Supporting Information for

Solvent Switchable Nanostructure and Function of a π-Amphiphile

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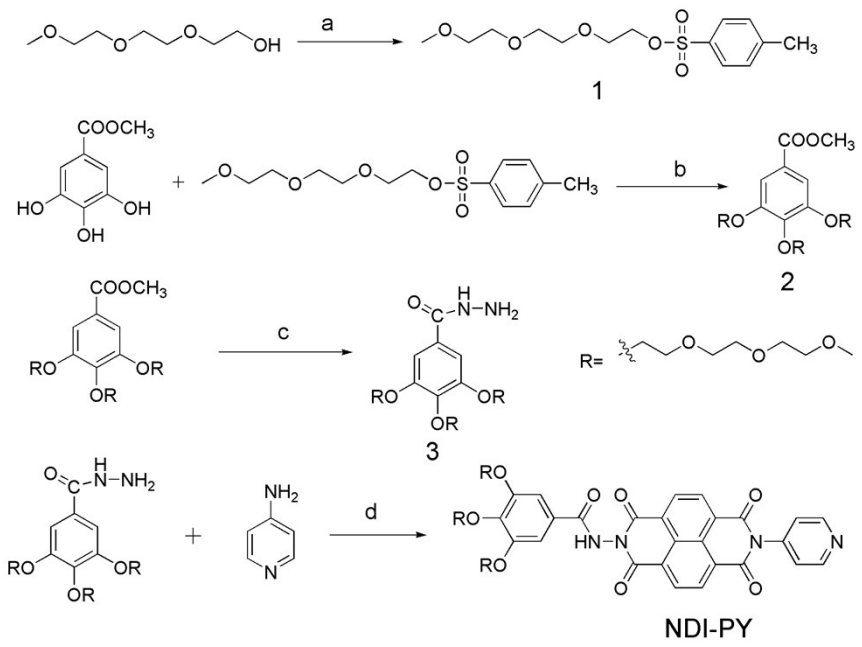
Materials: All reagents were purchased from Sigma Aldrich Chemical Co. and used without purification. Solvents were purchased from local chemical companies and purified by standard methods.[1] HPLC grade solvents were used for UV/Vis, IR and Fluorescence spectroscopy studies. NMR spectra were recorded on a Bruker DPX- 300 MHz, 400 MHz or 500 MHz NMR spectrometer and calibrated using TMS. Mass spectrometry data were obtained from a Q-tof-micro quadruple mass spectrometer by electron spray ionization (ESI) technique. UV/Vis spectra were recorded at a Perkin Elmer Lambda 25 spectrometer equipped with temperature variable external set up. Fluorescence spectra were taken in a Fluorolog spectrophotometer from Horiba Jobin Yvon. XRD data was recorded on a Seifert XRD3000P diffractometer with Cu Kα radiation (a = 0.15406 nm). TEM images were captured in JEOL-2010EX instrument operating at an accelerating voltage of 200 KV.

Synthesis and Characterization:

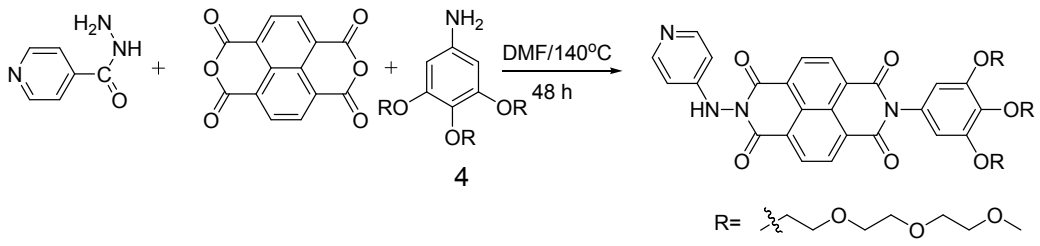
Synthesis of the compounds NDI-PY and NDI-PY-C was achieved in multiple steps using the protocol as outlined in Scheme S1 and S2. The compound has been characterized by ^{1}H NMR, ^{13}C NMR, UV/Vis, melting point and HRMS (ESI). Synthesis of compound 1, 2 and 3 has been described elsewhere.[2]



Reagents and conditions: (a) THF-water, 4-Toluenesulfonyl chloride, 0 ^{o}C, 5h, 75 %. (b) K_2CO_3, KI, CH_3CN, 80 ^{o}C 48 h, 68%. (c) Hydrazine hydrate, MeOH, 70 ^{o}C, 12h, 80% (d) DMF, 140 ^{o}C, 24h, 23%.

Scheme S1. Synthesis of **NDI-PY**

NDI-PY: Compound **3** (800 mg, 1.284 mM) and 1,4,5,8- napthalenetetracarboxylic bisanhydride (344 mg, 1.284 mM) were taken together in a round bottom flask containing 15 mL of dry DMF and the reaction mixture was stirred for 2 h at 140 ^{o}C. Then the reaction mixture was cooled and to it 4-amino pyridine (120 mg, 1.284 mM) was added. Reaction mixture was stirred for another 24 hr at 140 ^{o}C. Then reaction was stopped, cooled to room temperature and DMF was evaporated under reduced pressure. The pasty mass was partly dissolved in 30 mL of CH_2Cl_2 and the remaining solid was removed by filtration. The filtrate solution was washed with water (40 mL) and then with brine (40 mL) and dried over anhydrous Na_2SO_4. Solvent was evaporated to get the crude product as a brown solid. It was purified by column chromatography using silica gel as a stationary phase and CH_2Cl_2/ MeOH (95:5) as eluent to get the desired product as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$ and TMS): δ(ppm) = 9.67 (1H, s), 8.87 (2H, d), 8.84 (4H, s), 7.44 (2H, d), 7.35 (2H, d), 4.27 (6H, t), 3.85- 3.26 (39H, m). ^{13}C NMR ($CDCl_3$):166, 162.3, 160.94, 152.67, 151.38, 143, 142.65, 131.78, 127.38-126.28, 124.00, 109.14, 65.08, 59.11. HRMS (ESI): m/z calculated for $C_{47}H_{56}N_4O_{17}$ $[M + Na]^+$: 963.3637; found: 963.3634. m.p. 105 ^{o}C, UV-Visible (THF): λ_{max}= 376 nm (24200 M^{-1} cm^{-1}), 356 nm (22100 M^{-1} cm^{-1}), 338 nm (13500 M^{-1} cm^{-1}).



Scheme S2. Synthesis of NDI-PY-C

NDI-PY-C: Synthesis of compound 4 was published elsewhere.[3] Compound 4 (330 mg, 0.569mM), 1, 4, 5, 8-naphthalenetetracarboxylicacid bis-anhydride (153mg, 0.569 mM) and commercially available 4-Pyridinecarboxylic acid hydrazide (78 mg, 0.569 mM) were taken together in a 100 mL RB along with 10 mL dry DMF. It was stirred for 24h at 140 °C under N_2 atmosphere. Then the reaction was stopped and cooled to rt. DMF was evaporated under reduced pressure. The pasty mass was partly dissolved in 30 mL of CH_2Cl_2 and the remaining solid was removed by filtration. The filtrate solution was washed with water (40 mL) and then with brine (40 mL) and dried over anhydrous Na_2SO_4. Solvent was evaporated to get the crude product as a brown solid. It was purified by column chromatography using silica gel as a stationary phase and CH_2Cl_2/ MeOH (95:5) as eluent to get the desired product as orange solid. Yield: 30%. ^{1}H NMR (400 MHz, $CDCl_3$ and TMS): δ(ppm) = 8.85 (2H,d), 8.80 (4H, s), 7.85 (2H, d), 6.53 (2H, s), 4.27 (6H, t), 3.85- 3.26 (39H, m). ^{13}C NMR ($CDCl_3$):164.66, 162.97, 162.83, 153.30, 150.87, 138.74, 131.42, 129.84, 127.49, 127.21, 127.15, 126.59,121.77, 108.18, 72.34-70.46, 69.62, 68.93, 59.14. HRMS (ESI): m/z calculated for $C_{47}H_{56}N_4O_{17}$ $[M + Na]^+$: 963.3637; found: 963.3641. UV-Visible (THF): λ_{max}= 375 nm (19726 M^{-1} cm^{-1}), 355 nm (18446 M^{-1} cm^{-1}), 338 nm (11460 M^{-1} cm^{-1}).

Experimental Procedures:

Solution preparation: A stock solution of NDI-PY was made in THF (1.0 mM). Measured volume of the aliquot was taken in a vial and the solvent was evaporated. A thin yellow film obtained was dissolved in equal volume of water or 95:5 TCE: $CHCl_3$ to make the final concentration = 1.0 mM. The solutions were allowed to equilibrate for 30 min at room temperature before any physical studies.

To make acidic solution, equal volume of 1.0 mM aqueous solution of HCl was added to 2.0 mM aqueous solution of preformed vesicle of NDI-PY to make final concentration of 1.0 mM. For

(1:1) CT-complex, stock solution of NDI-PY and PY-A/ Pyrene were made in THF (10 mM). 0.2 mL aliquot each of donor and acceptor were mixed in a vial and the solvent was evaporated. The violet film was dissolved in 0.4 mL water to make the final concentration = 5.0 mM with respect to each component. The solution was equilibrated for 6h before any physical studies. For H-bonding driven encapsulation of the pyrene guest in the NDI membrane, firstly, a 2.0 mM (400 µl) aqueous solution of NDI-PY or NDI-PY-C were prepared. To this pre-formed vesicular assembly, 20.0 µl 40.0 mM THF solution of Py-A was added. UV/Vis spectra and images were taken 1h after the solutions were prepared.

Calcein encapsulation: A stock solution of Calcein in MeOH (20 µL, 1.0 mM) and NDI-PY in THF (100 µL, 5.0 mM) were mixed and solvent was evaporated. To this 500 µL aqueous solution was added and sonicated for a few minutes. Subsequently the solution was subjected to dialysis against de-ionised water by using 3500 Da MWCO membrane for 48 h to remove un-encapsulated Calcein. The fluorescence spectrum of the dialyzed solution was recorded and compared with absorption-normalized free Calcein solution in water.

Determination of association constant (K) of D-A CT-complex: UV/Vis spectra of 1:1 (D: A) solution were recorded as a function of concentration at a fixed temperature. K_a was determined by using equation 1, where c, A, l and Ɛ defines concentration, absorbance, optical path length and extinction coefficient, respectively.

$$\frac{C}{A} = \frac{1}{\sqrt{K\varepsilon l}} \times \frac{1}{\sqrt{A}} + \frac{1}{\varepsilon l} \quad \text{---------- (1)}$$

Job plot experiment: Series of NDI-PY + PY-A mixed solutions were prepared in water with varying ratios. The total chromophore concentration was kept fixed at 2.0 mM while the individual concentrations were varied. UV/Vis spectra were recorded for these solutions and the absorbance of the CT-band at 550 nm were plotted as a function of mole fraction of the acceptor to construct the Job's plot.

Powder X-Ray diffraction studies: A solution of NDI-PY (10 mM, 1.0 mL) in water or TCE (with 5% $CHCl_3$) were drop-casted repeatedly on a glass slide to make a thick film and air dried for 4 days before the data were recorded. For Figure S9, Powder X-ray diffraction analysis was

carried out using an X-ray diffractometer (Rigaku, MiniFlex600, λ = 1.54 Å) with a semiconductor detector (Rigaku, D/teX Ultra).

Micro Differential scanning Calorimetry (micro DSC): Calorimetric measurements were carried out in a micro-DSC from a TA instruments. All experiments were carried out between 10 and 70 °C with a scanning rate of 1 °C/min. Standard vessels were used with an average 400 μL sample volume (5.0 mM concentration). The same mass of sample and reference were weighted to minimize the differences in heat capacities between them. The samples were equilibrated at 25°C for 2 h before each scan.

Isothermal Titration Calorimetry (ITC): ITC measurements were performed at 25 °C using a MicroCal -200-ITC. Stirring rate was 700 rpm, injection volume was 2μL, concentration of the solution in syringe was 10 mM and injection interval was 160 seconds.

Flash-photolysis time-resolved microwave conductivity (FP-TRMC): The transient conductivity was measured by FP-TRMC technique at room temperature under air. Film samples on a quartz plate were prepared by dropcasting 1,1,2,2-tetorachloroethane or aqueous solution of NDI-PY, and dried under vacuum at room temperature. Transient charge carriers were generated upon photoexcitation by laser pulses of third harmonic generation (λ= 355 nm) from a Spectra Physics model INDI-HG Nd:YAG laser with a pulse duration of 5–8 ns. The photon density of a 355 nm pulse was 4.6×10^{15} photons cm^{-2}. The probing microwave frequency and power were set at 9.1 GHz and 3 mW, respectively. Photoconductivity transients were demodulated through a GaAs crystal-diode with Schottky-barriers (rise time < 1 ns), was monitored by a Tektronix model TDS3032B digital oscilloscope. The observed conductivities were normalized, given by a photocarrier generation yield (ϕ) multiplied by sum of the charge carrier mobilities ($\Sigma\mu$), according to the equation, $\phi\Sigma\mu = (A/eI_0F_{light})(\Delta P_r/P_r)$, where, e, A, I_0, F_{light}, P_r, and ΔP_r are unit charge of a single electron, sensitivity factor (S cm^{-1}), incident photon density of the excitation laser (photon cm^{-2}), correction (or filling) factor (cm^{-1}), and reflected microwave power and its change, respectively.

Bacterial preparation: *Staphylococcus aureus* (ATCC® 25923™) was grown aerobically in Nutrient Agar/ Broth and Luria-Bertani Agar/Broth at 37°C. Bacteria were harvested at the logarithmic growth phase with optical densities in between 0.6-1 at 600 nm.

Antimicrobial activity assay: In this study, the lowest concentration of NDI-PY required to inhibit growth of bacteria completely (defined as the MIC) was determined by a broth microdilution assay according to the procedures outlined by the National Committee for Clinical Laboratory Standards (Wayne, PA) with the modifications proposed by Weigand et al.[4] NDI-PY stock solutions were prepared in 0.01% aqueous acetic acid. Serial dilutions were made for getting different concentration of the sample in LB/NB with 0.01% acetic acid and finally 50 µL sample solutions were added to each well of a non-coated polystyrene 96-well plates containing 50 µL of bacteria with final inoculum of 5X 105 cfu ml^{-1}. The plates were incubated at 37 °C for 24 h with gentle shaking. MIC was taken as the lowest concentration of NDI-PY at which there was 100% reduction of growth. Broth alone and broth containing only cells were used as sterility control and growth control respectively. All experiments were performed in triplicate.

Zone inhibition test: A 100 µl volume of the *S. aureus* suspension at logarithmic phase was spread on LB agar plate to prepare lawns of *S. aureus*. Paper discs of 6 mm diameter soaked with 20 µL of NDI-PY dissolved in water (stock concentration: 60 µg/mL) were added to the agar plates and incubated at 37°C for 24h.

Hemolytic Activity Assay: HC50 value is a metric for measuring hemolytic activity of a compound which is defined as the concentration of antimicrobial compound that kills 50% red blood cells (RBCs). Human RBCs from healthy donor (1mL) were suspended in 9 mL of PBS buffer (pH 7.4) and centrifuged at 2000 rpm for 5 min. The supernatant was removed by pipetting and RBCs were re-suspended in PBS. This procedure was repeated for two additional times. Finally RBC pellet was dissolved in 10 ml of PBS buffer and again diluted four times to get stock solution where RBC is 2.5% v/v. Afterwards serial dilutions of NDI-PY in PBS (50 µL) were prepared on a 96-well sterile round bottomed polypropylene plate, the RBC suspension (150 µL) was added and incubated at 37°C with shaking at 180 rpm. Triton X-100 (0.1% v/v in water) was used as the positive lysis control and PBS was used as negative controls. Supernatant (100 µL) from each well was diluted with PBS buffer (100 µL) in a 96-well sterile flat-bottomed polystylene plate. The absorbance of the released haemoglobin at 414 nm was measured using

Varioskan microplate reader (Thermo Fisher). The percentage of hemolysis was calculated relative to the positive control and negative control solvents.

Cytotoxicity assay using MTT: For assessing the metabolic activity of HeLa cells using methylthiazolyldiphenyltetrazolium bromide (MTT) assay, cells were seeded in 96-well plates with a seeding density of 10,000 cells well-1. After 24 h incubation, the medium was replaced by the NDI-PY suspension at different concentrations upto 2.5 mg/mL. The cells were then incubated for 24h and 50 μL of freshly prepared MTT (5 μg mL-1 in 1X PBS) solution was added to each well. The medium with MTT solution was carefully removed after 4 h incubation in the 37 °C incubator. 200 μL DMSO was then added into each well and the plate was 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 by the ratio of absolute absorbance of the cells incubated with polymer suspension to that of the cells incubated with culture medium only.

Additional Figures:

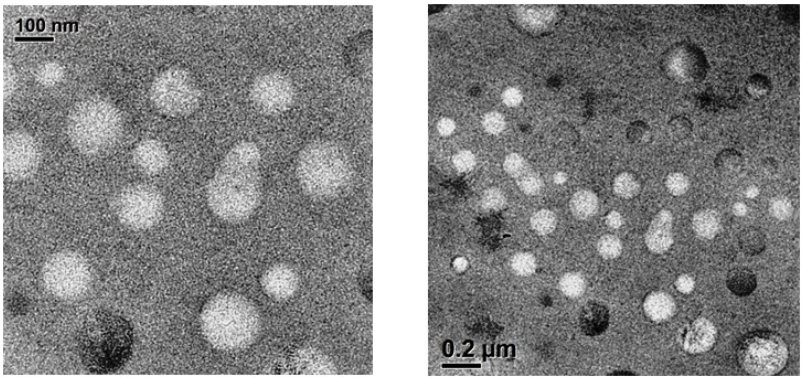


Figure S1. HRTEM images of NDI-PY in H_2O (C= 1.0 mM). Sample was prepared by drop-casting a solution on carbon coated Cu grid followed by drying the grid.

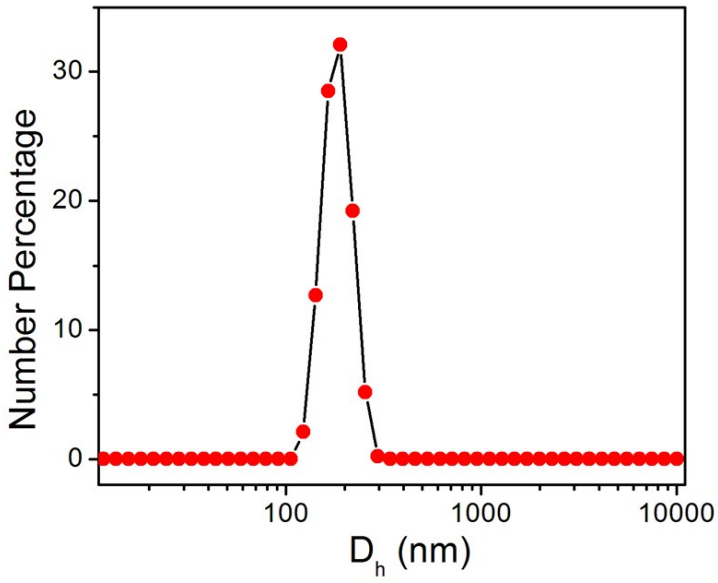


Figure S2. Number averaged size distribution from DLS measurements for NDI-PY solution in aqueous medium (C=1.0 mM, T=25^{o}C).

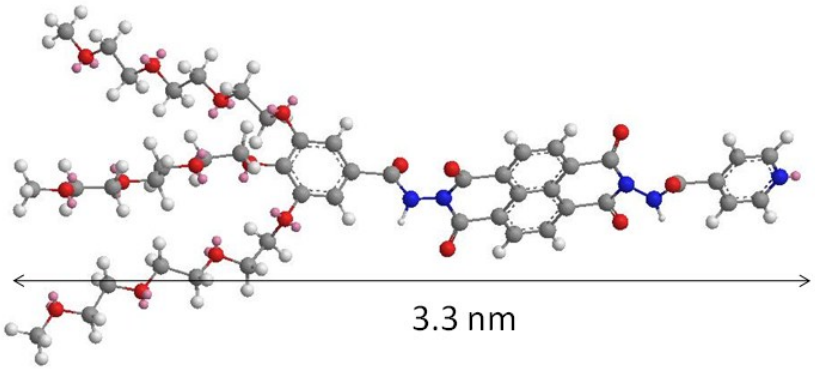


Figure S3. Structure of the fully stretched NDI-PY obtained from Chem Draw 3D software (blue= Nitrogen, red=Oxygen, grey= carbon, white= Hydrogen atom).

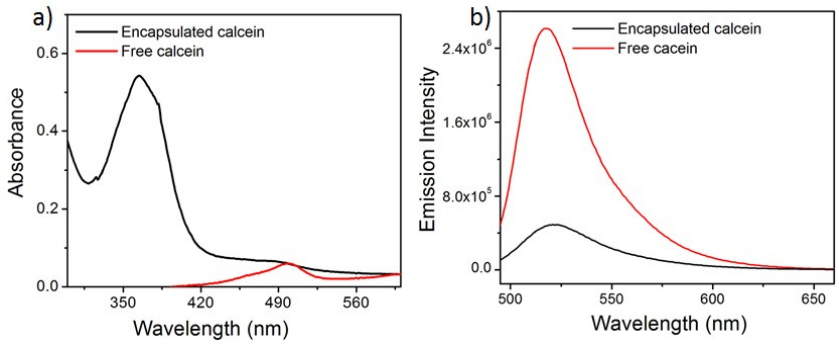


Figure S4. (a) UV-Visible spectra for Calcein encapsulated NDI-PY vesicle and free Calcein at the same concentration (Conc. of Calcein= 8.3×10^{-6}M, *l*=0.1cm, T= 25^{o}C) and (b) corresponding fluorescence spectra (λ_{ex}=480nm). Encapsulation Efficiency = (Conc. of Calcein after dialysis/ Conc. of Calcein added) × 100%. Calcein has molar extinction coefficient of 75000 M^{-1} cm^{-1} at 492 nm. Therefore the concentration of Calcein encapsulated inside the vesicles could be calculated from the absorption spectra.

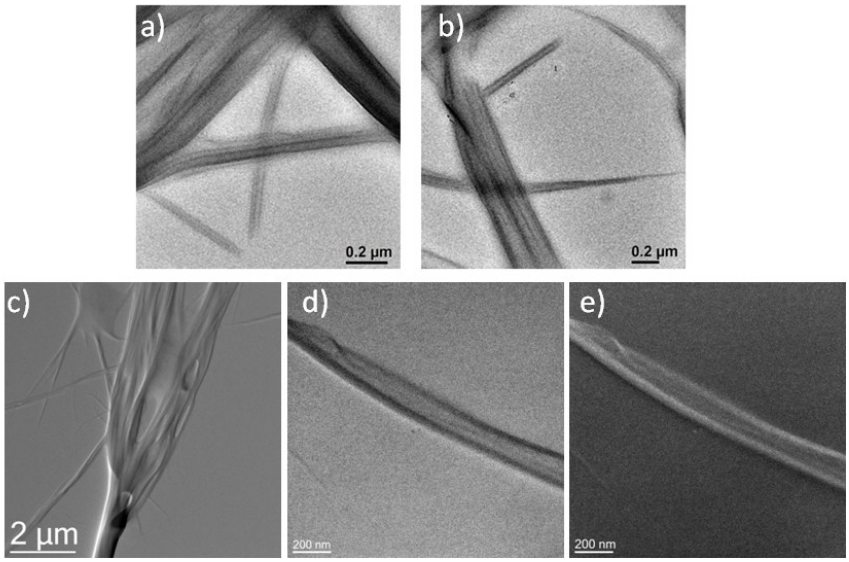


Figure S5. HRTEM images of NDI-PY in TCE with 5% $CHCl_3$ (C= 1.0 mM) prepared by drop-casting a solution on carbon coated Cu grid followed by drying the grid in air. Along with the tubular structures (a, b), a few images (c, d, e) reveal intermediate structures representing folded sheet prior to tube formation.

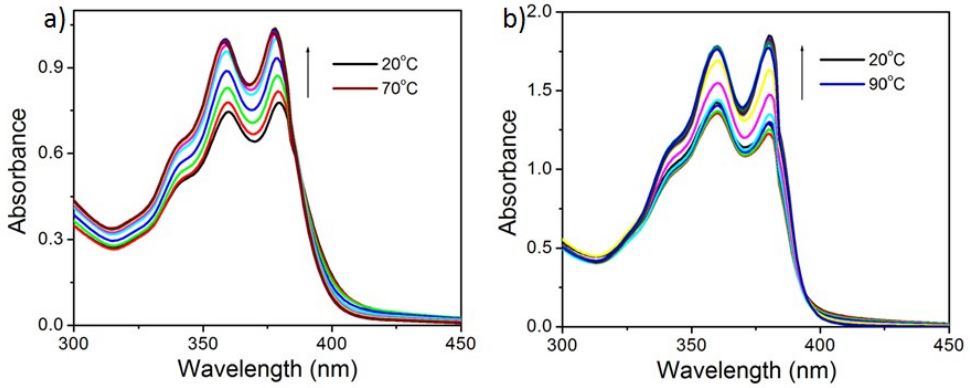


Figure S6. Temperature variable UV-Vis spectra of NDI-PY (a) in TEC with 5% $CHCl_3$ and (b) in water(c=1.0 mM, *l*=0.1 cm).

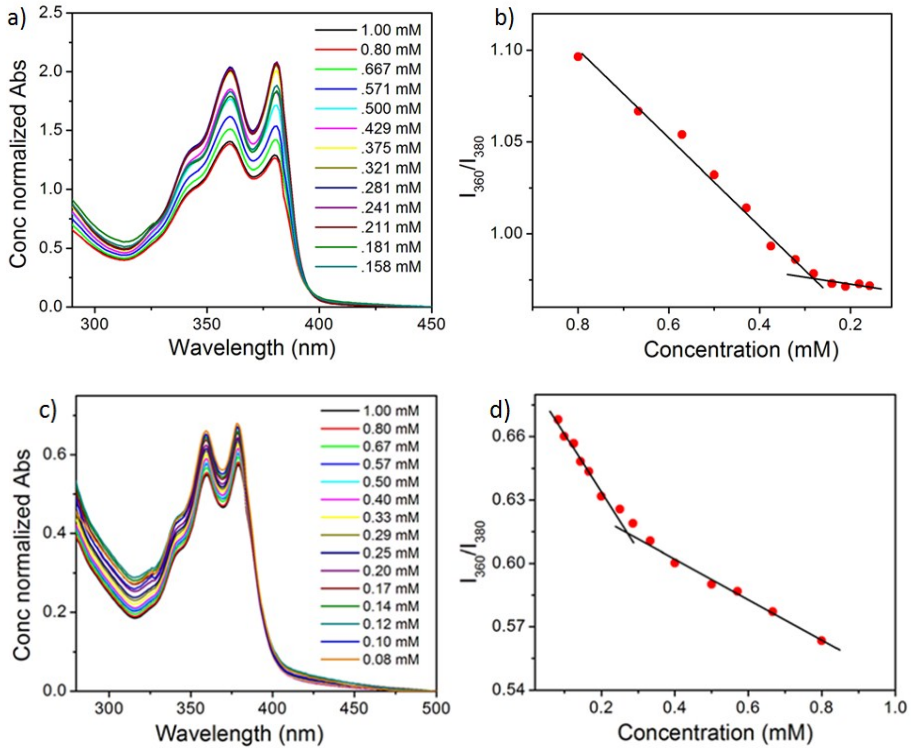


Figure S7. Concentration normalized absorption spectra of NDI-PY in (a) water and (c) TCE; Absorption intensity ratio (I_{360}/I_{380}) vs concentration plot for (b) water and (d) TCE. Inflection points at 0.30 mM and 0.28 mM reflect the CAC in water and TCE, respectively.

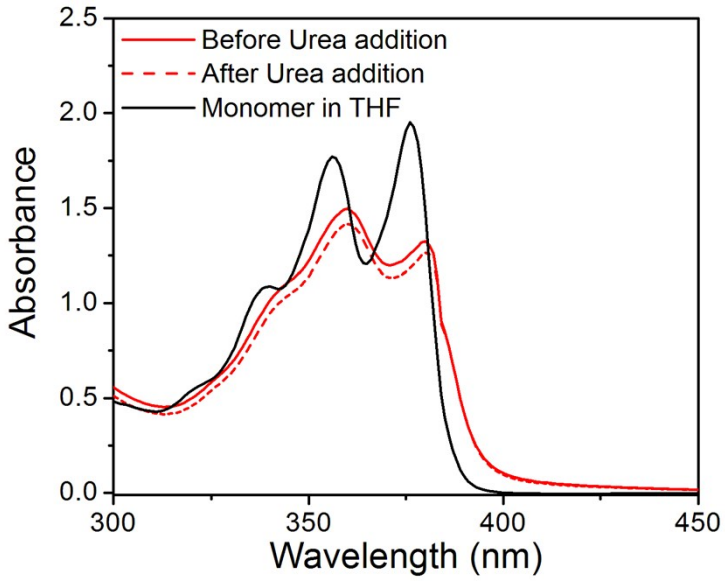


Figure S8. Effect of urea (10mM) on the UV/Vis spectra of an aqueous solution of NDI-PY (c=1 mM, *l*=0.1cm, T=25 °C). No disassembly upon urea addition was observed; only slight decrease in peak intensity is attributed to salting out effect.

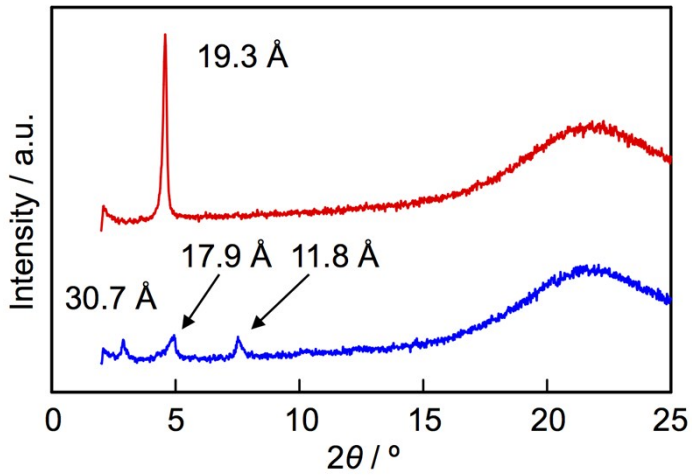


Figure S9. Powder X-ray diffraction patterns of dropcast films prepared from aqueous solution (red) and THF solution (blue) of NDI-PY at 10 mM that were used for FP-TRMC measurements. Corresponding d spacing values are described along with diffraction peaks.

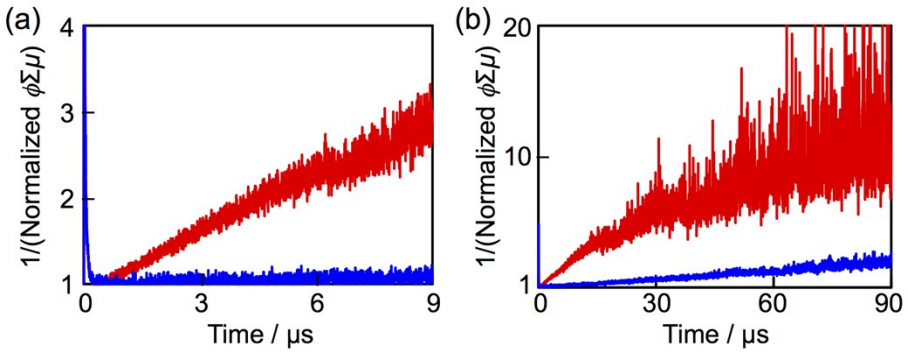


Figure S10. Reciprocal normalized conductivity transients in (a) 0–9 μs and (b) 0–90 μs time ranges observed for dropcast films from aqueous (red) and TCE solution (blue).

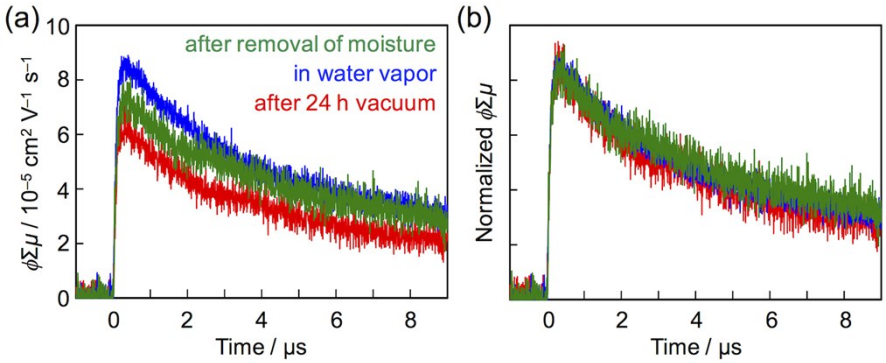


Figure S11. Kinetic traces of (a) conductivity transients and (b) normalized values at 0–9 μs upon exposure to 355 nm pulses at 4.6×10^{15} photons cm^{-2} observed for dropcast films from aqueous solution of NDI-PY after 24 h vacuum drying prior to measurement (red), in water moisture (blue), and under air soon after removal of moisture (green).

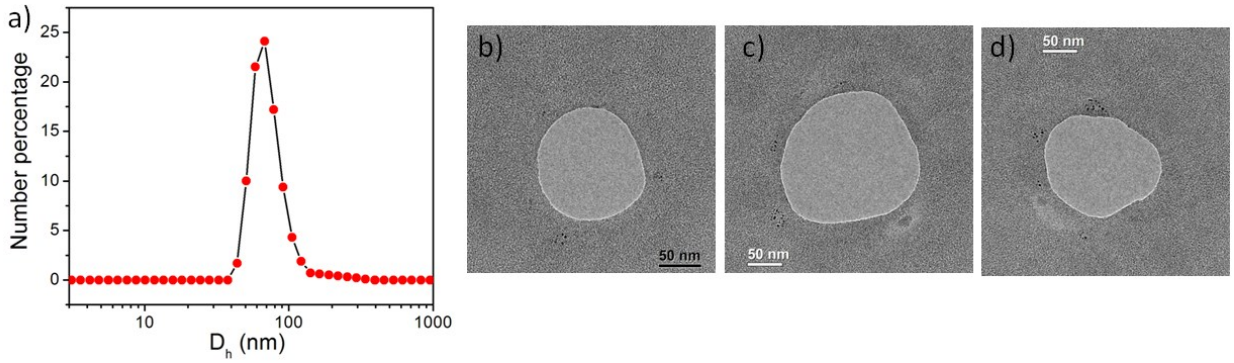


Figure S12. (a) Number averaged size distribution from DLS measurements for 1:1(NDI-PY +PY-A) CT solution in water (c=1.0 mM, T=25°C) (b) TEM images of 1:1, D-A CT solution in water obtained from different part of grid (c=1.0 mM).

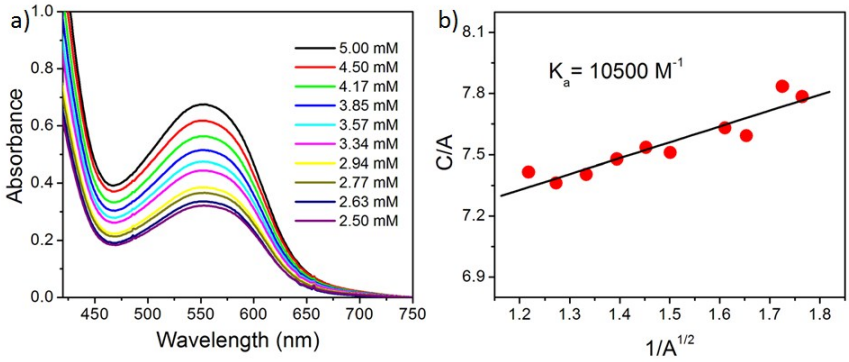


Figure S13. (a) Dilution experiment for 1:1 (NDI-PY+ PY-A) in water; (b) C/A Vs (1/A) $^{0.5}$ plot for K_a value determination. (K_a= 10500 M^{-1}).

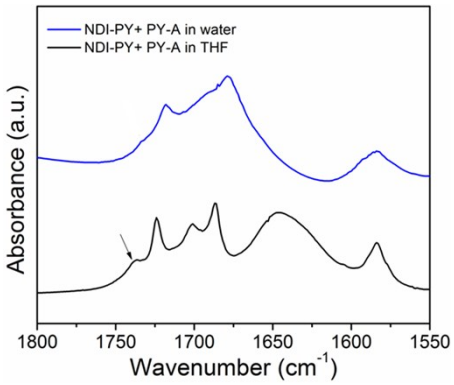


Figure S14.FT-IR spectra (selected region) of NDI-PY+ Py-A (1:1) in THF and D_2O. (C = 2.0 mM). The peak at 1736 cm^{-1} (black line, shown by arrow) is assigned to the stretching of C=O of carboxylic acid of Py-A which in D_2O shifts towards lower frequency and overlaps with NDI-imide carbonyl peaks suggesting H-bonding. Other peaks (black line) at 1724cm^{-1}, 1700cm^{-1} and 1686 cm^{-1} are assigned to imide unsymmetric stretching, hydrazide C=O stretching and imide symmetric stretching frequency, respectively.

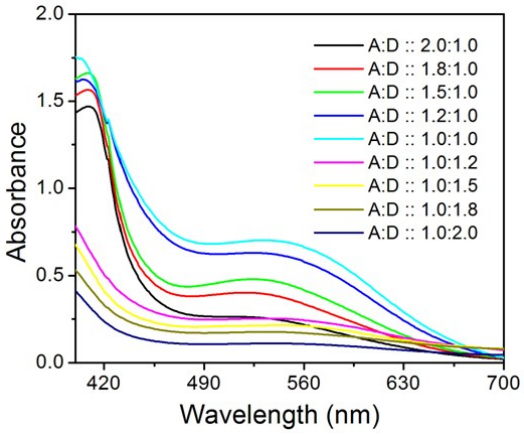


Figure S15: UV-Vis absorbance spectra of NDI-PY + PY-A mixture with different donor-acceptor ratio in water; Total chromophoric concentration = 5.0 mM.

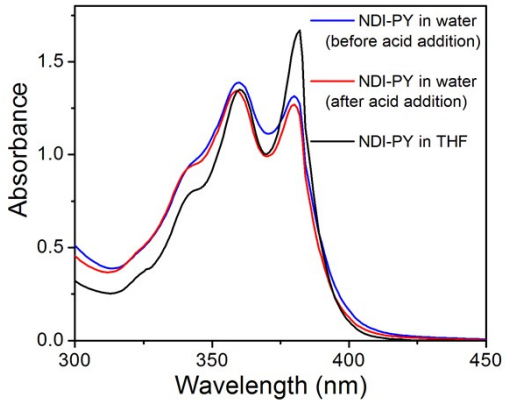


Figure S16: UV-Vis spectra of NDI-PY in neutral and acidic medium together with THF spectra for comparison (C=1mM, rt, l=0.1cm, pH=5.5).

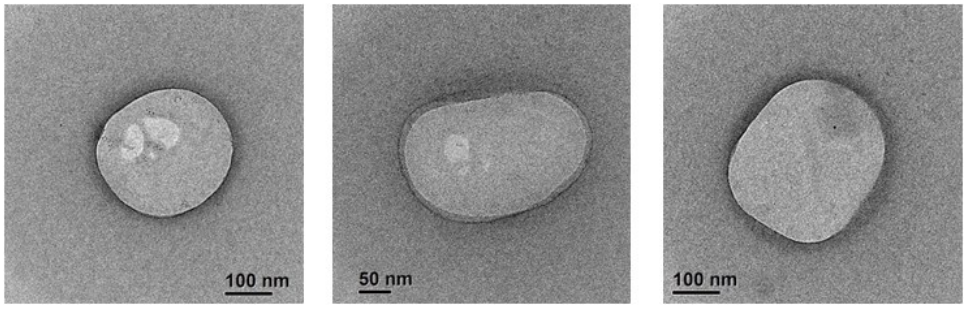


Figure S17: HRTEM images of 1.0 mM aqueous solution of NDI-PY in aqueous acidic medium (pH-5.5).

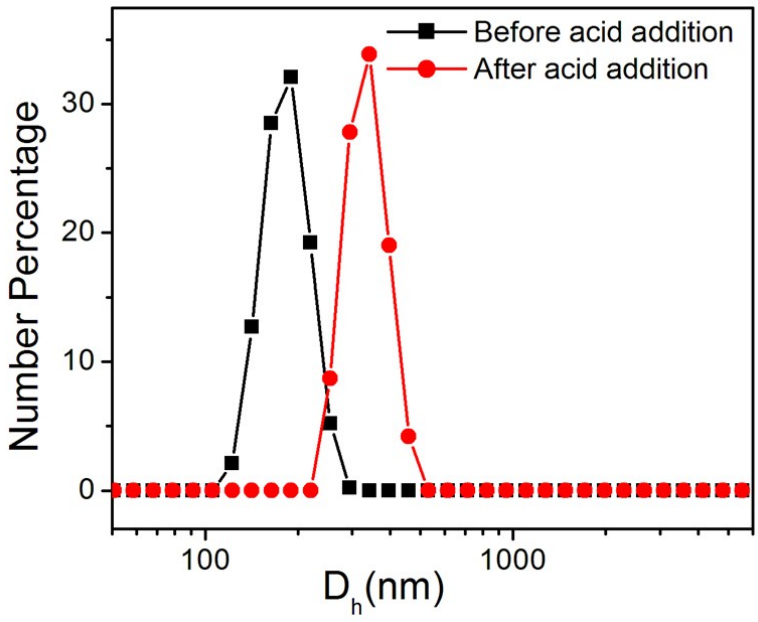


Figure S18. Number averaged size distribution from DLS measurements for NDI-PY solution in neutral medium and acidic medium (c=1.0 mM, T=25^{o}C).

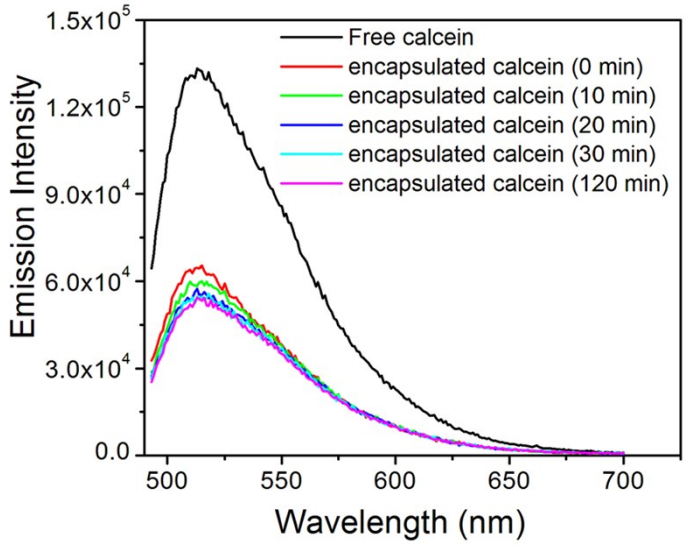


Figure S19. Absorption normalized emission spectra of free Calcein and Calcein that is encapsulated inside NDI-PY vesicles (λ_{ex}= 480nm).

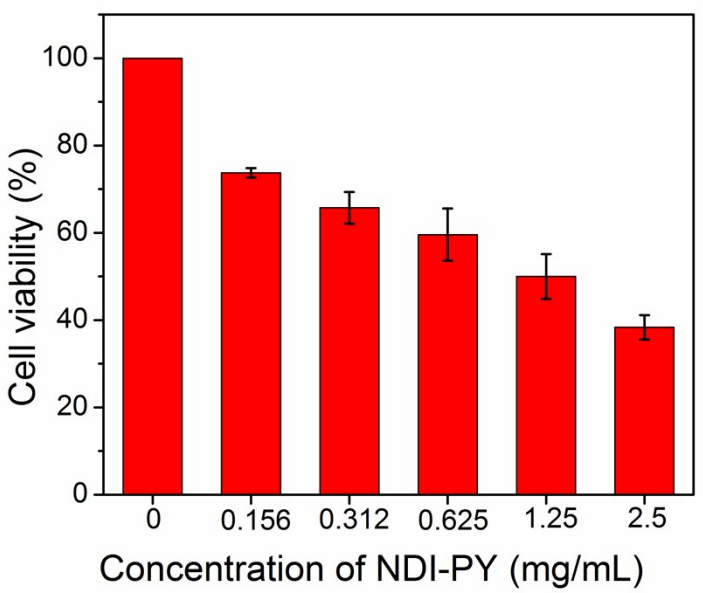


Figure S20. Cell viabilities of HeLa cells treated with different concentrations of NDI-PY for 24 h by MTT assay.

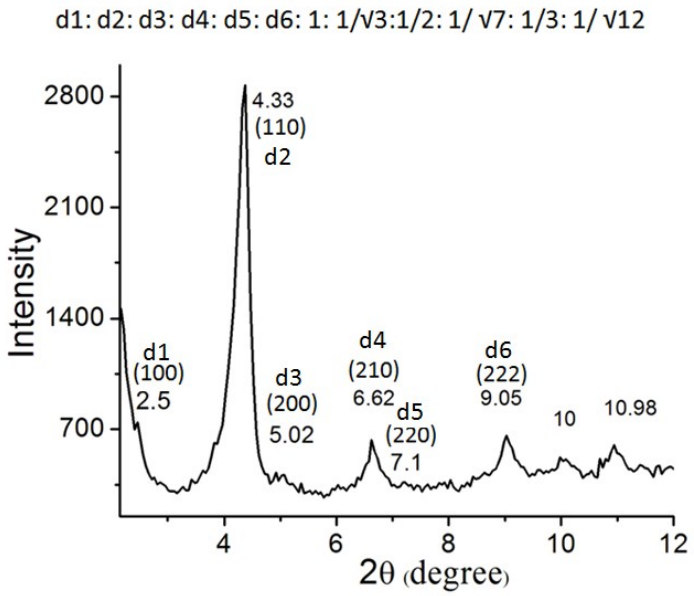


Figure S21. PXRD diffraction patterns of the dried samples of NDI-1 prepared from TCE. Multiple peaks those appear in the region of 2θ = 2-10° hold the relationship corresponding to hexagonal packing.

Reference

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