Electronic Supplementary Information

High affinity heparin detection by multivalent supramolecular polymers through aggregation induced emission

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SUPPORTING INFORMATION

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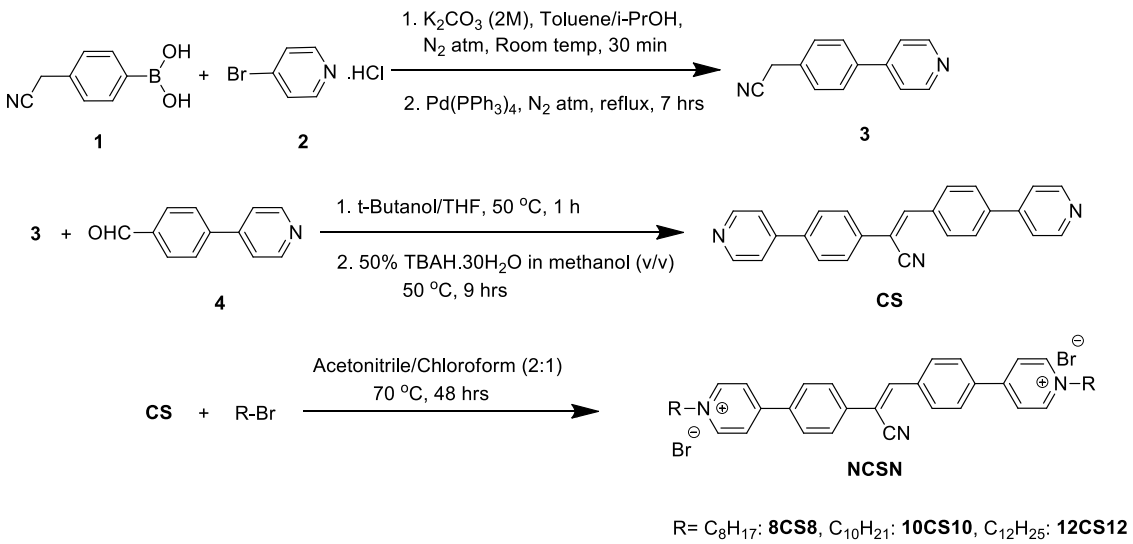
Experimental Procedures

1. Materials and Method:

All reagents were purchased from commercially available sources and used without further purification. 4-(pyridin-4-yl)benzaldehyde, (4-(cyanomethyl)phenyl)boronic acid and 4-bromopyridine hydrochloride were purchased from Combi-Blocks. Tris(hydroxymethyl)aminomethane, chondroitin sulfate sodium salt from shark cartilage, hyaluronic acid sodium salt from Streptococcus equi, protamine sulfate salt from salmon, tetrabutylammonium hydroxide 30-hydrate, 1-bromooctane, 1-bromodecane, 1-bromododecane, and tert-butanol were purchased from Sigma-Aldrich. Heparin sodium salt from hog intestine was purchased from TCI chemicals. Heparin sodium injection I.P. (1000 U/mL) was purchased from Biological E. Limited. Human serum was purchased from commercial source (Sigma-Aldrich) as well as extracted from the blood of a group of healthy males of age group 22 to 30. Citrated human plasma was extracted from the blood of a group of healthy males of age group 22 to 30. UV-Vis spectroscopic measurements were carried out in Agilent Technologies Cary 8454 spectrophotometer. Emission spectroscopic measurements were carried out in Horiba Fluoromax 4 spectrofluorometer. Absolute quantum yields were measure using Quanta-phi integrating sphere fitted with a Horiba Fluoromax 4 spectrofluorometer. Fluorescence images were taken under 365 nm UV lamp. DLS and zeta potential measurements were carried out using Malvern Zetasizer NanoZS. A Horiba Jobin Yvon Fluorocube instrument fitted with a 340 nm diode laser excitation source (with a temporal resolution of 70 ps) was used for the time-resolved fluorescence experiments applying the time correlated single photon counting (TCSPC) method. ^{1}H and ^{13}C NMR were performed on Jeol 400 MHz and Bruker 500 MHz spectrometers. Mass spectra were recorded in a Bruker mass spectrometer. TEM images were recorded in a JEOL JEM2100 PLUS instrument. FESEM images were recorded in a ZEISS instrument.

2. Synthetic Schemes and Characterization Data:



Scheme S1. Synthetic route for the preparation of **NCSN**s

A. Procedure for synthesis of CS: 2-(4-(pyridin-4-yl)phenyl)acetonitrile (**3**) was first synthesized from (4-(cyanomethyl)phenyl)boronic acid (**1**) and 4-bromopyridine hydrochloride (**2**) according to a literature procedure.[1] Then, (Z)-2,3-bis(4-(pyridin-4-yl)phenyl)acrylonitrile (**CS**) was prepared by a slight modification of the reported procedure.[1] 2-(4-(pyridin-4-yl)phenyl)acetonitrile (**3**) (360 mg, 1.86 mmol) and 4-(pyridin-4-yl)benzaldehyde (**4**) (340 mg, 1.86 mmol) were taken in a 10 mL round bottom flask and dissolved in 6 mL *tert*-butanol and 0.30 mL tetrahydrofuran mixture. The reaction mixture was stirred for 1 hour at 50 °C. 0.8 mL of 50% tetrabutylammonium hydroxide.30 H_2O (TBAH.30H_2O) in methanol (V/V) was then added dropwise over a period of 15 min at 50 ^{0}C until the reaction mixture assumed a maroon color. Stirring was continued for 9 more hours at 50 °C and then the reaction mixture was cooled to room temperature. The pale yellowish white precipitate formed was then filtered and washed with *tert*-butanol for 3-4 times and finally dried under vacuum (340 mg, 0.93 mmol). Yield = 50%. **^{1}H NMR (400 MHz, $CDCl_3$):** δ (ppm) = 8.71 (m, 4H), 8.05 (d, J = 8 Hz, 2H), 7.84 (d, J = 8 Hz, 2H), 7.76 (t, J = 8 Hz, 4H), 7.66 (s, 1H), 7.56 (m, 4H).

B. General procedure for the synthesis of NCSN (N = 8, 10, 12): CS (55 mg, 1.5 mmol) and corresponding alkyl halide (30 mmol) were taken in a 10 mL reaction tube and dissolved in 2 mL

acetonitrile and 1 mL chloroform solvent mixture. The reaction mixture was stirred at 70 ^{o}C under sealed condition for 48 hours and then allowed to cool at room temperature. The yellow precipitate formed was filtered and washed with cold chloroform for 3-4 times and finally dried under vacuum.

8CS8: From 55 mg of **CS,** 100 mg (0.134 mmol) of **8CS8** was obtained as a bright yellow powder. Yield = 89%.

^{1}H NMR (400 MHz, DMSO-D$_6$): δ(ppm) = 9.19 (t, J = 8 Hz, 4H), 8.63 (d, J = 8 Hz, 4H), 8.45 (s, 1H), 8.31 (t, J = 8 Hz, 4H), 8.24 (d, J = 8 Hz, 2H), 8.09 (d, J = 8 Hz, 2H), 4.61 (q, J= 8 Hz, 4H), 1.96 (br s, 4H), 1.31-1.26 (br s, 20H), 0.88 (t, J = 8 Hz, 6H)

^{13}C NMR (125 MHz, DMSO-D$_6$): δ(ppm) = 153.34, 153.31, 144.91, 144.86, 143.26, 136.72, 130.34, 129.02, 128.75, 127.05, 124.67, 124.58, 117.30, 111.26, 60.05, 60, 31.11, 30.66, 30.63, 28.43, 28.35, 25.43, 22.01, 13.90.

HRMS (ESI): m/z calculated for $C_{41}H_{51}N_3^{2+}$: 292.7036; found: 292.7036.

10CS10: From 55 mg of **CS,** 107 mg (0.133 mmol) of **10CS10** was obtained as a bright yellow powder. Yield = 89%.

^{1}H NMR (400 MHz, DMSO-D$_6$): δ(ppm) = 9.19 (t, J = 8 Hz, 4H), 8.63 (d, J = 8 Hz, 4H), 8.45 (s, 1H), 8.31 (t, J = 8 Hz, 4H), 8.24 (d, J = 8 Hz, 2H), 8.09 (d, J = 8 Hz, 2H), 4.61 (q, J= 8 Hz, 4H), 1.95 (m, 4H), 1.31-1.25 (br s, 28H), 0.85 (t, J = 8 Hz, 6H).

^{13}C NMR (125 MHz, DMSO-D$_6$): δ(ppm) = 153.35, 153.32, 144.91, 144.86, 143.25, 136.72, 130.33, 129.01, 128.74, 127.05, 124.66, 124.57, 117.19, 111.31, 60.08, 31.25, 28.96, 28.87, 28.75, 28.66, 28.36, 25.40, 22.05, 13.91.

HRMS (ESI): m/z calculated for $C_{45}H_{59}N_3^{2+}$: 320.7349; found: 320.7340.

12CS12: From 55 mg of **CS,** 90 mg (0.105 mmol) of **12CS12** was obtained as a bright yellow powder. Yield = 70%.

^{1}H NMR (400 MHz, DMSO-D$_6$): δ(ppm) = 9.17 (t, J = 8 Hz, 4H), 8.62 (d, J = 8 Hz, 4H), 8.44 (s, 1H), 8.31 (t, J = 8 Hz, 4H), 8.23 (d, J = 8 Hz, 2H), 8.08 (d, J = 8 Hz, 2H), 4.60 (q, J= 8 Hz, 4H), 1.95 (br s, 4H), 1.31-1.23 (br s, 36H), 0.85 (t, J = 8 Hz, 6H).

^{13}C NMR (125 MHz, DMSO-D$_6$): δ(ppm) = 153.34, 153.31, 144.92, 144.87, 143.26, 136.72, 130.34, 129.02, 128.75, 127.06, 124.67, 124.57, 117.30, 111.27, 60.06, 60.01, 31.24, 30.65, 28.84, 28.76, 28.61, 28.38, 25.42, 22.06, 13.92.

HRMS (ESI): m/z calculated for $C_{49}H_{67}N_3^{2+}$: 348.7662; found: 348.7662.

3. Preparation of Solutions:

A. Preparation of solution of NCSNs:

Initially, stock solutions of **NCSN**s (N = 8, 10, 12) were prepared by dissolving the solid powders in spectroscopic grade dimethyl sulfoxide (DMSO). These concentrated DMSO solutions were then diluted to 5 mM tris-HCl buffer made on Milli-Q water to get desired solutions having 1% DMSO as the final DMSO fraction. Solutions of **8CS8**, **10CS10** and **12CS12** in aqueous buffer were then equilibrated for 1.5 h.

B. Preparation of heparin solution:

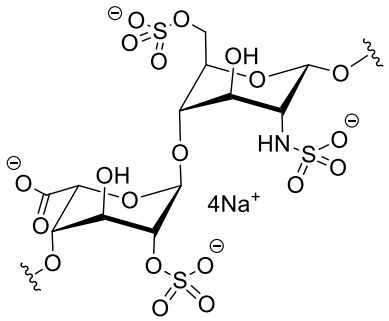


Fig. S1 A common repeat unit of heparin chain

The disaccharide unit shown in Fig. S1 is taken as the repeat unit of heparin for the molecular weight calculation. Though the supplied heparin contains only 30-40% materials with the active sequence of repeat units, the whole sample can still bind through the anionic polysaccharide unit irrespective of whether the repeat units are in active sequence or not. The molecular weight of the repeat unit is 665.40 g/mole. Heparin stock solutions were prepared in buffer and further diluted during titration.

C. Preparation of solution of other analytes:

Stock solutions of chondroitin-4-sulfate (**ChS**) and hyaluronic acid (**HA**) were prepared as 1.33 mg/mL in working buffer (2.7 mM for **ChS** and 3.3 mM for **HA**) and further diluted accordingly

during titration. Stock solution of protamine sulfate (**PS**) was prepared in buffer using its molecular weight as 4500 Daltons and further diluted during titration.

D. **Extraction of human serum:**

Blood samples were collected from a group of healthy males of age group 22-30 and then kept at room temperature for 1 hr. The samples were then centrifuged at 1500 rpm for 15 min at room temperature. The supernatant was collected and again centrifuged at 2500 rpm for 15 min at room temperature. Finally, the serum was collected and incubated at 4 ^{o}C overnight and stored at -20 ^{o}C.

E. Extraction of human plasma:

Blood samples from a group of healthy males (age group 22-30) were collected in collection tubes containing 3.8% sodium citrate solution (9 : 1 v/v blood : sodium citrate). The citrated samples were then centrifuged for 15 mins at 2500 rpm at 4 ^{o}C. The supernatant (platelet-free citrated plasma) was collected and stored at -20 ^{o}C.

4. Titration Procedures:

During the fluorometric titration, the addition of any of the analytes (heparin, chondroitin-4-sulfate, hyaluronic acid or protamine sulfate) was every time performed to a freshly equilibrated buffered solution of **NCSN**s to avoid any photoreaction of the cyanostilbene unit. After each addition of any analyte, 10 min equilibration time was given and then the spectrum was recorded.

Titration in buffer containing 25% human serum: To a 1.98 mL solution of sensor (**10CS10**, 25.25 µM) in buffer containing 25% human serum, 20 µL of heparin stocks in 100% human serum was added during each titration so that the final concentration of sensor (**10CS10**) became 25 µM.

Titration in buffer containing 50% human serum: To a 1.98 mL solution of sensor (**8CS8**, 45.45 µM) in buffer containing 50% human serum, 20 µL of heparin stocks in 100% human serum was added during each titration so that the final concentration of sensor (**8CS8**) became 45 µM.

Titration in buffer containing 50% human plasma: To a 1.98 mL solution of sensor (**8CS8**, 45.45 µM) in buffer containing 50% human plasma, 20 µL of heparin stocks in 100% human

plasma was added during each titration so that the final concentration of sensor (**8CS8**) became 45 μM.

Titration in buffer containing 75% human plasma: To a 1.98 mL solution of sensor (**8CS8**, 45.45 μM) in buffer containing 75% human plasma, 20 μL of heparin stocks in 100% human plasma was added during each titration so that the final concentration of sensor (**8CS8**) became 45 μM.

5. Quantum Yield Calculation Method:

Absolute quantum yield measurement of aggregated solutions:

Absolute quantum yields of **8CS8** (10 μM), **10CS10** (5 μM) and **12CS12** (5 μM) without and with heparin in aqueous buffer were measured using integrating sphere.

6. Activated Partial Thromboplastin Time (aPTT) Test Procedure:

50 mg of silica (100-200 mesh) and mono-n-dodecyl phosphate (0.4mg/mL stock, incubated at 37 ^{o}C for at least 2 min) were taken in a 2 mL glass vial. 0.1 mL of plasma or heparin containing plasma (incubated at 37 ^{o}C for 2 min) was added to it and after a gentle stirring the vial was kept at 37 ^{o}C for exactly 5 min. Then aqueous $CaCl_2$ solution (0.025 M, incubated at 37 ^{o}C for at least 2 min) was added to it and the vial was kept at 37 ^{o}C for 20 sec. Then in every 10 sec, clot formation was observed by gently tilting the vial. Clot formation time was noted down.

The above mentioned procedure was done using our lab heparin and a standard heparin solution. Subsequently, aPTT ratios were plotted against concentration of lab heparin or activity of standard heparin. From the calibration curve of standard heparin, activity (U/mL) of our lab heparin of different concentrations were calculated.

Results and Discussion

1. Transmission Electron Microscopic (TEM) Images:

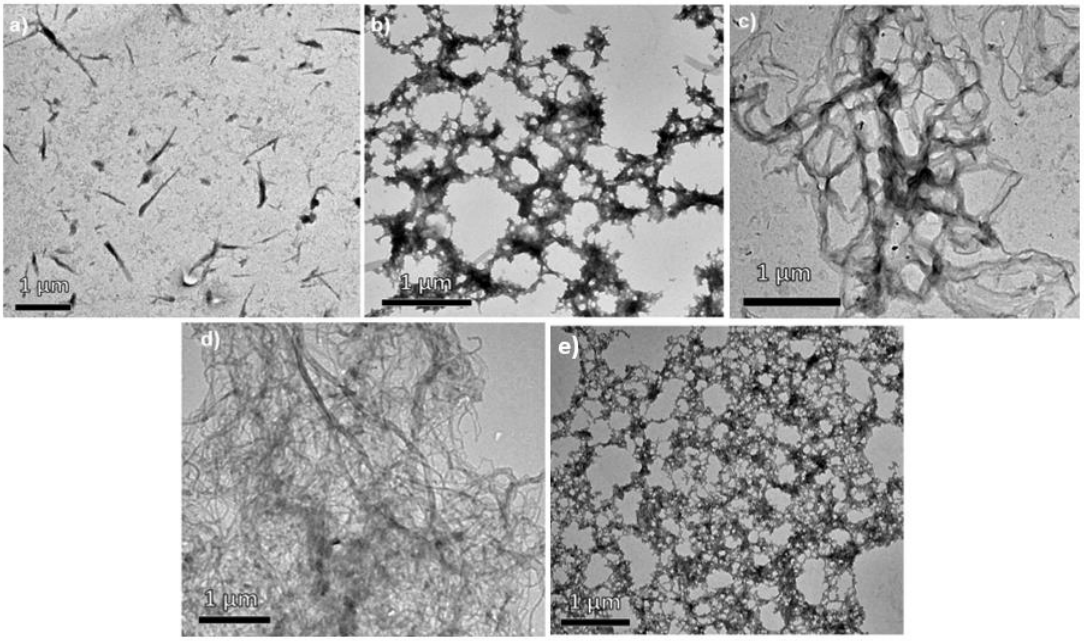


Fig. S2 TEM images of **8CS8** (10 µM) (a) without and (b) with 5 µM heparin (0.1 % uranyl acetate stain). TEM images of **10CS10** (5 µM) (c) without and (d) with 5 µM heparin. (e) TEM image of 12CS12 (2 µM) after 5 µM heparin addition (0.1 % uranyl acetate stain).

2. Heparin Sensing in Buffer:

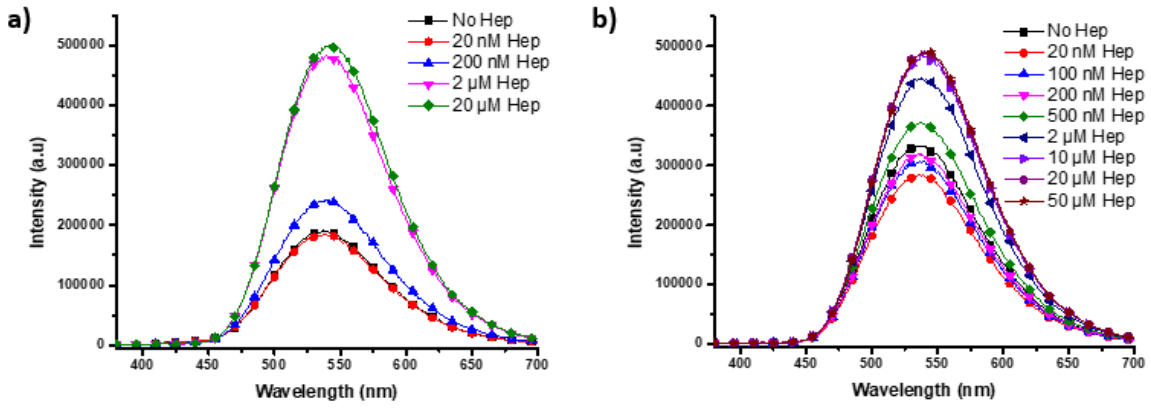


Fig. S3 Emission spectral changes of (a) **10CS10** (5 µM) and (b) **12CS12** (5 µM) upon addition of heparin in aqueous buffer (5 mM tris-HCl, 99:1 water/DMSO, pH 7.4).

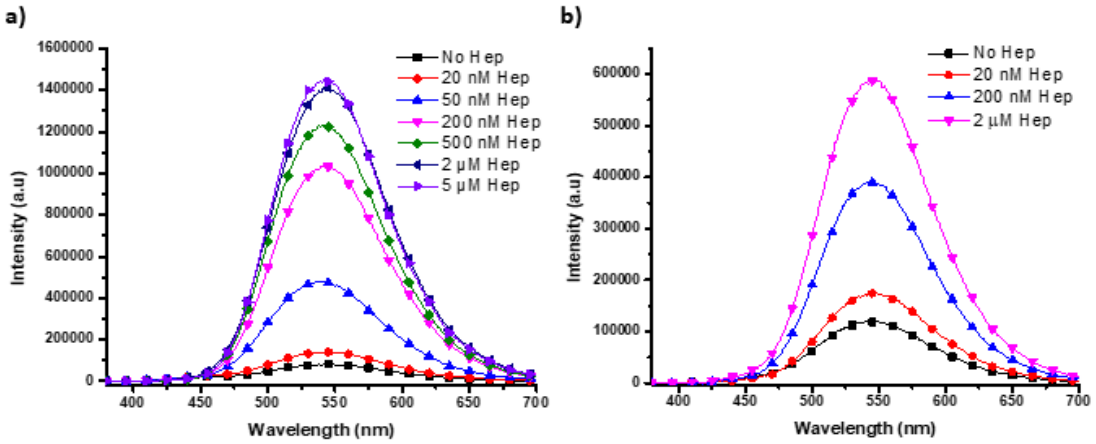


Fig. S4 Emission spectral changes of (a) **10CS10** (0.5 µM) and (b) **12CS12** (0.25 µM) upon addition of heparin in aqueous buffer (5 mM tris-HCl, 99:1 water/DMSO, pH 7.4).

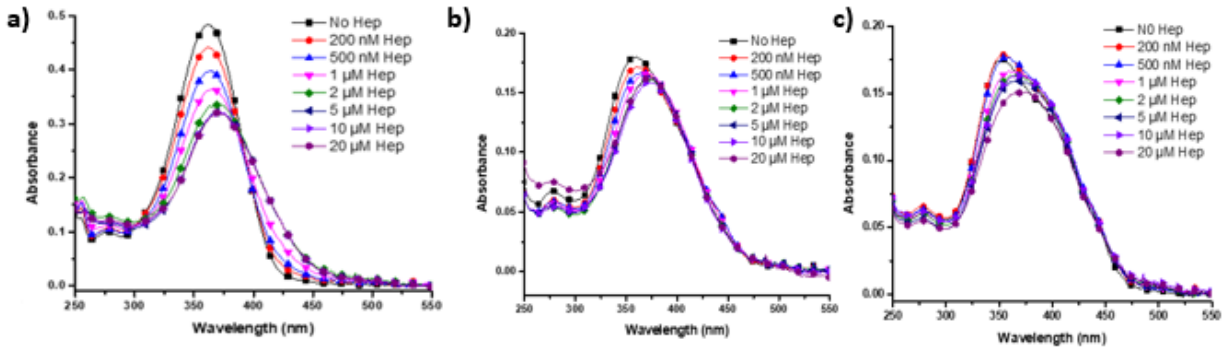


Fig. S5 Absorption spectral changes of (a) **8CS8** (10 µM), (b) **10CS10** (5 µM) and (c) **12CS12** (5 µM) upon addition of heparin in aqueous buffer (5 mM tris-HCl, 99:1 water/DMSO, pH 7.4).

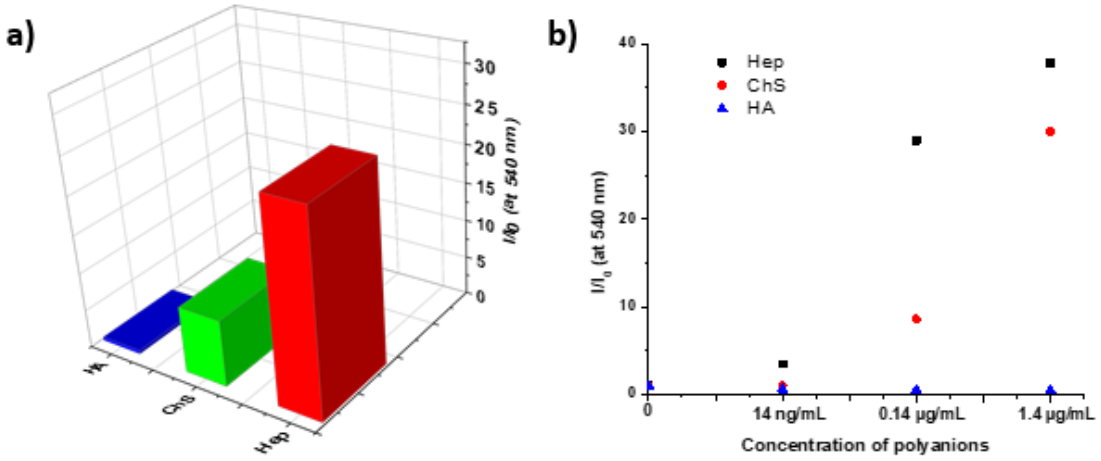


Fig. S6 (a) Comparative bar diagram of I/I_0 values of **8CS8** (0.5 µM) in aqueous buffer upon addition of heparin (**Hep**) (200 nM), chondroitin-4-sulfate (**ChS**) (272 nM) and hyaluronic acid (**HA**) (330 nM), separately. (b) Relative increment in the emission intensity upon addition of different amounts of heparin (**Hep**), chondroitin-4-sulfate (**ChS**) and hyaluronic acid (**HA**), to **8CS8** (0.5 µM) in aqueous buffer.

3. Heparin Sensing in Human Serum:

A. Heparin Sensing in 5% Human Serum:

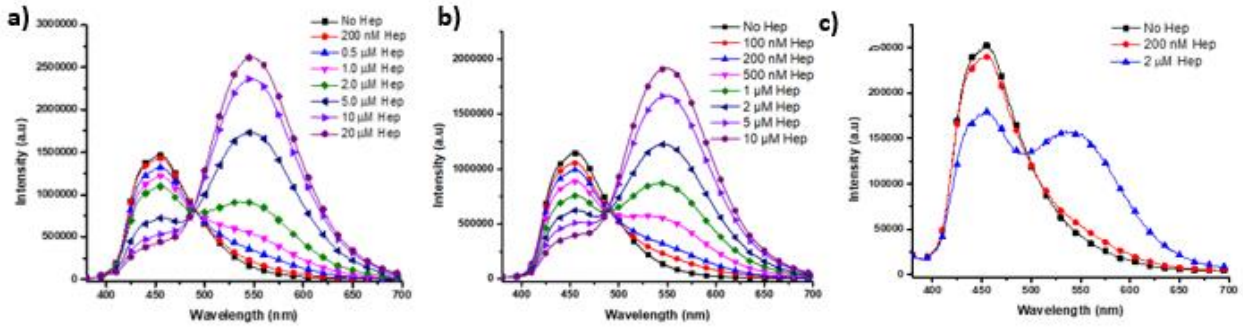


Fig. S7 Emission spectral changes of (a) **8CS8** (10 µM), (b) **10CS10** (4 µM) and (b) **12CS12** (1 µM) upon addition of heparin in aqueous buffer (5 mM tris-HCl, 99:1 water/DMSO, pH 7.4) containing 5% human serum.

B. Heparin Sensing in 25% Human Serum:

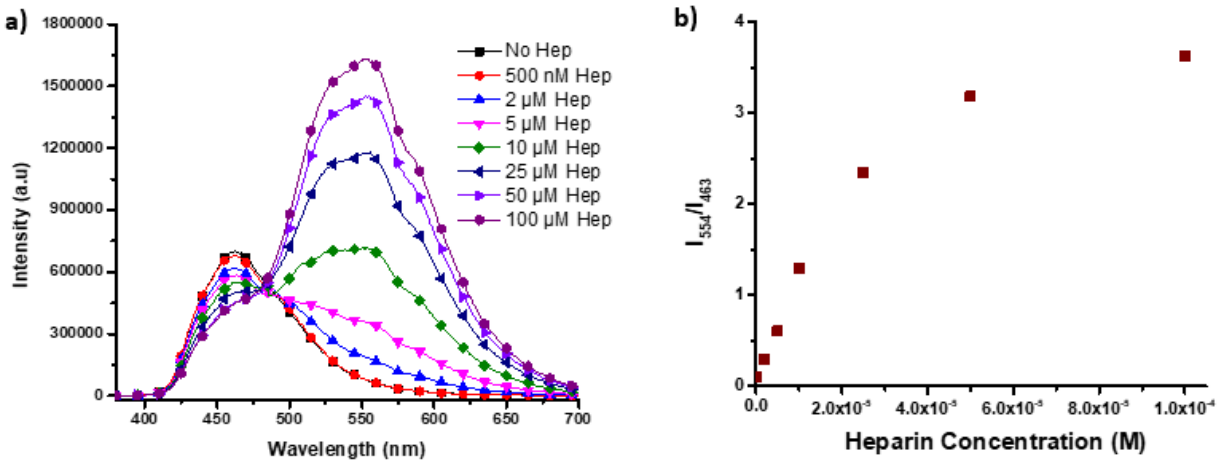


Fig. S8 (a) Emission spectral changes of **10CS10** (25 μM) upon addition of heparin (delivered in 100% human serum) in 25% human serum in buffer. (b) I_{554}/I_{463} vs. heparin concentration for (a).

C. Heparin Sensing in 50% Human Serum:

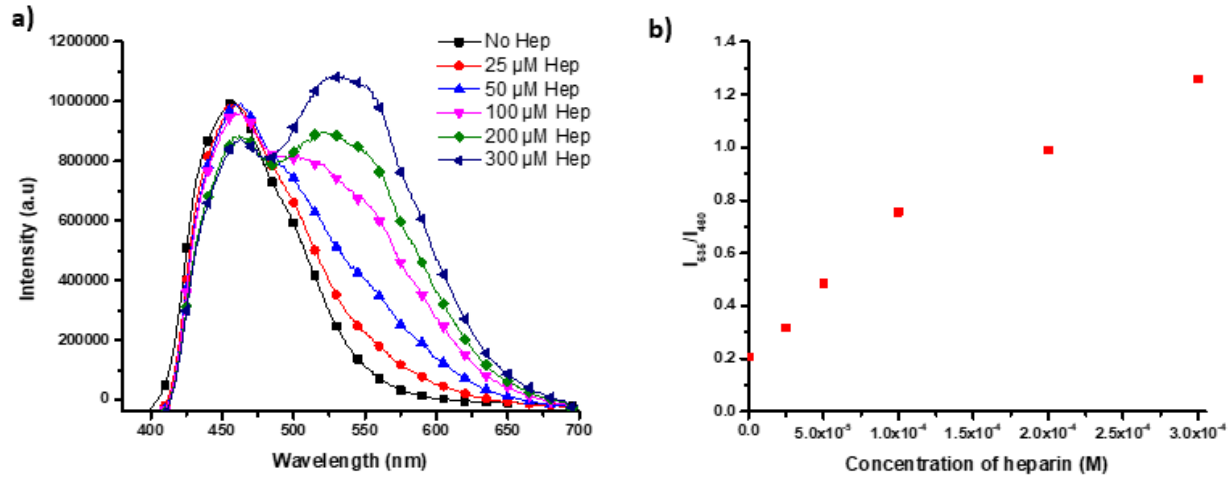


Fig. S9 (a) Emission spectral changes of **8CS8** (45 μM) upon addition of heparin (delivered in 100% human serum) in 50% human serum in buffer. (b) I_{535}/I_{460} vs. heparin concentration for (a).

4. Heparin Sensing in Human Plasma:

A. Heparin Sensing in 50% Human Plasma

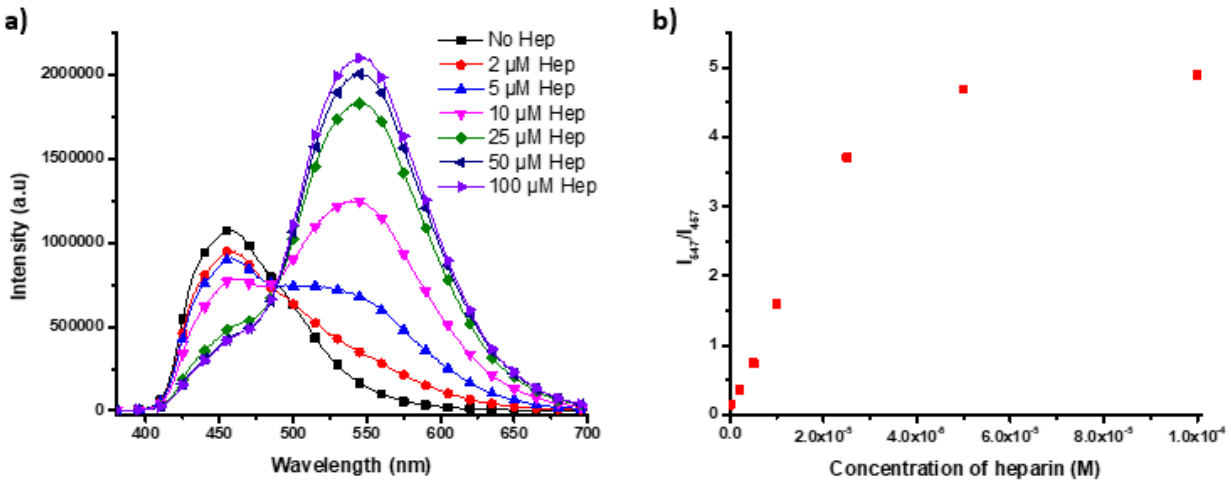


Fig. S10 (a) Emission spectral changes of **8CS8** (45 μM) upon addition of heparin (delivered in 100% human plasma) in 50% human plasma in buffer. (b) I_{547}/I_{457} vs. heparin concentration for (a).

B. Sensing of Heparin in Heparin Injection (5000 U/mL) in 50% Human Plasma:

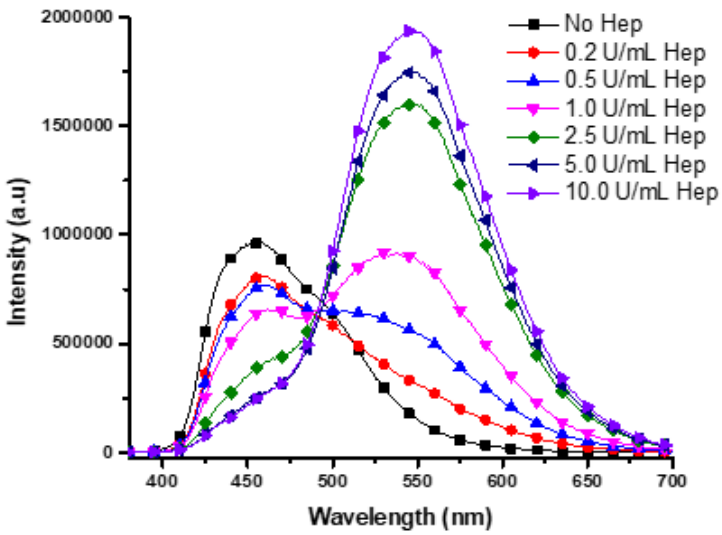


Fig. S11 Emission spectral changes of **8CS8** (45 μM) upon addition of heparin injection solution in 50% human plasma in buffer.

5. Determination of Activity of Lab Heparin by Fluorometric Titration in 50% Human Plasma:

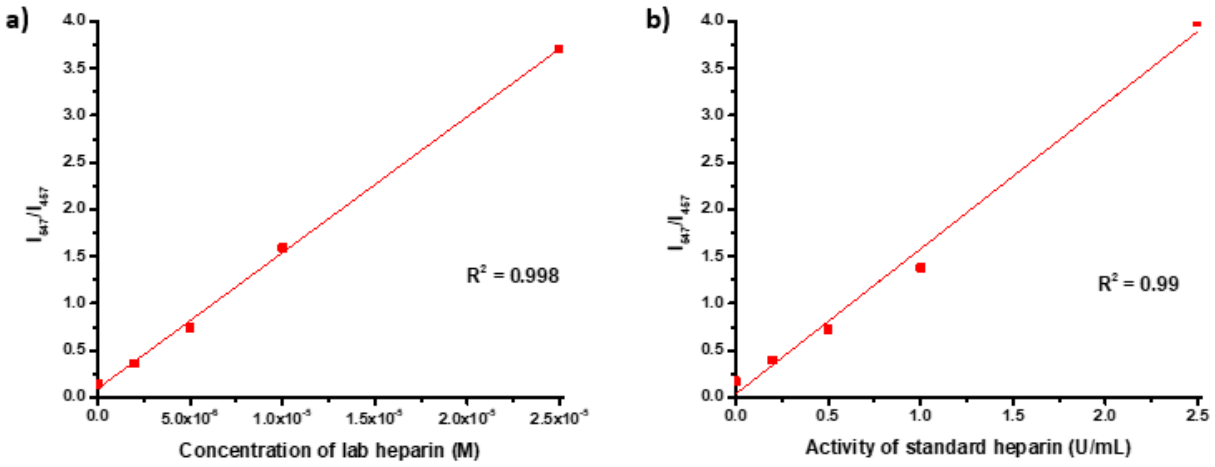


Fig. S12 (a) I_{547}/I_{457} vs. concentration of lab heparin and (b) I_{547}/I_{457} vs. activity of heparin injection in 50% human plasma.

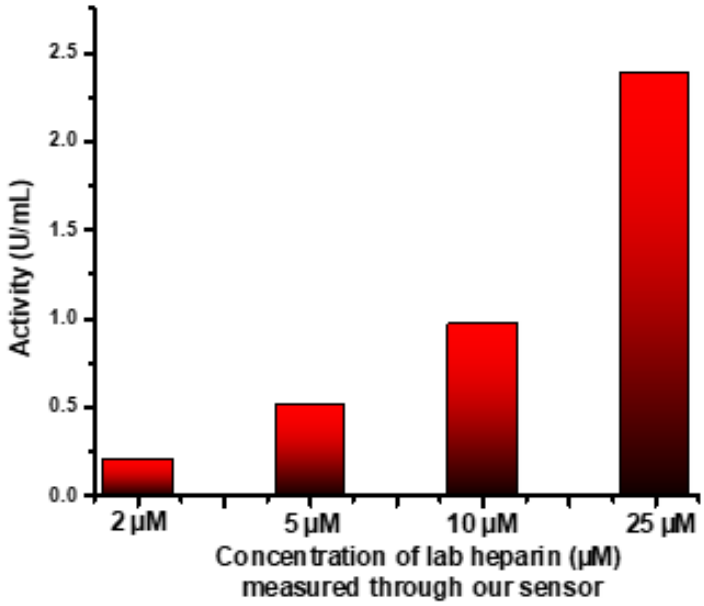


Fig. S13 Activity vs. concentration of lab heparin measured by fluorometric titration.

6. Determination of Activity of Lab Heparin by Activated Partial Thromboplastin Time (aPTT) Test:

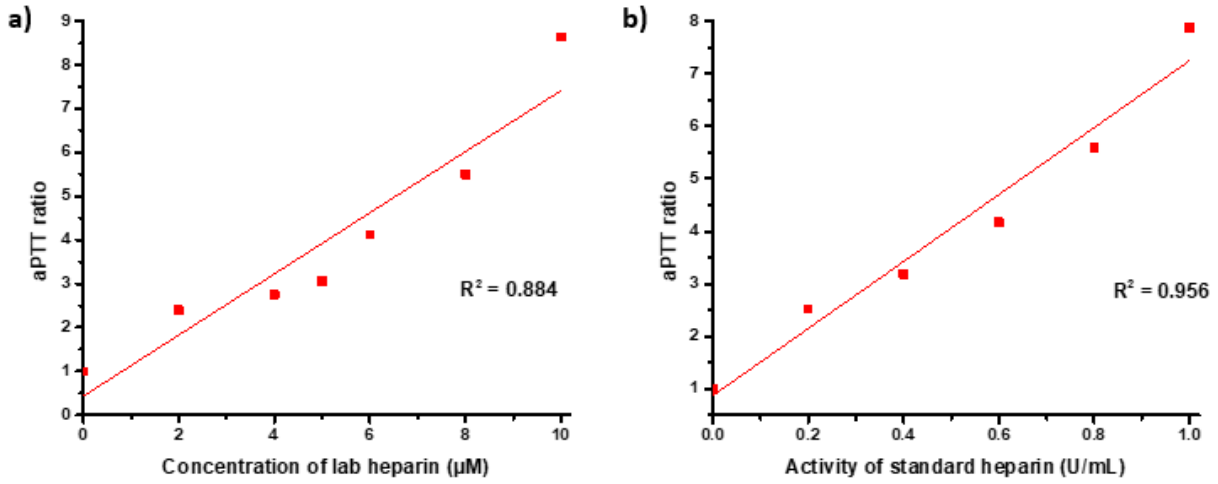


Fig. S14 (a) aPTT ratio (with and without heparin) vs. concentration of lab heparin and (b) aPTT ratio vs. activity of heparin injection in 50% human plasma.

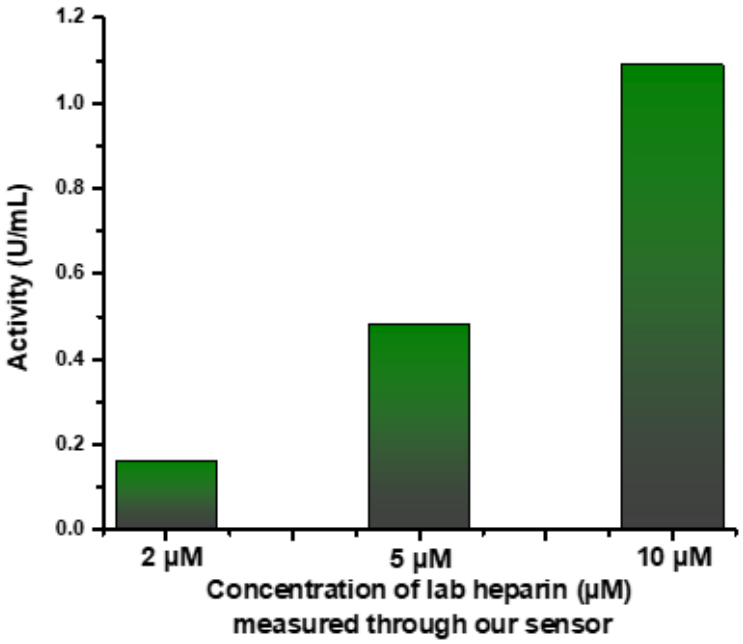


Fig. S15 Activity vs. concentration of lab heparin measured by aPTT test.

Table S1. Activity vs. concentration correlation from emission studies and aPTT measurements

Concentration of Lab Heparin (μM)	**Activity from emission Measurements (U/mL)**	**Activity from aPTT Measurements (U/mL)**
2	0.21	0.16
5	0.51	0.48
10	0.97	1.08
25	2.38	-

Heparin Activity:

Typically to obtain a heparin solution of 1 U/mL activity, ~0.002 mg/mL is required. Taking the molecular weight of the disaccharide unit as 665.40 g/ mol, this would correspond to a heparin concentration of 3 μM. However, the fluorescence measurements and aPTT tests (Table S1) clearly showed that to obtain a heparin solution of 1 U/mL activity, around 10 μM of the heparin is needed. This suggests that only around one-third of all heparin molecules are biologically active.

7. Comparison of Heparin Binding with Protamine Sulfate:

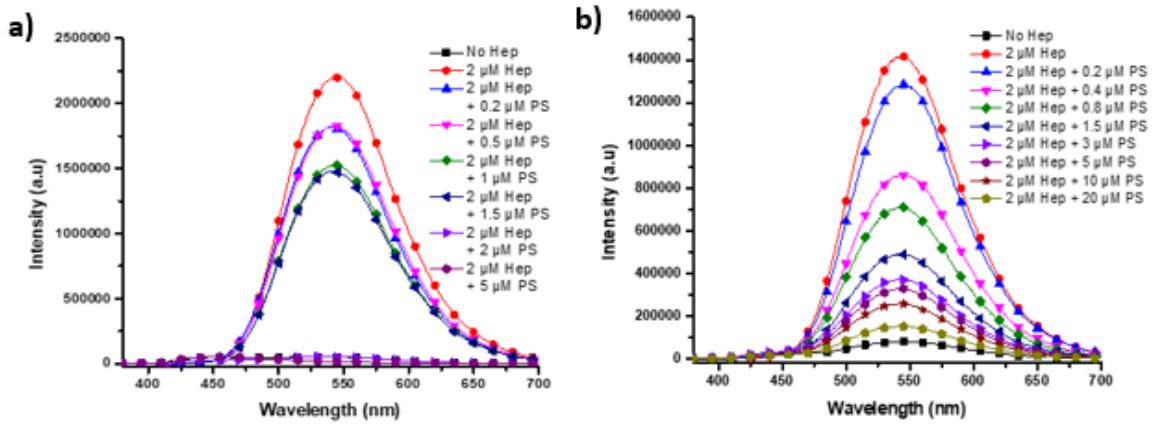


Fig. S16 Emission spectral changes of (a) **8CS8** (0.5 μM) (λ_{ex} = 365 nm) and (b) **10CS10** (0.5 μM) (λ_{ex} = 365 nm) upon addition of heparin (2 μM) and subsequent addition of protamine sulfate (**PS**) in aqueous buffer.

8. Quantum Yield Measurement:

Table S2. Absolute quantum yield (Φ) of **CS** derivatives[a] without and with heparin in aqueous buffer (5 mM tris-HCl, 99:1 water/DMSO, pH 7.4)

CS derivative	Φ (%) w/o-heparin	Φ (%) w/- heparin (5 μM)
8CS8 (10 μM)	0.85	24.26
10CS10 (5 μM)	7.87	24.8
12CS12 (5 μM)	10.52	22.36[b]

[a] λ_{ex} = 365 nm, [b] 10 μM heparin.

9. Time-correlated Single Photon Counting (TCSPC) Measurement:

Table S3. Average lifetime (τ_{avg}) of **NCSN**s without and with different amount of heparin in aqueous buffer as measured by time-correlated single photon counting (TCSPC) (excitation wavelength = 340 nm).

CS derivative	τ_{avg} (ns) w/o-heparin	τ_{avg} (ns) w/-heparin
8CS8 (10 µM)	0.19[a], 2.49[b]	15.42[d] (200 nM heparin), 20.29[d] (2µM heparin), 22.45[d] (5 µM heparin)
10CS10 (5 µM)	4.3[c]	12.96[d] (200 nM heparin), 16.30[d] (2µM heparin), 17.90[d] (5 µM heparin)
12CS12 (10 µM)	6.97[c]	10.15[e] (2µM heparin), 10.74[e] (10 µM heparin), 11.22[e] (20 µM heparin)

[a] λ_{em} = 453 nm, [b] λ_{em} = 536 nm, [c] λ_{em} = 538 nm, [d] λ_{em} = 540 nm, [e] λ_{em} = 542 nm.

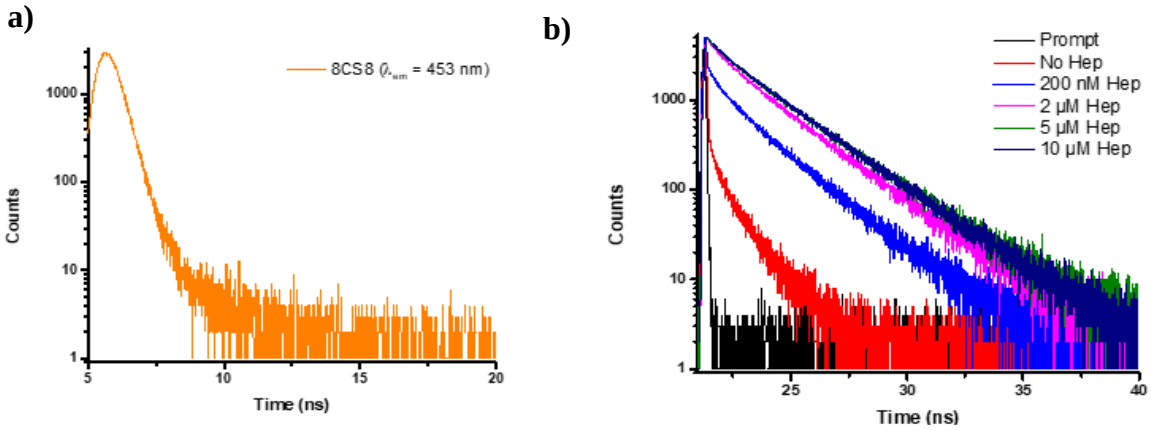


Fig. S17 Time-dependent decay curve of (a) **8CS8** (10 µM) without heparin (monomeric) (λ_{em} = 453 nm) and (b) without (excimeric) (λ_{em} = 536 nm) and with heparin (λ_{em} = 540 nm).

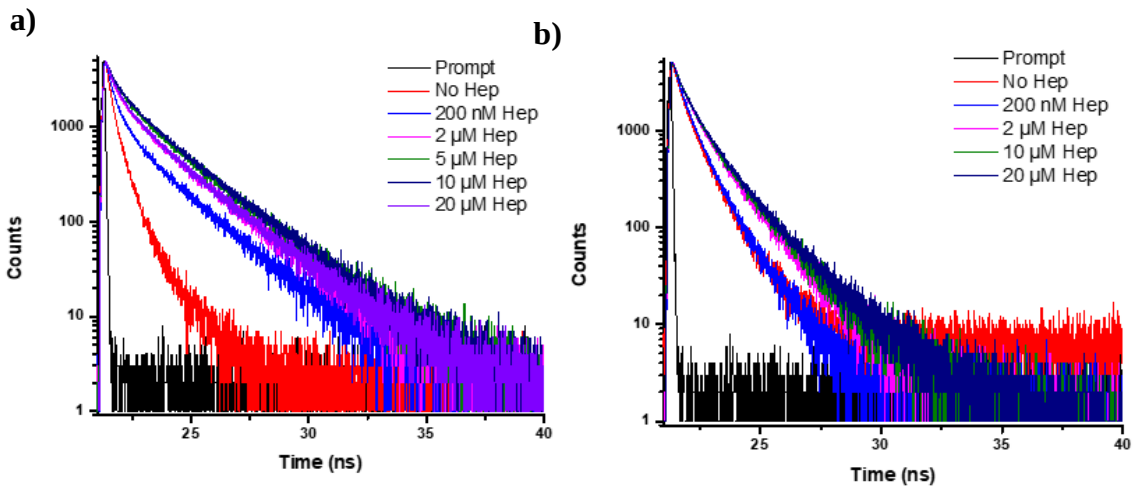
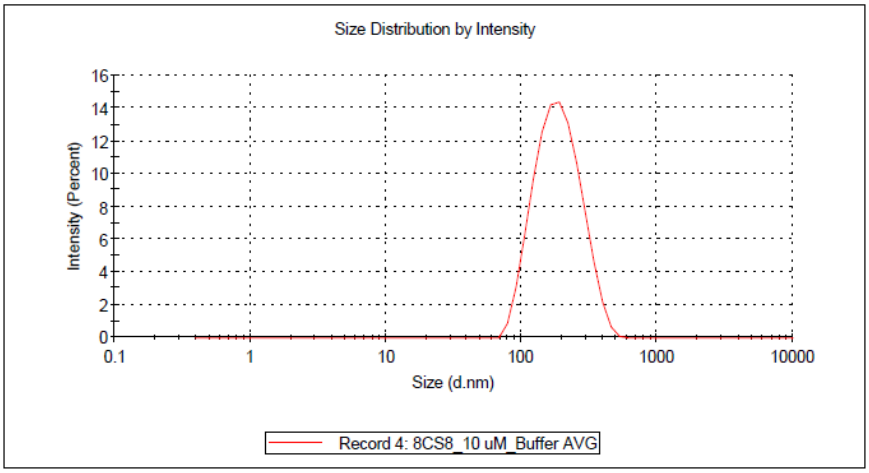


Fig. S18 Time-dependent decay curve of (a) **10CS10** (5 µM) without (λ_{em} = 538 nm) and with heparin (λ_{em} = 540 nm) and (b) **12CS12** (5 µM) without (λ_{em} = 538 nm) and with heparin (λ_{em} = 542 nm)

10. DLS Measurement:

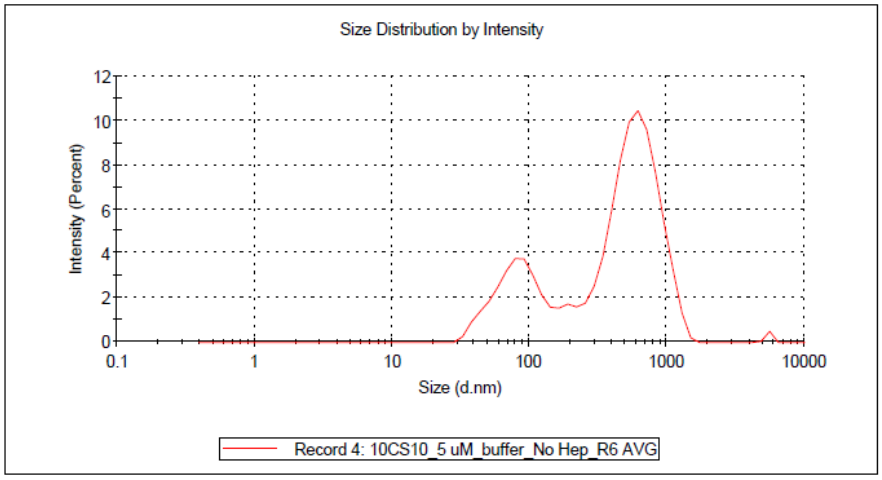
A. 8CS8 (10 µM) in buffer without heparin:

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	170.1	Peak 1:	197.0	100.0	75.02
PdI:	0.154	Peak 2:	0.000	0.0	0.000
Intercept:	0.885	Peak 3:	0.000	0.0	0.000
Result quality :	Good				



B. 10CS10 (5 µM) in buffer without heparin:

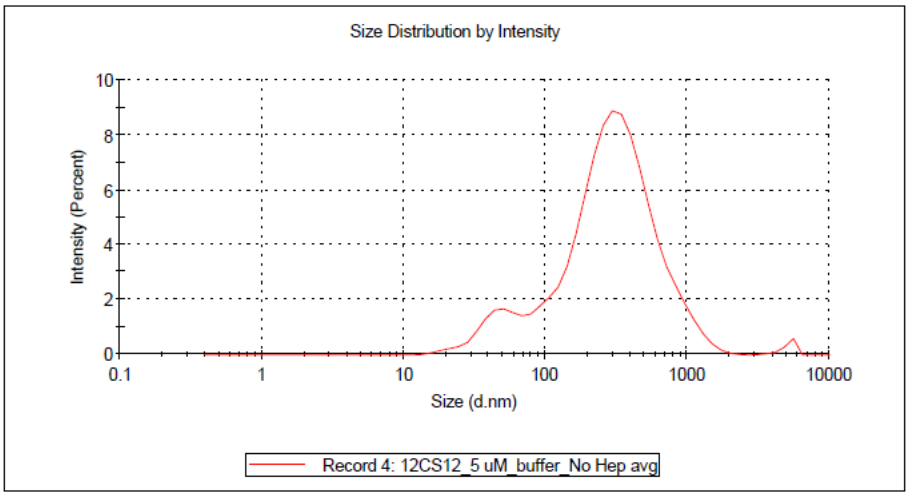
			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	287.9	Peak 1:	621.5	69.5	240.9
PdI:	0.676	Peak 2:	87.27	25.2	33.53
Intercept:	0.933	Peak 3:	191.8	4.7	22.53
Result quality :	Refer to quality report				



C. 12CS12 (5 µM) in buffer without heparin:

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	207.5	Peak 1:	374.3	89.5	255.9
PdI:	0.449	Peak 2:	46.60	9.5	13.88
Intercept:	0.894	Peak 3:	5114	1.0	603.2

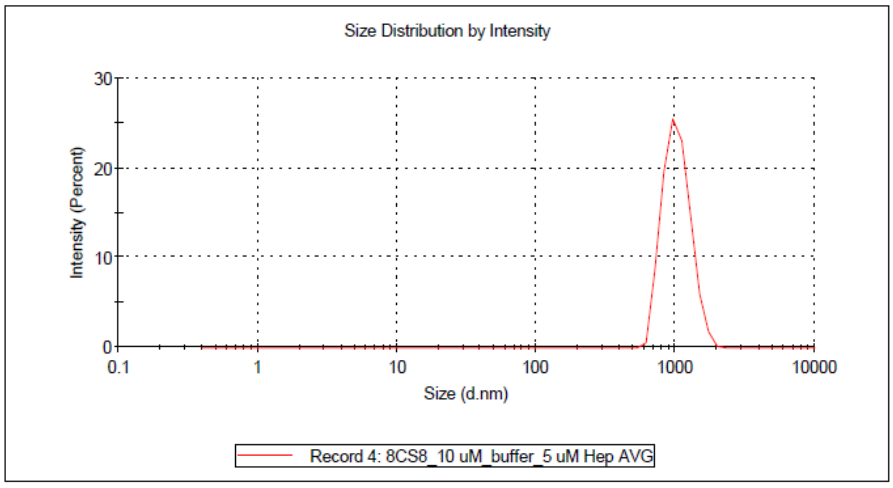
Result quality : Refer to quality report



D. 8CS8 (10 µM) in buffer with 5 µM heparin:

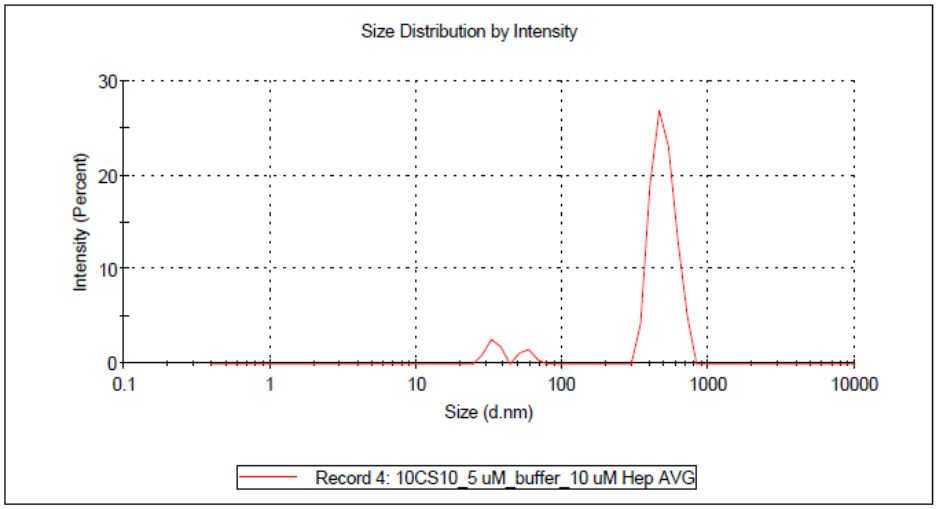
			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	952.0	Peak 1:	1036	100.0	229.1
PdI:	0.159	Peak 2:	0.000	0.0	0.000
Intercept:	0.904	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report



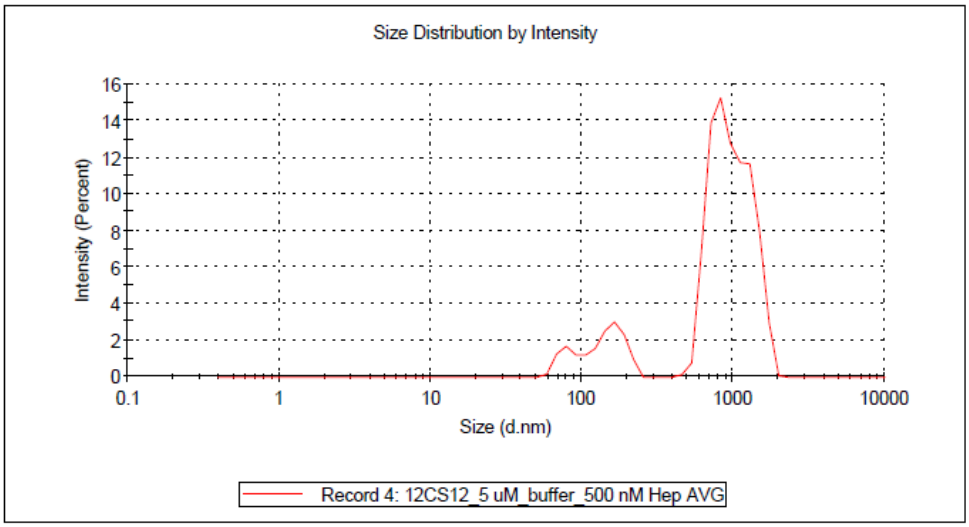
E. 10CS10 (5 µM) in buffer with 10 µM heparin:

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	1636	Peak 1:	495.3	91.4	92.69
PdI:	0.934	Peak 2:	33.61	5.4	3.420
Intercept:	1.01	Peak 3:	57.17	3.2	5.804
Result quality :	Refer to quality report				



F. 12CS12 (5 µM) in buffer with 500 nM heparin:

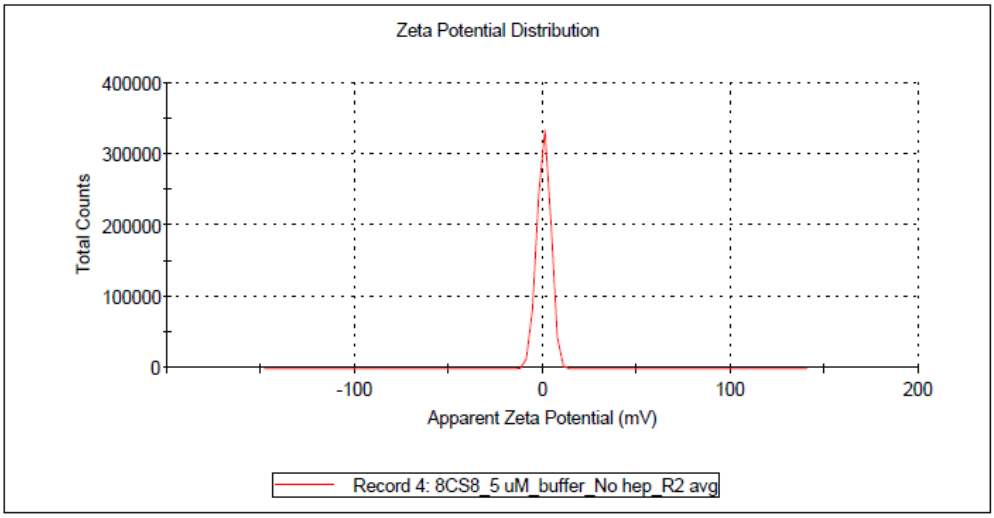
			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	1186	Peak 1:	1003	83.0	296.7
PdI:	0.756	Peak 2:	157.4	11.5	32.37
Intercept:	1.02	Peak 3:	84.26	5.5	14.18
Result quality :	Refer to quality report				



11. Zeta Potential (ζ) Measurement:

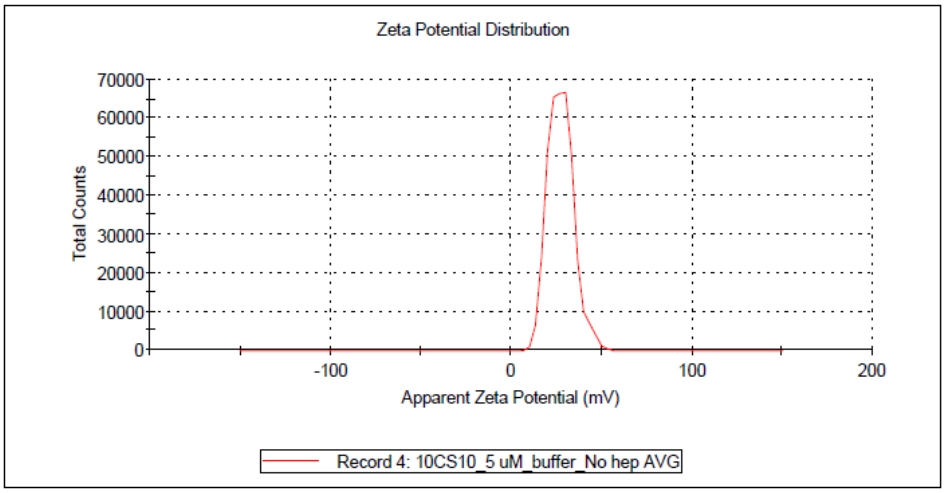
A. 8CS8 (10 µM) in buffer without heparin:

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	0.508	Peak 1:	0.503	100.0	3.59
Zeta Deviation (mV):	3.48	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.294	Peak 3:	0.00	0.0	0.00
Result quality :	Good				



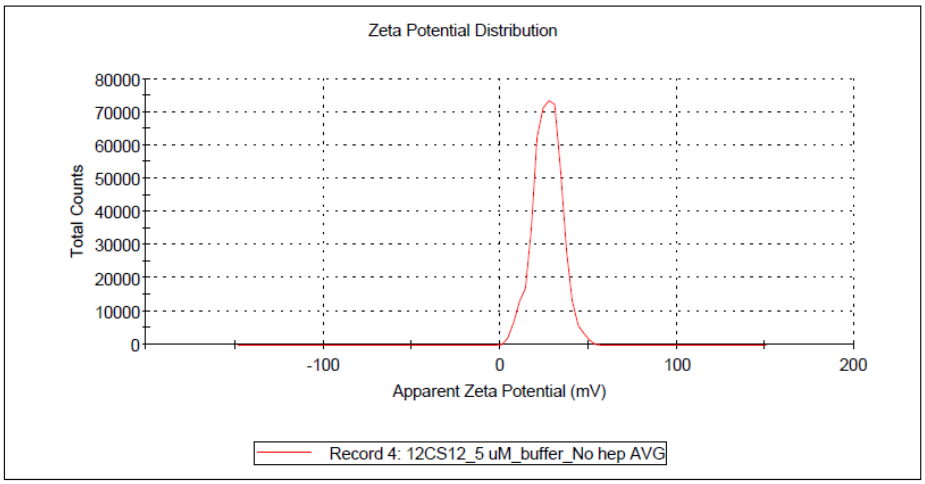
B. 10CS10 (5 µM) in buffer without heparin:

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	27.3	Peak 1:	27.4	100.0	6.98
Zeta Deviation (mV):	6.71	Peak 2:	50.7	1.5	1.81
Conductivity (mS/cm):	0.280	Peak 3:	0.00	0.0	0.00
Result quality :	Good				



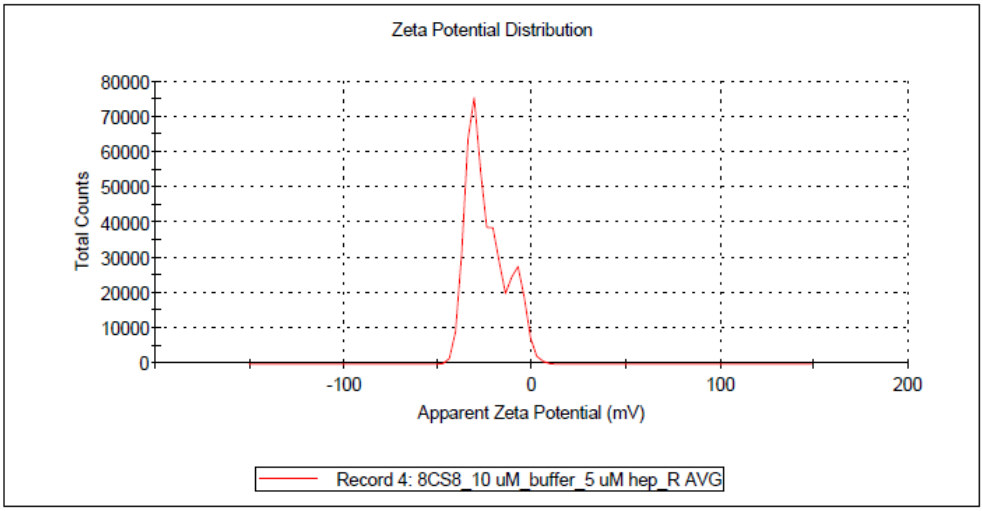
C. 12CS12 (5 µM) in buffer without heparin:

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): 27.0	Peak 1:	26.7	100.0	7.98
Zeta Deviation (mV): 7.44	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.282	Peak 3:	0.00	0.0	0.00
Result quality : Good				



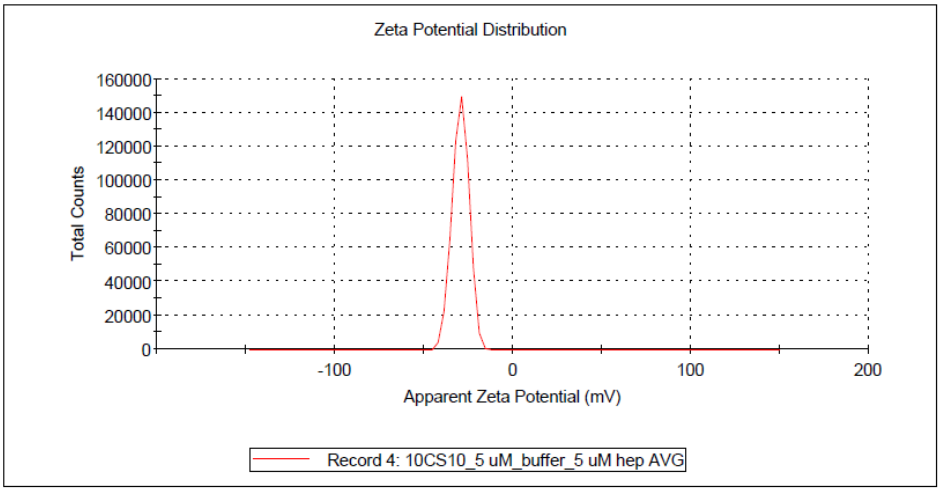
D. 8CS8 (10 µM) in buffer with 5 µM heparin:

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -24.0	Peak 1:	-27.9	86.3	6.89
Zeta Deviation (mV): 10.5	Peak 2:	-4.83	13.7	3.13
Conductivity (mS/cm): 0.308	Peak 3:	0.00	0.0	0.00
Result quality : Good				



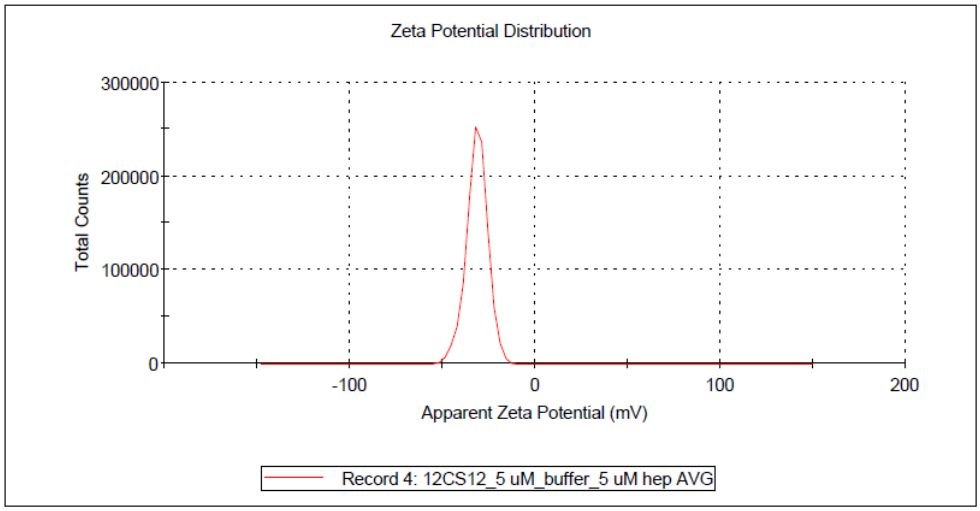
E. 12CS12 (5 µM) in buffer with 5 µM heparin:

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-29.4	Peak 1: -29.4	100.0	4.62
Zeta Deviation (mV):	4.51	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm):	0.284	Peak 3: 0.00	0.0	0.00
Result quality :	Good			



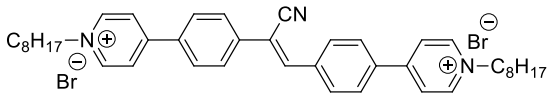
F. 12CS12 (5 µM) in buffer with 5 µM heparin:

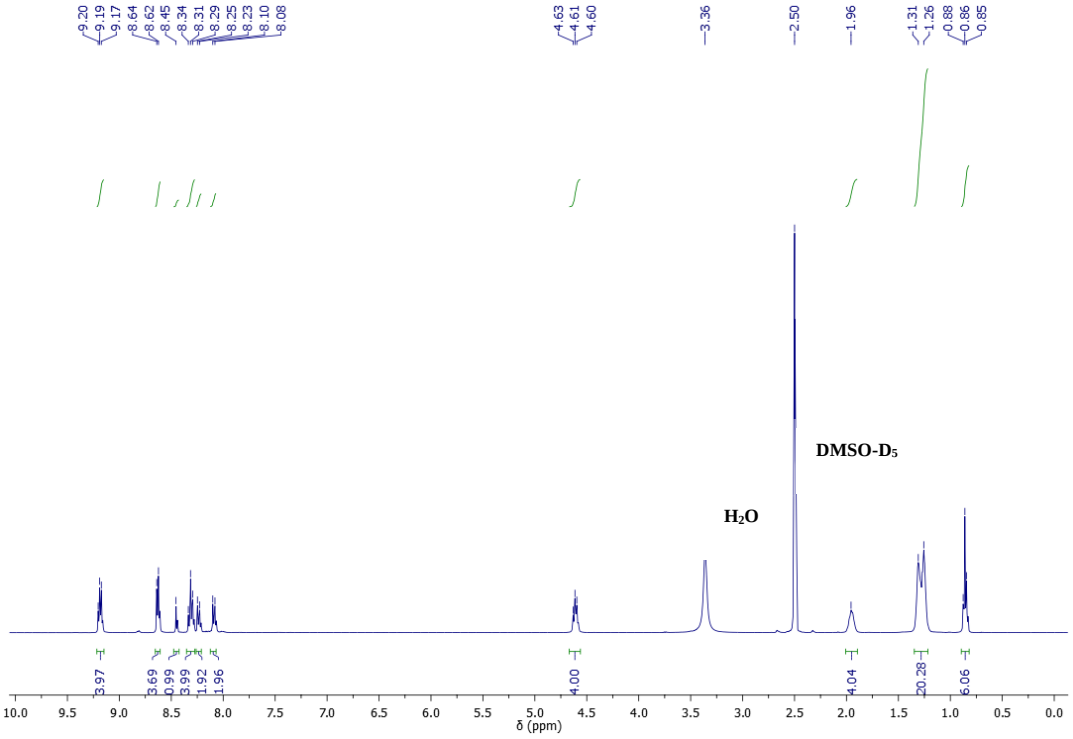
		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-31.4	Peak 1: -31.5	100.0	5.80
Zeta Deviation (mV):	5.81	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm):	0.287	Peak 3: 0.00	0.0	0.00
Result quality :	Good			



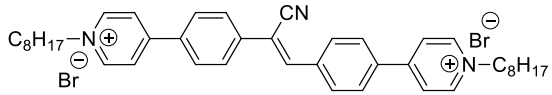
12. NMR Characterization:

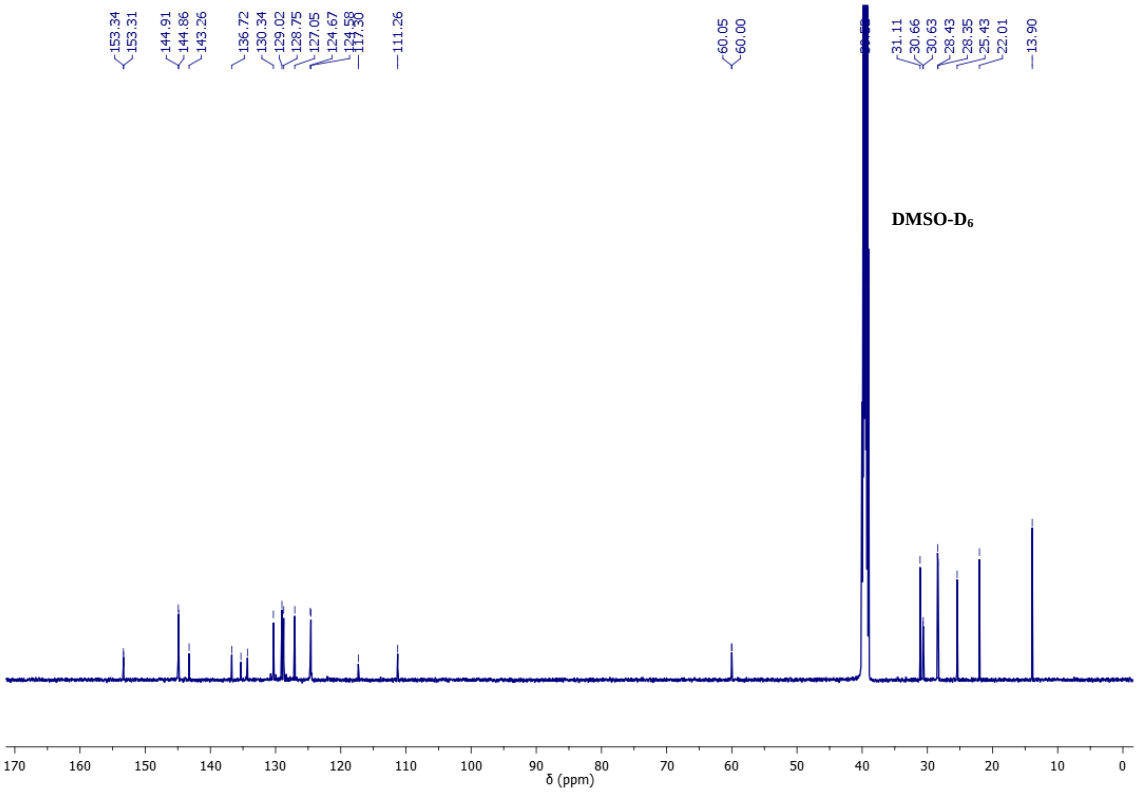
A. ^{1}H NMR spectra of 8CS8 (400 MHz, DMSO-D_6):



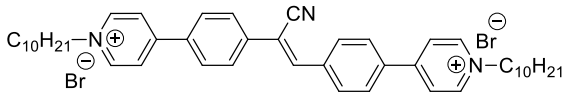


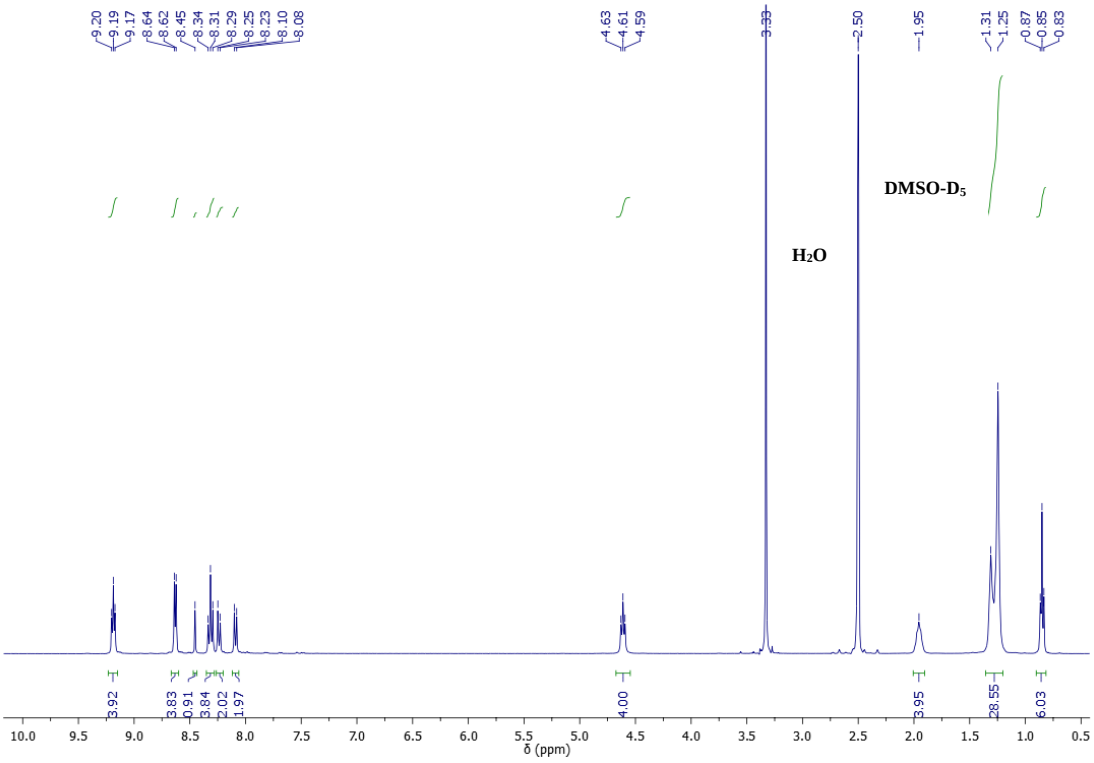
B. ^{13}C NMR spectra of 8CS8 (125 MHz, DMSO-D$_6$):



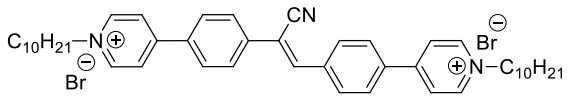


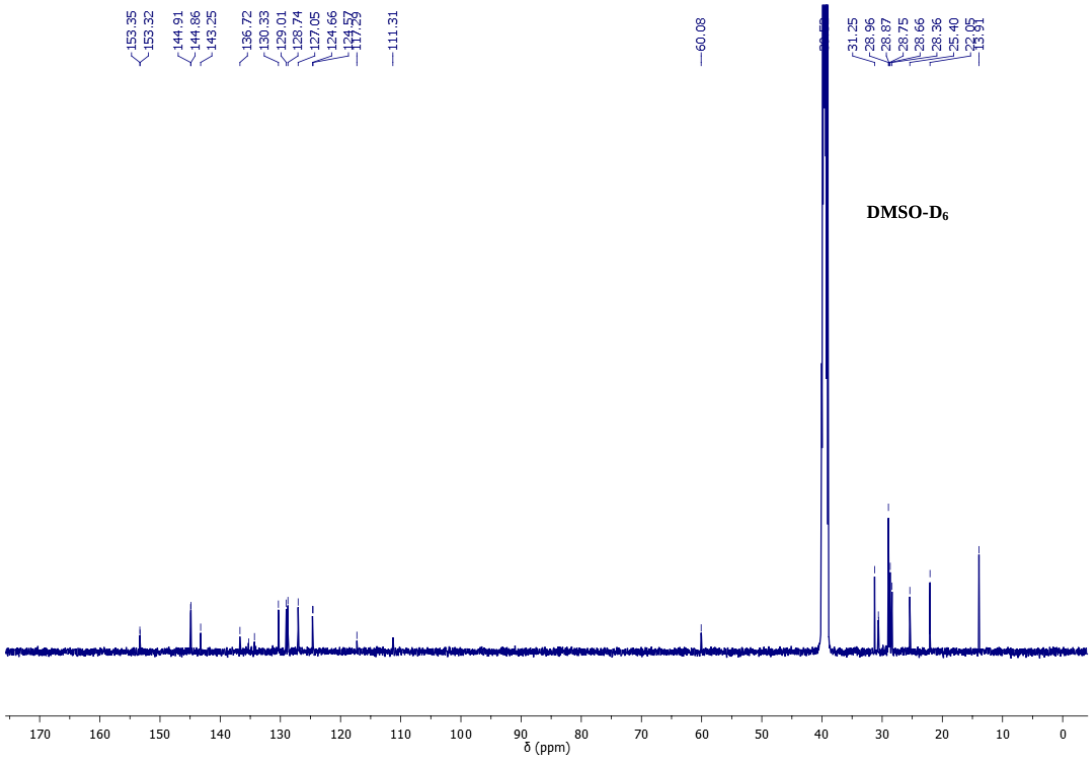
C. ^{1}H NMR spectra of 10CS10 (400 MHz, DMSO-D$_6$):



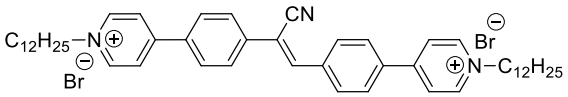


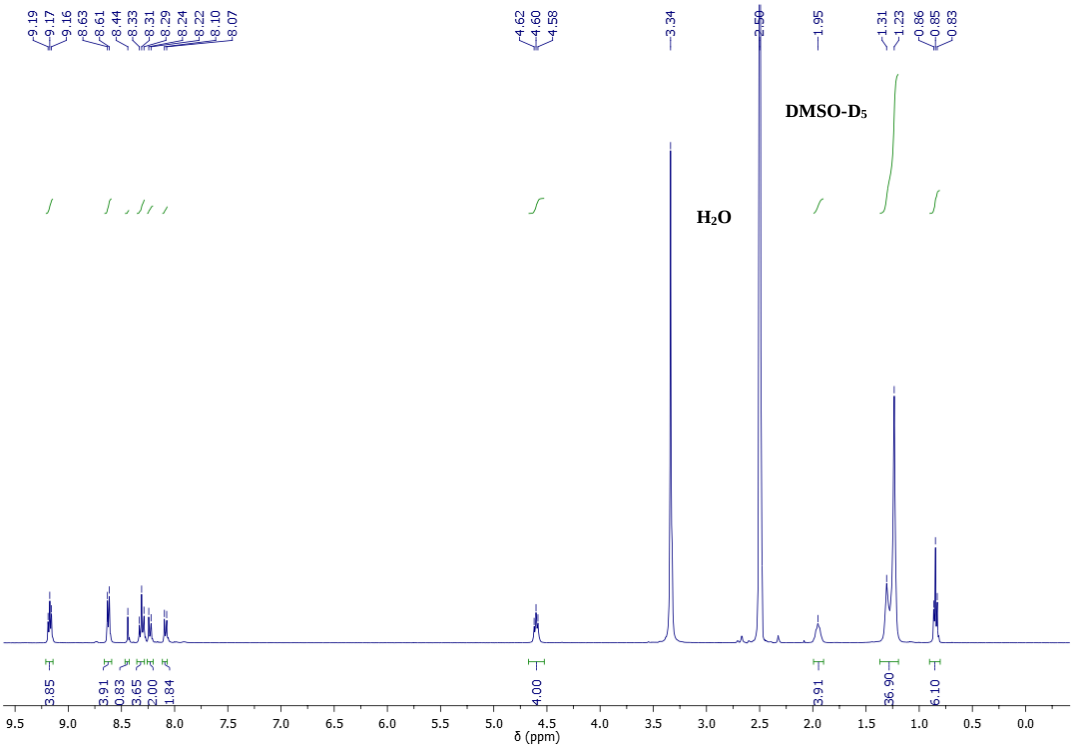
D. ^{13}C NMR spectra of 10CS10 (125 MHz, DMSO-D$_6$):



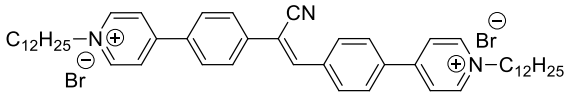


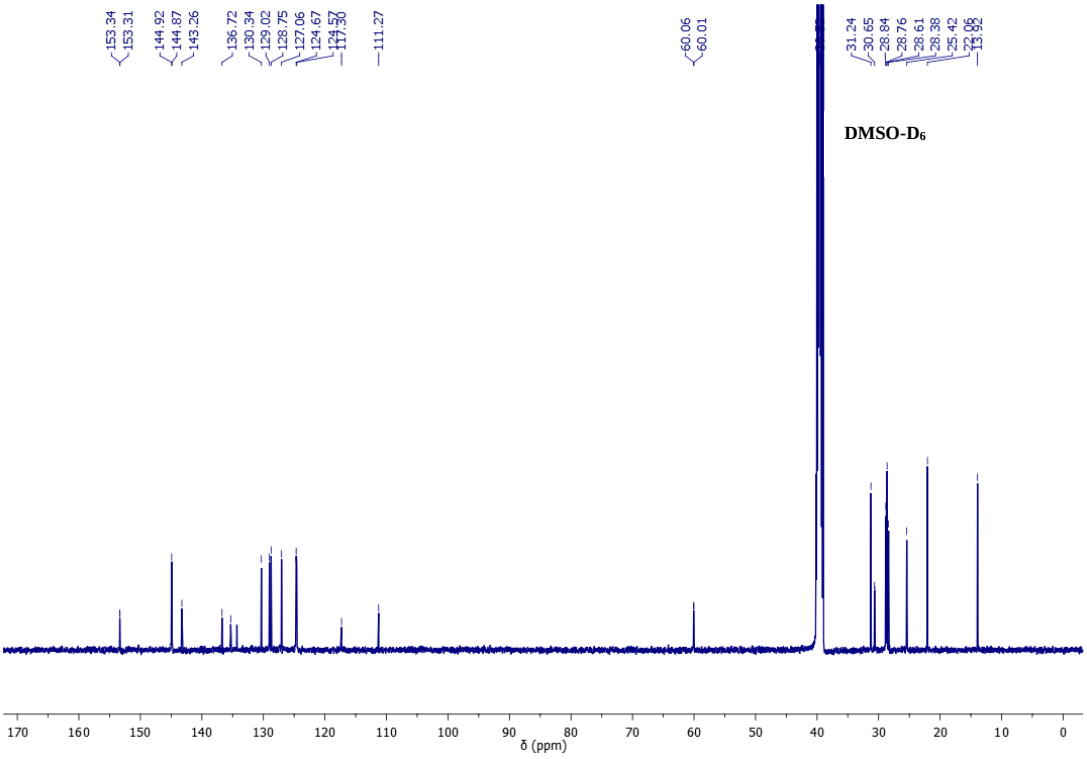
E. ^{1}H NMR spectra of 12CS12 (400 MHz, DMSO-D$_6$):





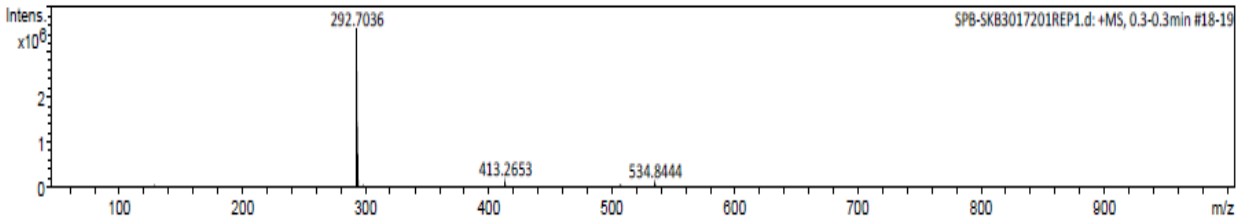
F. ^{13}C NMR spectra of 12CS12 (125 MHz, DMSO-D$_6$):



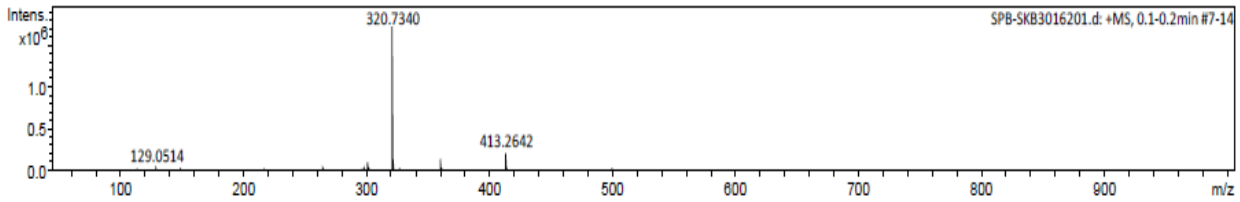


13. Characterization by Mass Spectrometry:

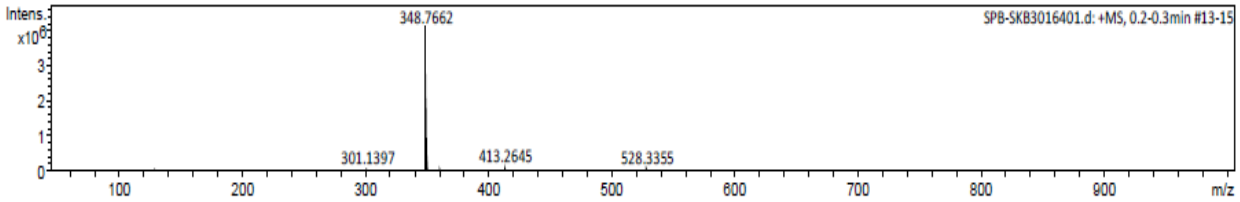
A. 8CS8:



B. 10CS10:



C. 12CS12:



References

1. *(a)* Y. You, H. Yang, J. W. Chung, J. H. Kim, Y. Jung and S. Y. Park, *Angew. Chem. Int. Ed.*, 2010, **49**, 3757; *(b)* S. K. Bhaumik and S. Banerjee, *Chem. Commun.*, 2020, **56**, 655.