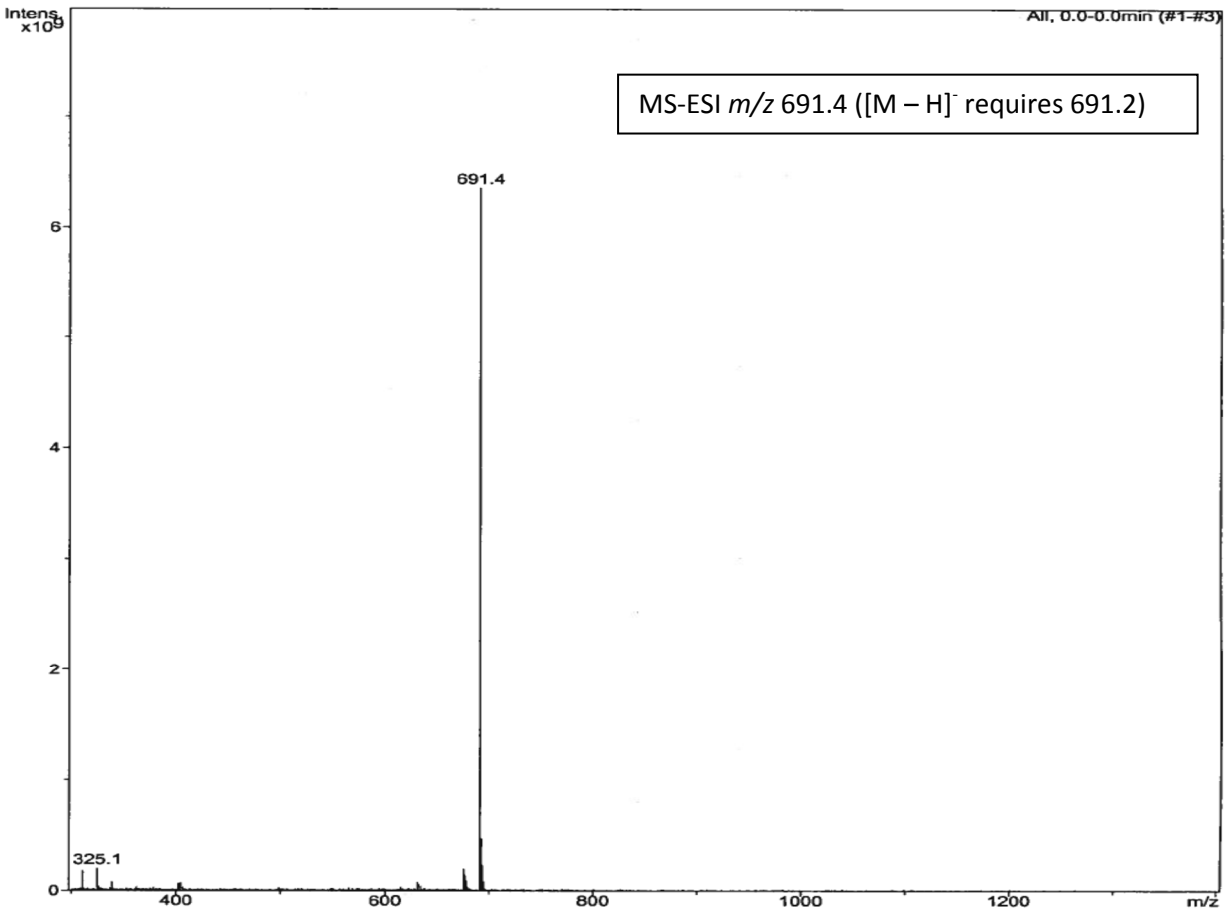
mero60-BME (33% yield)



mero61-BME (46% yield)



mero62-BME (52% yield)



mero87-BME (25% yield)