Electronic supplementary information for:

# Multichannel Dual Protein Sensing Using Amphiphilic Supramolecular Assemblies

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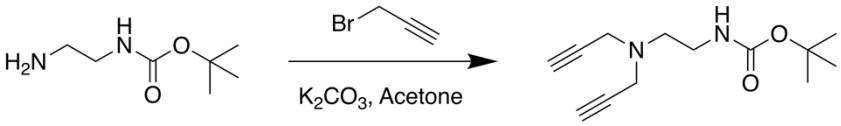
**Materials and Methods**:

All chemicals and reagents were purchased from commercial sources and were used as received, unless otherwise mentioned. $^{1}$H NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer using the residual proton resonance of the solvent as the internal standard. Chemical shifts are reported in parts per million (ppm). $^{13}$C NMR spectra were proton decoupled and recorded on a 100 MHz NMR spectrometer using the carbon signal of the deuterated solvent as the internal standard. Fluorescence spectra were obtained from Molecular Devices Spectramax iD5 plate reader. Dynamic light scattering (DLS) measurement were performed using Malvern Zetasizer Nano ZS with a 637 nm laser source with non-invasive backscattering technology detected at 173°. The sizes were measured after 2 minutes of temperature stabilization and reported as hydrodynamic diameters.

**Synthesis and Characterizations**

Synthesis of compound **5**

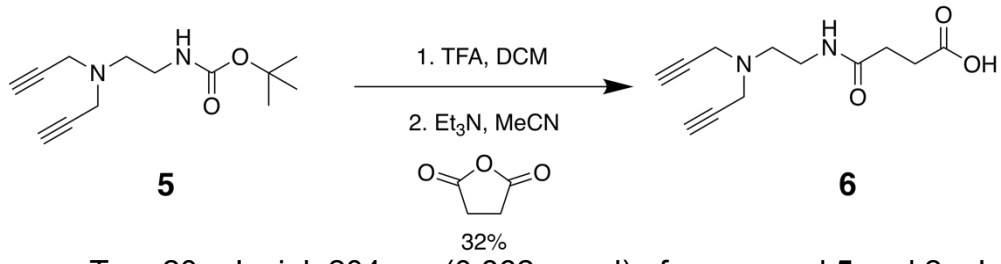



**4** **5**

To a 100-mL round bottom flask, 0.69 mL (700 mg, 4.369 mmol) of compound **4**, 1.81 g (13.11 mmol) of dry potassium carbonate, and 15 mL of acetone were added. While stirring the mixture at room temperature, 0.99 mL (1.56 g, 13.11 mmol) of allyl bromide was added dropwise over the course of 30 minutes. The reaction was stirred at room temperature overnight. After filtering out the solid, the acetone solvent in the solution

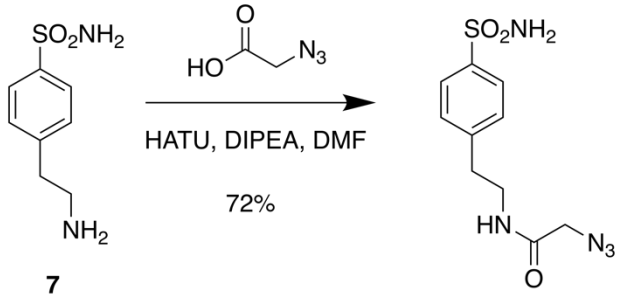
was removed under reduced pressure. Ethyl acetate (EA) was added to redissolve the mixture, which was then subjected to three cycles of brine washing. The organic phase was dried with sodium sulfate before purified with silica gel flash column (0 - 30% EA in hexanes). The obtained product **5** is colorless oil (170 mg) with 16% yield. $^{1}$H NMR (400 MHz, $CDCl_3$) δ: 4.94 (bs, 1H), 3.44 (d, *J* = 2.4 Hz, 4H), 3.22 (q, *J* = 5.7 Hz, 2H), 2.68 (t, *J* = 5.9 Hz, 2H), 2.23 (t, *J* = 2.4 Hz, 2H), 1.44 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 155.96, 79.22, 78.47, 73.27, 51.91, 42.08, 37.66, 28.42.

Synthesis of compound **6**




To a 20-mL vial, 204 mg (0.862 mmol) of compound **5** and 2 mL dichloromethane (DCM) were combined. When the solution became clear, 2 mL of trifluoroacetic acid was added and left to react at room temperature overnight. Excess DCM and trifluoroacetic acid were removed under reduced pressure by co-evaporating with toluene three times. The obtained oil was redissolved in 10 mL acetonitrile. Then, 420.5 μL (3.017 mmol) of triethylamine was introduced and let stir at 50 °C for 30 minutes. After that, 129 mg (1.293 mmol) of succinic anhydride was added to the reaction mixture and left to react at 50 °C overnight. The mixture was concentrated *in vacuo* followed by purification with silica gel flash column (0 - 4% methanol in DCM). The obtained **6** product is colorless oil (65 mg) with 34% yield. $^{1}$H NMR (400 MHz, Acetone-$d_6$) δ: 7.15 (bs, 1H), 3.45 (d, *J* = 2.3 Hz, 4H), 3.31 (q, *J* = 6.4 Hz, 2H), 2.71 (t, *J* = 2.5 Hz, 2H), 2.64 (t, *J* = 6.4 Hz, 2H), 2.56 (t, *J* = 6.4 Hz, 2H), 2.47 (t, *J* = 6.5 Hz, 2H); $^{13}$C NMR (101 MHz, Acetone) δ: 173.90, 172.26, 79.55, 74.46, 52.66, 42.44, 37.53, 30.94.

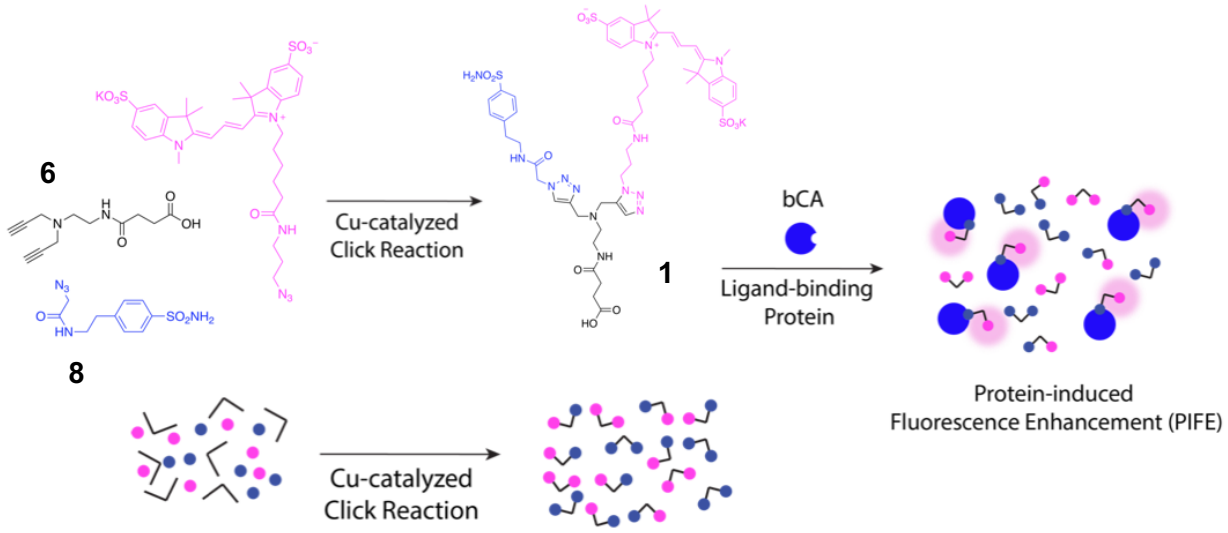
Synthesis of compound **8**




**7** **8**

To a 20-mL vial, 134.6 μL (181.7 mg, 1.798 mmol) of azidoacetic acid, 339.2 μL (251.7 mg, 1.947 mmol) of diisopropylethylamine (DIPEA), and 5 mL dimethyl formamide (DMF) were combined. After letting stir at room temperature for 15 minutes, 740.5 mg (1.947 mmol) of hexafluorophosphate azabenzotriazole tetramethyl uranium (HATU) was added to the mixture. The reaction was left to react for additional 30 minutes. In a separate vial, 300 mg (1.498 mmol) of compound **7** was dissolved in 5 mL DMF. The obtained solution was added dropwise to the reaction mixture and let stir overnight at room temperature. The reaction was dried *in vacuo*, redissolved in EA, filtered. The final mixture was purified with silica gel flash column (0 - 70% EA in dichloromethane). The obtained product **8** is white solids (304 mg) with 72% yield. $^{1}$H NMR (400 MHz, Acetone-$d_6$) δ: 7.81 (d, $J$ = 8.3 Hz, 2H), 7.42 (d, $J$ = 8.4 Hz, 2H), 6.53 (d, $J$ = 4.8 Hz, 1H), 3.85 (s, 2H), 3.52 (t, $J$ = 7.2 Hz, 2H), 2.92 (t, $J$ = 7.2 Hz, 2H). $^{13}$C NMR (101 MHz, Acetone) δ: 167.94, 144.79, 143.12, 130.12, 127.06, 52.33, 40.93, 35.98.

Synthesis of PIFE-based probe and measurement of PIFE due to protein presence




Supplementary Scheme 1: PIFE-based Probe Synthesis and PIFE-based sensing experiment

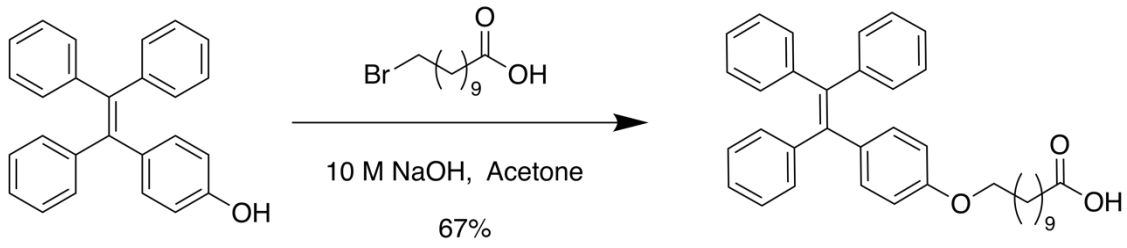
To 5-mL vial, 11.78 μL of compound **6** solution (4 mg/ml in acetone), 22.56 μL of compound **8** solution (2 mg/ml in acetone), 11.74 μL of sulfo-Cy3 azide solution (10 mg/mL in water), and 800 μL of DI water were added and mixed well. 21.64 μL of Cu-THPTA solution (4 mg/mL in water) and 78.92 μL of sodium ascorbate solution (1 mg/mL in water) were mixed in a separate vial, before adding to the reaction mixture. The reaction was left to react at room temperature in an open container overnight. The volume of the probe **1** solution was then adjusted to 4 mL.

For the specificity experiment, 100 μL of the probe **1** solution was placed in each well of a 96-well microplate. 10 μL of 100 mol% of each protein, including bCA, bovine serum albumin (BSA), horseradish peroxidase (HRP), and lysozyme was added to the probe solution. In the case of PTP1B and COX2 specificity experiment. Less amount of probe **1** and proteins were used due to protein availability issues. 20 μL of the probe **1** solution was placed in each well of a 96-well microplate, followed by addition of 80 μL DI water. 2 μL of 100 mol% of each protein was added to the probe solution. For each protein addition, the experiment was triplicated for the statistical significance. The microplate was

shaken for 5 minutes before Cy3 fluorescence measurement was carried out by monitoring emission intensity at 579 nm after excited at 520 nm.

For the concentration variation experiment, 100 μL of the probe **1** solution was placed in each well of a 96-well microplate. 5 μL of 0, 50, 75, 100, 125, and 150 mol% of bCA was added to the probe solution. For each protein addition, the experiment was triplicated for the statistical significance. The microplate was shaken for 5 minutes before Cy3 fluorescence measurement was carried out by monitoring emission intensity at 579 nm after excited at 520 nm.

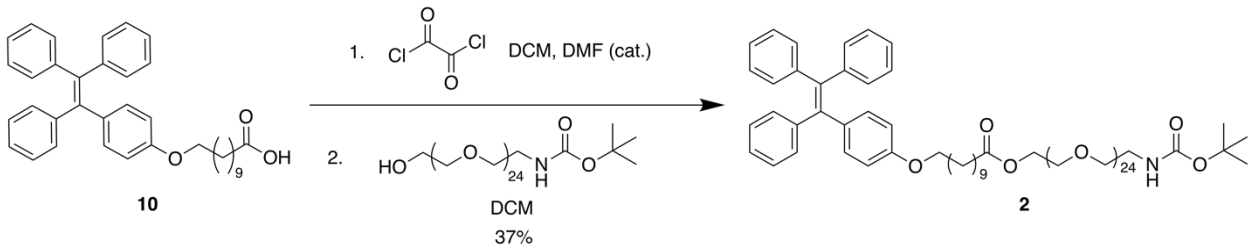
Synthesis of compound **10**




**9** **10**

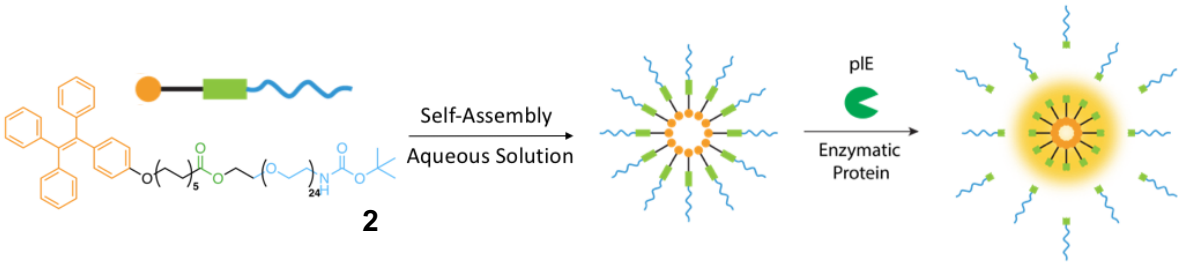
To a 250-mL round bottom flask, 1.50 g (4.305 mmol) of compound **9** was dissolved in 30 mL acetone, followed by addition of 0.95 mL 10 M NaOH solution in DI water. The mixture was refluxed for 30 minutes. In a separate container, 1.37 g (5.166 mmol) of 11-bromoundecanoic acid was dissolved in 15 mL acetone. The solution was added dropwise to the reaction mixture and refluxed overnight. Solvents were removed under reduced pressure, followed by redissolving back in EA and water mixture. The mixture was washed with brine three times. The organic layer was dried with sodium sulfate, then purified with silica gel flash column (0 - 5% EA in hexanes). The obtained product **10** is yellow solids (1.53 g) with 67% yield. $^{1}$H NMR (400 MHz, $CDCl_3$) δ: 7.18 – 6.96 (m, 15H), 6.92 (d, *J* = 8.7 Hz, 2H), 6.62 (d, *J* = 8.8 Hz, 2H), 3.87 (t, *J* = 6.6 Hz, 2H), 2.35 (t, *J* = 7.5 Hz, 2H), 1.78 – 1.58 (m, 4H), 1.49 – 1.21 (m, 12H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ: 180.16, 157.90, 144.30, 144.26, 140.83, 140.19, 136.10, 132.72, 131.63, 131.60, 131.56, 127.93, 127.81, 126.55, 126.42, 113.79, 68.02, 34.25, 29.70, 29.61, 29.58, 29.53, 29.44, 29.27, 29.25, 26.28, 24.90.

Synthesis of compound **2**




In a 20-mL vial under argon atmosphere, 190.0 mg (0.357 mmol) of compound **10** was dissolved in 2 mL DCM. Two drops of dry DMF was added, followed by addition of 42.8 μL (63.4 mg, 0.499 mmol) of oxalyl chloride. The reaction was left to react at room temperature for 30 minutes before being concentrated under reduced pressure to obtained acyl chloride version of compound **10**. In a separate vial, 167.5 mg (0.143 mmol) of HO-PEG24-NHBoc was dissolved in 2 mL DCM. The solution was passed through a sodium sulfate column packed in a Pasteur pipette. The column was washed with another 2 mL DCM and combined with the previous solution. The dry solution of HO-PEG24-NHBoc was added to another dry reaction mixture under argon atmosphere. The reaction was let stir at room temperature overnight. The final mixture was purified with silica gel flash column (0 - 5% methanol in DCM). The obtained product **2** is yellow oil (85 mg) with 37% yield. $^{1}$H NMR (400 MHz, $CDCl_3$) δ: 7.19 – 6.99 (m, 15H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.64 (d, *J* = 8.8 Hz, 2H), 4.29 – 4.19 (m, 2H), 3.88 (t, *J* = 6.6 Hz, 2H), 3.79 – 3.61 (m, 46H), 3.56 (t, *J* = 5.2 Hz, 2H), 3.33 (d, *J* = 5.5 Hz, 2H), 2.35 (t, *J* = 7.6 Hz, 2H), 1.80 – 1.70 (m, 2H), 1.65 (d, *J* = 9.2 Hz, 6H), 1.47 (s, 9H), 1.31 (s, 10H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ: 173.98, 157.82, 144.21, 144.17, 140.74, 140.10, 136.00, 132.63, 131.54, 127.71, 126.34, 113.70, 70.78, 70.73, 70.39, 70.01, 69.37, 67.94, 67.45, 63.51, 42.35, 39.32, 37.12, 34.35, 29.85, 29.65, 29.54, 29.46, 29.41, 29.28, 28.59, 26.21, 25.05.

Homogenous assembly formation of TPE-based amphiphiles and measurement of AIE due to protein presence




Supplementary Scheme 2: TPE-based Amphiphiles Synthesis

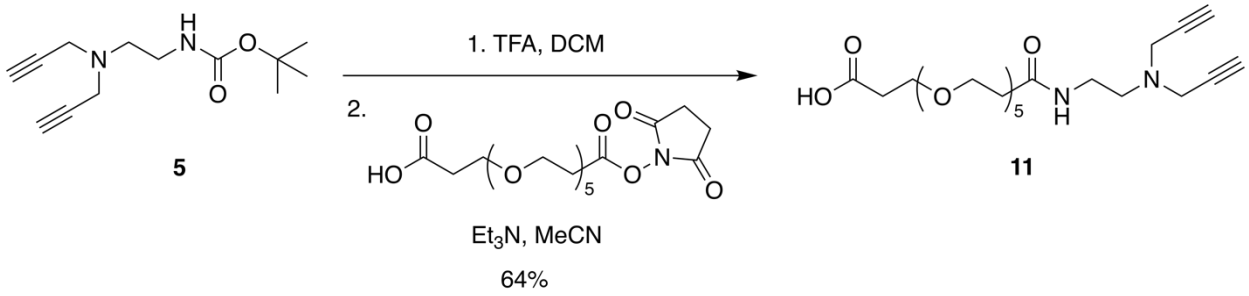
To a 5-mL vial, 1.13 mg of compound **2** was dissolved in 100 μL of acetone. 2.26 mL of DI water was added to the solution and let stir in an open container at room temperature overnight. The volume was adjusted to 5.52 mL to ensure the assembly concentration of 0.25 mg/mL.

For the specificity experiment, 100 μL of the probe solution was placed in each well of a 96-well microplate. 5.45 μL of 1 mol% of each protein, including plE, bCA, BSA, HRP, and lysozyme was added to the assembly solution. For each protein addition, the experiment was triplicated for the statistical significance. The microplate was shaken for 5 minutes before 12 hour incubation at room temperature. TPE fluorescence measurement was carried out by monitoring emission intensity at 450 nm after excited at 360 nm.

For the concentration variation experiment, 100 μL of the probe solution was placed in each well of a 96-well microplate. 2 μL of 0.1, 0.25, 0.5, and 1 mol% of plE was added to the probe solution. For each protein addition, the experiment was triplicated for the statistical significance. The microplate was shaken for 5 minutes before 12 hour incubation at room temperature. TPE fluorescence measurement was carried out by monitoring emission intensity at 450 nm after excited at 360 nm.

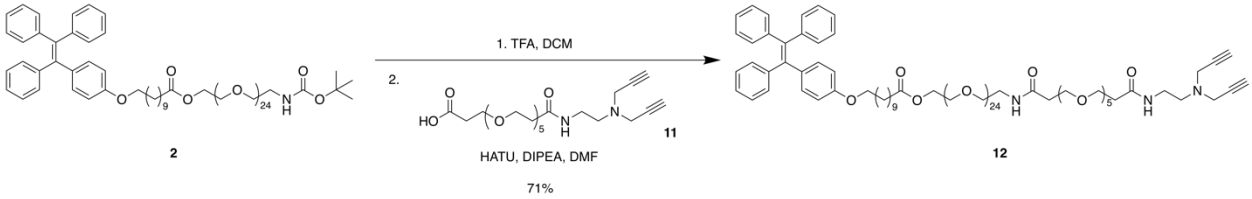
For the DLS study, 200 μL of the assembly was diluted with 800 μL DI water before adding to a cuvette. 33.3 μL of 4.45 mg plE/mL (1 mol% plE) was added, then the DLS was collected observed the size change over time.

Synthesis of compound **11**



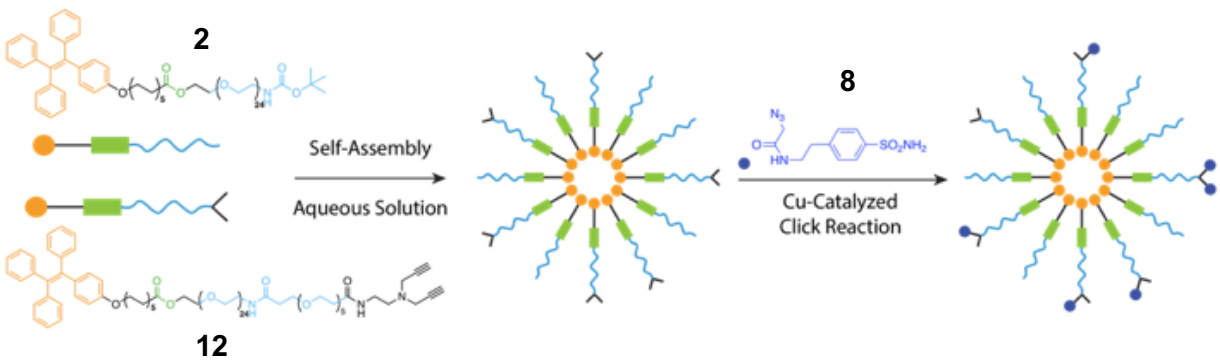

To 20-mL vial, 32.6 mg (0.138 mmol) of compound **5** was dissolved in 2 mL DCM, followed by addition of 2 mL trifluoroacetic acid. The reaction was stirred at room temperature for 3 hours. Excess DCM and trifluoroacetic acid were removed under reduced pressure by co-evaporating with toluene three times. The obtained oil was redissolved in 3 mL acetonitrile with 35.3 μL (25.6 mg, 0.253 mmol) of triethylamine. 50 mg (0.115 mmol) of HOOC-PEG5-NHS in 2 mL acetonitrile was mixed into the previous reaction mixture and left to react at room temperature overnight. The final mixture was purified with silica gel flash column (0 - 10% methanol in EA). The obtained product **11** is white solid (33.4 mg) with 64% yield. $^{1}$H NMR (400 MHz, $CDCl_3$) δ: 3.80 – 3.73 (m, 4H), 3.70 (d, *J* = 2.5 Hz, 4H), 3.68 – 3.62 (m, 16H), 3.49 (q, *J* = 5.7 Hz, 2H), 2.98 (t, *J* = 5.9 Hz, 2H), 2.60 (t, *J* = 5.9 Hz, 2H), 2.50 (t, *J* = 5.9 Hz, 2H), 2.40 (t, *J* = 2.4 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ: 174.08, 172.58, 76.06, 75.76, 70.74, 70.69, 70.63, 70.60, 70.44, 70.42, 70.32, 67.24, 66.85, 51.78, 42.34, 36.86, 36.12, 35.28.

Synthesis of compound **12**



To 20-mL vial, 41.2 mg (0.0244 mmol) of compound **2** was dissolved in 2 mL DCM, followed by 0.4 mL trifluoroacetic acid. The reaction proceeded at room temperature for 20 minutes. Excess DCM and trifluoroacetic acid were removed under reduced pressure by co-evaporating with toluene three times. The mixture was redissolved in 2 mL DMF. In a separate vial, 33.4 mg (0.0730 mmol) of compound **11** was dissolved in 2 mL DMF with 23.4 μL (17.3 mg, 0.134 mmol) of DIPEA. After 30 minutes of mixing at room temperature, 30.6 mg (0.0805 mmol) of HATU was added and left to react for another 30 minutes. The two DMF solutions were combined and let stir at room temperature overnight. Solvents were removed *in vacuo*, followed by redissolving in 2 mL DCM. The solution was purified by dialysis with 1kDa cut-off in 4:1 DCM:methanol solution. The obtained mixture was further purified with silica gel flash column (0 - 7% methanol in DCM). The obtained product **12** is yellow oil (35.9 mg) with 71% yield. $^{1}$H NMR (400 MHz, $CDCl_3$) δ: 7.18 – 6.97 (m, 15H), 6.92 (d, *J* = 8.7 Hz, 2H), 6.75 (bs, 1H), 6.63 (d, *J* = 8.8 Hz, 2H), 6.41 (bs, 1H), 4.27 – 4.19 (m, 2H), 3.87 (t, *J* = 6.6 Hz, 2H), 3.77 - 3.58 (m, 114H), 3.56 (dd, *J* = 5.6, 4.5 Hz, 2H), 3.51 – 3.40 (m, 6H), 3.39 – 3.32 (m, 2H), 2.71 (t, *J* = 6.0 Hz, 2H), 2.53 (s, 4H), 2.34 (t, *J* = 7.6 Hz, 2H), 2.27 (t, *J* = 2.4 Hz, 2H), 1.81 – 1.68 (m, 2H), 1.67 – 1.56 (m, 2H), 1.30 (q, *J* = 12.2, 8.7 Hz, 12H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ: 174.04, 172.46, 157.87, 144.26, 144.22, 140.79, 140.15, 136.05, 132.68, 131.59, 131.56, 131.52, 127.89, 127.77, 126.51, 126.39, 113.75, 78.70, 73.61, 70.81, 70.74, 70.69, 70.67, 70.39, 70.05, 69.41, 67.99, 63.56, 56.19, 51.66, 42.38, 39.49, 36.93, 34.40, 31.90, 31.78, 29.69, 29.59, 29.51, 29.46, 29.33, 26.26, 25.10.

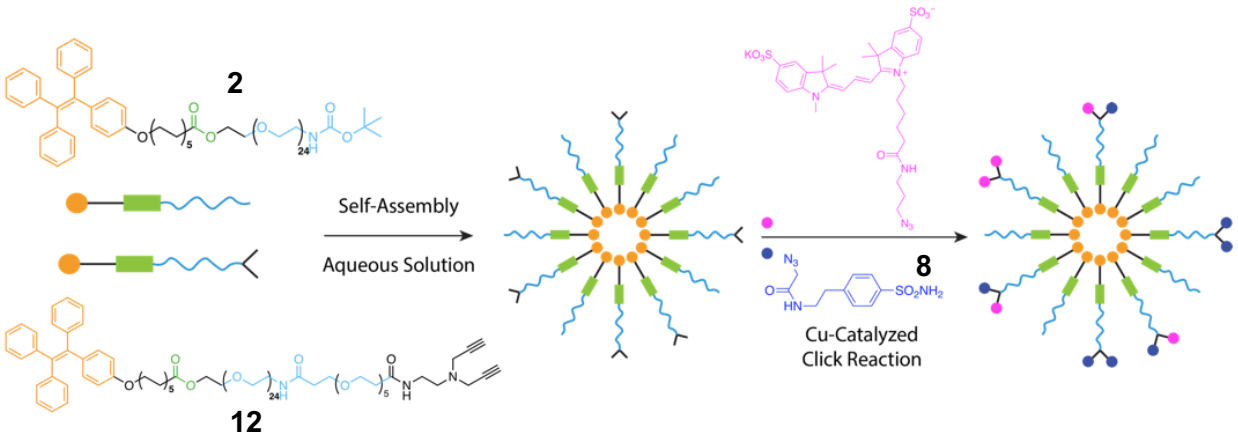
Mixed assembly formation of TPE-based amphiphiles with ligand modification and size distribution measurement due to addition of proteins



Supplementary Scheme 4A: Ligand Modification of the Mixed Assemblies

To a 20-mL vial, 3.00 mg of compound **2** and 3.00 mg of compound **12** were dissolved in 100 μL of acetone. 6.0 mL of DI water was added to the solution and let stir in an open container at room temperature overnight. The volume was adjusted to 6.0 mL to ensure the assembly concentration of 0.5 mg/mL. 600 μL of the assembly was taken out for the modification. 10.27 μL of compound **8** solution (2 mg/ml in acetone), 7.88 μL of Cu-THPTA solution (4 mg/mL in water) and 14.37 μL of sodium ascorbate solution (1 mg/mL in water) were mixed in a separate vial, before adding to the assembly solution. The reaction was left at room temperature overnight, before being purified by dialysis against DI water with 8 kDa cut-off membrane. For each sample, one-third of the assembly solution was diluted to 1 mL with DI water before adding to a cuvette. 33.3 μL of a protein solution was added, then the DLS was collected observed the size change. Protein solutions used included 18.10 mg bCA/mL (50 mol% bCA), 4.45 mg plE/mL (1 mol% plE), and PBS.

Mixed assembly formation of TPE-based amphiphiles with ligand and dye modification and fluorescence measurement due to addition of proteins




Supplementary Scheme 4B: Ligand and Dye Modification of the Mixed Assemblies

To a 20-mL vial, 3.00 mg of compound **2** and 3.00 mg of compound **12** were dissolved in 100 μL of acetone. 6.0 mL of DI water was added to the solution and let stir in an open container at room temperature overnight. The volume was adjusted to 6.0 mL to ensure the assembly concentration of 0.5 mg/mL. 2.0 mL of the assembly was taken

out for the modification. 17.80 μL of sulfo-Cy3 azide solution (10 mg/mL in water), 34.23 μL of compound **8** solution (2 mg/ml in acetone), 26.24 μL of Cu-THPTA solution (4 mg/mL in water) and, 47.85 μL of sodium ascorbate solution (1 mg/mL in water) were mixed in a separate vial, before adding to the assembly solution. The reaction was left at room temperature overnight, before being purified by dialysis against DI water with 8 kDa cut-off membrane. For each set of triplicates, 100 μL of the assembly was added to each well, followed by adding 2.0 μL of a protein solution. Protein solutions used included 4.45 mg plE/mL (1 mol% plE), 18.10 mg bCA/mL (50 mol% bCA), and PBS. For the bCA-then-plE samples, the bCA solution was added 4 times in total to reach 200 mol% concentration. The addition was done every 4 hours. After each addition, the fluorescence was monitored at immediately, 30 minutes, 1 hour, 2 hours, and 4 hours after each addition. The plE solution was added afterwards and monitored for another 48 hours. For the plE-then-bCA samples, the addition and monitoring time points are similar to the bCA-then-plE samples, except that the plE solution was added first, and the bCA solution was added 48 hours afterwards. The fluorescence intensity at 455 and 579 nm were collected by Molecular Devices Spectramax iD5 plate reader using 360 and 520 nm excitation wavelength respectively.

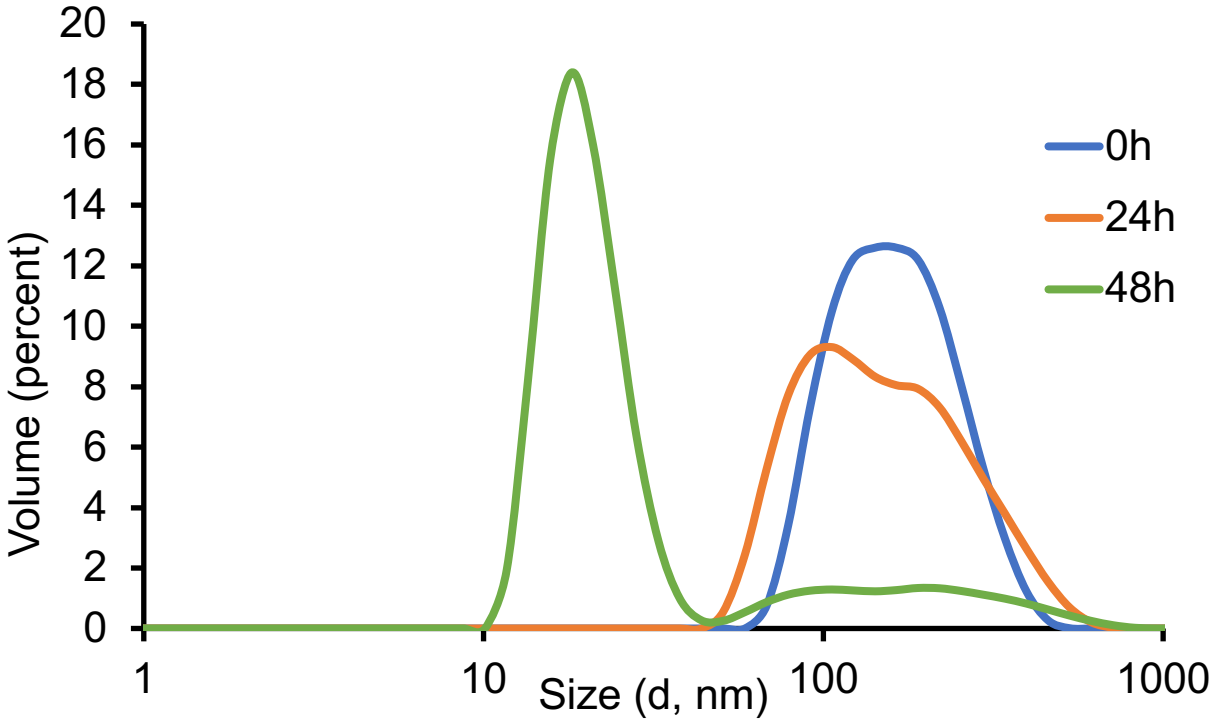



Figure S1 Size distribution of the TPE-amphiphile **2** assembly by volume after treated with 1 mol% pIE for 0, 24, and 48 hours

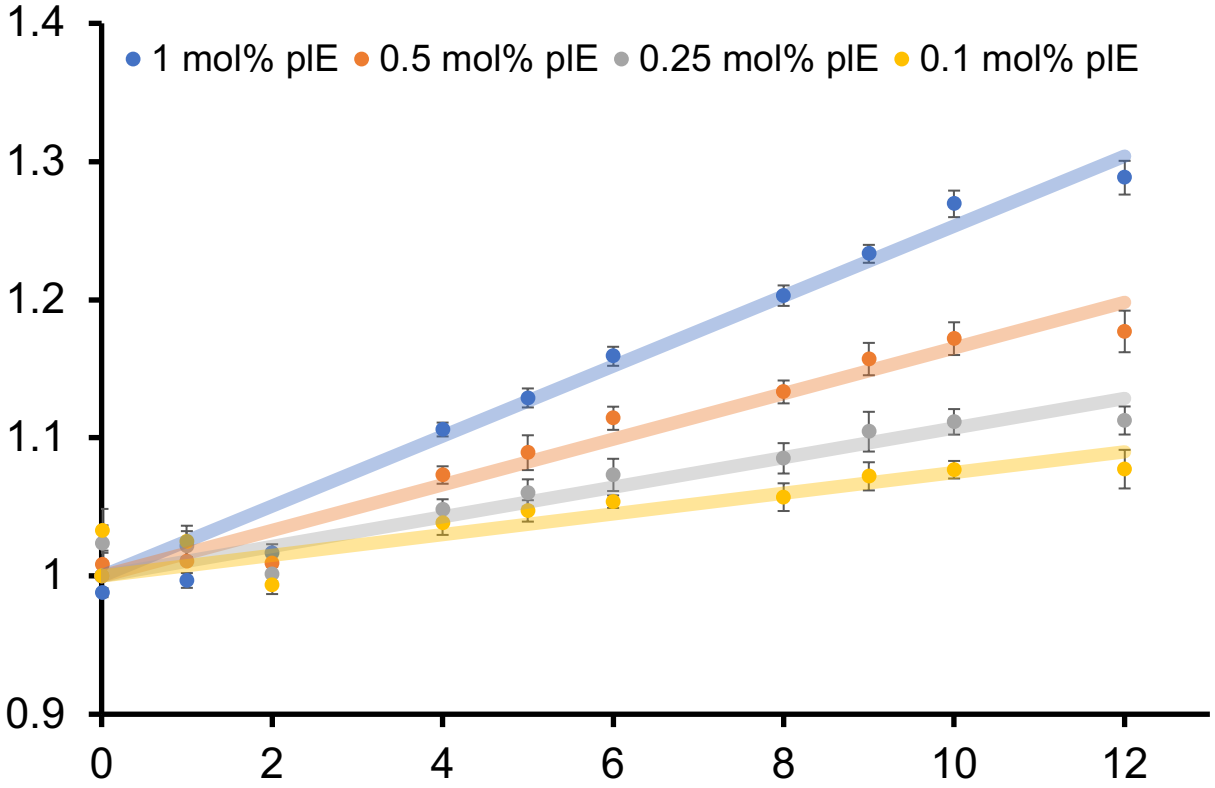


Figure S2 TPE fluorescence evolution at 450 nm of the TPE-amphiphile **2** assembly after treated with 0.1 - 1 mol% pIE for 12 hours

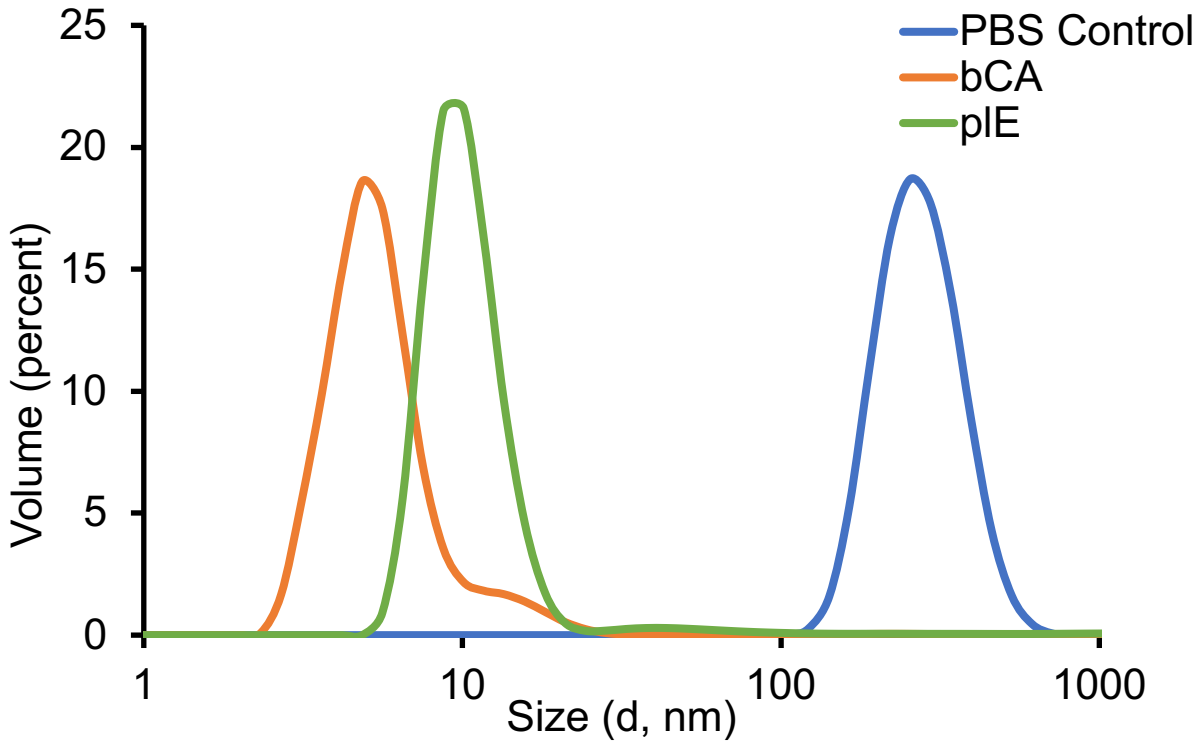



Figure S3 Size distribution of the phenyl sulfonamide-modified assembly from molecule **2** and **12** by volume after 50 mol% bCA, 1 mol% pIE, or PBS control were introduced

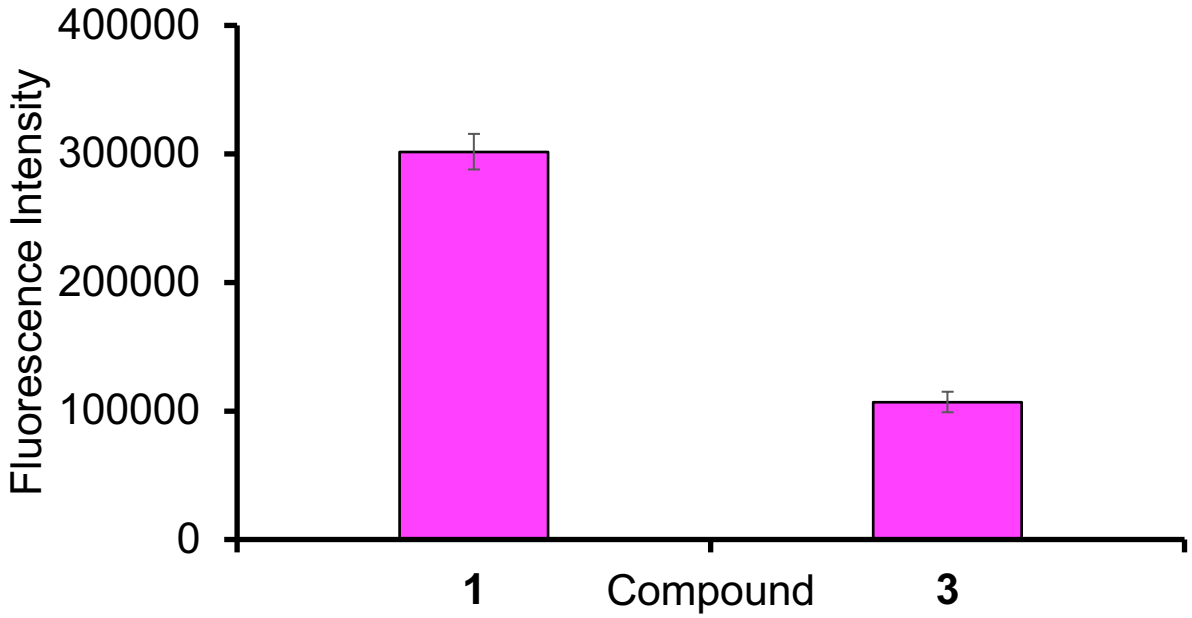



Figure S4 Cy3 fluorescence comparison between compound **1** and assembly of compound **2** and **3** (1:1) ratio, showing Cy3 quench when modified on assembly surface