Supporting Information for:

Excimer-monomer fluorescence changes by supramolecular disassembly for protein sensing and quantification

Hongxu Liu,[a] Jenna Westley,[a] S. Thayumanavan*[abc]

[a] Department of Chemistry, University of Massachusetts Amherst, Amherst, Massachusetts 01003, United States

[b] Department of Biomedical Engineering, University of Massachusetts Amherst, Amherst, Massachusetts 01003, United States

[c] Center for Bioactive Delivery, the Institute for Applied Life Sciences, University of Massachusetts Amherst, Amherst, Massachusetts 01003, United States

Experiment Section

1. Materials and Instruments: All chemicals were obtained from commercial sources and were used without further purification, unless otherwise stated. ^{1}H and ^{13}C NMR were recorded on a Bruker Avance-400 MHz or Bruker Avance-500 MHz NMR spectrometer. Chemical shifts are reported in ppm with TMS or the residual solvent peak as standard. ^{13}C NMR spectra are reported with the internal chloroform signal at 77.05 ppm as standard. Mass spectrometric data were recorded by a Bruker ESI-TOF Mass Spectrometer (Micro mass). Dynamic light scattering (DLS) measurements were performed using a Malvern Zetasizer Nano ZS. Fluorescence spectra were recorded on a PerkinElmer LS 55 spectrofluorimeter.

2. Methods:

2.1 Preparation of the supramolecular assemblies: 200 nmol the **D1, D2,** or **D3** oligomer in 200 μL acetone was added dropwise into 1 mL (**D1**) or 2 mL (**D2** and **D3**) deionized H_2O while stirring. The solution was stirred at room temperature in an uncapped vial for overnight, allowing the organic solvent

to evaporate. The obtained solution was diluted and sonicated for 1 min before DLS and fluorescence measurement unless otherwise stated.

2.2 Dynamic Light Scattering: DLS was performed on a Malvern Zetasizer Nano ZS instrument with a 637 nm laser source with non-invasive backscattering technology detected at 173°. All the sizes were reported as hydrodynamic diameter and recorded for three replicates. The samples were equilibrated under certain temperature for 10 min before size measurements.

2.3 Fluorescence measurements: Fluorescence of assemblies were recorded by a PerkinElmer LS 55 spectrofluorimeter with a 420 nm excitation wavelength for coumarin. The samples (with or w/o proteins) were pre-equilibrated for 10 minutes at ambient temperature before fluorescence measurements. The kinetics study of protein quantification was performed right after mixing without equilibration time. The experiments were performed for three replicates.

2.4 Critical aggregation concentration (CAC) measurements: The synthesized oligomer (200 nmol) was dissolved in 150 μL acetone and added dropwise into 1 mL deionized H_2O while stirring. The solution was stirred at room temperature in an uncapped vial overnight, evaporating the organic solvent. Then a calculated 5 wt% Nile red (compared to oligomer) solution in acetone (1 $mg \cdot mL^{-1}$) was added into the prepared assembly solution and stirred for another 6 h to for Nile red encapsulation and acetone evaporation. Next, the unencapsulated Nile red was removed by passing through a cotton-plugged glass Pasteur pipette[1] and the assembly solution was calibrated to 1 mL with deionized H_2O. The fluorescence was recorded with the excitation at 545 nm and the sample was diluted 1.5 times for next measurement. Finally, the fluorescence emission intensity was plotted against the oligomer concentration and the concentration at the inflection point was reported as the corresponding CAC.

2.5 Competitive protein-binding assays: The assemblies of molecule **D1** (200 μM) was made following the afore mentioned procedure in Section 2.1. The stock solution of DNSA (10 mM in DMSO) and bCA (100 μM in aqueous solution) was prepared. Next, DNSA and bCA was mixed to a diluted aqueous

solution (10.53 μM for each, 760 μL) and incubated at room temperature for 30 min. Next, 40 μL **D1** solution (200 μM) or water was added to the sample to obtain a 10 μM concentration solution for each component. For the control experiment, a 10 μM **D1** solution without DNSA or bCA was used directly. Finally, the fluorescence of the final sample was recorded by fluorimeter at different time points with 280 nm as excitation wavelength.

2.6 The response of assemblies to proteins: The stock solution of assemblies with 100 μM of target molecules was first prepared following the aforementioned procedures in Section 2.1. A stock solution of bCA was prepared with 0.6 $mg \cdot mL^{-1}$ concentration. Next, certain amount of the assembly solution was mixed with bCA (or mixtures with other proteins) and diluted to 800 μL with 25 μM target molecule concentration. The solution was applied for measurement of fluorescence on a PerkinElmer LS 55 spectrofluorimeter for three replicates.

2.7 Nile Red encapsulation and release in response to bCA: The dye encapsulation was performed following the procedure of CAC measurement (100 μM). The guest release was measured immediately after mixing the protein and assemblies. The final concentration of assemblies was 25 μM and the concentrations of proteins were 5 (0.2 equiv.) and 25 μM (1 equiv.) respectively. The fluorescence was recorded at 631 nm using a PerkinElmer LS 55 spectrofluorimeter (excitation at 545 nm) for at least two replicates.

2.8 The anti-quenching study to Fe^{3+}: A 100 μM **D2** assembly solution of was prepared following the afore mentioned procedures in section 2.1. The stock solution of $FeCl_3$ was made with 200 mM concentration. Next, certain amount of the $FeCl_3$ solution was mixed with the assembly solution to obtain a 25 μM **D2** concentration. The solution was equilibrated for 1 h before applied for fluorescence measurement on a PerkinElmer LS 55 spectrofluorimeter. Three replicates were performed for all groups.

3. Supplementary Figures and Table

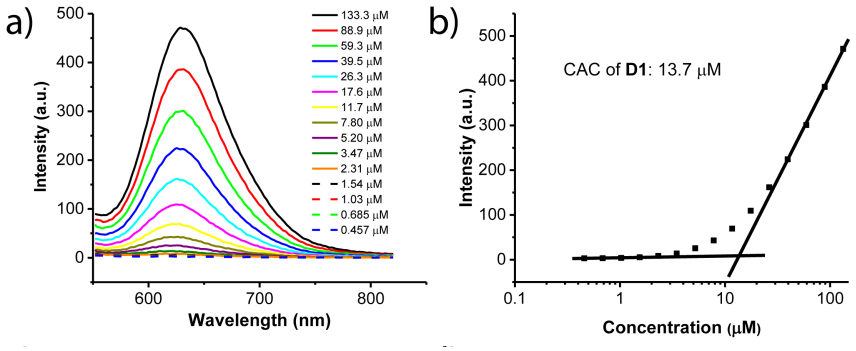


Figure S1. (a) Fluorescence of assembly **D1** for CAC measurement. (b) Curve for CAC calculation.

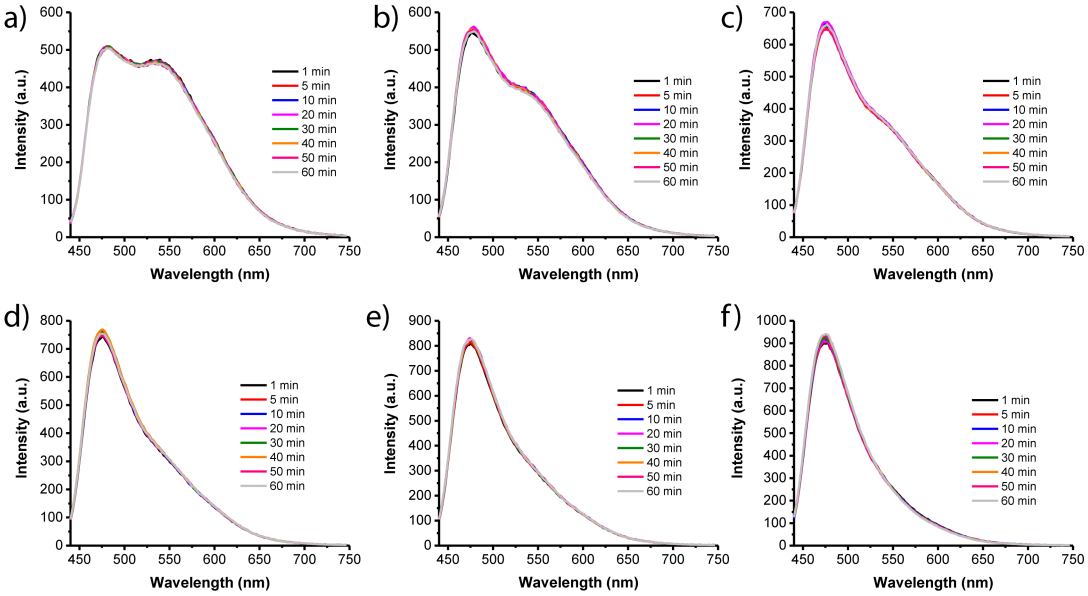


Figure S2. (a) Fluorescence of assembly **D2** (25 μM) in response to (a) no bCA, (b) 5 μM bCA, (c) 10 μM bCA, (d) 15 μM bCA, (e) 18.75 μM bCA, (f) 25 μM bCA.

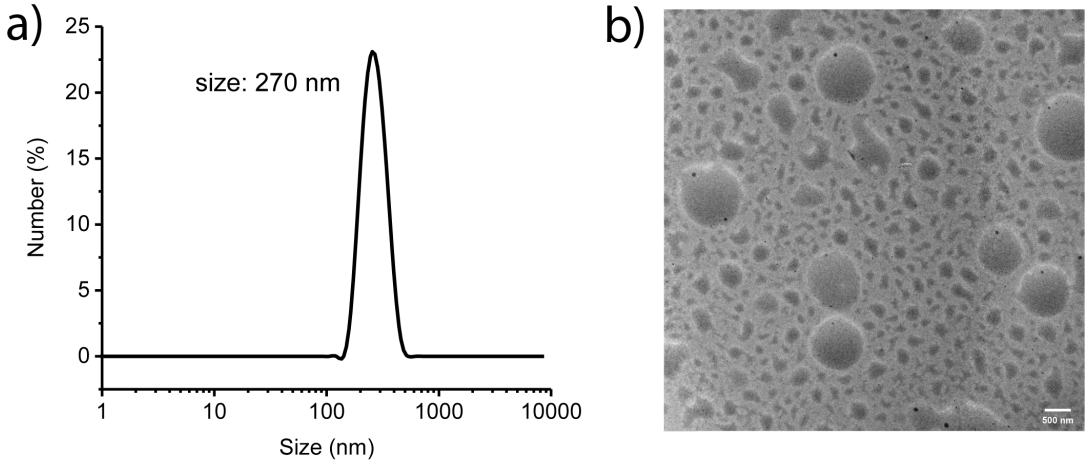


Figure S3. (a) Size distribution from DLS. (b) TEM of particle from **D2**.

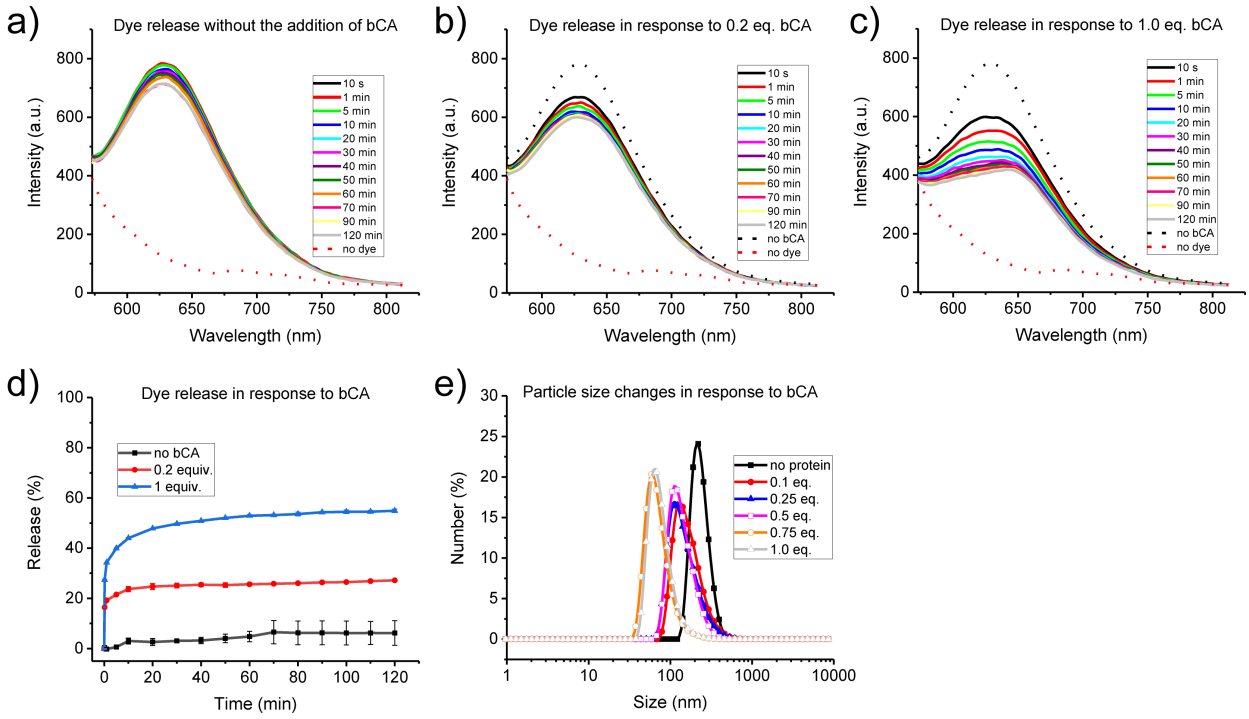


Figure S4. (a) Fluorescence of Nile Red without the addition of bCA. (b) Fluorescence of Nile Red in response to 0.2 equiv. of bCA. (c) Fluorescence of Nile Red in response to 1 equiv. of bCA. (d) Nile Red release in response to 0.2 equiv. and 1 equiv. of bCA. (e) Change of assembly sizes in response to different amount of bCA.

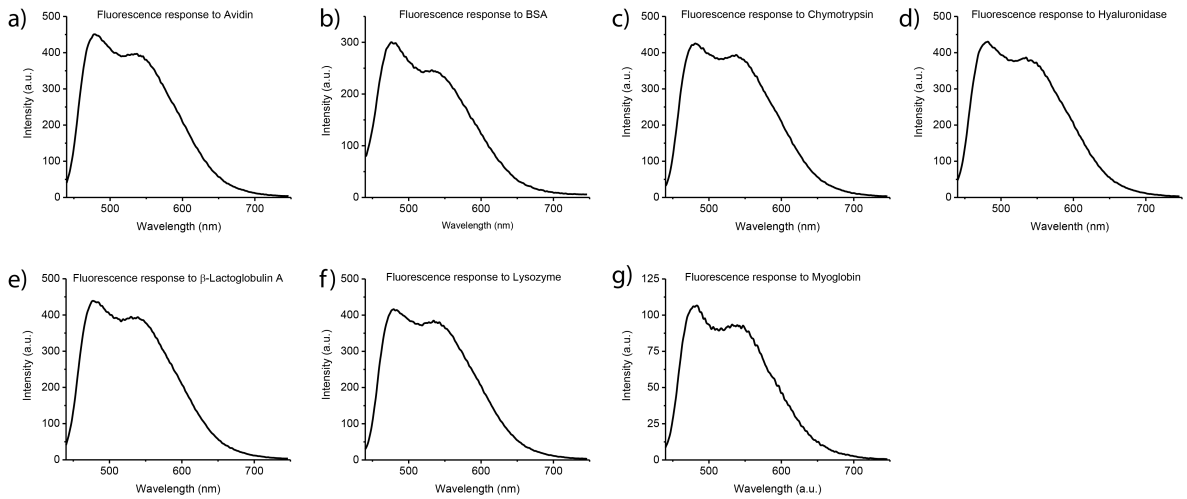


Figure S5. (a) Fluorescence of assembly (25 μM) in response to 25 μM protein (a) Avidin, (b) BSA, (c) Chymotrypsin, (d) Hyaluronidase, (e) β-Lactoglobulin A, (f) Lysozyme, (g) Myoglobin.

Table S1. Molecular weight and pI of proteins

Protein	Molecular weight (kDa)	pI
Avidin	66.0	10
BSA	66.3	4.8
Chymotrypsin	25.0	8.8
Hyaluronidase	55.0	5.5
β-Lactoglobulin A	18.4	5.1
Lysozyme	14.4	11.4
Myoglobin	16.7	7.2
bCA	30.0	6.4

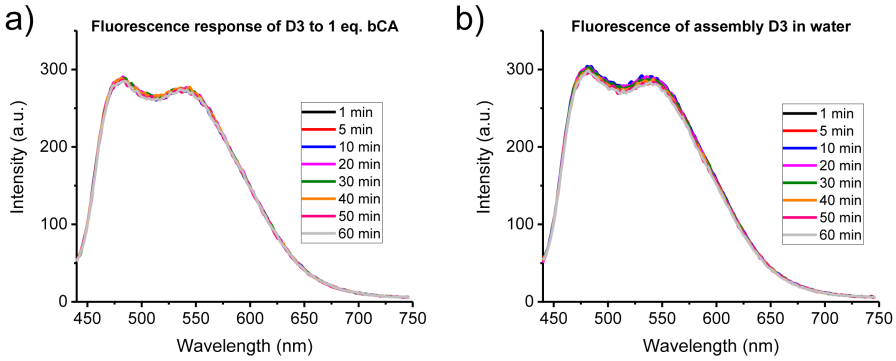


Figure S6. Fluorescence of assembly **D3** (25 μM) (a) in response to bCA, (b) in water.

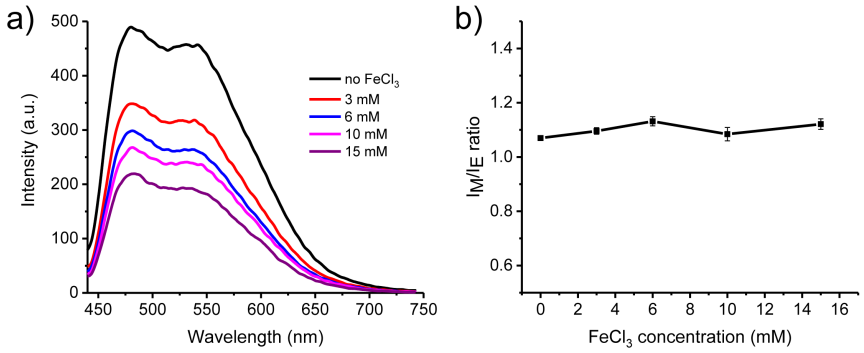


Figure S7. (a) Fluorescence and (b) I_M/I_E ratio of probe **D2** in different concentration of Fe^{3+}.

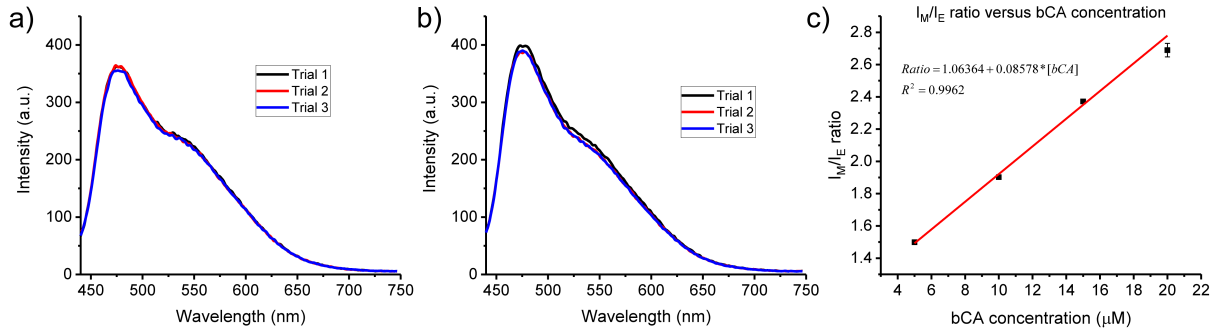


Figure S8. (a) Fluorescence of assembly **D2** in response to a mixture of chymotrypsin (5 μM) and bCA (5 μM). (b) Fluorescence of assembly **D2** in response to a mixture of chymotrypsin (7.5 μM), lysozyme (7.5 μM), β-lactoglobulin A (2.5 μM) and bCA (7.5 μM). (c) Standard calibration curve of I_M/I_E ratio to bCA concentration.

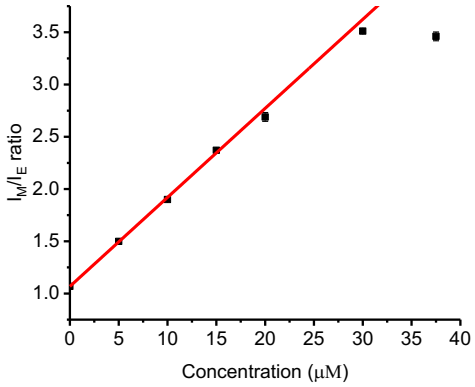
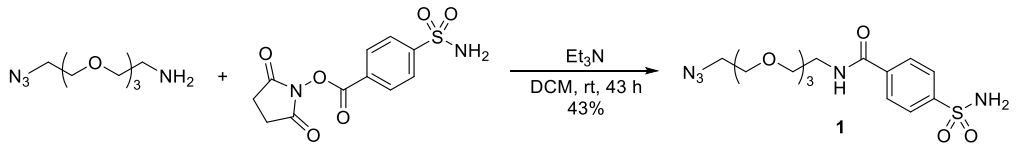
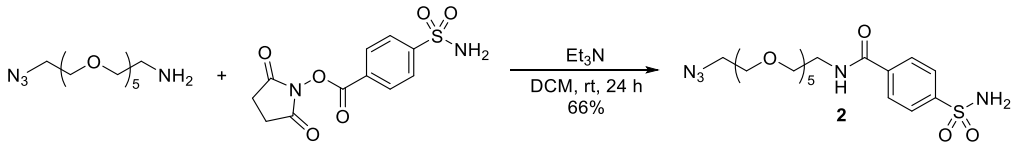


Figure S9. Test of concentration limit for linear relationship between bCA concentration and I_M/I_E.

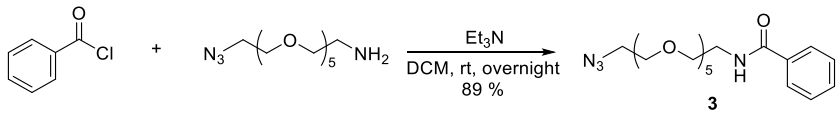
4. Synthetic Procedures of the Probes



Azido-PEG3-amine (804 mg, 3.68 mmol) and 4-Sulfamoylbenzoic acid *N*-hydroxysuccinimide ester (1.00 g, 3.35 mmol) were first mixed in 5 mL of dry DMF. Next, 560 μL Et_3N (4 mmol) was slowly added. The mixture was stirred at room temperature for 43 h until completion. The final solution was diluted with ethyl acetate (50 mL) and washed with brine (50 mL, twice) and 1M HCl (50 mL, twice). Then the organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel (0~30% ethyl acetate in hexane) to obtain the final product as colorless oil (580 mg, 43% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.37 (t, 2H, *J* = 5.02 Hz), 3.54-3.63 (m, 8H), 3.65-3.76 (m, 6H), 5.88 (s, 2H), 7.35 (t, 1H, *J* = 5.18 Hz), 7.80-7.87 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.1, 50.5, 69.8, 69.9, 70.30, 70.35, 70.4, 70.6, 126.3, 127.9, 138.1, 144.9, 166.3; ESI-MS m/z calcd for $C_{15}H_{23}N_5O_6S$ $[M + Na]^+$: 424.13; found: 424.19.

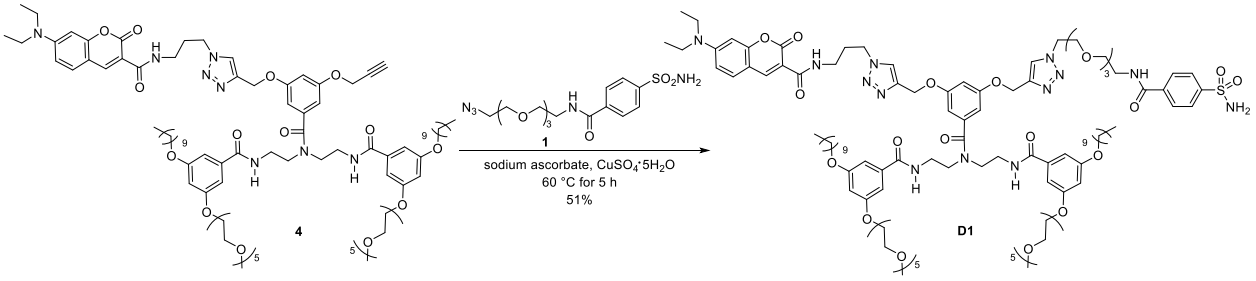


Azido-PEG5-amine (674 mg, 2.2 mmol) and 4-sulfamoylbenzoic acid *N*-hydroxysuccinimide ester (597 g, 2 mmol) were first mixed in 3 mL of dry DMF. Next, 335 μL of Et_3N (2.4 mmol) was slowly added. The mixture was stirred at room temperature for 24 h until completion. The final solution was diluted with ethyl acetate (50 mL) and washed with brine (50 mL, twice) and 1M HCl (50 mL, twice). Then the organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel (0~3.5% MeOH in dichloromethane) to obtain the final product as colorless oil (642 mg, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.33-3.45 (m, 6H), 3.50-3.54 (m, 4H), 3.56-3.72 (m, 13H), 3.74-3.79 (m, 2H), 6.17 (s, 2H),), 7.56 (t, 1H, J = 4.82 Hz), 7.75-7.84 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.3, 50.7, 69.6, 69.9, 70.1, 70.21, 70.23, 70.4, 70.45, 70.48, 70.6, 126.2, 127.9, 138.3, 145.0, 166.7; ESI-MS m/z calcd for $C_{19}H_{31}N_5O_8S$ $[M + Na]^+$: 512.18; found: 512.24.

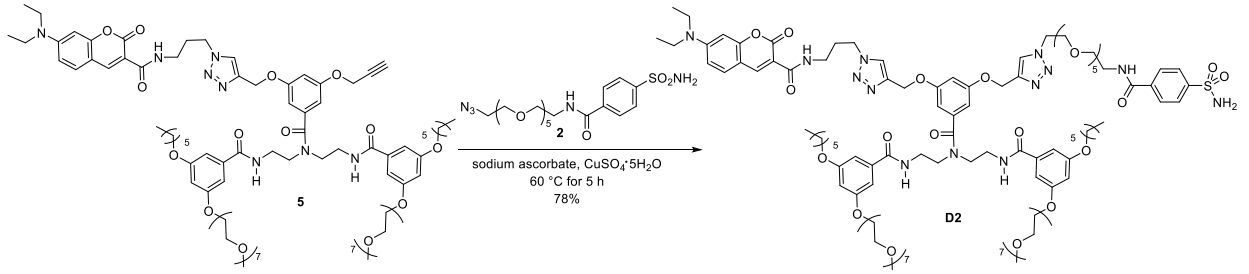


3

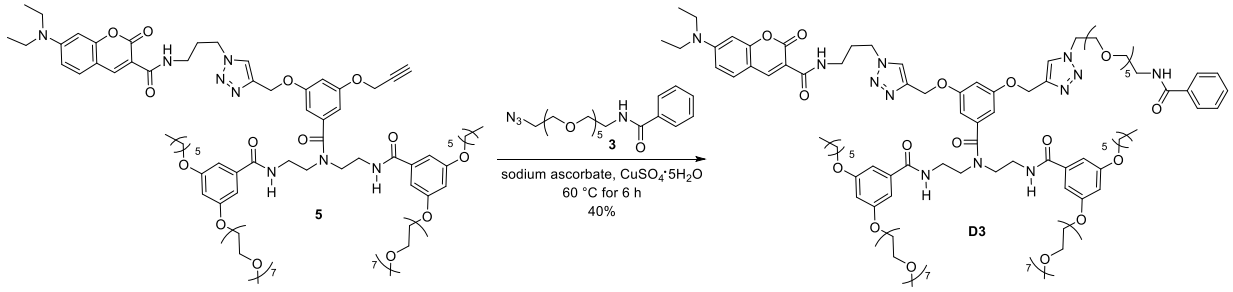
The compound **3** was synthesized as reported before.[2] 460 mg of azido-PEG5-amine (1.5 mmol) was first dissolved in 5 mL dry DCM. Next, 174 μL of benzoyl chloride (1.5 mmol) and 418 μL of Et_3N (3 mmol) were added subsequently. The mixture was stirred at room temperature overnight. Ethyl acetate was next added, and the solution was washed with brine. The resulted organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel. The final product was obtained as colorless oil in 89% yield (550 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.36 (t, 2H, J = 5.02 Hz), 3.60-3.69 (m, 22H), 7.03 (s, 1H), 7.40-7.52 (m, 3H), 7.80-7.85 (m, 2H). This molecule was then carried over to the synthesis of **D3**.



Compound **4** was synthesized as previously reported procedure and confirmed by NMR.[2] An aqueous solution (1 mL) of sodium ascorbate (4.90 mg, 0.025 mmol) and copper sulfate pentahydrate (6.1 mg, 0.025 mmol) was added into the THF solution (2 mL) of compound **1** (9.90 mg, 0.025 mmol) and **4** (20.7 mg, 0.012 mmol). The mixture was heated to 60 ℃ for 5 h and monitored to be completed. Then ethyl acetate was added, and the mixture was washed with brine. The organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product **D1** as a yellow oil (13 mg, 51% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84-0.90 (m, 6H), 1.21-1.31 (m, 30H), 1.34-1.42 (m, 4H), 1.65-1.75 (m, 8H), 2.15-2.26 (m, 2H), 3.35 (s, 6H), 3.42-3.69 (m, 48H), 3.72-3.95 (m, 14H), 4.06 (s, 4H), 4.35-4.52 (m, 4H), 4.83-5.00 (m, 4H), 6.24-6.37 (m, 2H), 6.43-6.55 (m, 5H), 6.65 (d, 1H, J = 7.72 Hz), 6.90-7.05 (m, 4H), 7.28-7.37 (m, 1H), 7.43 (d, 1H, J = 8.88 Hz), 7.60-7.72 (m, 2H), 7.76-7.87 (m, 5H), 8.67 (s, 1H), 8.94 (t, 1H, J = 5.20 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.5, 14.2, 22.7, 26.0, 29.2, 29.36, 29.44, 29.6, 29.7, 31.9, 36.4, 39.9, 45.2, 47.9, 50.4, 59.0, 61.5, 61.6, 67.6, 68.4, 69.3, 69.5, 69.6, 70.1, 70.4, 70.46, 70.53, 70.7, 71.9, 72.1, 96.6, 104.3, 104.9, 105.0, 105.3, 105.5, 105.58, 105.64, 106.0, 106.1, 108.4, 109.7, 110.1, 124.0, 124.6, 126.2, 127.9, 131.3, 136.1, 136.2, 137.9, 138.0, 143.15, 143.20, 145.5, 148.4, 152.8, 157.7, 159.15, 159.21, 159.8, 160.0, 160.4, 160.5, 162.8, 163.9, 166.4, 167.7, 167.8, 173.0; ESI-MS m/z calcd for $C_{105}H_{157}N_{13}O_{28}S$ $[M + Na]^+$: 2104.09; found: 2104.01.



Compound **5** was synthesized as previously reported procedure and confirmed by NMR.[2] An aqueous solution (1 mL) of sodium ascorbate (3.4 mg, 0.017 mmol) and copper sulfate pentahydrate (4.3 mg, 0.017 mmol) was added into the THF solution (1 mL) of compound **2** (8.4 mg, 0.017 mmol) and **5** (15 mg, 0.0086 mmol). The mixture was heated to 60 ℃ for 3h and monitored to be completed. Then EA was added, and the mixture was washed with brine. The organic phase was combined, dried with anhydrous Na_2SO_4, concentrated *in vacuo* and purified by flash column on silica gel to obtain the final product **D2** as a yellow oil (15.7 mg, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-0.90 (m, 6H), 1.20-1.32 (m, 14H), 1.35-1.42 (m, 4H), 1.65-1.76 (m, 4H), 2.14-2.25 (m, 2H), 3.35 (s, 6H), 3.41-3.54 (m, 20H), 3.56-3.68 (m, 52H), 3.74-3.94 (m, 12H), 4.04-4.11 (m, 4H), 4.43 (t, 2H, J = 6.76 Hz), 4.50 (t, 2H, J = 4.76 Hz), 4.98 (d, 3H, J = 9.12 Hz), 5.11 (s, 1H), 6.32 (s, 1H), 6.48-6.57 (m, 5H), 6.65 (dd, 1H, J = 2.32, 9.00 Hz), 6.94-7.03 (m, 4H), 7.39-7.49 (m, 2H), 7.67-7.76 (m, 1H), 7.82-7.93 (m, 3H), 8.67 (s, 1H), 8.94 (t, 1H, J = 5.98 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.4, 14.06, 14.14, 22.6, 22.7, 25.7, 29.1, 29.4, 29.7, 30.2, 30.6, 31.6, 31.9, 36.4, 40.0, 45.1, 47.9, 50.3, 56.0, 59.0, 61.6, 67.6, 68.3, 69.3, 69.56, 69.63, 70.1, 70.28, 70.34, 70.38, 70.43, 70.5, 70.6, 70.7, 71.9, 96.6, 104.3, 104.9, 105.0, 105.3, 105.5, 105.7, 105.9, 106.2, 108.4, 109.7, 110.1, 124.0, 124.8, 126.3, 128.0, 131.3, 136.1, 136.2, 137.9, 138.0, 143.2, 145.4, 148.3, 157.7, 159.21, 159.24, 159.8, 159.9, 160.3, 160.4, 162.8, 163.8, 167.6, 167.7, 173.0; ESI-MS m/z calcd for $C_{109}H_{165}N_{13}O_{34}S$ $[M + Na]^+$: 2256.12; found: 2256.03.



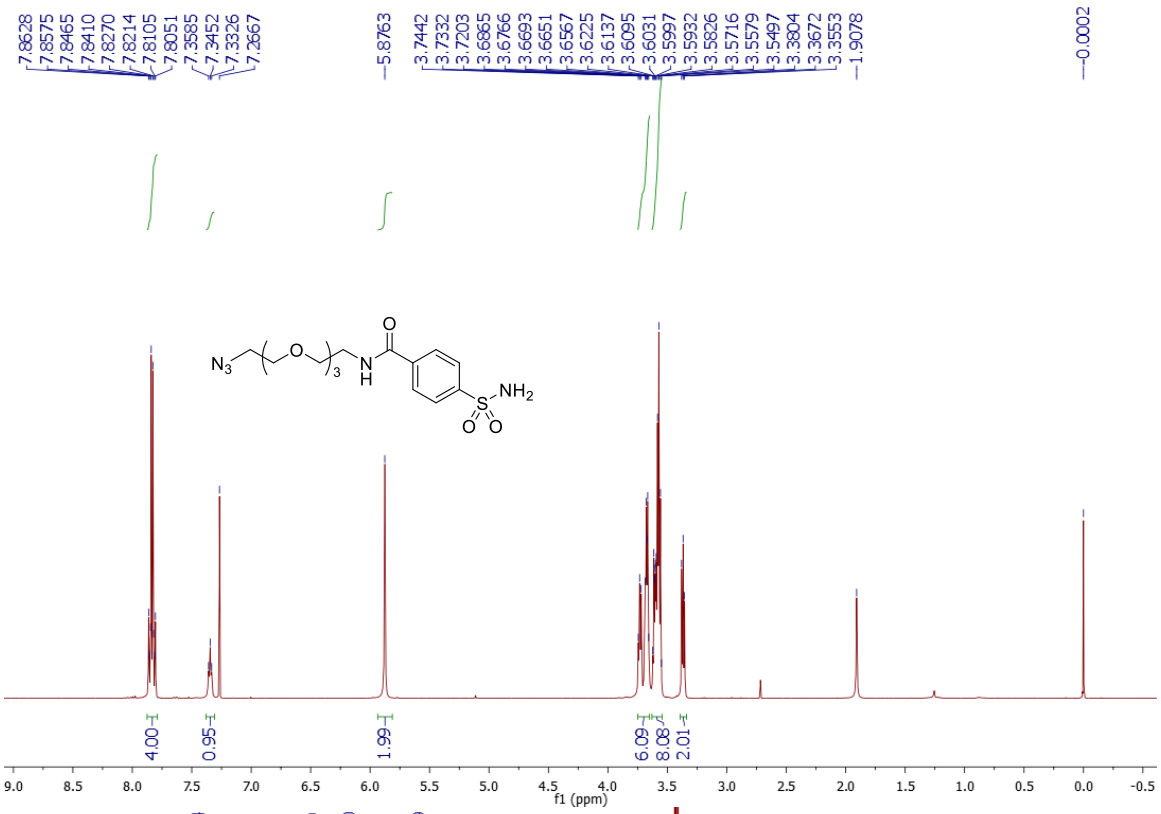
A mixture of sodium ascorbate (2.1 mg, 0.0103 mmol) and copper sulfate pentahydrate (2.6 mg, 0.0103 mmol) was dissolved into 1 mL of H_2O and added into 2 mL of the THF solution of compound **5** (9 mg, 0.0052 mmol) and **3** (4.2 mg, 0.0103 mmol). The solution was then stirred at 60 ℃ in a preheated oil bath for 2 h until completion. EA was next added, and the solution was washed with brine. The organic phase was combined, dried with Na_2SO_4, concentrated *in vacuo* and purified by flash column chromatography on silica gel. The product **D3** was obtained as a yellow oil (4.1 mg, 37% yield). NMR was consistent with previous report.[2] ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86-0.90 (m, 6H), 1.21-1.33 (m, 14H), 1.36-1.42 (m, 4H), 1.65-1.78 (m, 4H), 2.16-2.26 (m, 2H), 3.34-3.38 (m, 6H), 3.41-3.49 (m, 6H), 3.51-3.55 (m, 12H), 3.58-3.70 (m, 56H), 3.75-3.95 (m, 12H), 4.08 (s, 4H), 4.43 (t, 2H, J = 6.74 Hz), 4.50 (t, 2H, J = 4.84 Hz), 5.00-5.13 (m, 4H), 6.44-6.58 (m, 5H), 6.65 (dd, 1H, J = 2.06, 8.92 Hz), 6.95-7.04 (m, 4H), 7.07 (s, 1H), 7.36-7.49 (m, 4H), 7.73 (s, 1H), 7.78-7.84 (m, 3H), 8.66 (s, 1H), 8.93 (t, 1H, J = 5.86 Hz).

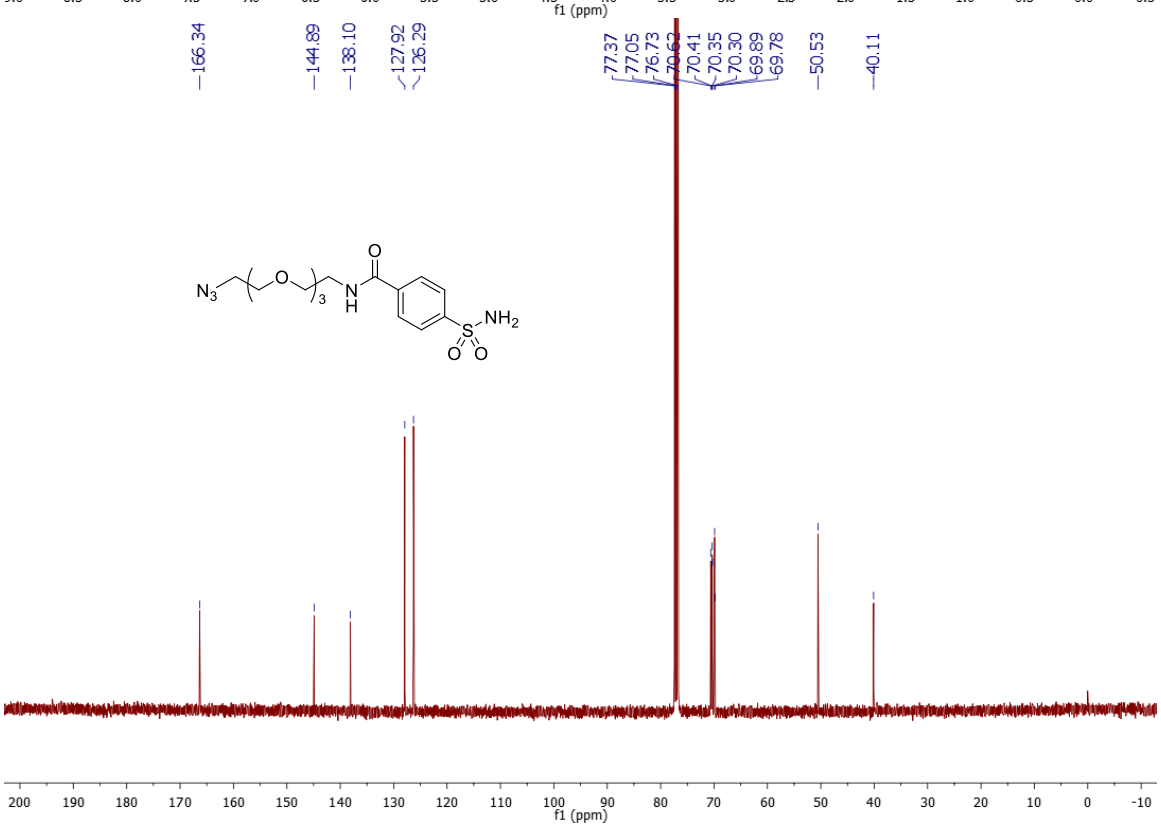
5. References

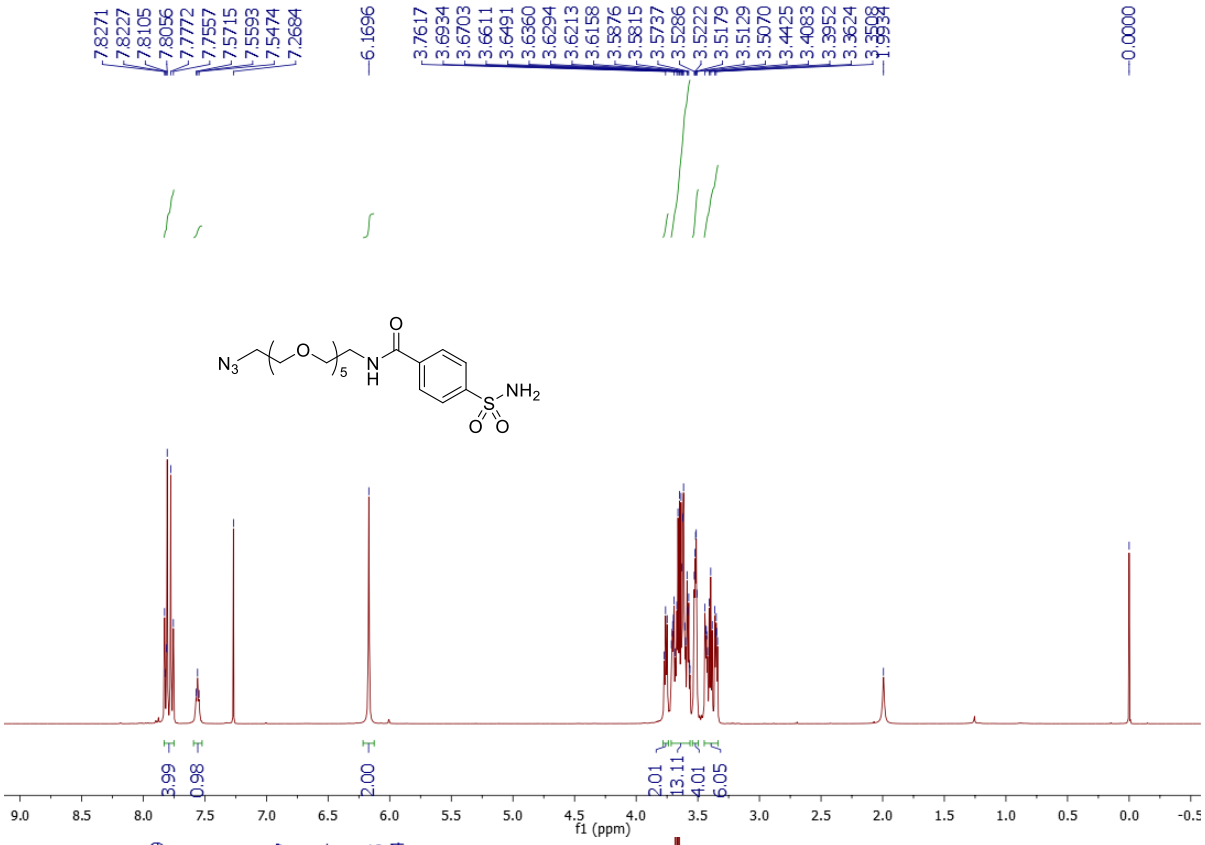
1 Z. Jiang, H. Liu, H. He, A. E. Ribbe and S. Thayumanavan, *Macromolecules*, 2020, **53**, 2713–2723.

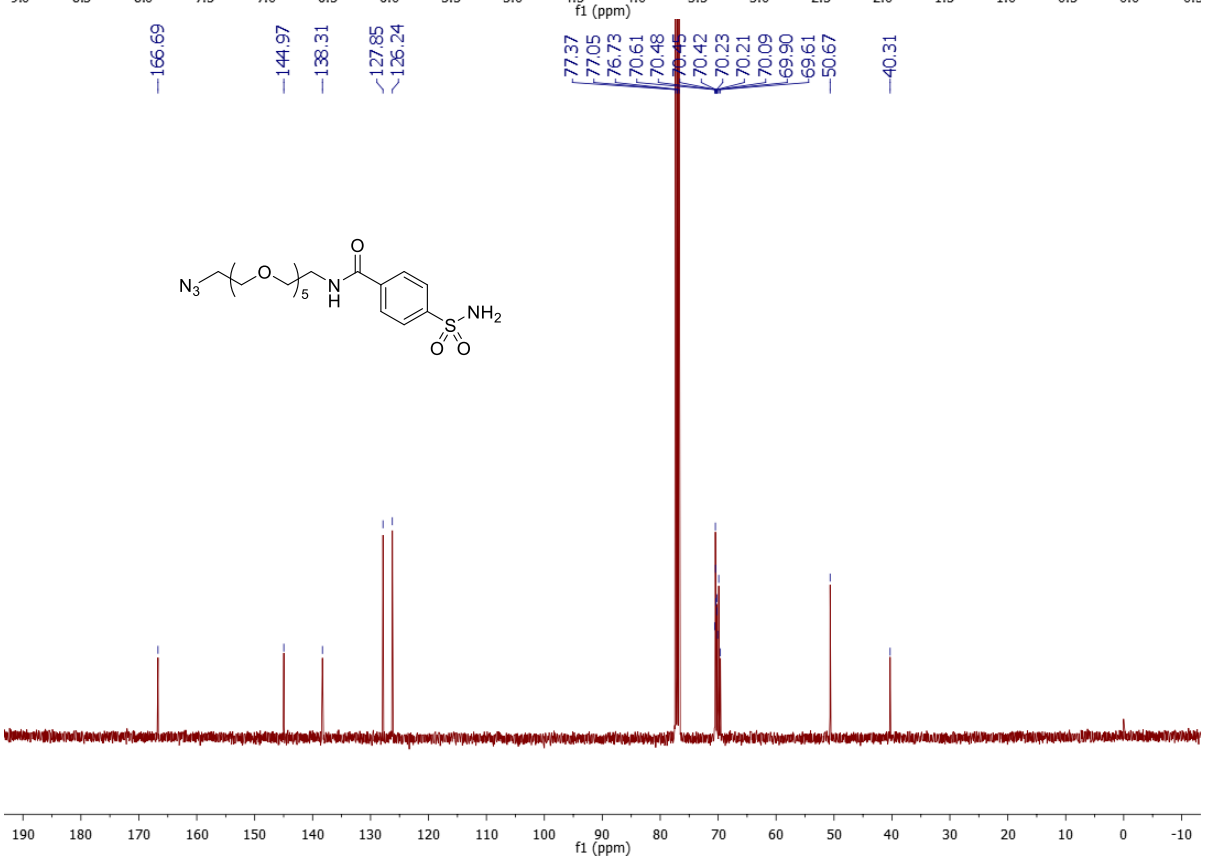
2 H. Liu, C. Lionello, J. Westley, A. Cardellini, U. Huynh, G. M. Pavan and S. Thayumanavan, *Nanoscale*, 2021, **13**, 11568–11575.

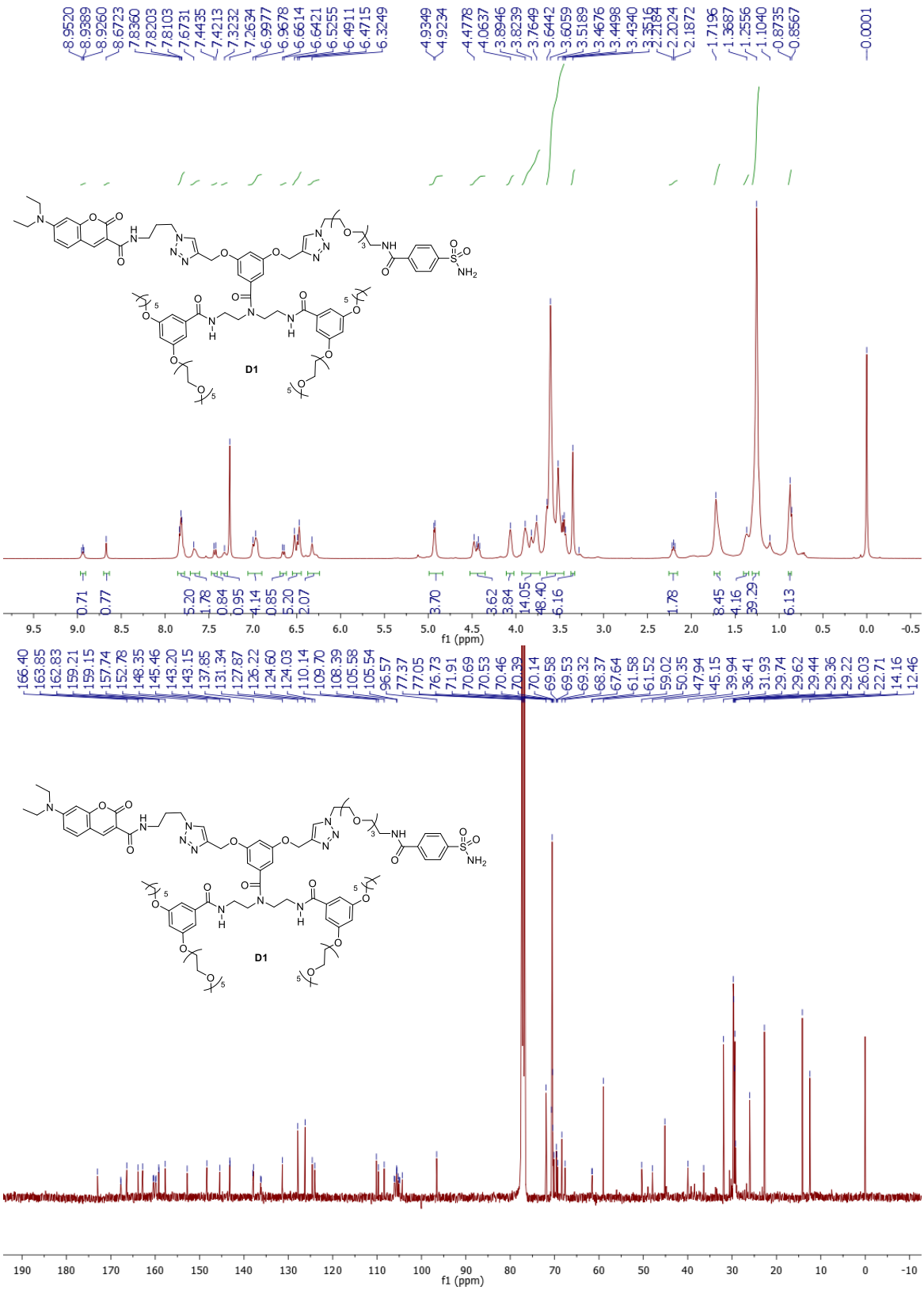
6. NMR Spectra

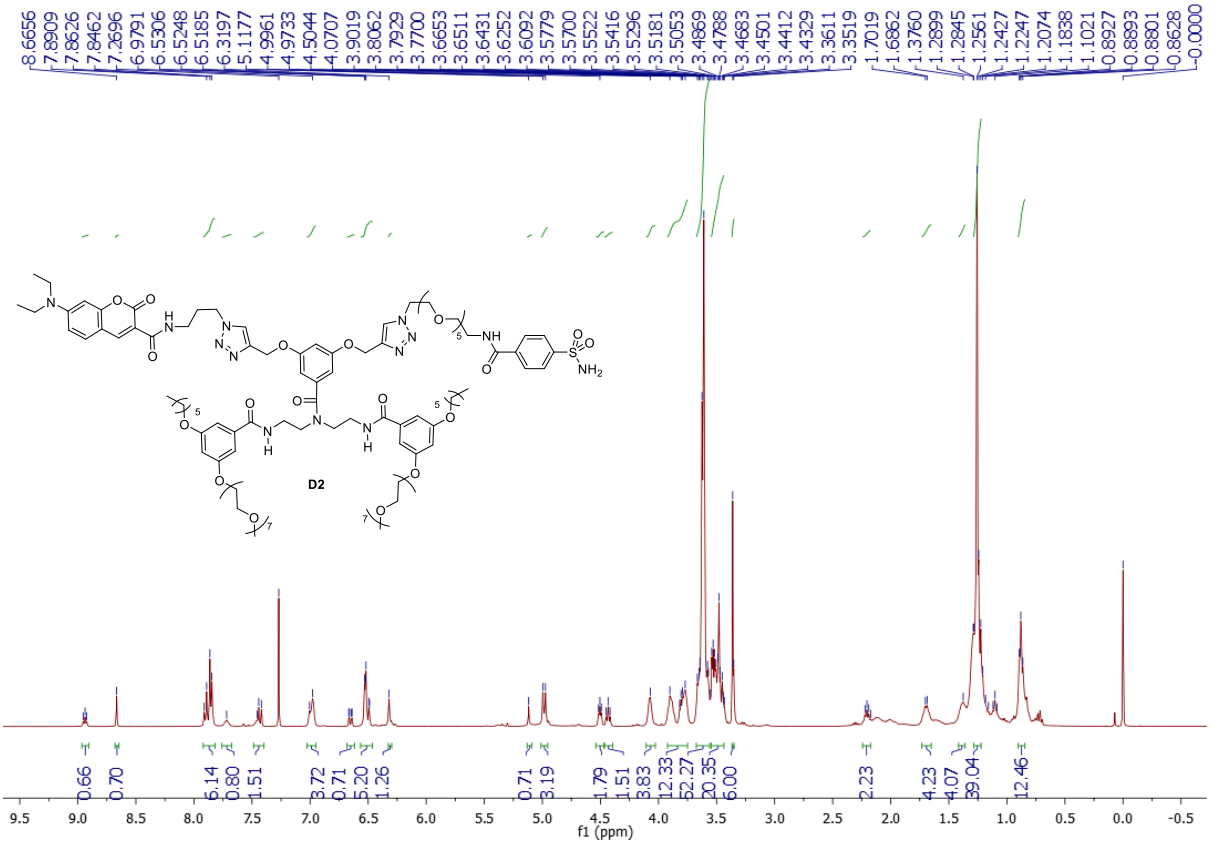


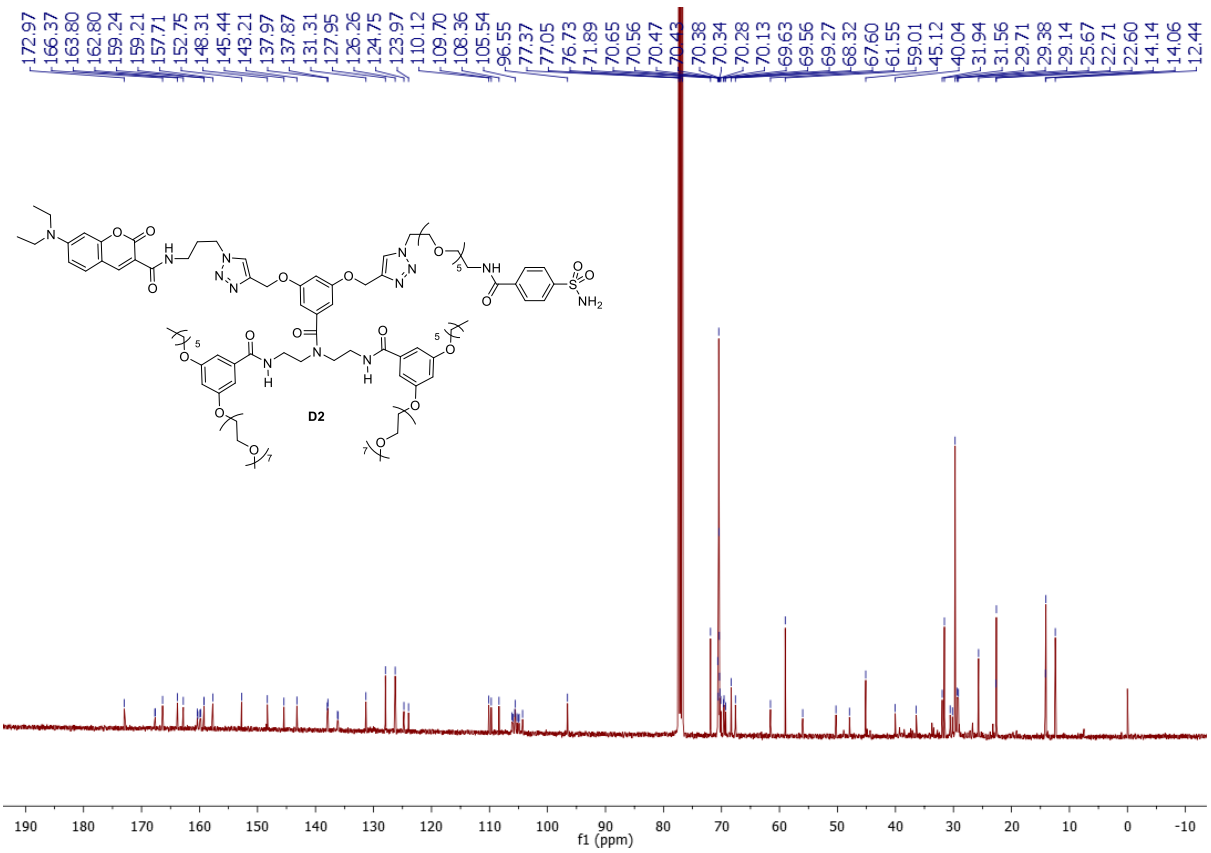



7.8271
7.8227
7.8105
7.8056
7.7772
7.7557
7.5715
7.5593
7.5474
7.2684
6.1696
3.7617
3.6934
3.6703
3.6611
3.6491
3.6360
3.6294
3.6213
3.6158
3.5876
3.5737
3.5286
3.5222
3.5179
3.5129
3.5070
3.4425
3.4083
3.3952
3.3624
1.9934
0.0000
3.99
0.98
2.00
2.01
13.11
4.01
6.05
f1 (ppm)


166.69
144.97
138.31
127.85
126.24
77.37
77.05
76.73
70.61
70.48
70.45
70.42
70.23
70.21
70.09
69.90
69.61
50.67
40.31
f1 (ppm)


D1
f1 (ppm)
D1
f1 (ppm)


D2
f1 (ppm)


D2
f1 (ppm)