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Pyrene-based heparin sensors in competitive aqueous media – the role of self-assembled multivalency (SAMul)

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SUPPORTING INFORMATION

Contents

1. Materials and Methods

All reagents were obtained from commercial sources and used without further purification. Sodium salt heparin from porcine intestinal mucosa with a molecular weight between 15,000 ± 2,000 Da (1 KU = 1000 units) was obtained from Calbiochem®. Trizma® hydrochloride (Tris HCl) and Human Serum (from human male AB plasma) were obtained from Sigma Aldrich. Preparative gel permeation chromatography (GPC) was performed on Biobeads SX-1 supplied by Bio-Rad. Thin layer chromatography (TLC) was performed on Merck aluminum-backed plates, coated with 0.25 nm silica gel 60. ^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC NMR were recorded on a JEOL ECX400 (^{1}H 400 MHz, ^{13}C 100 MHz) spectrometer. ESI and HR-ESI mass spectra were recorded on a Bruker Daltonics MicroTOF mass spectrometer. IR spectra were measured on a PerkinElmer Spectrum Two™ IR Spectrometer with ATR-IR. Dynamic light scattering data were measured at 1 mg/mL using a Zetasizer Nano (Malvern Instruments Ltd., Worcestershire, UK), based on the principle of measurement of the backscattered light fluctuations at an angle of 173° and the calculation of an autocorrelation function. Data were recorded from 15–20 runs per single measurement, each of which was carried out at 25°C using folded capillary cells (DTS 1060). Monomer solutions were freshly prepared by dissolving an appropriate amount of dry compound in filtered aqueous media (e.g. 10 mM Tris HCl, 150 mM NaCl). All samples were agitated and incubated at 25°C for 10 minutes prior to measurement. Data are reported based on volume distribution.

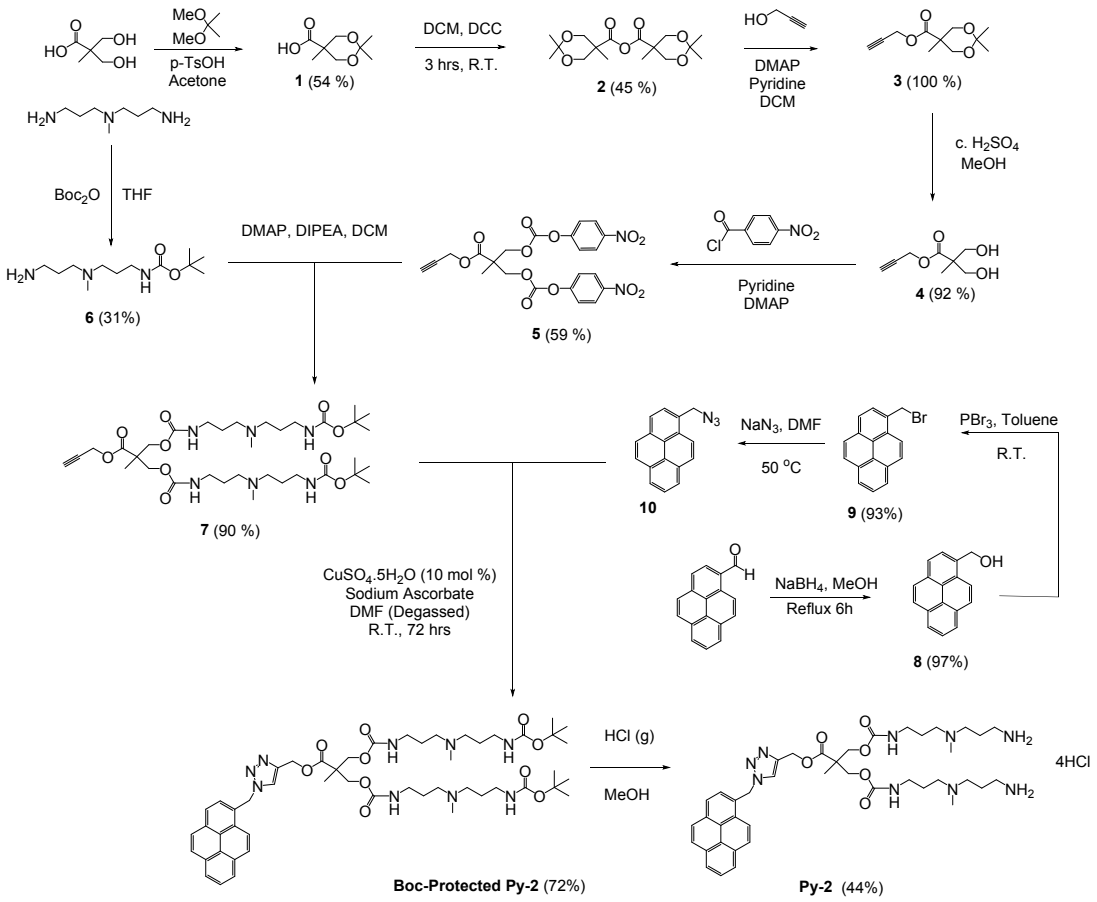
2. Synthesis and Characterisation Data

Precursor Boc-Protected Py-1. 1-Pyrenecarboxylic acid (100 mg , 0.406 mmol) was dissolved in DCM (40 ml) and TBTU (130 mg, 0.406 mmol) and Et_3N (5 ml) were added. The mixture was stirred for 5 min, then mono-Boc-protected *N*,*N*-di-(3-aminopropyl)-*N*-methylamine (99.6 mg, 0.406 mmol) was dissolved in CH_2Cl_2 (20 ml) and added to the mixture. The solution was left stirring overnight. The solvent was evaporated *in vacuo* and the product purified by column chromatography (SiO_2 in MeOH : DCM 1:9). The product was obtained as pale yellow solid (0.156 g, 81%, 0.33 mmol). R_f= 0.25 (1:9 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.38-7.90 (m, 9H, Ar-*H*); 6.18 (br s, N*H*, 2H); 3.40 (d, CH_2NH, *J* = 6.8 Hz, 2H); 3.00 – 2.91(m, CH_2N(CH_3), 4H) 2.90-2.82 (m, CH_2N(CH_3), 2H); 2.54 (s, N(CH_3), 3H); 1.91, 1.61 (q, C$H_2$$CH_2$NH, *J* = 6.8 Hz, 4H); 1.40 (s, C(CH_3)$_3$, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.42 (*C*=O); 156.90 (*C*=ONH); 132.29, 130.76, 130.22, 129.53, 128.49, 128.39, 128.15, 126.88, 126.25, 125.81, 125.65, 124.89, 124.21, 124.09, 123.94, 123.82 (Ar-*C*); 79.61 (*C*(CH_3)$_3$); 54.05, 53.93 (*C*H_2N(CH_3)); 39.51 (N(*C*H_3)); 37.14, 36.93 (*C*H_2NH); 28.30 (C(*C*H_3)$_3$); 24.64, 24.30 (*C*$H_2$$CH_2$NH). IR (cm^{-1}): 3283*w* (N-H), 2929*w* (C-H), 1691*s* (C=O), 1633*s*, 1519*s* (CONH), 1363*s*, 1246*s*, 1163*s*, 844*s*, 712*m*. HRMS *m/z*: Calcd. ($C_{29}H_{36}N_3O_3$) $[M+H]^+$ 474.2751; Obs. $[M+H]^+$*m/z* 474.2736 (100%).

Sensor Py-1. Precursor **Boc-Protected Py-1** (100 mg, 0.211 mmol) was dissolved in MeOH (20 ml) and HCl gas was bubbled through the solution for 20 s. The reaction mixture was stirred at room temperature for 3 h. The solvent was removed *in vacuo* to afford the product as a brown oil (89 mg, 200 mmol, 95%). R_f= 0.00 (NH_4OH). ^{1}H NMR (400 MHz, MeOD-d_4) δ: 8.01-7.55 (m, 9H, Ar-*H*); 3.70-3.55 (m, CH_2NH, 2H); 3.20-2.90 (m, CH_2N(CH_3), CH_2N(CH_3), N(CH_3) 9H); 2.30-2.00 (m, C$H_2$$CH_2$NH, 4H) – peaks were

relatively broad owing to aggregation at NMR concentrations. ^{13}C NMR (100 MHz, MeOD-d_4) δ: 171.89 (*C*=O); 132.56, 131.02, 130.46, 130.02, 128.50, 128.20, 126.86, 126.32, 125.82, 125.63, 125.03, 124.27, 124.19, 123.89 (Ar-*C*); 54.45, 53.16 (*C*H$_2$N(CH$_3$)); 39.61 (N(*C*H$_3$)); 37.05, , 36.89 (*C*H$_2$NH); 24.39, 23.33 (*C*H$_2$CH$_2$NH) IR cm^{-1}: 3385*m* (N-H), 2958*m* (C-H), 1708*s* (C=O), 1624*m*, 1528*s* (CONH), 1459*s*, 1244*m*, 1050*s*, 847*s*, 708*m*. HRMS *m/z*: Calcd. [M+H]$^+$ ($C_{24}H_{28}N_3O$) 374.2227; Found [M+H]$^+$374.2227 (100%).

The synthesis of **Py-2** is outlined in Scheme 1. Compounds **1-7** were prepared as previously reported using methodologies from Fréchet,[1] Sharpless & Hawker,[2] and our own research group.[3] Compounds **8-10** were synthesised as previously reported in the literature.[4]



Scheme S1 – Synthesis of **Py-2**.

Precursor Boc-Protected Py-2. Compound **7** (0.100 g, 0.14 mmol) was dissolved in degassed DMF (10 ml) along with 1-azidomethyl pyrene **10** (0.036 g, 0.14 mmol), $CuSO_4.5H_2O$ (3.49 mg, 14 µmol, 10 mol%) and sodium ascorbate (2.66 mg, 28 µmol, 20 mol%). The reaction mixture was stirred for 72 h at room temperature under N_2. The DMF was then removed under high vacuum at room temperature and the residue taken up in CH_2Cl_2 (20 ml). The solution was washed with H_2O (2 x 10 ml), dried over $MgSO_4$ and the solvent removed *in vacuo* to leave the crude product (102 mg). The crude product was purified by GPC (Biobeads SX-1, CH_2Cl_2) affording the desired product as a yellow solid (98 mg, 0.101 mmol, 72 %). ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.38-7.90 (m, 9H, Ar-*H*); 7.45 (br s, CH triazole, 1H); 6.21 (s, 2H, ArC*H*$_2$); 5.80 (br s, N*H*, 2H); 5.38 (br s, N*H*, 2H); 5.11 (s, C*H*$_2$O triazole, 2H); 4.22-4.12 (m, CH_2O, 6H); 3.24-3.05 (m, C*H*$_2$NH, 8H); 2.39-2.31 (m, C*H*$_2$N(CH_3), 8H); 2.15 (s, N(CH_3), 6H; 1.61 (app. q, C*H*$_2$CH$_2$NH, *J* = 6.8 Hz, 8H); 1.40 (s, C(CH_3)$_3$, 18H). ^{13}C NMR (100MHz, $CDCl_3$) δ: 173.1(*C*ONH); 156.1 (O*C*ONH); 155.9 (O*C*ONH); 142.35 (C triazole); 131.7, 131.2, 130.7, 129.2, 128.4, 128.2, 127.9, 127.4, 127.3, 126.2, 125.6, 125.5, 125.0, 124.6, 122.6 (Ar-C); 123.47 (CH triazole); 78.79(C(*C*H$_3$)$_3$); 65.60 (CH_2O); 58.3 (CH_2O triazole) 56.06, 55.64 (*C*H$_2$N(CH_3)); 52.0 (Ar-*C*H$_2$N triazole); 46.88 (C_{quat}); 41.67 (N(*C*H$_3$)); 40.08, 39.15 (*C*H$_2$NH); 28.37 (C_{quat}(*C*H$_3$)); 26.42, 26.40 (*C*H$_2$CH$_2$NH); IR (cm^{-1}): 3378*m* (N-H), 2923*m* (C-H), 1697*s* (C=O), 1601*w,* 1530*m* (CONH), 1461*m*, 1248*s*, 1128*s*, 1022*m*, 845*m*, 708*m*. HRMS: Calcd. $[M+2H]^{2+}$ ($C_{51}H_{75}N_9O_{10}$) *m/z* = 486.7813; Found $[M+H]^+$ *m/z* = 486.7808 (100%).

Sensor Py-2. Precursor **Boc-Protected Py-2** (98 mg, 101 µmol) was dissolved in MeOH (20 ml) and HCl gas was bubbled through the solution for 20 s. The reaction mixture was stirred at room temperature for 3 h. The solvent was removed *in vacuo* to afford the product as a yellow foam (41 mg, 45 µmol, 44%). ^{1}H NMR (400 MHz,

MeOD-d_4) δ: 7.90-8.38 (m, 9H, Ar-*H*); 7.45 (br s, CH triazole, 1H); 6.21 (s, 2H, ArCH_2); 5.80 (br s, N*H*, 2H); 5.38 (br s, N*H*, 2H); 5.19 (br s, CH_2O triazole, 2H); 4.22-4.12 (m, CH_2O, 6H); 3.05-3.24 (m, CH_2NH, 8H); 2.31-2.39 (m, $CH_2N(CH_3)$, 8H); 2.15 (s, $N(CH_3)$, 6H); 1.61 (app q, CH_2CH_2NH, J = 6.8 Hz, 8H). ^{13}C NMR (100 MHz, MeOD-d_4) δ: 173.1(*C*=O); 156.1 (O*C*ONH); 142.35 (*C* triazole); 131.7, 131.2, 130.7, 129.2, 128.4, 128.2, 127.9, 127.4, 127.3, 126.2, 125.6, 125.5, 125.0, 124.6, 122.6 (Ar-*C*); 123.47 (*C*H triazole); 65.60 (*C*H_2O); 58.3 (*C*H_2O triazole) 56.06, 55.64 (*C*$H_2N(CH_3)$); 56.0 (Ar-*C*H_2N triazole); 46.88 (C_{quat}); 41.67 (N(*C*H_3)); 40.08, 39.15 (*C*H_2NH); 28.37 (C_{quat}(*C*H_3)); 26.42, 26.40 (*C*H_2CH_2NH); IR (cm^{-1}): 3362*w* (N-H), 2957*m* (C-H), 1708*s* (C=O), 1600*m*, 1529*m* (CONH), 1462*w*, 1246*m*, 1131*m*, 1084 *w*, 846*w*, 707*w* HRMS *m/z*: Calcd. $[M+2H]^{2+}$ ($C_{41}H_{59}N_9O_6$) 386.7289; Found $[M+H]^{2+}$ 386.7285. (100%)

3. Dynamic Light Scattering Data

Py-1

			Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm):	74.84	Peak 1:	14.21	100.0	28.20
PdI:	0.504	Peak 2:	0.000	0.0	0.000
Intercept:	0.688	Peak 3:	0.000	0.0	0.000
Result quality :	Good				

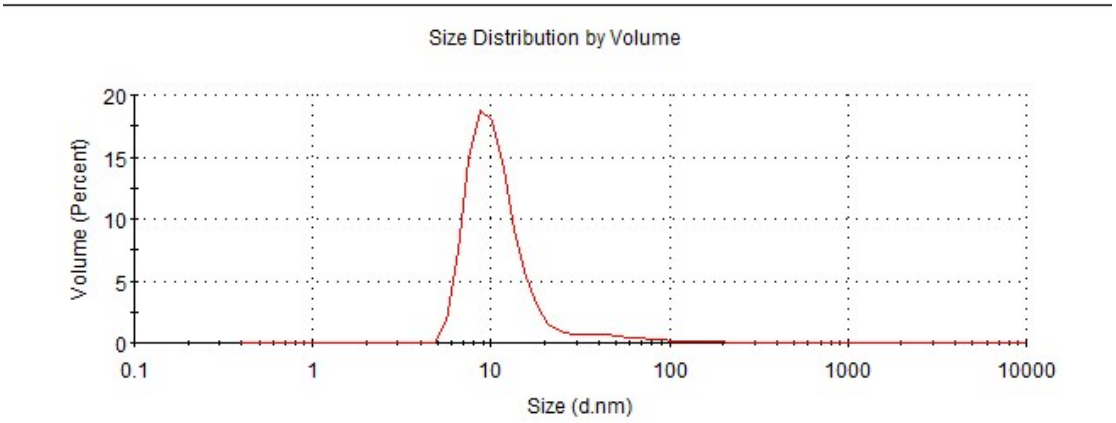


Fig. S1. DLS data for **Py-1** measured at 1 mg/mL.

Py-2

			Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm):	67.31	Peak 1:	101.7	2.2	52.04
PdI:	0.553	Peak 2:	12.25	97.8	3.709
Intercept:	0.765	Peak 3:	0.000	0.0	0.000
Result quality :	Refer to quality report				

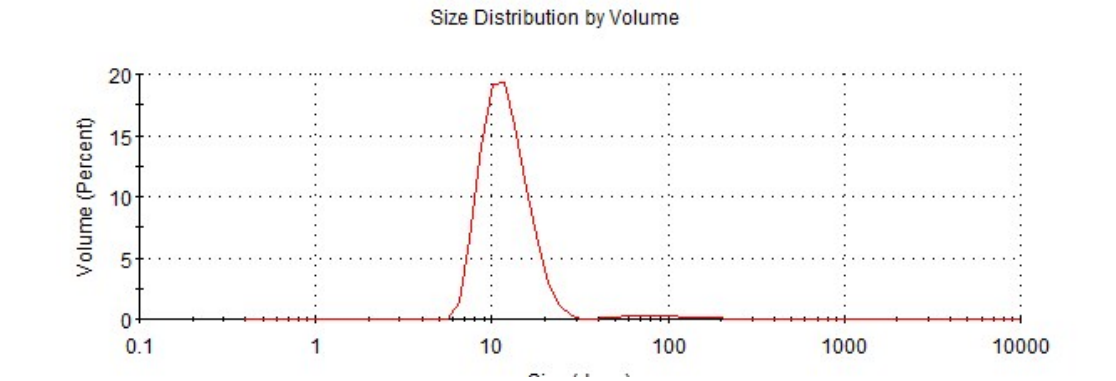


Fig. S2. DLS data for **Py-1** measured at 1 mg/mL.

4. Transmission Electron Microscopy (TEM) Images

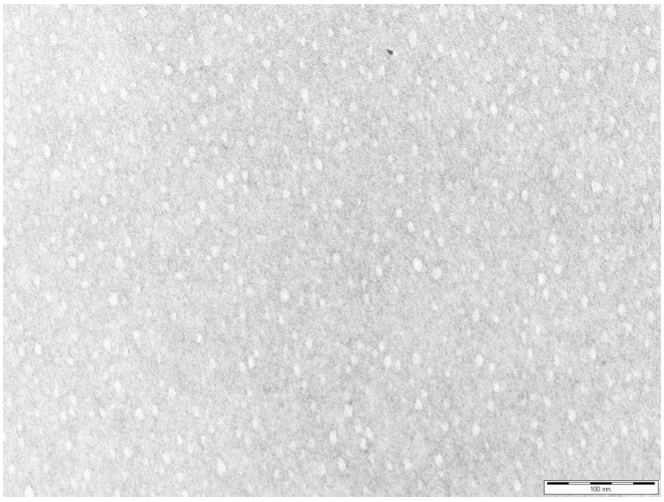


Fig. S3. TEM image of **Py-1** dried from aqueous solution (1 mg/mL). Scale bar = 100 nm.

5. Assay Methods

5.1 Critical Aggregation Concentration Assay

A stock solution of **Py-1**/**Py-2** was made up in PBS buffer at various concentrations. Aliquots of the stock solution were taken and diluted with PBS to the desired concentration in a 1 ml assay volume. The fluorescence emission was measured on a Hitachi F-4500 spectrofluorimeter using an excitation wavelength of 363 nm. The fluorescence intensity of the excimer band was recorded at 495 nm. The discontinuity in excimer intensity with concentration was considered to represent the critical aggregation concentration. Experiments were performed in triplicate.

5.2 Heparin binding assay

Py-1 (0.28 mg, 60 μM) /**Py-2** (0.27 mg, 30 μM) were made up to 10 ml in volumetric

flasks with 10 mM Tris HCl with 150 mM NaCl buffer solution or 12.5% Human Serum in 20mM Tris HCl buffer or 100% Human Serum to yield a stock solution of sensor. 5 mL of this solution was then added to heparin (0.27 mg) to yield a stock solution of stock sensor/heparin (80 μM). 1 ml of stock sensor solution was added in the cuvette and titrated with stock sensor/heparin solution until the cuvette ended up with a total 2 ml volume. The fluorescence emission was measured on a Hitachi F-4500 spectrofluorimeter using an excitation wavelength of 363 nm. Fluorescence intensity was recorded at 383 and 495 nm. Experiments were performed in triplicate.

6. Fluorescence Spectra

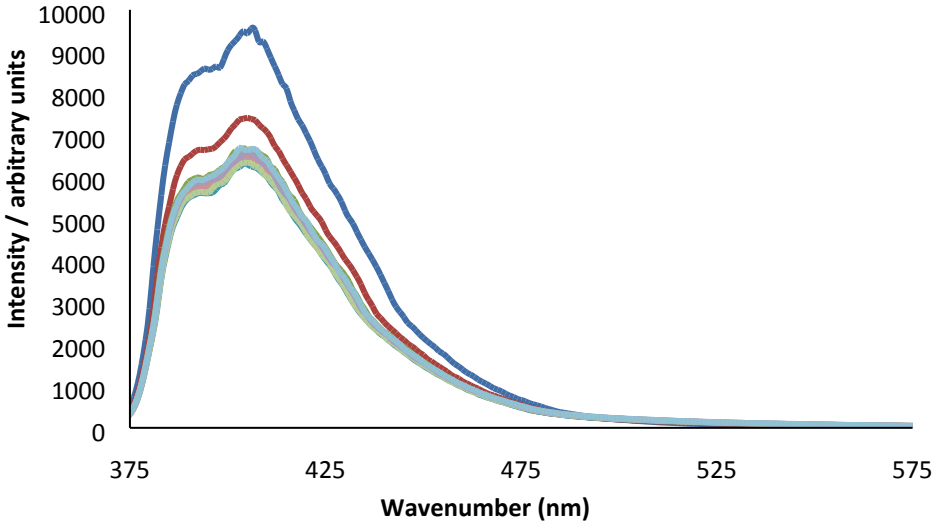


Fig. S4. Sensor **Py-1** (60 μM) titrated with heparin in buffer showing no SAMul-type response.

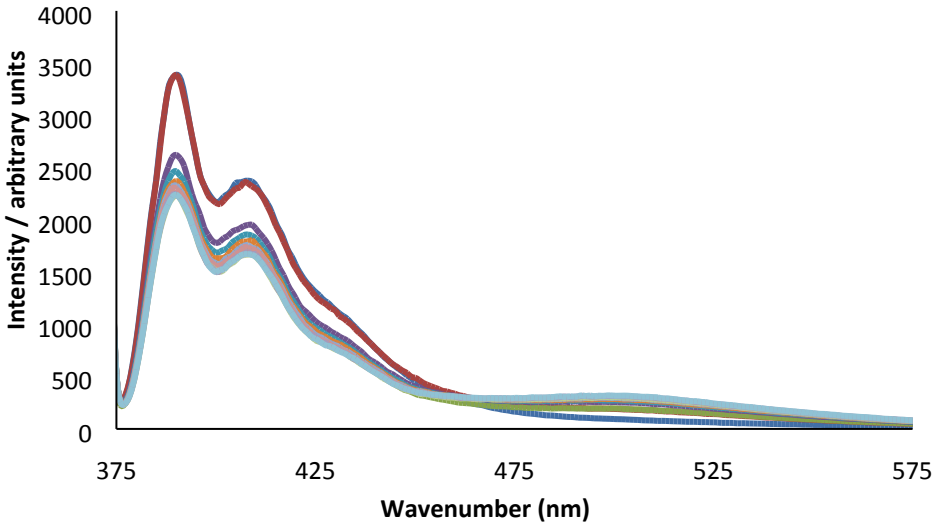


Fig. S5. Sensor **Py-1** (60 μM) titrated with heparin in 12.5% serum showing a small SAMul binding response.

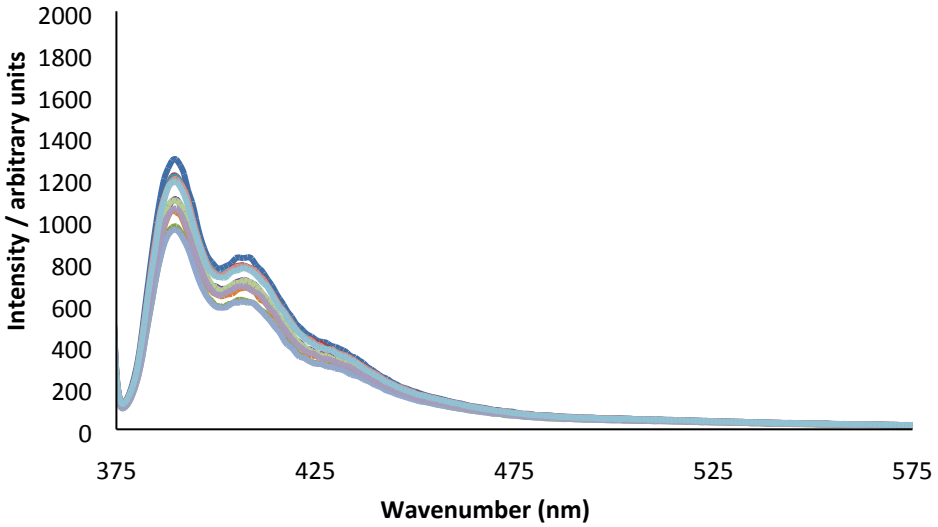


Fig. S6. Sensor **Py-1** (60 μM) titrated with heparin in 100% serum showing no binding response.

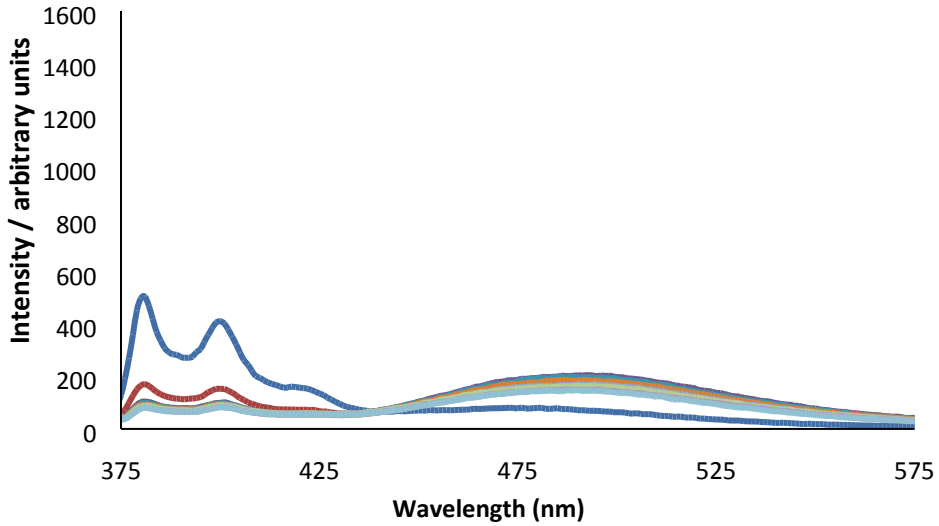


Fig. S7. Sensor **Py-2** (30 μM) titrated with heparin in buffer showing clear SAMul sensing response.

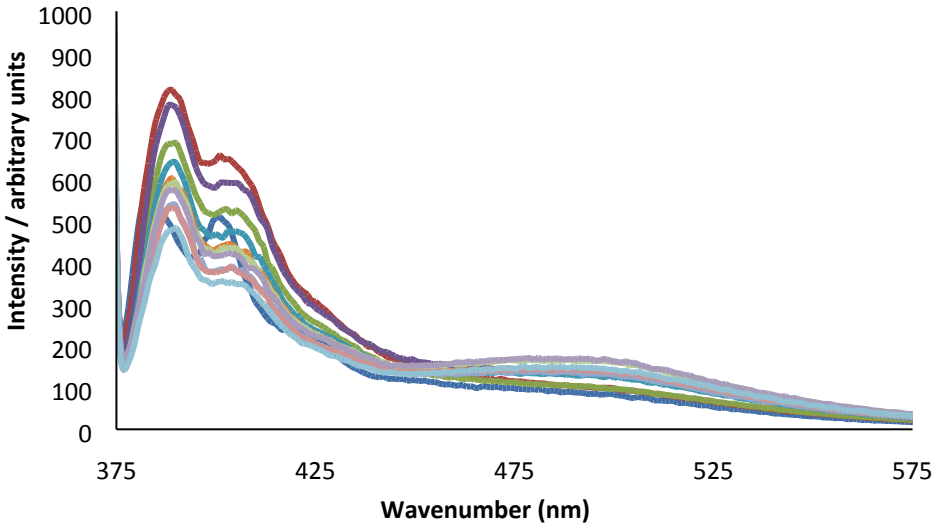


Fig. S8. Sensor **Py-2** (30 μM) titrated with heparin in 12.5% serum showing a small SAMul binding response.

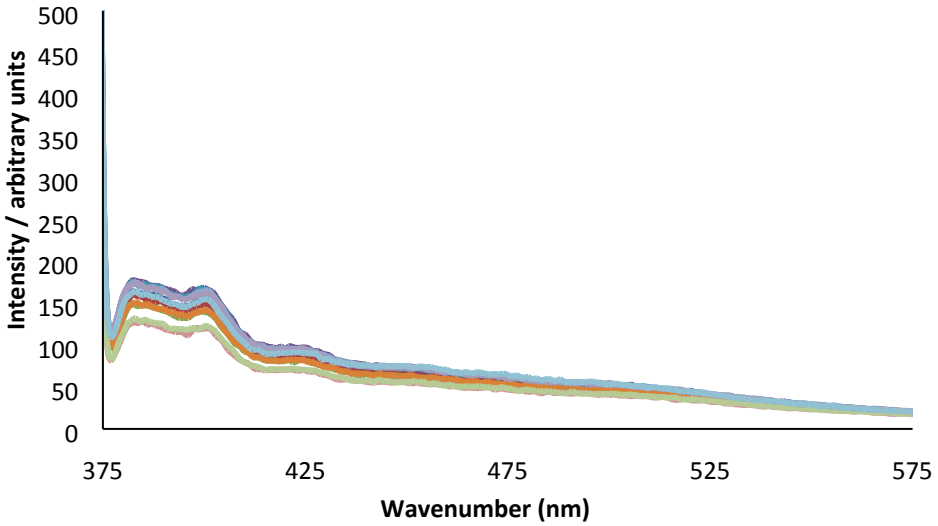


Fig. S9. Sensor **Py-2** (30 μM) titrated with heparin in 100% serum showing no binding response.

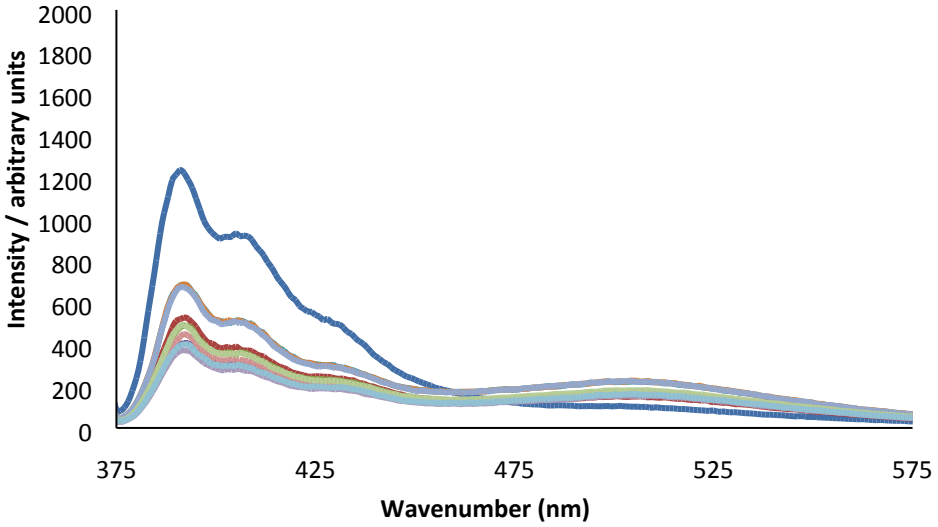


Fig. S10. Sensor **Py-1** at elevated concentration (426 μM) titrated with heparin in 100% serum, showing some SAMul binding response under these conditions.

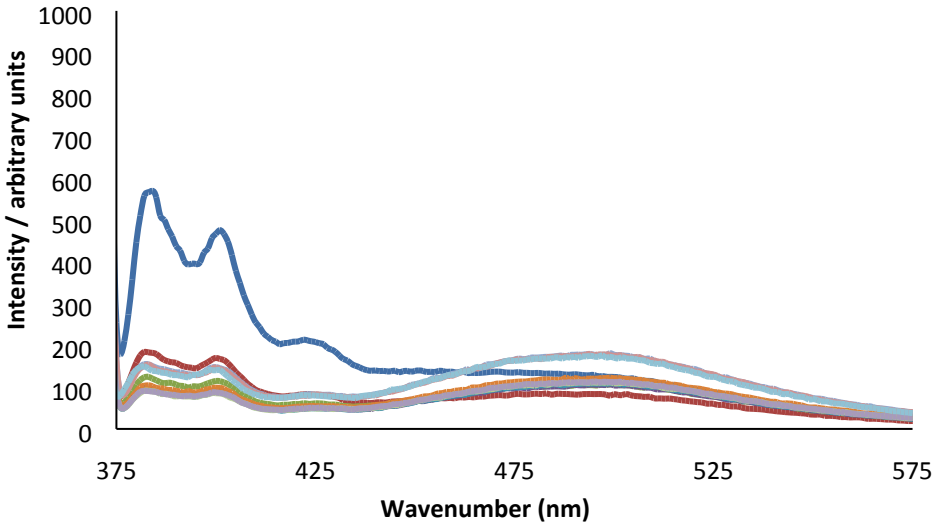


Fig. S11. Sensor **Py-2** at elevated concentration (213 μM) titrated with heparin in 100% serum, showing significant SAMul binding response under these conditions

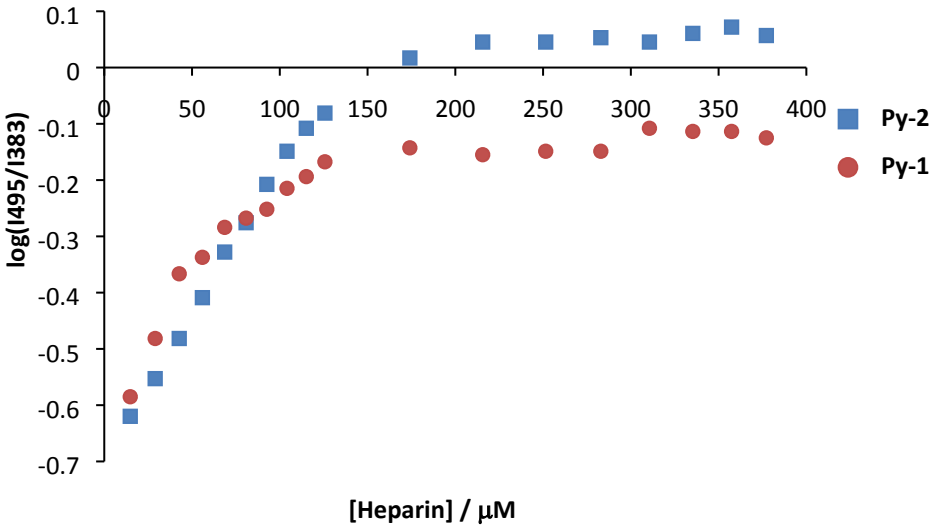


Figure S12. The changes of the fluorescence intensity ratio of **Py-G1** and **Py-G2** ($\log(I_{495}/I_{383})$) as extracted from Figs. S7 and S8, plotted against increasing heparin concentration in 100% serum.

7. References

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