Supporting Information for

Tunable Nanostructures by Directional Assembly of Donor-Acceptor Supramolecular Copolymer and Antibacterial Activity

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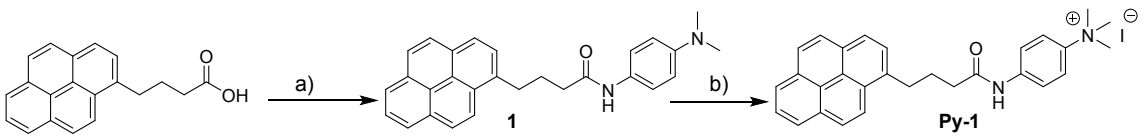
Materials and Methods:

Solvents and reagents were purchased from commercial sources [1] and used as it is unless otherwise mentioned. ^{1}H NMR spectra and ^{1}H ROESY spectrum were recorded on a Bruker DPX- DPX-500 MHz NMR spectrometer and all the spectra were calibrated using TMS as the internal standard. UV/Vis absorption spectra were recorded on a PerkinElmer Lamda 25 spectrophotometer. FT-IR spectra were recorded in a PerkinElmer Spectrum 100FT-IR spectrometer. DLS and zeta potential measurements were carried out in a Malvern instrument. Fluorescence experiments were performed with a FluoroMax-3 spectrophotometer from HORIBA Jobin Yvon. TEM measurements were carried out in a Technai G2 (FEI) microscope with an accelerating voltage of 200kV. Atomic force microscopy (AFM) images were taken in an Innova instrument from Bruker. Fluorescence microscopic images were obtained by microscope (Olympus IX73, Tokyo, Japan). SEM images of bacteria were with JEOL-JSM-7500F field-emission scanning electron microscopy (SEM).

Synthesis and characterization: Synthesis of NDI-1 has been reported by us earlier. [2] Py-1 was prepared in a few steps as shown in Scheme S1.



Reagents and Conditions: a) N,N-Dimethyl-p-phenylenediamine, DMAP (cat), EDC, DMF, 24 h, rt, b) MeI, MeOH, 36h, 80^{o}C.

Scheme S1: Synthesis scheme of Py-1

Compound 1: 1-pyrene butyric acid (200 mg, 0.69 mmol) and *N*,*N*-Dimethyl-*p*-phenylenediamine (114 mg, 0.83 mmol) were dissolved in dry DMF (4mL). 4-dimethylaminopyridine (17 mg, 0.13 mmol) was added to the reaction mixture and it was stirred for 10 min at 0 °C. After that, a solution of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (160.0mg, 1.03 mmol) in dry CH_2Cl_2 (1 mL) was slowly added to the reaction mixture and it was stirred for 24 h at rt under N_2 atmosphere. Afterthat, organic layer was washed with aqueous HCl (1N) solution (3 x 10 mL) followed by brine (1 x 10 mL) and dried over anhydrous Na_2SO_4. Excess solvent was evaporated to get the crude product. It was further purified by column chromatography using silica gel as the stationary phase and 1% MeOH/DCM as the eluent to get the pure product as a light yellow solid. Yield: 210 mg (73%). ^{1}H NMR (500 MHz, $CDCl_3$, TMS): 8.33 (s, 1H), 8.16 (d, 1H), 8.10 (d, 2H) 8.04 (t, 2H), 7.97 (t, 2H), 7.88 (d, 1H), 7.30 (d, 2H), 6.91 (s, 1H), 6.69 (d, 2H), 3.45 (t, 2H, J = 15 Hz), 3.45 (t, 2H), 2.90 (s, 6H), 2.40 (t, 2H, J = 15 Hz), 2.33-2.27 (m, 2H). m/z calc for $C_{28}H_{26}N_2O$ $[M+Na]^+$: 429.1943; found: 429.2585.

Py-1: In a sealed tube, compound **1** (100 mg, 0.24 mmol) and methyl iodide (300 µL, 4.8 mmol) were taken and dissolved in methanol (5 mL). Reaction mixture was stirred at 80 °C for 36h in sealed condition. Then solvent and excess methyl iodide was evaporated to get yellowish crude compound which was further purified by column chromatography using 10% MeOH/DCM as an eluent to get the desired product as a yellow solid. Yield: 240 g (72%). ^{1}H NMR (500 MHz, DMSO-d_6, TMS): δ (ppm): 10.24 (s, 1H), 8.39 (s, 1H), 8.29-8.18 (m, 4H), 8.12 (d, 2H) 8.04 (t, 1H), 7.95 (d, 1H), 7.85 (d, 2H), 7.76 (d, 2H), 3.5 (s, 9H), 3.45 (t, 2H), 3.39 (t, 2H, J = 15), 2.16-2.06 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6, TMS): 171.57, 166.5, 139.8, 136.3, 130.8, 130.4, 129.3, 128.2, 127.5, 127.4, 127.2, 126.5, 126.1, 124.9, 124.8, 124.2, 123.5, 56.5, 35.9, 32.1, 27.0. m/z calculated for $C_{29}H_{29}N_2O^+$ $[M+Na]^+$: 444.2172; found: 444.4859, M.P. = 118 °C.

Additional experimental Section:

Determination of association constant for D-A complex: Py-1 and NDI-1 were prepared in THF (C = 10.0 mM). Measured amount (400 μl) of each stock solution was transferred to a screw capped vial, mixed together and solvent was evaporated by blowing air which produced a red film. To this 400 μl of water was added and the sample was subjected to a few cycles of sonication and gentle heating which produced a clear red solution consist of 10.0 mM concentration of each component. It was gradually diluted with a known volume of water and intensity of the CT-band (λ_{max} = 550 nm) was monitored after each dilution. Association constant (K_a) was estimated by fitting the experimental data to the following equation.

$$\frac{C}{A} = \frac{1}{\sqrt{K\varepsilon l}} \times \frac{1}{\sqrt{A}} + \frac{1}{\varepsilon l}$$ ----------Equation 1

Where c, A, l and Ɛ indicates concentration, absorbance, optical path length and extinction coefficient, respectively.

Effect of salt on donor-acceptor assembly: To an aqueous solution (2.0 mL, 0.5 mM) of Py-1 + NDI-1 (1:1), a measured amount of 0.5 M NaCl solution was gradually added and the CT-band intensity was monitored as a function of salt concentration. The change in the absorption intensity due to dilution was corrected with the change observed in a control experiment involving addition of equal volume of water without any salt.

Effect of pH on donor-acceptor assembly: To an aqueous solution (2.0 mL, 0.5 mM) of Py-1 + NDI-1 (1:1), aqueous HCl solution (0.1 M) was added in steps and the CT-band intensity was monitored as a function of solution pH. Likewise in another experiment, instead of HCl, measured amounts of NaOH solution (0.1 M) were added to the aqueous solution of Py-1 + NDI-1 (1:1). Before each spectral recording, the pH of the solution was checked using a pH

meter and finally the effect of dilution was corrected with the change observed in a control experiment involving addition of equal volume of water without any acid or base.

Cell culture condition: Human cervical cancer cell line (HeLa) was cultured in a high glucose Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and 1% L-Glutamine-penicillin-streptomycin at 37 °C in a humidified environment containing 5% CO_2. Cells were maintained by passaging them regularly at ~ 85% confluency.

MTT assay: HeLa cells were seeded in 96-well plates with a seeding density of 10,000 cells/ well. After 24 h incubation, the medium was replaced by different concentration of Py-1+ NDI-1 (95:5). The cells were then incubated for 12h. Then 50 μL of freshly prepared 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (2.5 mg/mL in DMEM) solution was added into each well. The medium with MTT solution was removed after 4 h incubation at the 37 ˚C. 100 μL DMSO was then added into each well and the plates were gently shaken for 10 min at room temperature to dissolve all precipitates formed. The absorbance of MTT at 570 nm was monitored by the microplate reader (VARIOSKAN, Thermo Fisher). Cell viability was expressed as a function of concentration of Py-1 by the ratio of absolute absorbance of the cells incubated with Py-1+ NDI-1 (95:5).

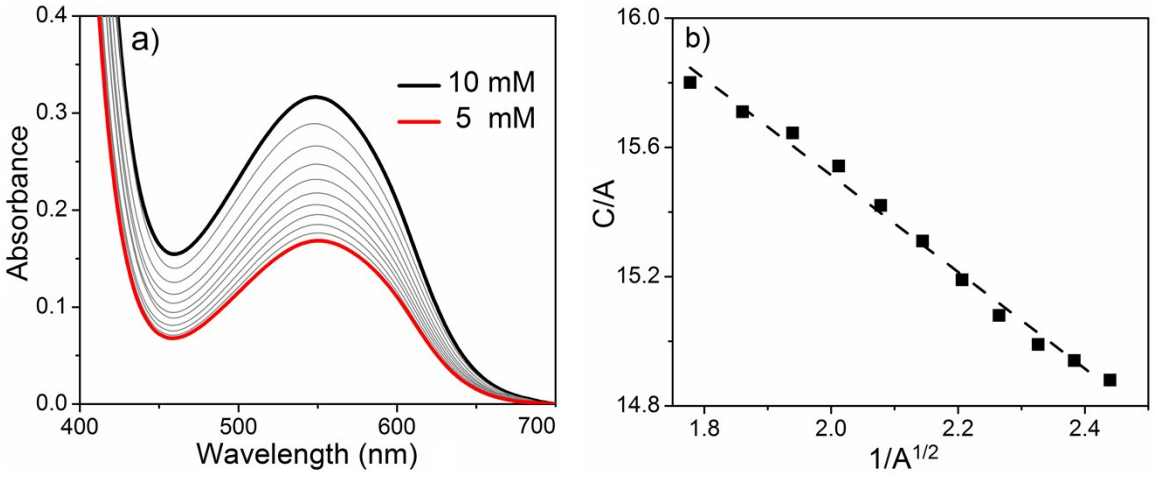


Figure S1: a) Concentration dependent UV/Vis spectra of fresh solution of NDI-1+Py-1 (1:1) in water; b) Fitting of the data using Equation-1 to determine the association constant.

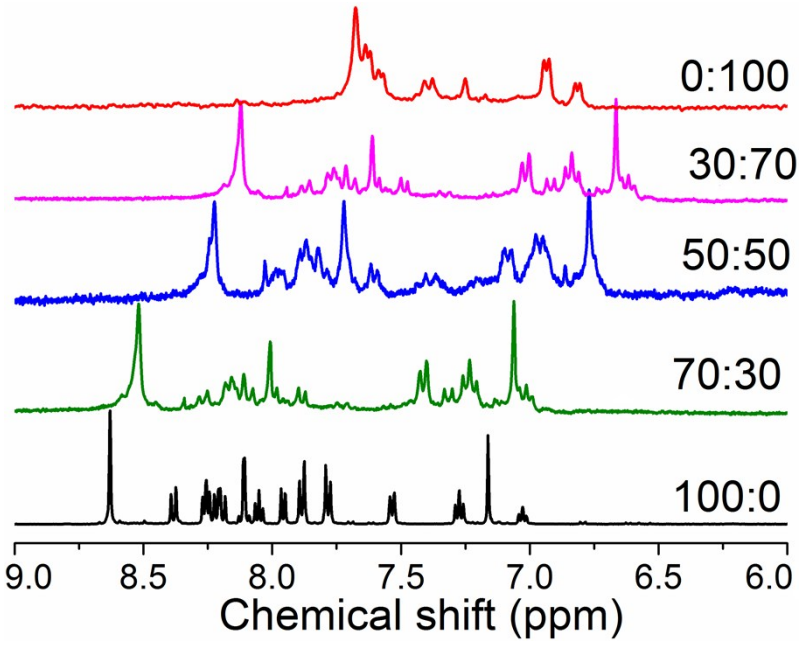


Figure S2: ^{1}H NMR spectra of Py-1+NDI-1 (1:1) (selected region) in varying ratio of DMSO:D_2O (numbers mentioned in the figure) . c = 5mM

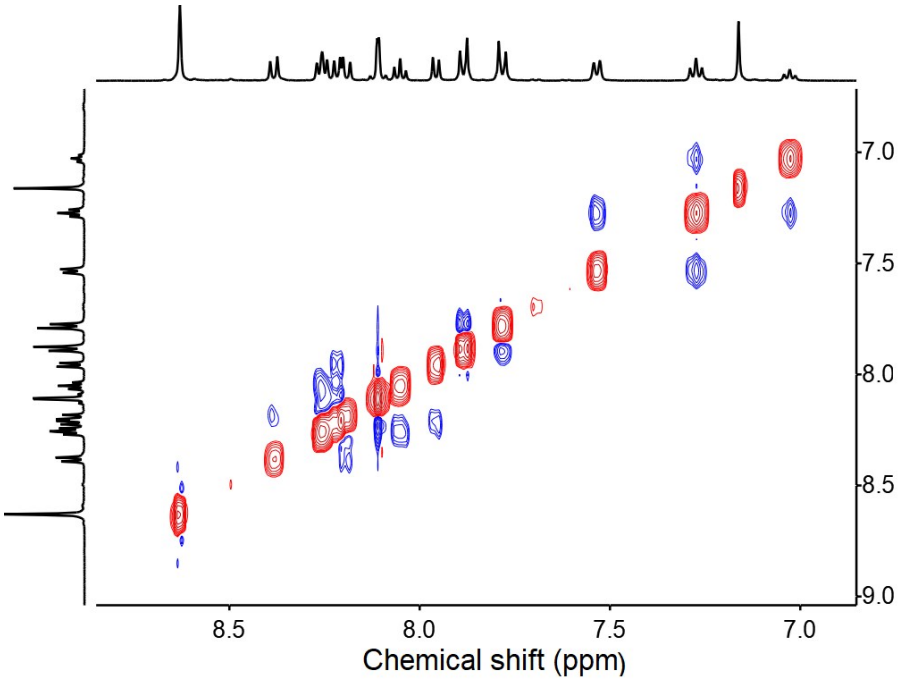


Figure S3: ROESY NMR spectrum (DMSO-d_6, 500 MHz, 20 mM, 293 K) of Py-1+NDI-1 (1:1), where diagonal blue and red spots correspond to intramolecular coupling.

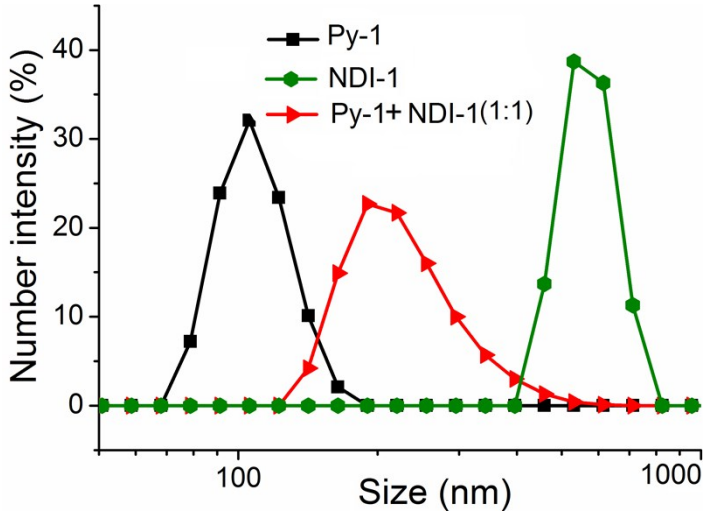


Figure S4: DLS profile of the individual components and their 1: 1 mixture (C = 0.2 mM) in water.

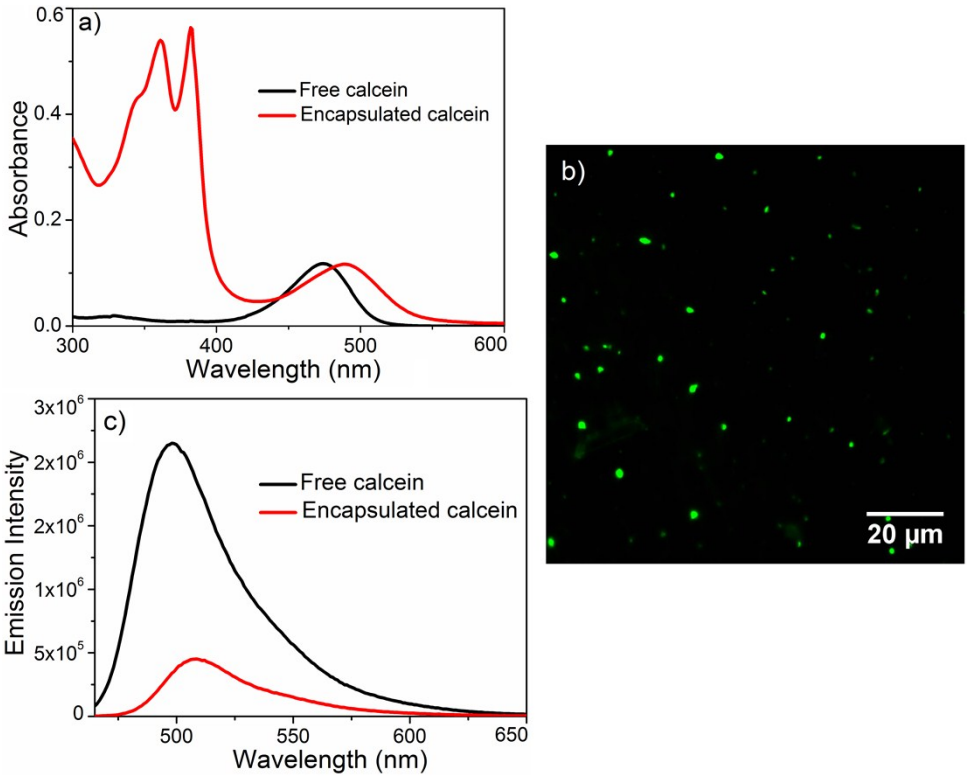


Figure S5: a) UV-Visible spectra for calcein encapsulated Py-1+NDI-1 (1:1) vesicle and free calcein (appropriate dilution was made so that the absorbance for the Calcein peak is almost equal. Bathochromic shift in the encapsulated spectrum possibly originated from aggregation of the dye in confined water inside polymersome); b) Fluorescence microscopy images (λ_{ex} = 450 nm) of Calcein encapsulated in Py-1+NDI-1 (1:1) plymersome, c) Absorbance matched normalized emission spectra (λ_{ex} = 450 nm) of Calcein encapsulated in Py-1+NDI-1 (1:1) plymersome and free dye in water (λ_{ex}=450nm, slit=3).

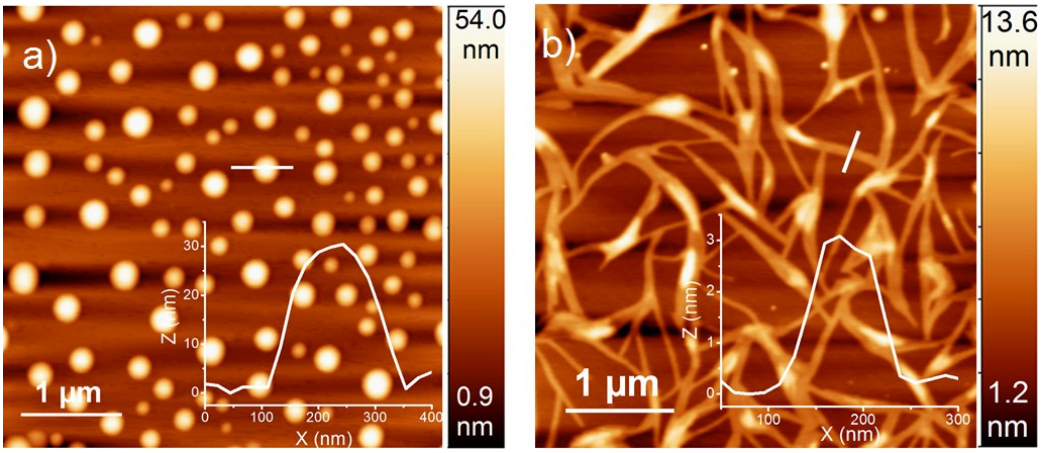


Figure S6: Tapping mode AFM images of drop-casted NDI-1+Py-1 (1:1) from water solution: a) fresh (after 1 h), b) aged (after 12 h), on mica surface; c = 0.2 mM. Graphs in the inset show height-width profile of part a and b in corresponding image which are processed by WSxM 5.0 software.

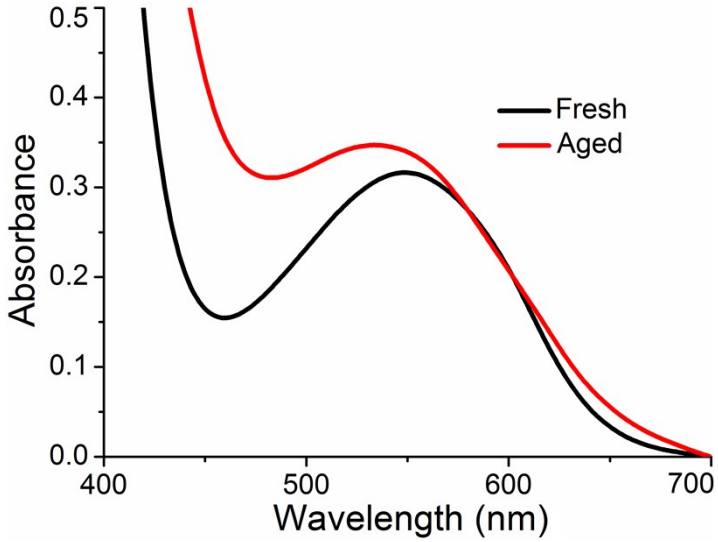


Figure S7: Change in charge transfer band intensity with aging the sample of Py-1+NDI-1 (1:1), c = 10 mM. Observed shift in the absorption maxima and band intensity are attributed to chromophoric re-organization as a consequence of spherical to elongated morphology transition.

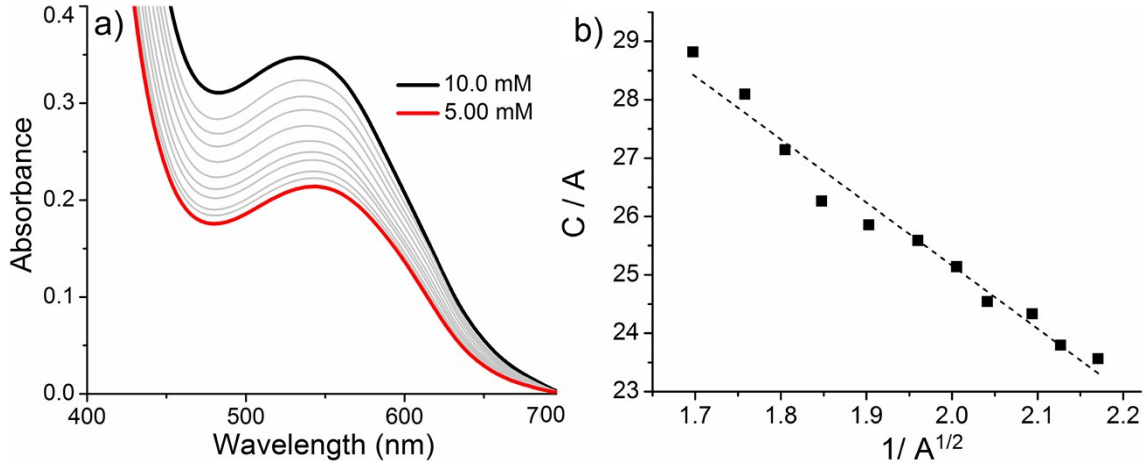


Figure S8: a) Concentration dependent UV/Vis spectra of aged (for 12h) solution of NDI-1+Py-1 (1:1) in water; b) Fitting of the data using Equation-1 to determine the association constant.

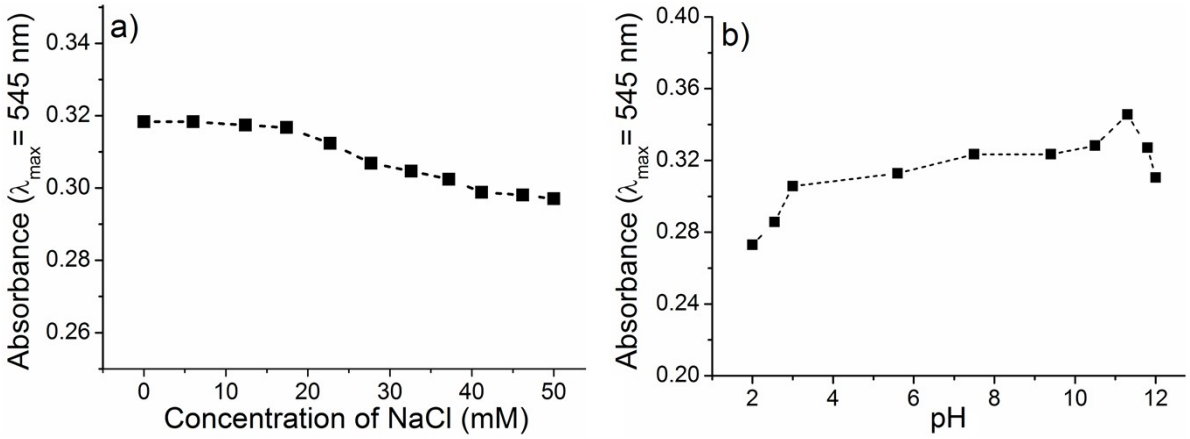
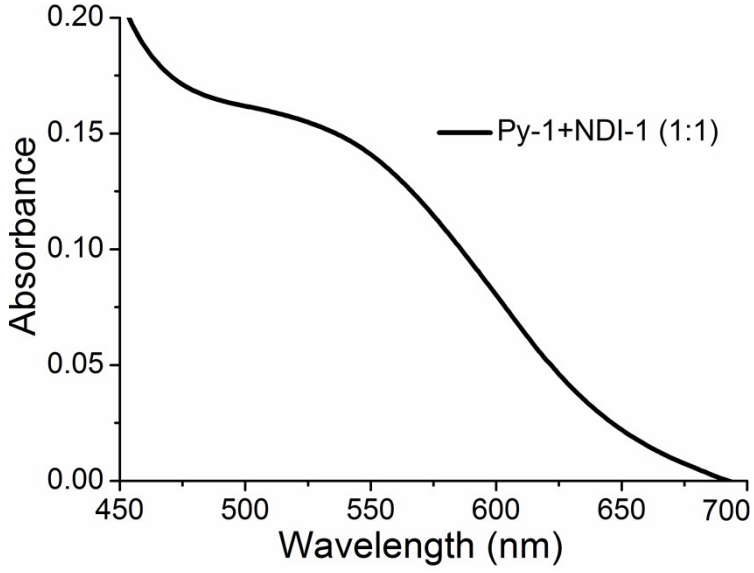


Figure S9: Variation in the CT-band intensity (545 nm) of equimolar ratio of NDI-1+Py-1 (C = 0.5 mM) in water as a function of a) NaCl concentration and b) solution pH.



F**igure S10:** UV/Vis absorption spectra (selected region) of NDI-1 + Py-1 (1:1) showing the appearance of the CT-band in water at MIC; c = 0.11 mM**,** *l* = 1 cm.

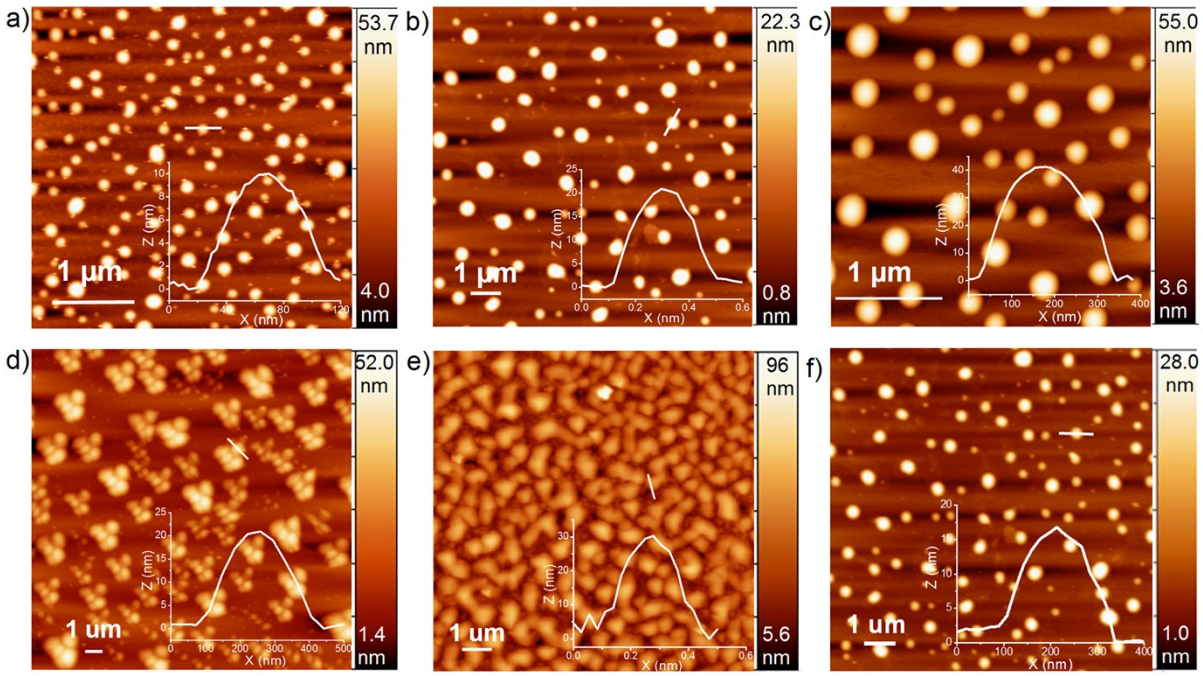


Figure S11: AFM images of various stoichiometry of Py-1:NDI-1; a) 100: 0, b) 95: 5, c) 90: 10, d) 80:20, e) 70:30, f) 60:40; In all cases aqueous solution of the compound (s) (c = 0.2 mM) was drop-casted on a mica surface and dried before imaging.

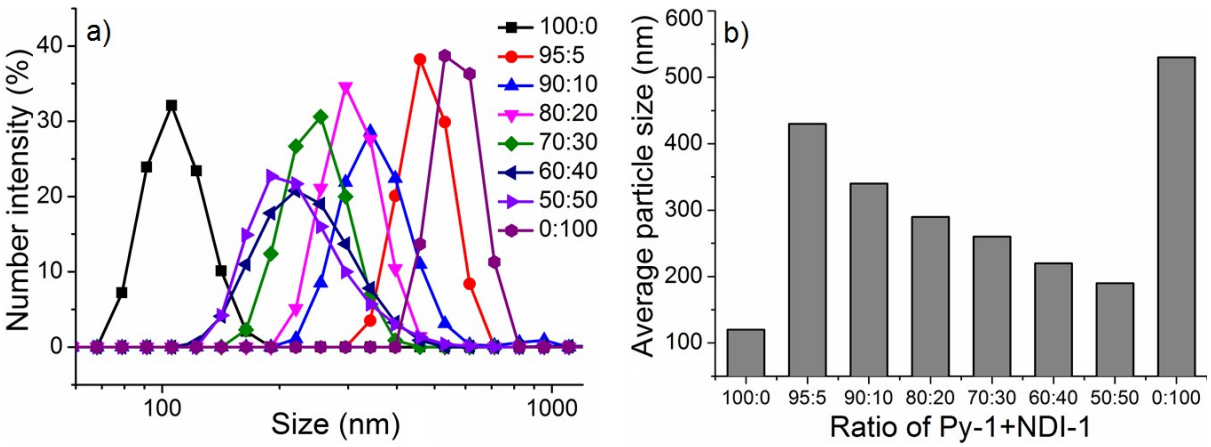


Figure S12: a) Stoichiometry dependent DLS profile of Py-1: NDI-1 in water; c = 0.2 mM, b) Plot of average particle size vs various ratio of Py-1+NDI-1.

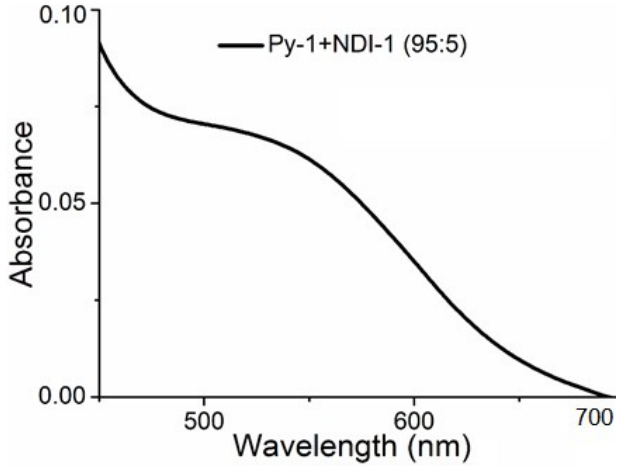


Figure S13: UV/Vis absorption spectra (selected region) of Py-1+ NDI-1 (95:5) showing the appearance of the CT-band in water at C = 0.5 mM**,** *l* = 1 cm.

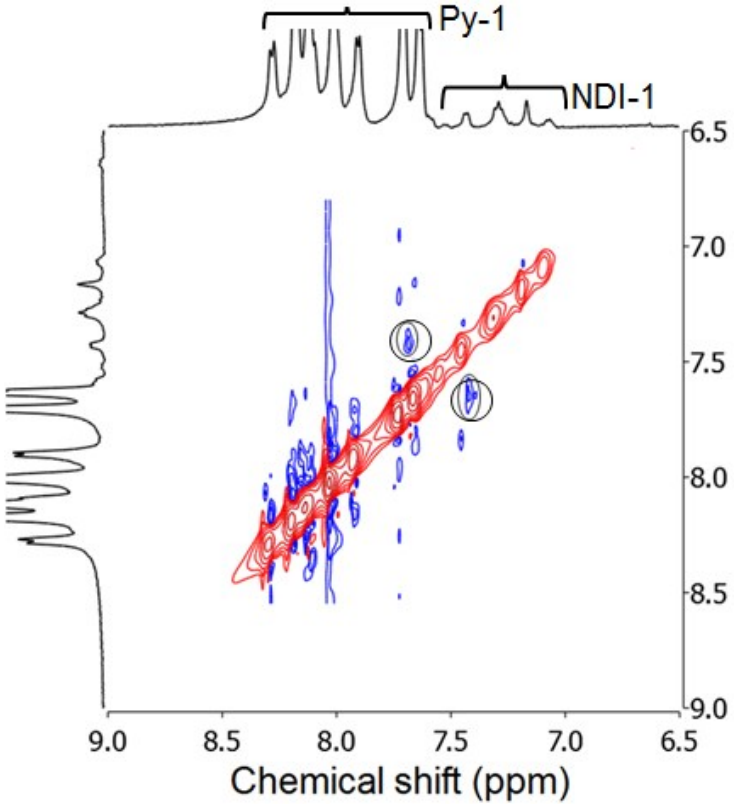


Figure S14: ROESY NMR spectrum (500 MHz, 20 mM, 293 K) of Py-1+NDI-1 (95:5), where diagonal circled blue spots correspond to the intermolecular coupling between terminal benzene ring protons of Py-1 and NDI-1 molecule. As NDI-1 was only 5 % in the mixture, to have reasonable peak intensity total concentration of the mixture had to be increased to 20.0 mM. However, at this high concentration the mixture was not soluble fully in only D_2O and therefore 40% DMSO-d6 had to be added to the NMR sample solution. Nevertheless, the red colour still persisted indicating intact CT-complex.

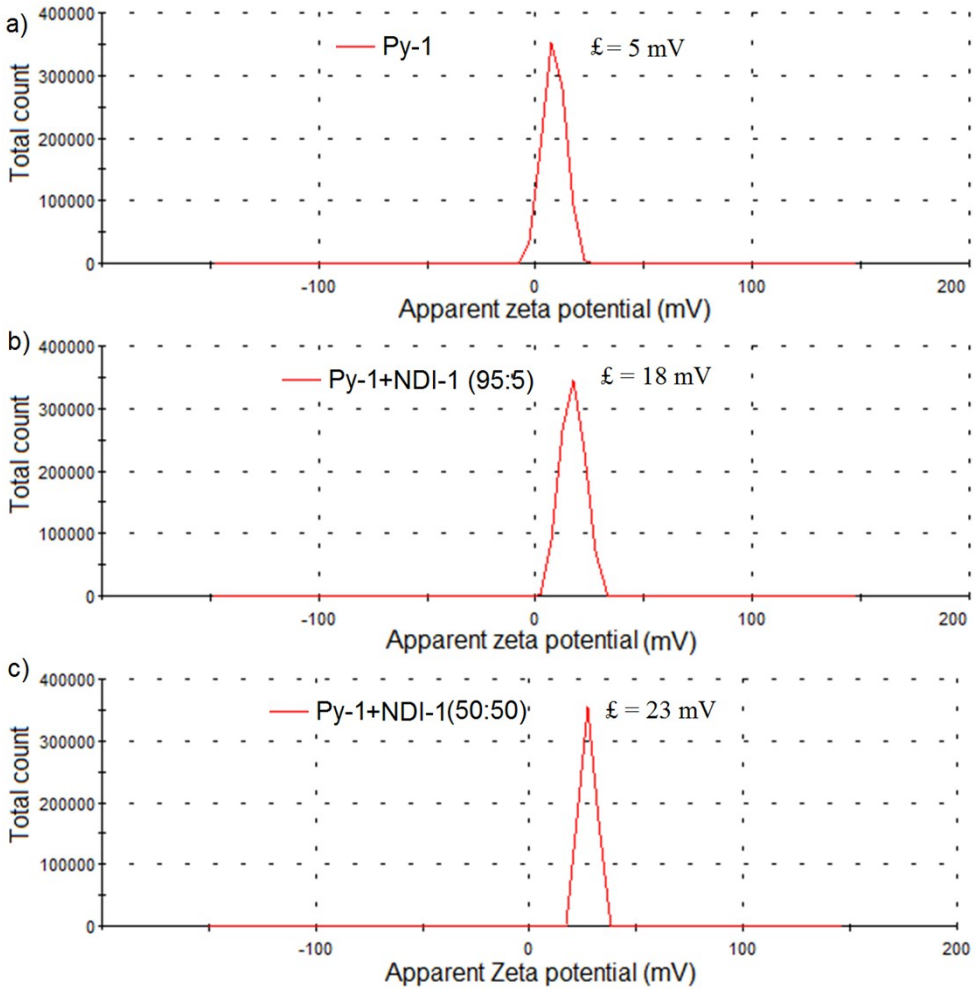


Figure S15: Total count vs apparent zeta potential (mV) plot for aqueous solution of a) Py-1, b) Py-1+NDI-1 (95:5) and Py-1+NDI-1 (50:50); c = 0.2 mM.

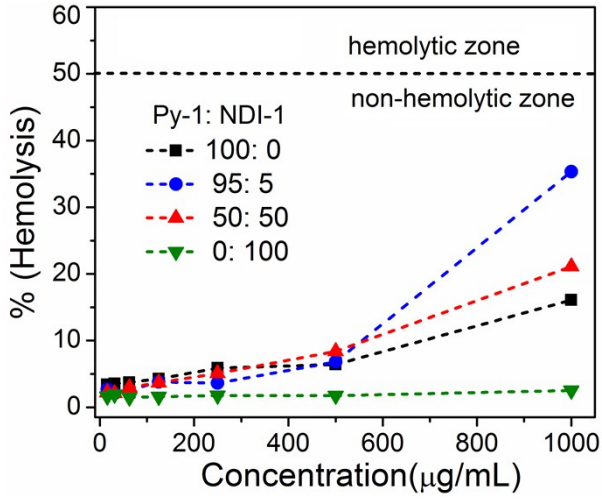


Figure S16: Graph showing percentage of RBC hemolysis with varying concentration and ratio of Py-1+NDI-1.

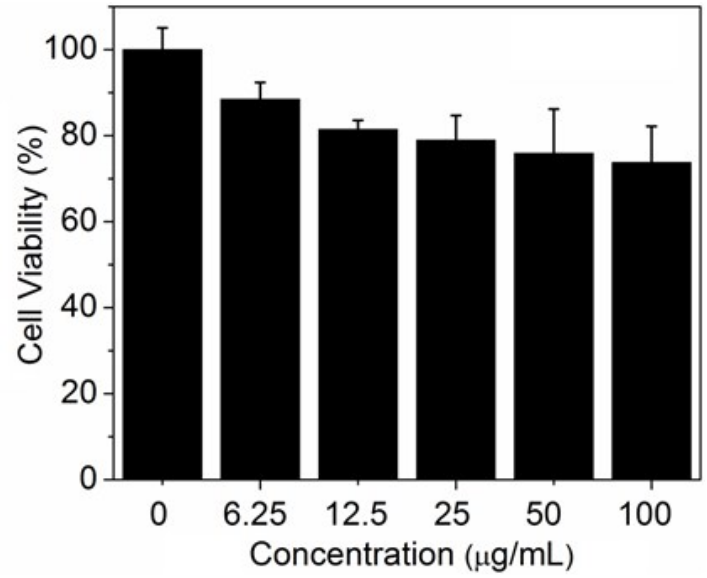


Figure S17: Cell viability of Py-1+NDI-1 (95:5) against HeLa cell as obtained from the MTT assay after incubation for 12h.

References:

1 D. D. Perrin, W. L. F. Armarego and D. R. Perrin, Purification of Laboratory Chemicals, 2nd ed., Pergamon, Oxford, **1980**.

2 P. Rajdev, S. Chakraborty, M. Schmutz, P. Mesini and S. Ghosh, *Langmuir* 2017, **33**, 4789.