Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Heparin Sensing Based on Multisite-Binding Induced Highly Ordered Perylene Nanoaggregates

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1. Experimental Procedures

Materials and Instruments

Unless otherwise stated, all solvents and chemicals were purchased commercially (e.g. Sigma-Aldrich, TCI chemicals, and Alfa Aesar Chemicals) and used without further purification. Cucurbit[8]uril (CB[8]) was purchased from Strem Chemicals Inc. (Newburyport, MA) and dried at 110 ºC for 24 hours before use. Heparin (heparin sodium porcine mucosa having molecular weights ranging from 6,000 to 30,000 Daltons, with most chains in the range of 17,000 to 19,000 Daltons) and human serum (from human male AB plasma, USA origin, sterile-filtered) were purchased from Sigma-Aldrich.

^{1}H- and ^{13}C-NMR spectra were recorded on a Varian 400 MHz spectrometer in DMSO-d_6, or D_2O. Mass spectra were recorded on a Bruker Autoflex3 Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometer (MALDI-TOF MS).

UV-vis spectra were recorded with a dual-beam Perkin Elmer Lambda 950 Spectrophotometer using UV-WIN Lab version 5.1.5 software. Fluorescence spectra were acquired using a Jobin-Yvon Horiba Fluorolog 3-222 Fluorescence Spectrophometer. 1-cm quartz cuvettes or polystyrene (PS) plastic cuvettes were used for UV-vis and fluorescence studies. Quantum yields were determined using a previously reported water-soluble perylenemonoimide as a standard (QY=0.50 in methanol).[1,2]

The optical images of samples were acquired using a Canon D60 digital camera under room light. The fluorescence images were acquired using a Canon D60 digital camera in a dark room when samples were excited using a blue laser pointer (405 nm).

DLS analyses were performed using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK).

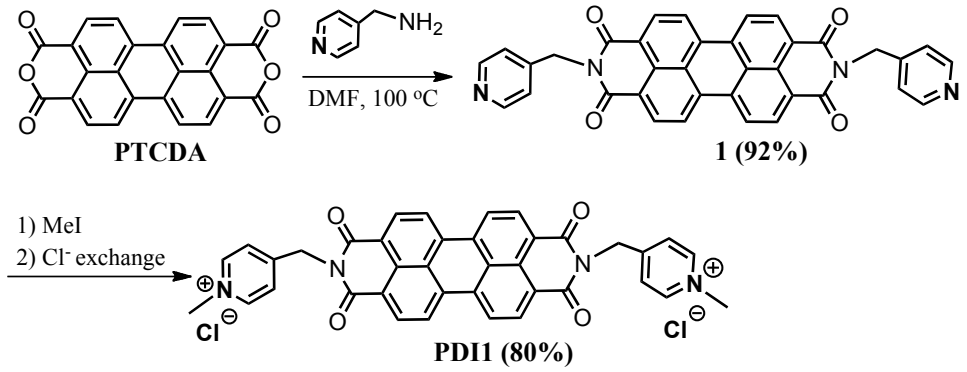
Binding Constant Determination

The binding constants of CB[8]:**PDI1** complexes were determined by fluorescence titration and calculated with a 1:1 binding model using the Origin program and the equation (Int=Igh+(Ig-Igh)*(((Go-Ho-1/Kg)/2+sqrt(((Ho+Go+1/Kg)^2)/4-Ho*Go))/Go), where Ig is the fluorescence intensity of guest, Igh is the intensity of host-guest complexes, Go is the concentration of the guest, and Kg is the binding constant.[3]

Cryo-EM Imaging

3 μL of each sample ($2x10^{-4}$ M, in water) was applied onto a glow-discharged holey carbon grid (Quantifoil, 1.2/1.3 300 mesh) followed by a 60 sec incubation and blotting for 9 sec at 20 °C with 100% humidity and flash-freezing in liquid ethane using a FEI Vitrobot Mark III. Cryo-EM image acquisition was performed on a 200 kV ThermoFisher Glacios electron microscope equipped with a Gatan K3 detector at UC Davis BioEM Core Facility. Micrographs were recorded at a nominal magnification of x45,000, resulting in a pixel size of 0.88 $Å^2$ using SerialEM.[4] The dose rate was 20 e $Å^2$ /sec with accumulated dose of 60 e in the 3 sec exposure.

Synthesis



Scheme S1: Synthesis of **PDI1**.

Synthesis of 1: A mixture of 3, 4, 9, 10-perylenetetracarboxylic dianhydride (PTCDA) (0.20 g, 0.51 mmol) and 4-(Aminomethyl) Pyridine (0.21 g, 2.04 mmol) were added into 6 mL of DMF in a round-bottom flask. The mixture was stirred at 100 °C for 12 hours under nitrogen. The reaction mixture was cooled down to room temperature and precipitate out using excess acetone. The resulting precipitate was collected by centrifugation at 6000 RPM and washed with excess acetone. The solid was dried under vacuum to yield **1** (0.27 g, 92%). ^{1}H NMR (Varian 400 MHz, CF_3COOD) δ 9.0 (d, J = 8 Hz, 4H), 8.95 (d, J = 8 Hz, 4H), 8.84 (d, J = 8 Hz, 4H), 8.31 (d, J = 8 Hz, 4H), 5.92 (s, 4H); ^{13}H NMR (Varian 100 MHz, CF_3COOD) δ = 166.3, 141.0, 136.3, 133.2, 129.4, 126.7, 124.3, 121.4 ppm; MALDI-TOF: m/z = 575.07 $[M+3H]^+$ (calc'd. 575.15 for $C_{36}H_{20}N_4O_4$).

Synthesis of PDI1: Compound **1** (0.1 g, 0.175 mmol) and methyl iodide (0.5 g, 3.52 mmol) were added in toluene (5 mL) in a round bottom flask. The resulting mixture was stirred at 100 °C for 12 hours under nitrogen. The reaction mixture was cooled down to room temperature and precipitate out using excess acetone. The resulting precipitate was collected by centrifugation at 6000 RPM and washed with acetone. The solid was dried under vacuum at 110 ^{O}C overnight. The dry solid was dissolved in 5 mL DMSO and aqueous HCl (1.5 mL, 4M) was added for anion exchange. The resulting mixture was stirred at 50 °C for 4 hours and it was cooled down to r. t. followed by precipitate with excess acetone. The precipitate was collected by centrifugation and washed with acetone. The solid was dried under vacuum to yield **PDI1** (94 mg, 80%). ^{1}H NMR (Varian 400 MHz, DMSO-d_6) δ 8.95 (d, J = 8 Hz, 4H), 8.88 (d, J = 8 Hz, 4H), 8.56 (d, J = 8 Hz, 4H), 8.16 (d, J = 8 Hz, 4H), 5.51 (br, 4H), 4.27 (s, 6H); ^{13}H NMR (Varian 100 MHz, CF_3COOD) δ = 169.3, 161.6, 157.0, 145.0, 136.4, 139.3, 129.4, 127.5, 124.4, 121.5, 47.5, 43.1 ppm; MALDI-TOF: m/z = 604.25 $[M+2H]^+$ (calc'd. 604.20 for $C_{38}H_{24}N_6N_4O_4$).

2. Supplementary Figures

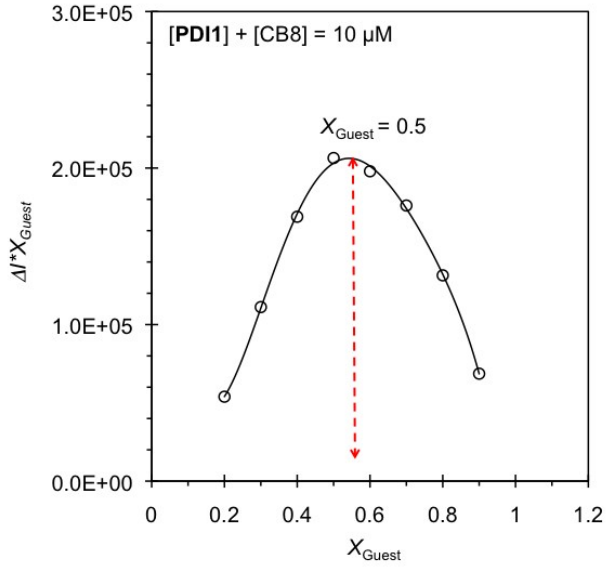


Figure S1. The Job plot for CB[8]:**PDI1** (the total concentration of the guest **PDI1** and the host CB8 was fixed at 10 μM).

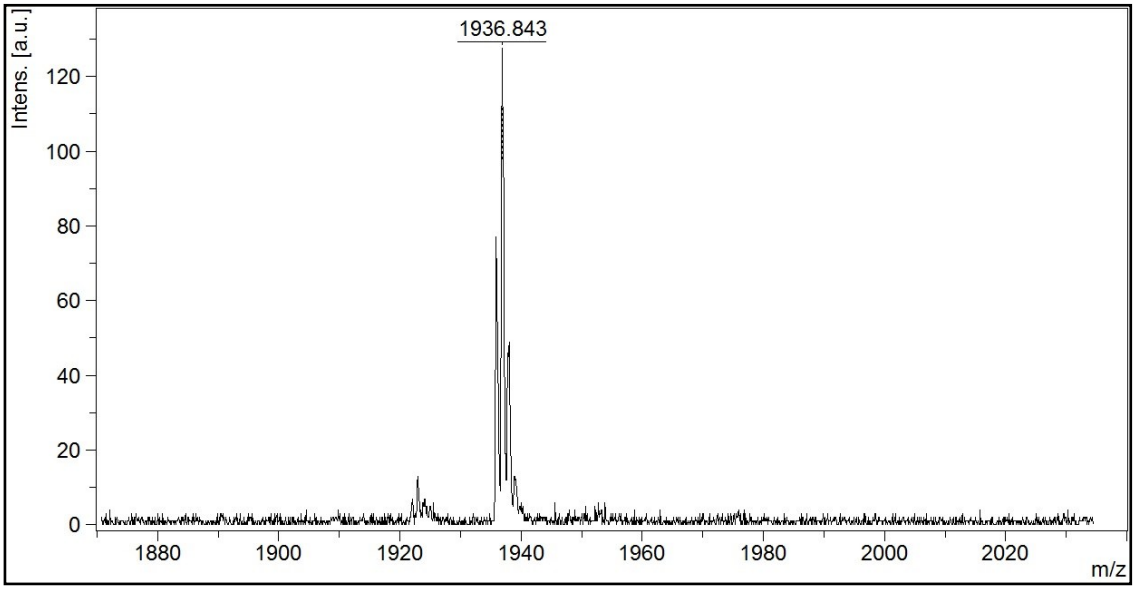


Figure S2. MALDI mass of CB[8]:**PDI1** using α-Cyano-4-hydroxycinnamic acid as a matrix. [CB[8]:**PDI1** + 3H]: Observed mass 1936.84 Da, calculated mass 1936.29Da.

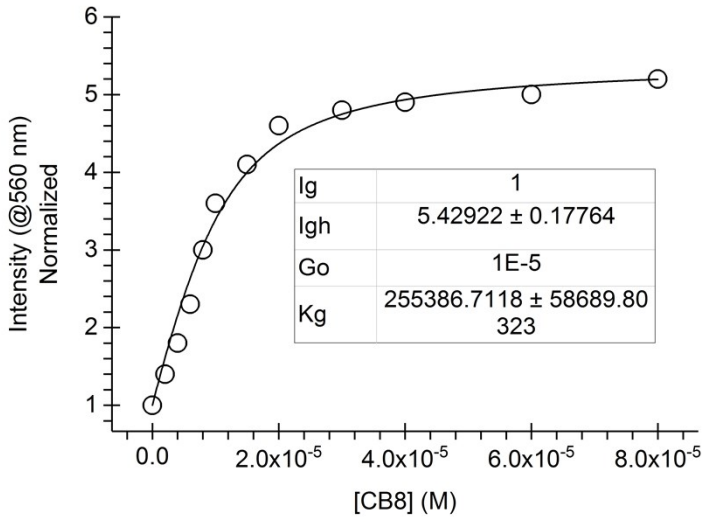


Figure S3. The binding curve for CB[8]:**PDI1** (The fluorescence titration experiment of **PDI1** (10 μM) in ultrapure water in the presence of CB[8] and the fluorescence intensity was plotted against the concentration of CB[8]). The solid line represents the best fitting of the data according to a 1:1 binding.

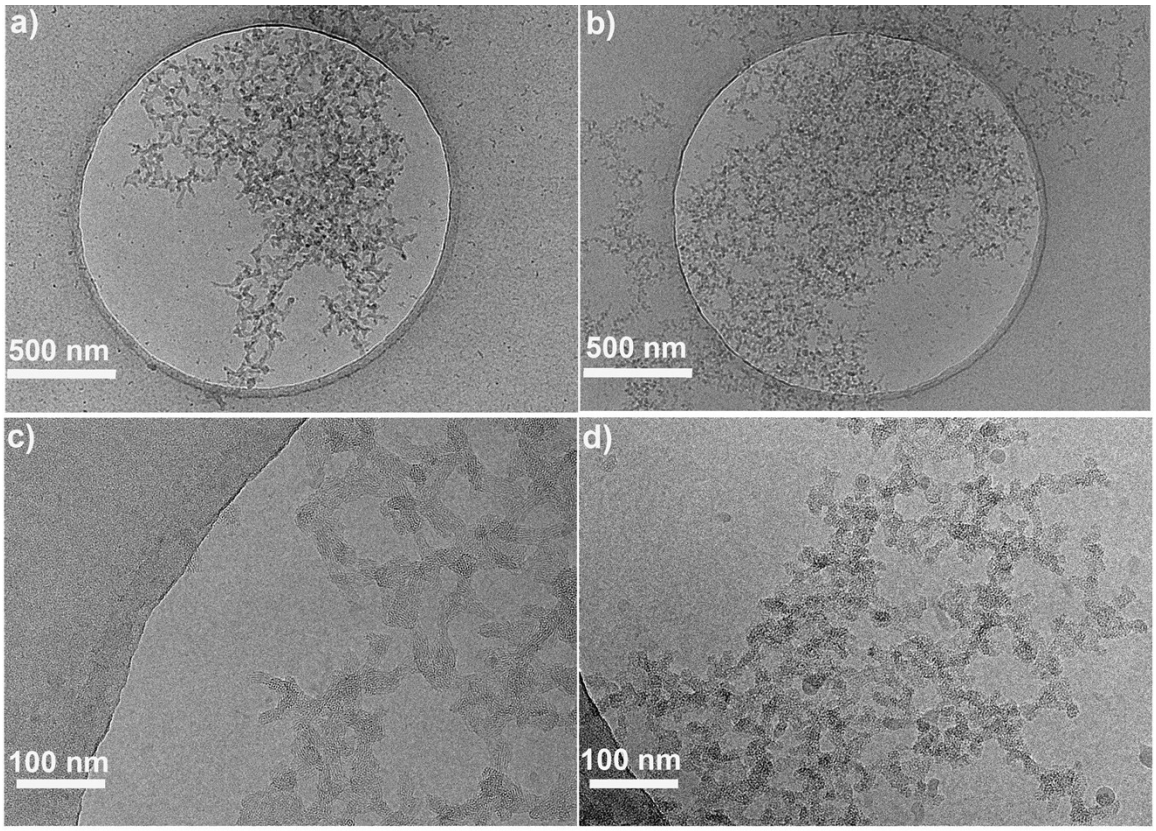


Figure S4. (a) and (c) Cryo-EM images of **PDI1** ($2x10^{-4}$ M) with heparin in water. (b) and (d)) Cryo-EM images of CB[8]:**PDI1** (1:1, $2x10^{-4}$ M **PDI1**) with heparin in water.

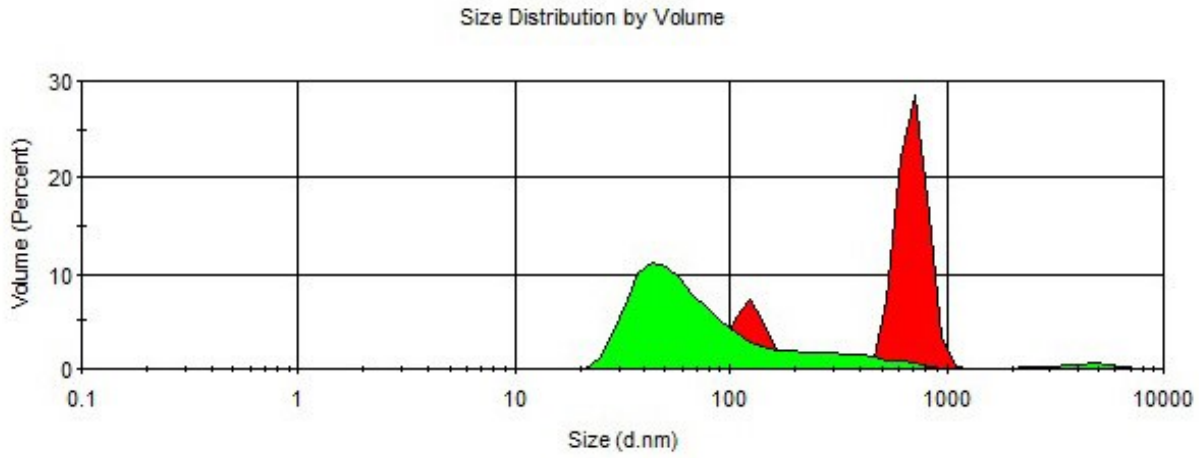


Figure S5. The size distribution by volume of **PDI1** ($2x10^{-4}$ M) (red) and HEP:**PDI1** (0.2 mg/mL HEP, $2x10^{-4}$ M **PDI1**) (green) in ultra-pure water.

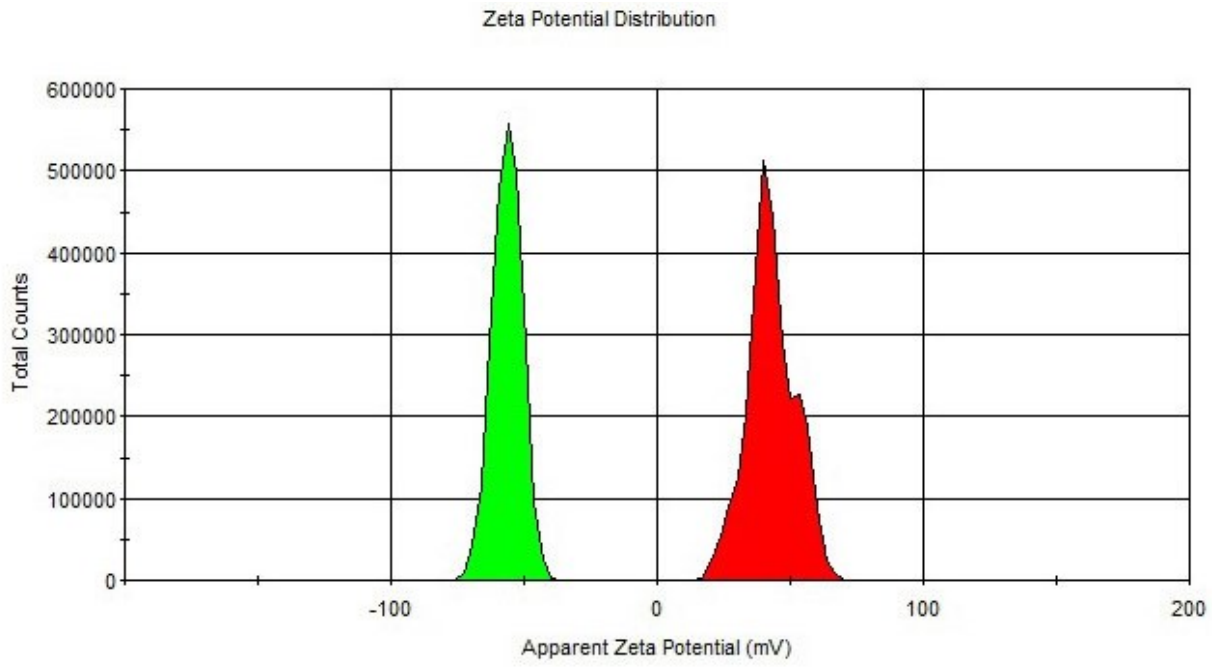


Figure S6. The zeta potential distribution of **PDI1** ($2x10^{-4}$ M) (red) and HEP:**PDI1** (0.2 mg/mL HEP, $2x10^{-4}$ M **PDI1**) (green) in ultra-pure water.

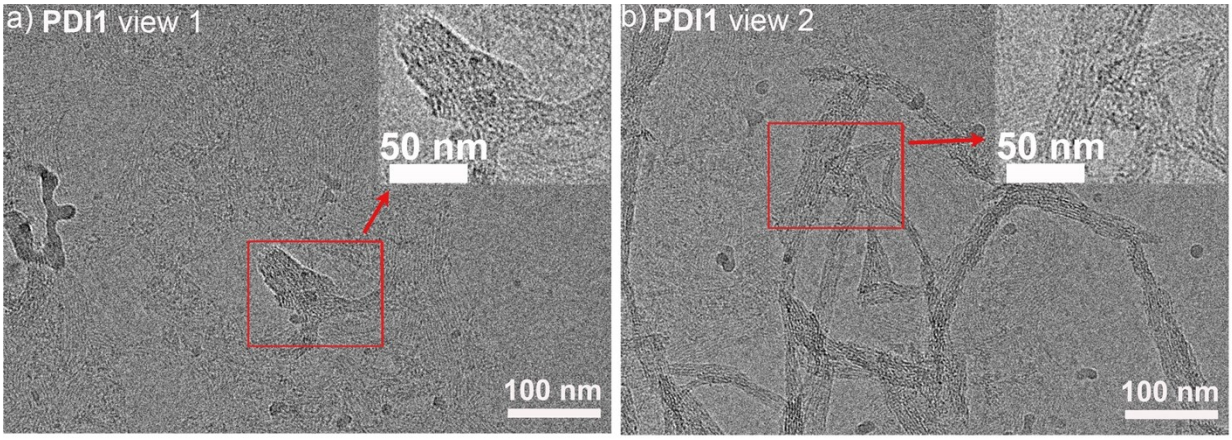


Figure S7. Low power views of cryo-EM images of aggregates of **PDI1** ($2x10^{-4}$ M) in water. Inset: the magnified cryo-EM images of **PDI1** aggregates.

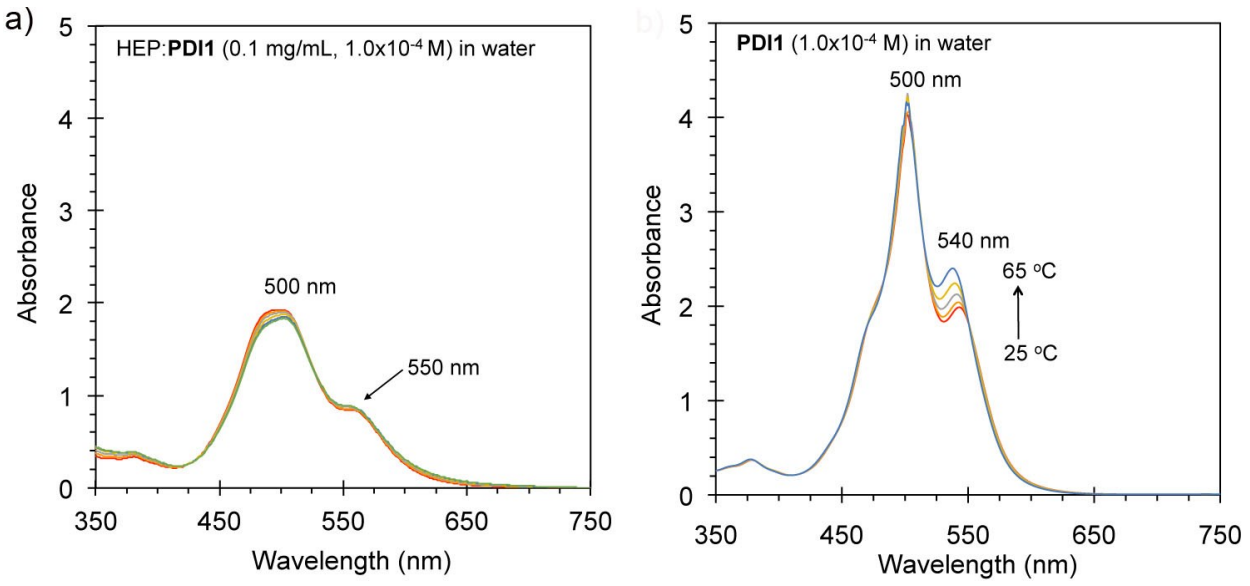


Figure S8. (a) The absorbance of HEP:**PDI1** (0.1 mg /mL HEP and 1.0x10^{-4} M **PDI1**) in water with temperatures from 25 °C to 65 °C. (b) The absorbance of HEP:**PDI1** (1.0x10^{-4} M) in water with temperatures from 25 °C to 65 °C.

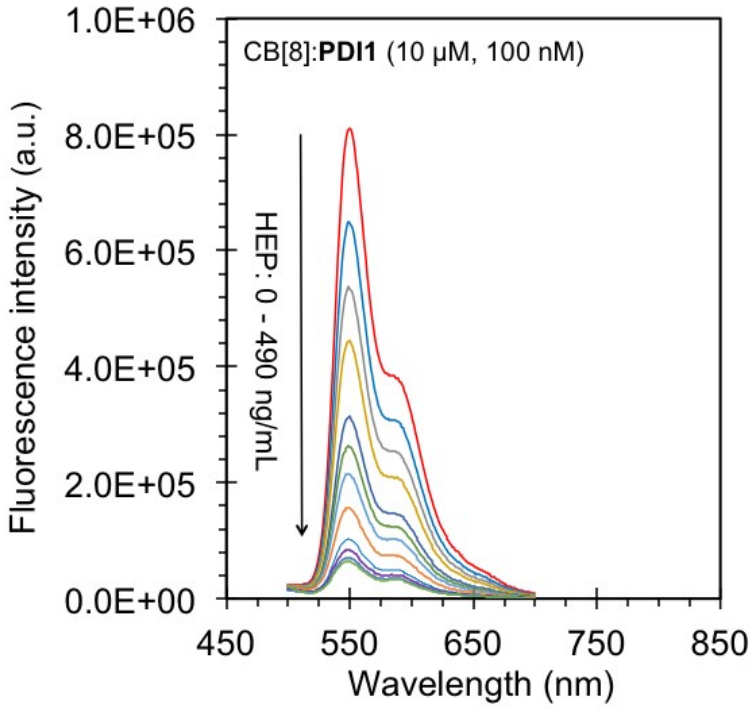


Figure S9. The fluorescence emission spectra of CB[8]:**PDI1** (10 μM CB[8], 100 nM **PDI1**) in Tris buffer (pH 7.4) in the presence of HEP from 0 to 490 ng/mL.

3. Detection Limit

The limit of detection (LOD) was calculated from the fluorescence titration using following equation.[5]

$$\mathrm{LOD} = 3\,\boldsymbol{\sigma}/\mathrm{K} \approx 2.4\ \mathrm{ng/mL}$$

Where $\boldsymbol{\sigma}$ is the standard deviation (the standard deviation of the fluorescence changes of the CB[8]:**PDI1** (10 μM, 100 nM) in Tris buffer (pH 7.4) in the presence of 9.8 ng/mL of HEP with three duplicates. $\boldsymbol{\sigma}$ was calculated to be 0.011) and K (equals to 0.0138) is the slope between the fluorescence intensity of CB[8]:**PDI1** versus HEP concentration.

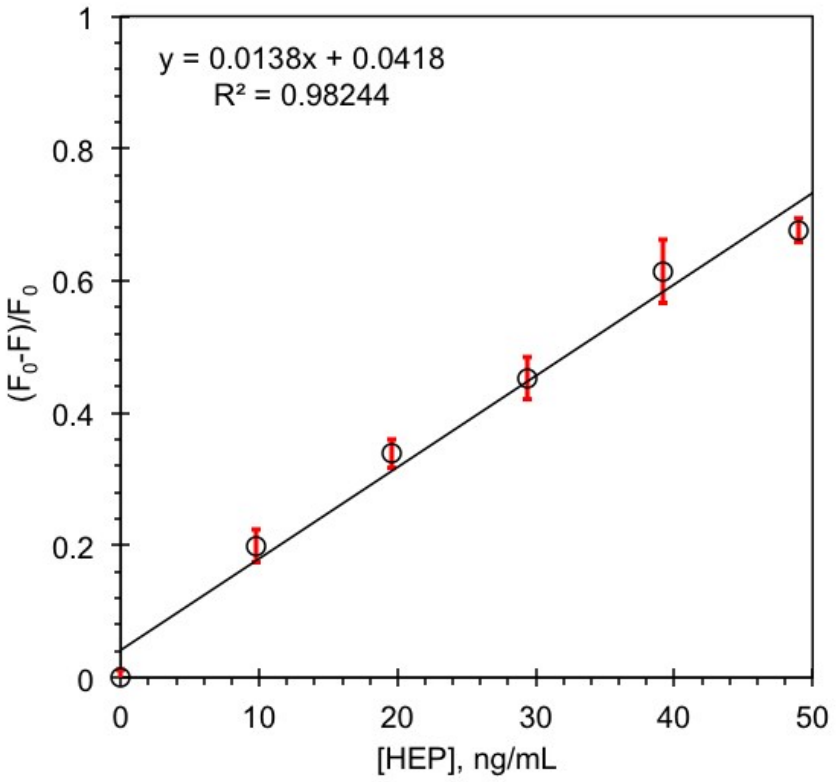


Figure S10. A linear plot of $(F_0\text{-}F)/F_0$ vs. HEP in the concentration range of 0 to 49 ng/mL. Where F_0 and F are the fluorescence intensity of CB[8]**:PDI1** in Tris buffer (pH 7.4).

4. Comparison of Fluorescent Sensors for Heparin

Probes	Response type	LOD in buffer	Application	Ref.
Pseudoisocyanine	Turn on	2 nM	3% serum	8a
Salicyladehyde azine derivatives	Turn on	8.64 mU/mL	1% horse serum	8b
Tetraphenylethene derivatives	Turn on	1.53 ng/mL	1% serum	8c
Pyrene derivative/GO	Turn-on	0.046 U/mL	Not measured	8d
Polyadenosine-coralyne complex	Turn-off	4 nM	2.5% plasma	8e
CuInS2 quantum dots	Turn-off	12.36 nM	Not measured	8f
Rhodamine B-labelled peptides	Turn-off	7.5 pM	216-fold dilution	8g
Perylene derivative/host-guest complex	Turn off	2.4 ng/mL (or 0.13 nM or 0.5 mU/mL)	1% serum	This work

Table S1. Comparison of reported fluorescent sensors for heparin.

5. ^{1}H-NMR, ^{13}C-NMR and Mass Spectra

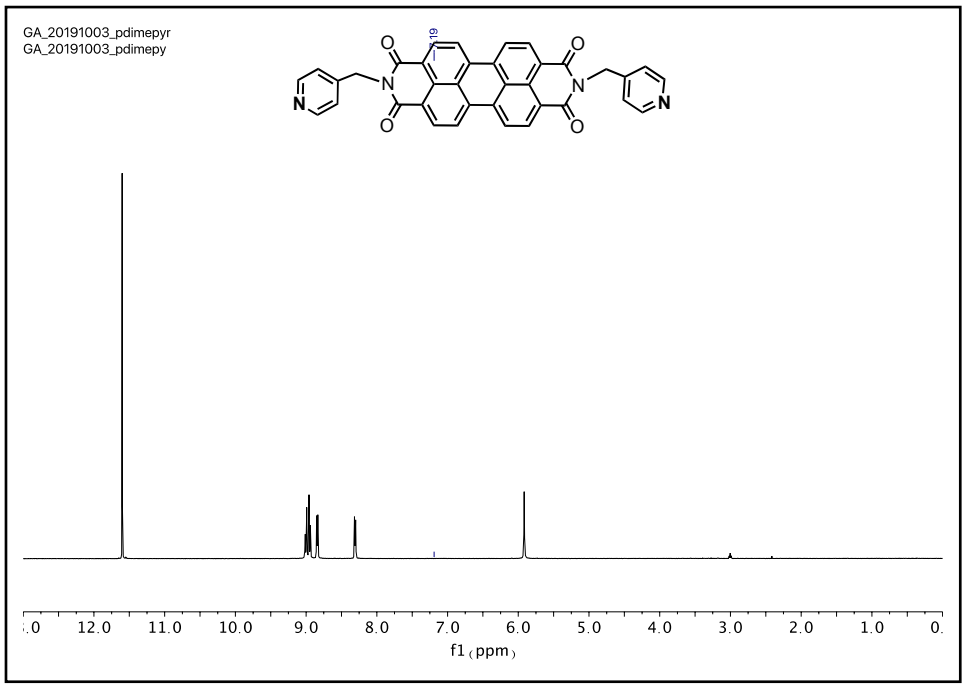


Figure S11. ^{1}H-NMR of **1** in CF_3COOD.

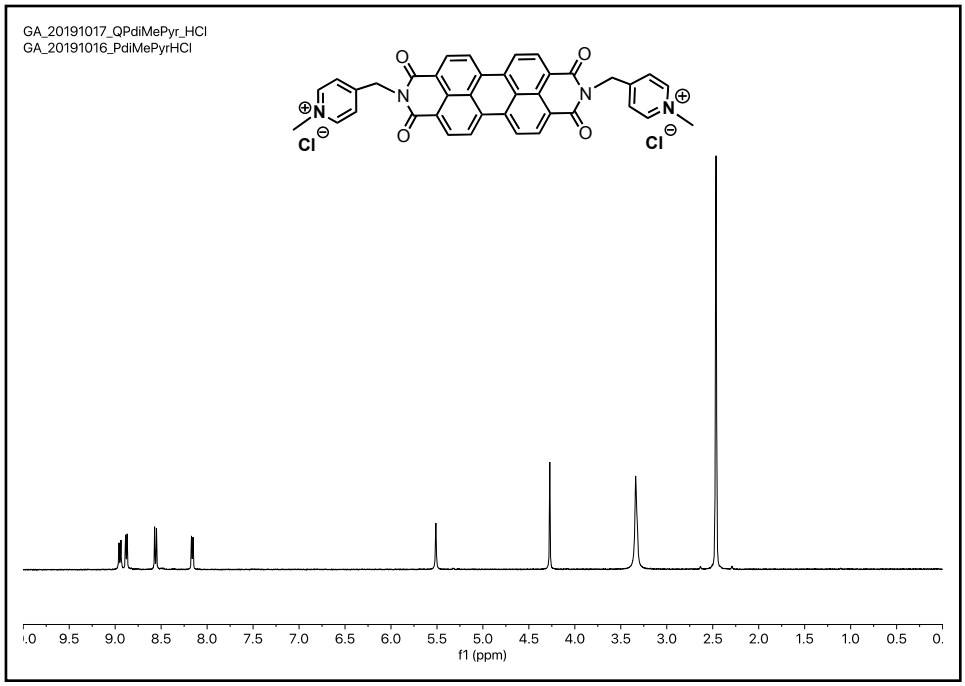


Figure S12. ^{1}H-NMR of **PDI1** in DMSO-d_6

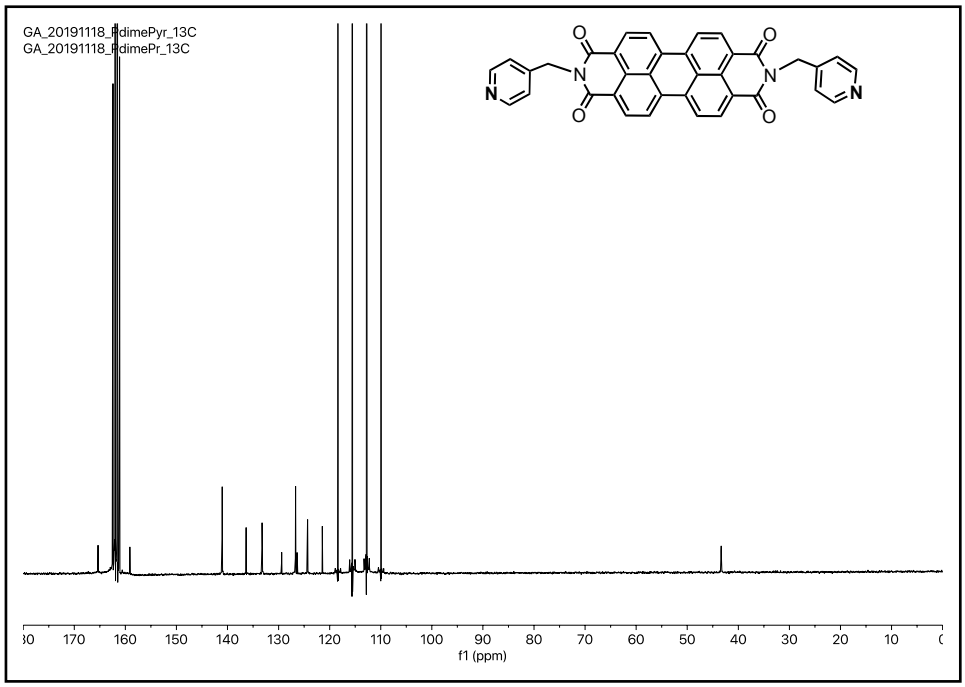


Figure S13. ^{13}C-NMR of **1** in CF_3COOD.

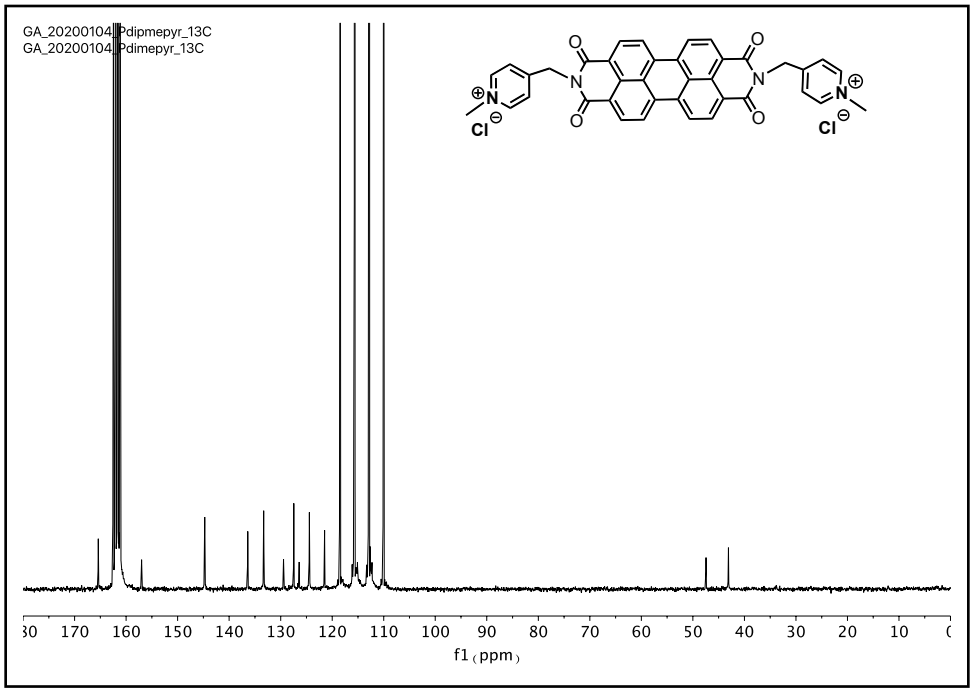


Figure S14. ^{13}C-NMR of **PDI1** in CF_3COOD.

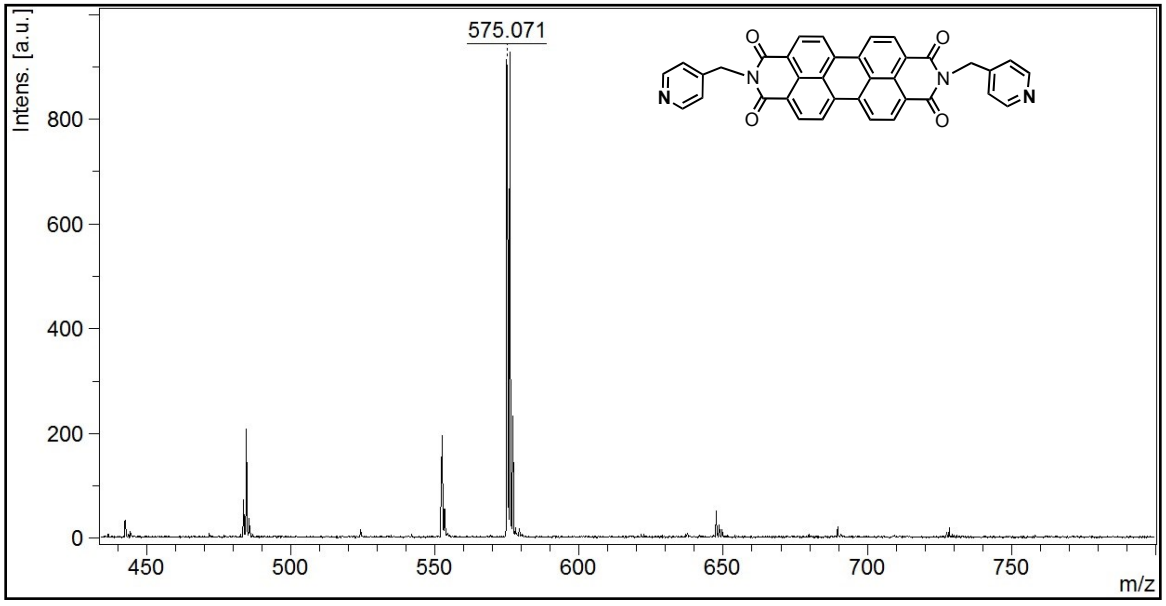


Figure S15. MALDI mass of **1** using 2-5-dihydroxy benzoic acid acid as a matrix. [**1**+3H]$^+$: Observed mass 575.07 Da, calculated mass 575.15Da.

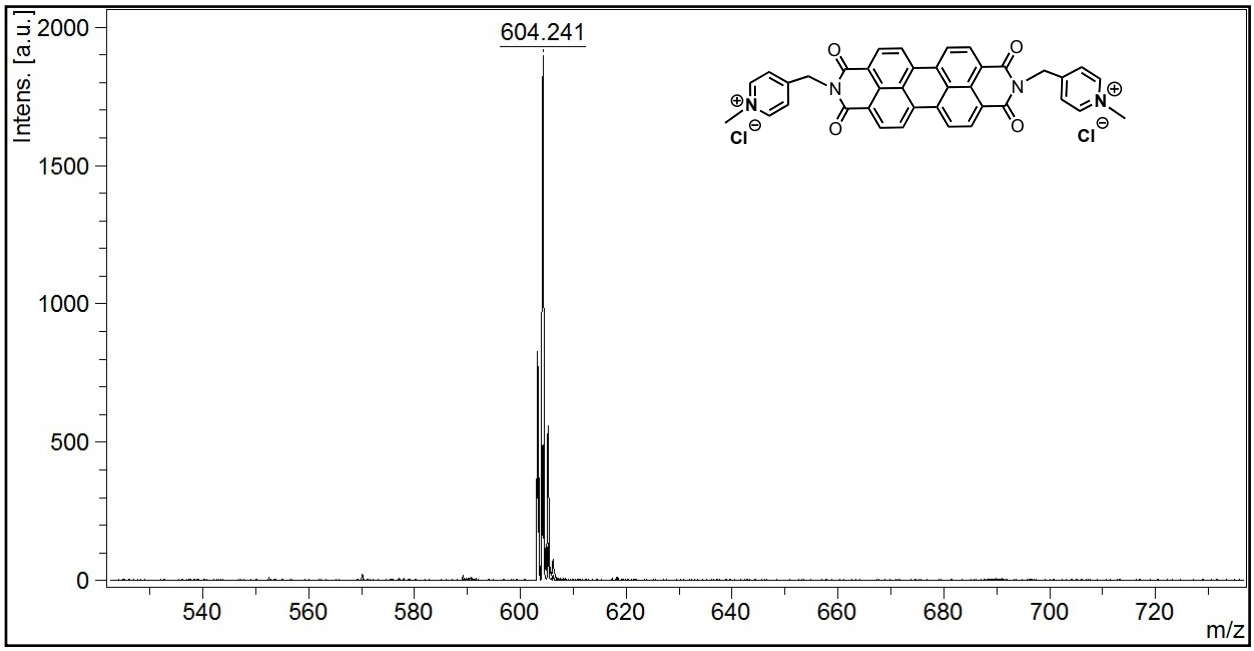


Figure S16. MALDI mass of **PDI1** using α-Cyano-4-hydroxycinnamic acid as a matrix. [**PDI1**+2H]$^{+}$: Observed mass 604.24 Da, calculated mass 604.20Da.

6. References

1. L. Huang, S.-W. Tam-Chang, W. Seo and K. Rove, *Adv. Mater.,* 2007, **19**, 4149-4152.
2. L. Huang and S.-W. Tam-Chang, *J. Fluoresc.,* 2011, **21**, 213-222.
3. W. M. Nau and X. Zhang, *J. Am. Chem. Soc.,* 1999, **121**, 8022–8032.
4. D. N. Mastronarde, *J. Struct. Biol.,* 2005, **152**, 36-51.