Supplementary Information (SI) for Chemical Science.
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Supporting Information

Unravelling denaturation, temperature and cosolvent-driven chiroptical switching in peptide self-assembly with switchable piezoelectric responses

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Experimental Procedures

1. Materials and methods

Chemicals and reagents: The required chemicals were purchased from Sigma-Aldrich, TCI and Spectrochem. Unless otherwise specified, all the chemicals were used without further purification, and the solvents were dried and purified following standard procedures before use.[1] Spectroscopy-grade solvents were used for physical studies.

NMR spectroscopy: Avance II 400 and 600 from Bruker were used to record ^{1}H NMR spectra by using tetramethylsilane (TMS) as an internal reference. Multiplicities for proton signals are abbreviated as s, d, t, q and m for singlet, doublet, triplet, quadruplet and multiplet, respectively.

Mass spectrometry: MALDI was performed on a Bruker Daltonics Autoflex Speed MALDI TOF system (GT0263G201) spectrometer.

UV-Vis spectroscopy: UV/Vis absorption spectra were recorded on a JASCO V 750 spectrophotometer with a spectral bandwidth of 1.0 nm and a scan rate of 500 nm min^{-1}. The instruments were outfitted with Julabo F250 water circulation units and Peltier cells. Ramp rates of 1K min^{-1} were used for variable temperature measurements. Solvents of spectroscopic grade were used in all measurements. Quartz cuvettes with optical paths of 1 cm was used in all the experiments.

Fluorescence spectroscopy: Fluorescence spectra were recorded on a Horiba Jobin Yvon, model FL-1039/40 spectrofluorometer. Sample preparation for fluorescence spectroscopy was the same as for UV-Vis spectroscopy.

FT-IR spectroscopy: FT-IR studies were carried out using a Perkin Elmer Spectrum spectrometer. Deuterated solvents, DMSO d_6 and D_2O were used for FT-IR measurements.

Atomic Force Microscopy: AFM images were recorded on Agilent Technologies AFM instrument (model:5000) and MFP-3D Origin by Oxford Instrument.

CD spectroscopy: A JASCO J-810 spectropolarimeter was used to perform CD experiments. This instrument is equipped with a Peltier module as a temperature controller. Cuvettes with a path length of 1.0 cm were used.

Dynamic Light Scattering Studies: DLS measurements were carried out using a Zetasizer ULTRA Malvern.

Analysis of temperature-dependent spectroscopic data by nucleation-elongation model

Ten Eikelder, Markvoort, and Meijer's Nucleation-Elongation model can be used to cooperatively describe the equilibrium between monomeric and supramolecular species.[3,4] The self-assembly of NDI-PEP-L, which displays a non-sigmoidal cooling curve as demonstrated in temperature-dependent UV-Vis, and CD experiments, is described by this

model. From the experimental melting curves, a non-linear least-squares analysis can yield the values T_e, $\Delta H°_{nucl}$, $\Delta H°$, and $\Delta S°$. Equations 1 and 2 can be used to calculate the equilibrium constants related to the nucleation and elongation phases.

$$K_n = e^{\left(\frac{-(\Delta H^o - \Delta H^o_{nucl}) - T\Delta S^o}{RT}\right)} \text{----------(1)}$$

$$K = e^{\left(\frac{(\Delta H^o - T\Delta S^o)}{RT}\right)} \text{----------(2)}$$

$$\sigma = \frac{K_n}{K_e} = e^{\left(\frac{\Delta H^o_{nucl}}{RT}\right)} \text{----------(3)}$$

Where, $\Delta H^o{}_{nucl}$= nucleation enthalpy; ΔH^o = enthalpy difference; ΔS^o = entropy difference; T_e= elongation temperature; K_{nucl} = equilibrium constant of the nucleation process; K_{el} = equilibrium constant of the elongation process; σ = degree of cooperativity (K_{nucl}/K_{el}). Gibbs free energy and all the equilibrium constants were calculated at 298 K.

2. Synthesis

3.1 Synthesis procedure of NDI-A: Naphthalene dianhydride (500 mg, 1.86 mmol), β-alanine (165 mg, 1.85 mmol), and dodecylamine (345 mg, 1.86 mmol) were taken in a Schleck round bottom flask and degassed three times with argon and vacuum. 5 ml dry dimethylformamide (DMF) along with triethylamine (20 μL) were added to the mixture and further degassed it for another two times. The complete mixture was then heated at 140°C for 12 h under argon atmosphere. After completion of the reaction, it was cooled down to room temperature and refrigerated for 2 hours for precipitation of the solid component. It was filtered and to the filtrate crushed ice was added to precipitated down the second residue. The mixture was filtered and washed with ice-cold water several times. The resulting second residue was dissolved in chloroform, washed with acid water (HCl), dried over Na_2SO_4, and then concentrated to obtain the crude product. It was purified by column chromatography using silica gel (60-120 mesh size) as the stationary phase. Pure NDI-A was collected with the 1% MeOH/DCM mixture as the eluent (Yield = 34%). NDI-A: ^{1}H-NMR ($CDCl_3$, 400 MHz, TMS), d(ppm): 8.76 (4H, s); 4.17-4.21 (4H, t); 1.71-1.76 (4H, m); 1.25 (16H, s); 0.86-0.89 (5H, t); MALDI (-ve mode): m/z calc. for $C_{20}H_{15}N_2O_6$ $[M-H]^-$: 505.242; found: 505.118.

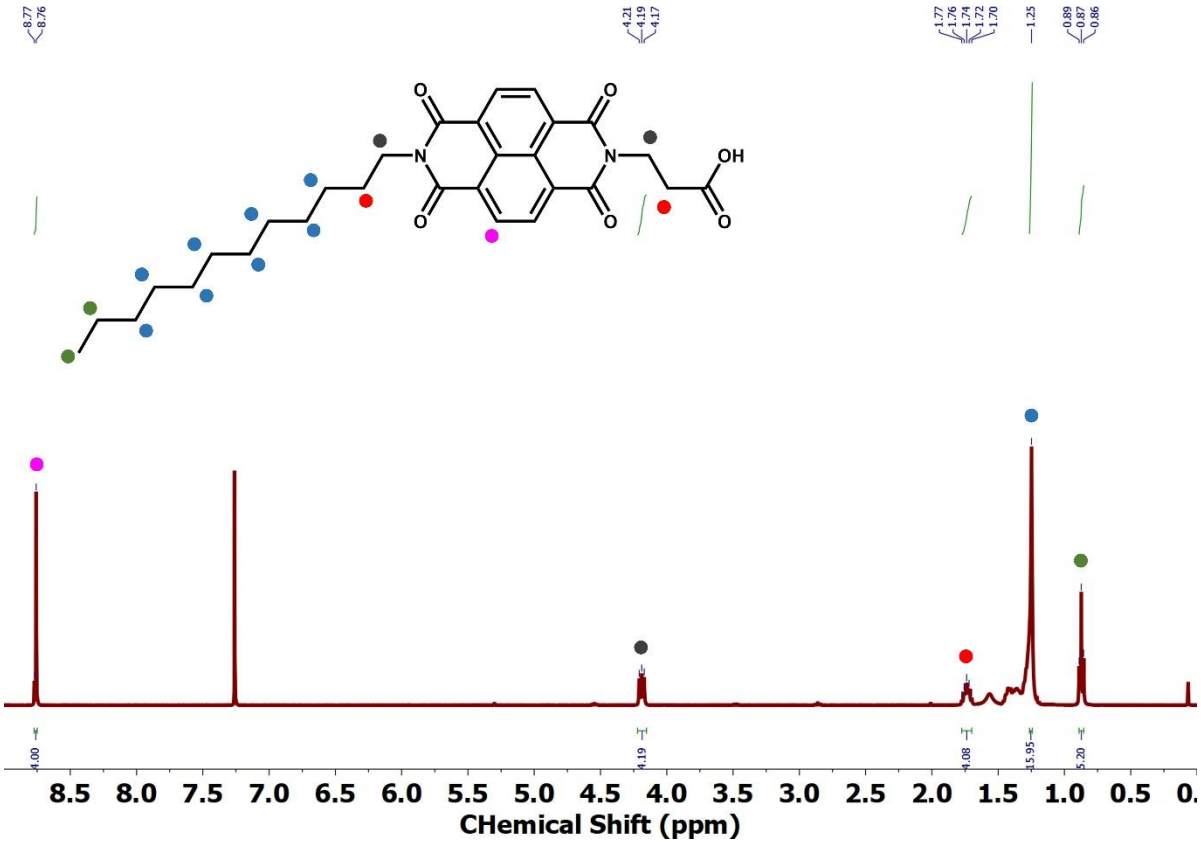


Figure S1. ^{1}H-NMR spectra of **NDI-A** in $CDCl_3$.

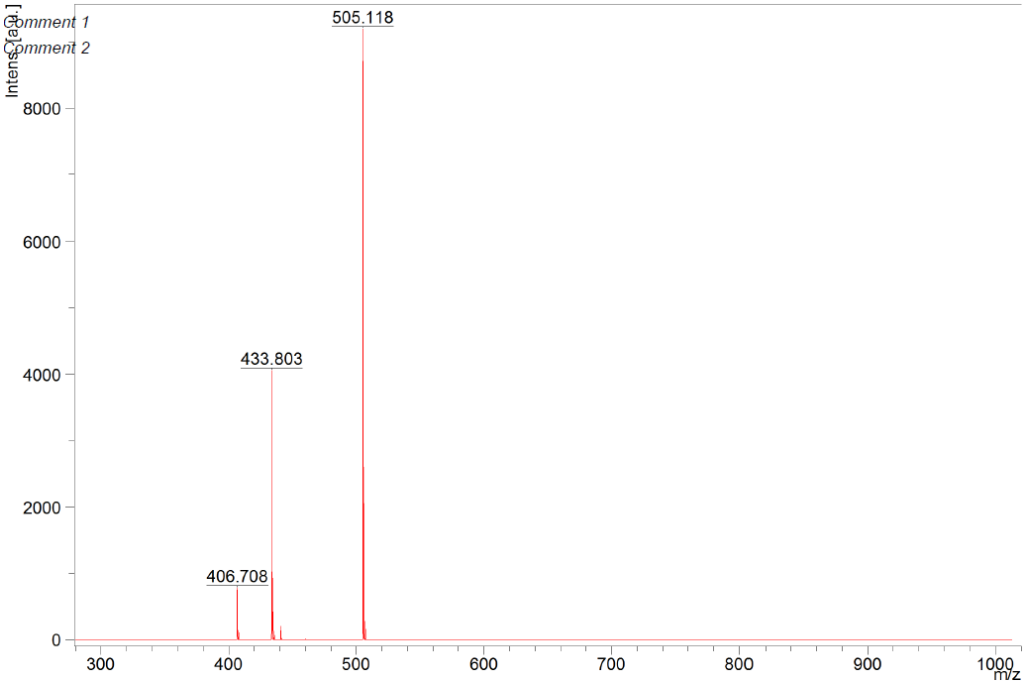
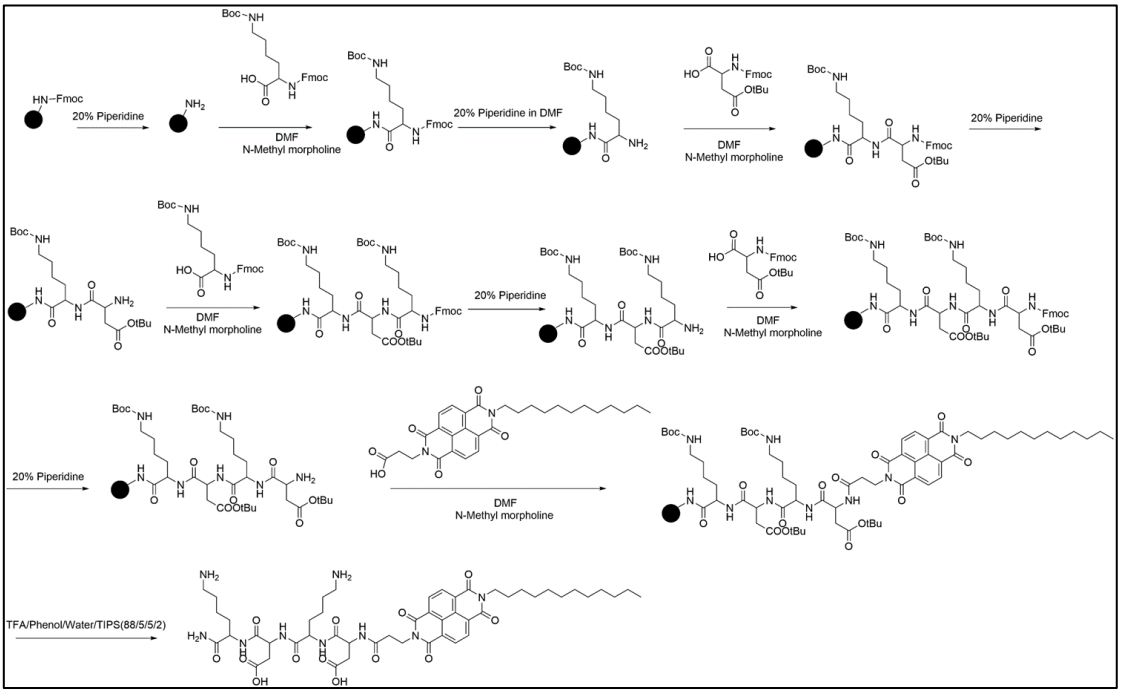


Figure S2. MALDI mass analysis of **NDI-A**.

3. **Scheme for peptide synthesis**



Scheme S1. Synthesis Scheme of NDI-PEP.

3.2 Synthetic procedure of NDI-PEP-L: Using a specialized glass apparatus and Fmoc chemistry, the tetra peptide was manually synthesized in a solid phase under a nitrogen atmosphere. The standard protocols were followed from a recent report by Ghosh et al.[4] Fmoc-protected rink amide resin (0.45 g, 0.5 mmol) was used as solid support. At first, the resin was soaked in 10 ml of DMF for two hours and then transferred to the special peptide synthesising apparatus. Four different steps were followed to obtain the peptide such as i) deprotection of Fmoc group by 20% piperidine in DMF, ii) After deprotection of Fmoc- group, coupling of Fmoc-protected amino acid (4 mM) was performed by using HBTU (4 mM, 0.76 g) as the coupling agent and N-methyl morpholine (8 mM) as a base. The same procedures were repeated until the desired peptide was obtained. Cleavage of the final peptide was performed by applying the cocktail (TFA/phenol/water/TIPS 88/5/5/2). The solution was drained off and concentrated to dryness in a desiccator. The peptide was washed several times with cold ether. The purity of NDI-PEP-L was confirmed by NMR spectroscopy which was further checked by MALDI-TOF mass spectrometry.

^{1}H-NMR (DMSO-D6, 400 MHz, TMS), δ(ppm): δ 8.67 (s, 4H), 8.38 (d,1H), 8.13 (t, 2H), 7.68 (d, 2H), 7.13 (d, 2H), 4.55 (m, 2H), 4.27 (t, 2H), 4.09 (m, 4H), 2.76 (m, 8H), 2.56 (m, 2H), 1.65 (m, 4H), 1.53 (m, 6H), 1.33 (m, 8H), 1.23 (m, 16H), 0.84 (m, 3H).

13C NMR (151 MHz, DMSO-D6), δ(ppm): 173.87, 172.27, 170.69, 163.15, 130.96, 126.92, 118.82, 116.82, 52.82, 50.29, 37.32, 36.39, 33.73, 31.82, 29.52, 27.89, 27.05, 22.61, 14.48.

MALDI (-ve mode): m/z calc. for $C_{49}H_{69}N_9O_3$ $[M-H]^-$:991.50; found:991.45

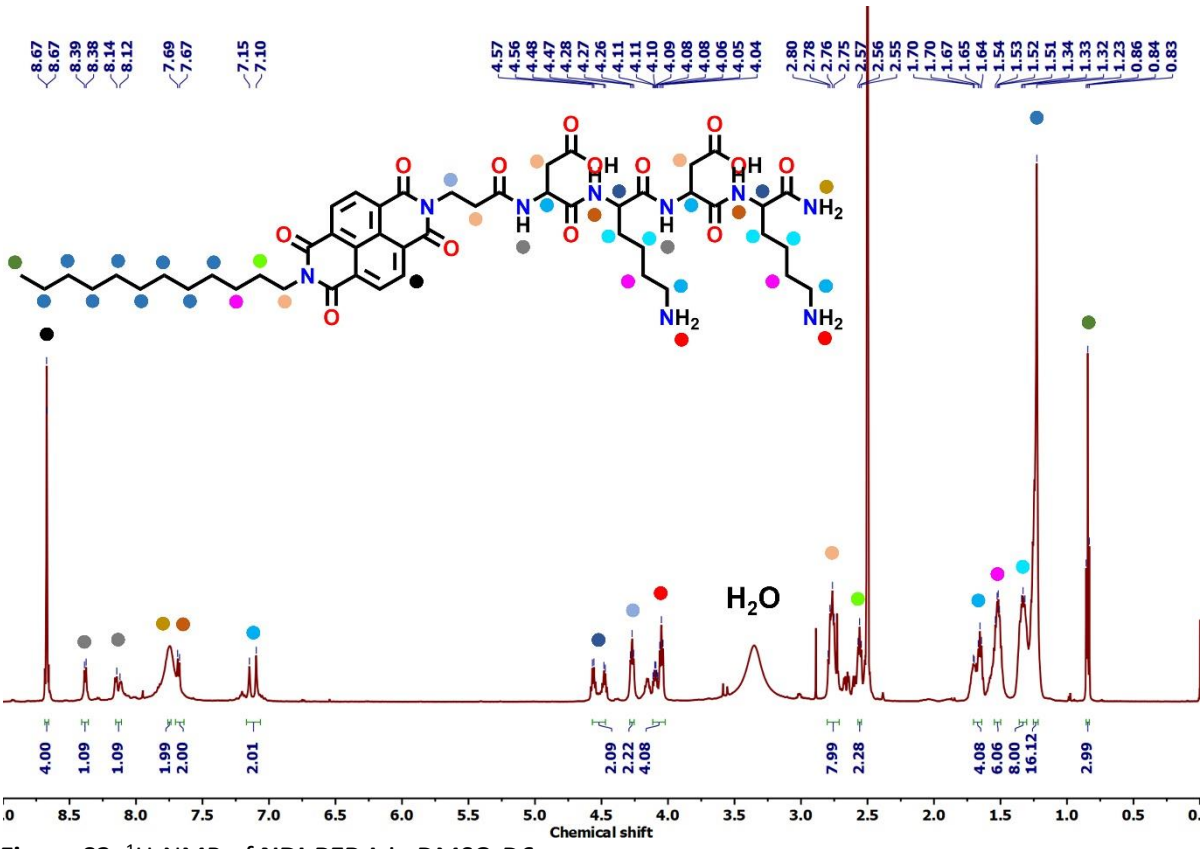


Figure S3. ^{1}H-NMR of **NDI-PEP-L** in DMSO-D6.

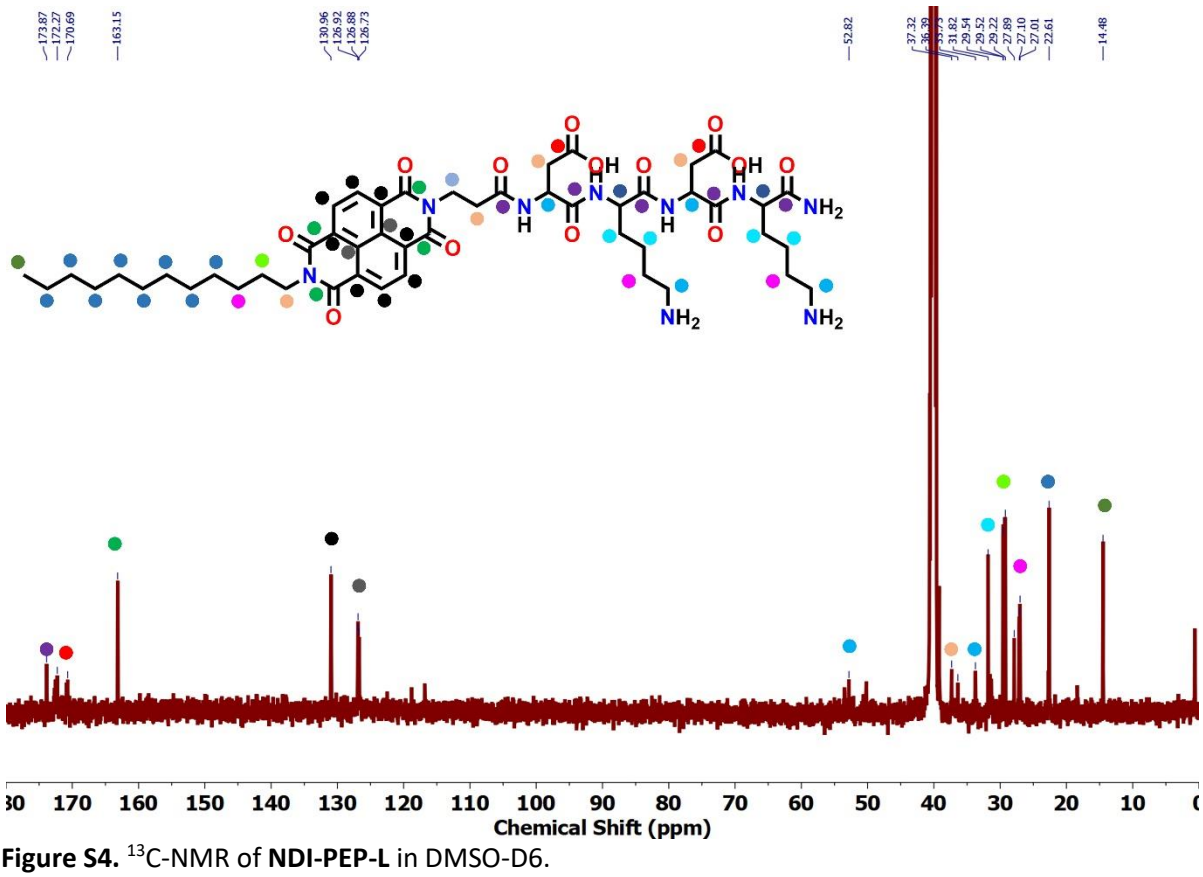


Figure S4. ^{13}C-NMR of **NDI-PEP-L** in DMSO-D6.

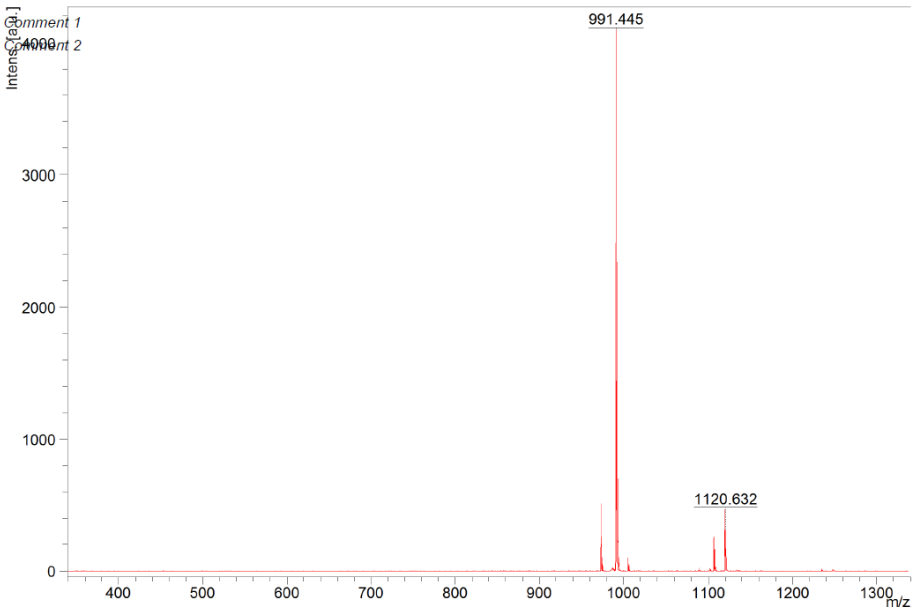


Figure S5. MALDI mass analysis of **NDI-PEP-L.**

3.3 Synthetic procedure of NDI-PEP-D: Similar synthetic protocol was employed for the synthesis of NDI-PEP-D. In the case of NDI-PEP-D, D amino acids were used instead of L amino acids for the synthesis process.

^{1}H NMR (600 MHz, DMSO-*D*$_6$), δ(ppm): δ 8.67 (s, 4H), 8.36 (d,1H), 8.19 (t, 2H), 7.65 (d, 2H), 7.1 (d, 2H), 4.55 (m, 2H), 4.27 (t, 2H), 4.05 (m, 4H), 2.78 (m, 8H), 2.56 (m, 2H), 1.67 (m, 4H), 1.53 (m, 6H), 1.33 (m, 8H), 1.23 (m, 16H), 0.84 (m, 3H).

13C NMR (151 MHz, DMSO-D6), δ(ppm): 173.85, 172.5, 170.83, 163.15, 130.96, 126.91, 118.8, 116.82, 52.81, 50.3, 37.31, 36.35, 33.74, 31.82, 29.54, 27.89, 27.05, 22.61, 14.47.

MALDI (-ve mode): m/z calc. for $C_{49}H_{69}N_9O_3$ $[M-H]^-$:991.50; found:992.34

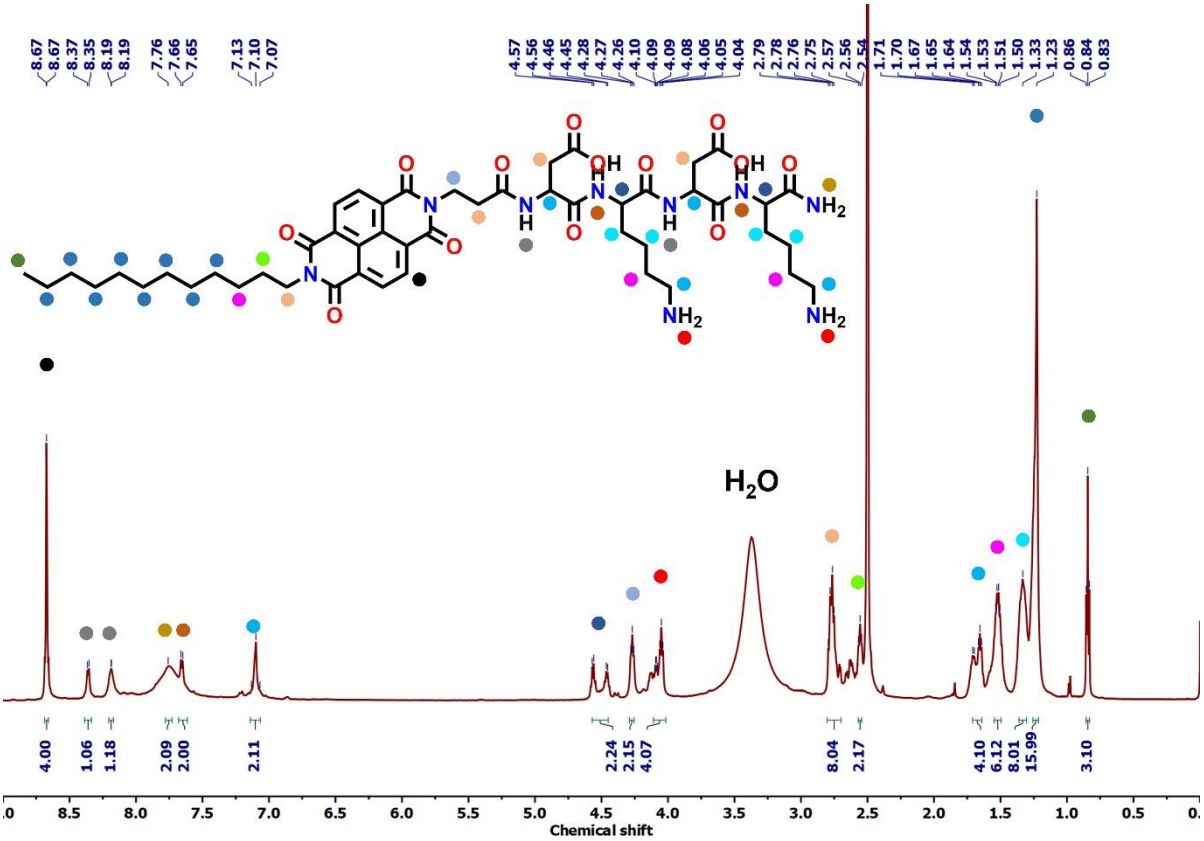


Figure S6. ^{1}H-NMR of NDI-PEP-D in DMSO-D6.

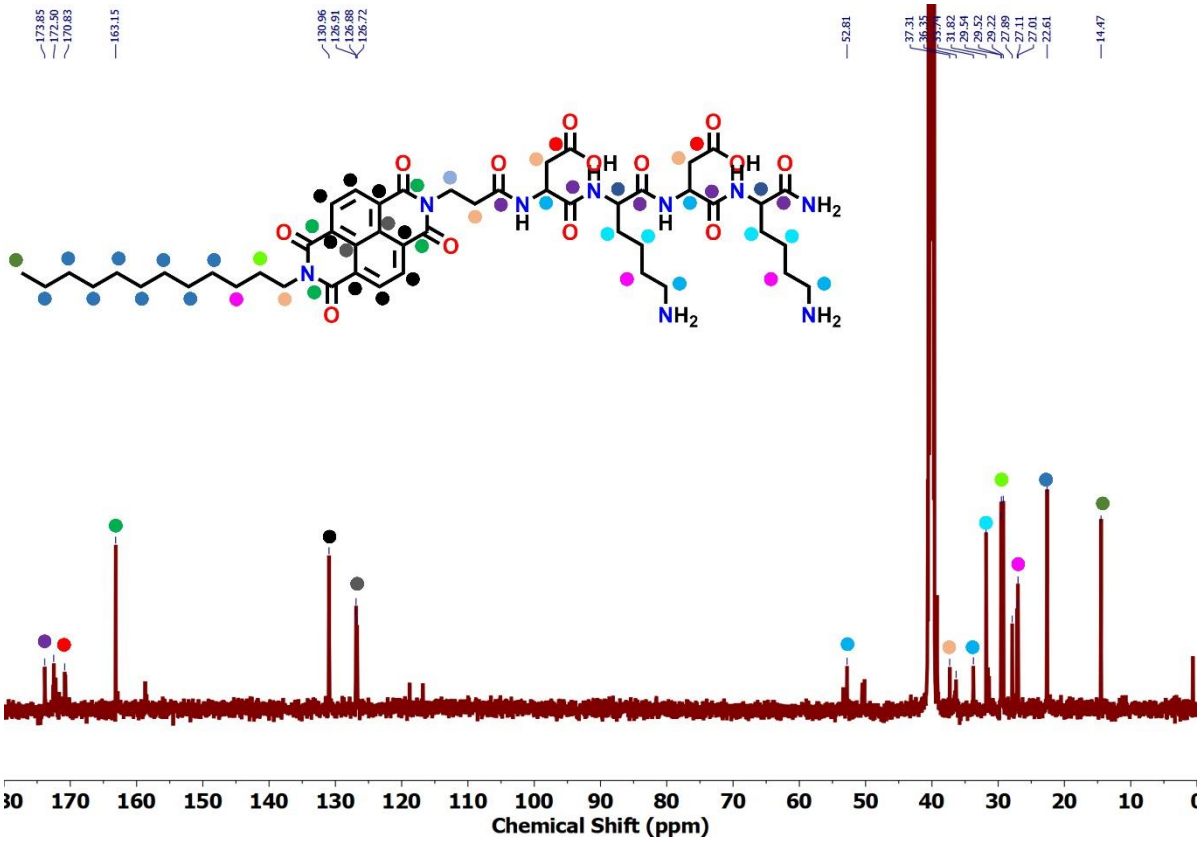


Figure S7. ^{13}C-NMR of NDI-PEP-D in DMSO-D6.

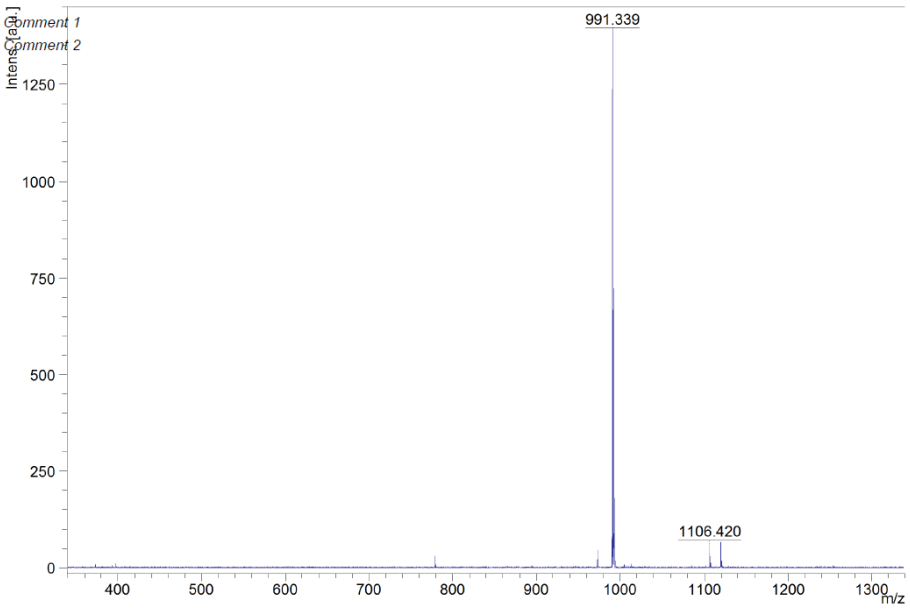


Figure S8. MALDI mass analysis of NDI-PEP-D.

4. Experimental Section

UV Vis spectroscopic studies: A 1 mM stock solution of NDI-PEP-L was prepared in DMSO solvent and a 0.5 mM stock solution was prepared in H_2O. The desired amount of the stock solutions was transferred to a glass vial, and then the required amount of solvents were added to it to make the final desired concentration. The solution was then transferred into a quartz cuvette of path length 1 cm and spectra were recorded. The measured wavelength range was from 200-600 nm.

For the variable temperature experiment, the solutions were transferred to a quartz cuvette, after that the temperature was gradually raised from 293 K to 363K at a rate of 1 K/min. The spectra were recorded during cooling with the same temperature interval 1 K/min.

FTIR spectroscopic studies: The deuterated solvents, DMSO d_6 and D_2O were used for FT-IR measurements. A high concentration (1 mM) of solutions was prepared using the deuterated solvents. The solutions were placed in CaF_2 windows with a 0.2 mm spacer.

Atomic Force Microscopic (AFM) studies: For the AFM experiment, 10 µl of aqueous solution (concentration = 0.01 mM) of NDI-PEP-L was drop casted and followed by spin coated on a mica surface, further allowed to air dry overnight under vacuum before the images were taken. Images were captured in tapping mode.

Dynamic Light Scattering Studies: Samples were prepared in the same manner as for the UV studies by diluting the stock solution with the appropriate solvent ratio to achieve a final concentration of 0.01 mM.

Photoluminescence (PL) studies: The same stock prepared for UV studies was used for PL studies also. A sample of NDI-PEP-L in DMSO (C= 0.01 mM) and H_2O (C= 0.01 mM) was taken

in a fluorescence cuvette of path length 1 cm. The excitation wavelength of 340 nm was maintained for NDI-PEP-L.

Circular Dichroism (CD) studies: CD spectra were recorded using a quartz cuvette with a path length of 1 cm, a scan speed of 50 nm min^{-1}, and a response time of 1 s. The spectra were averaged over three scans to minimise signal noise. The measurements were done in the wavelength range of 200-500 nm. A sample of NDI-PEP-L (C = 0.01 mM) in water was taken in a stoppered cuvette, and the sample was heated from 298 K to a higher temperature (363 K). The CD spectra were recorded at 1 K intervals, and the aqueous solution was equilibrated for 1 minutes after reaching the desired temperature. The melting curve was obtained by plotting the CD magnitude (at a fixed wavelength) vs. temperature. The sample was cooled back from 363 K to 298 K and the CD spectra were recorded to get the cooling curve.

Linear Dichroism (LD) studies: A quartz cuvette with a path length of 1 cm was used to record the LD spectra, with a response time of one second and a scan speed of 50 nm min^{-1}. To minimize the noise in the signals, the spectra were averaged over three scans. A wavelength range of 200–500 nm was used for the measurements.

5. **Additional Figures:**

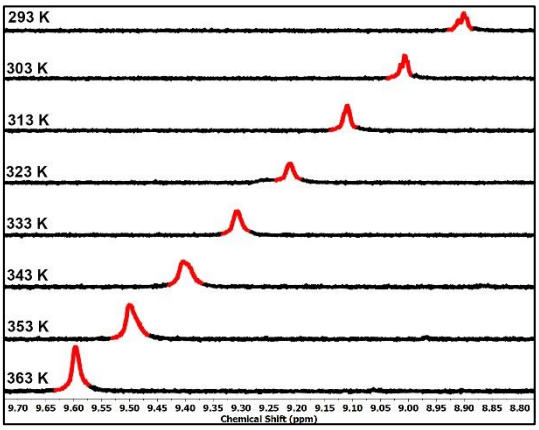


Figure S9. Variable temperature partial ^{1}H-NMR spectra of **NDI-PEP-L** at 1 mM concentration in D_2O:DMSO-D_6 (1:1) condition.

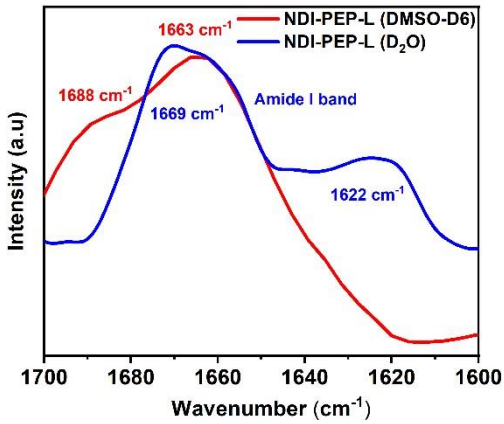


Figure S10. FTIR spectra (C=O stretching region) of **NDI-PEP-L** (*C* = 1 mM) in different solvent condition showing involvement of the amide carbonyl unit in H-bonding during the assembly process.

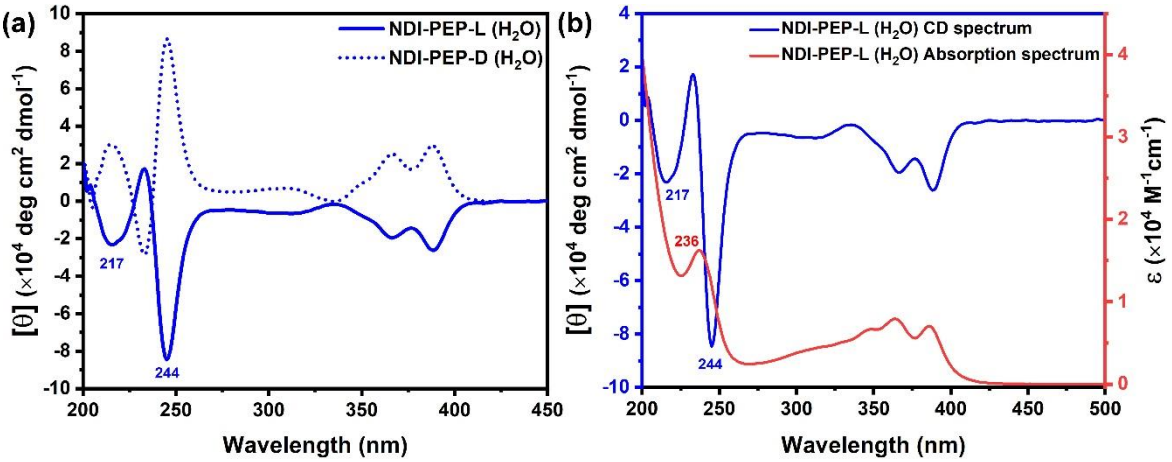


Figure S11. (a) CD spectra of **NDI-PEP-L** and **NDI-PEP-D** (*C* = 0.01 mM) in H_2O showing a signal at 217 nm corresponding to the β-sheet secondary structure of the peptide backbone. (b) A comparison CD (*C* = 0.01 mM) and UV-vis spectra of **NDI-PEP-L** (*C* = 0.01 mM) in H_2O showing the band (300-400 nm) corresponds to the absorption spectra of NDI chromophore, and a bisignated band crossing zero at 236 nm corresponding to the band II of NDI chromophore.[5]

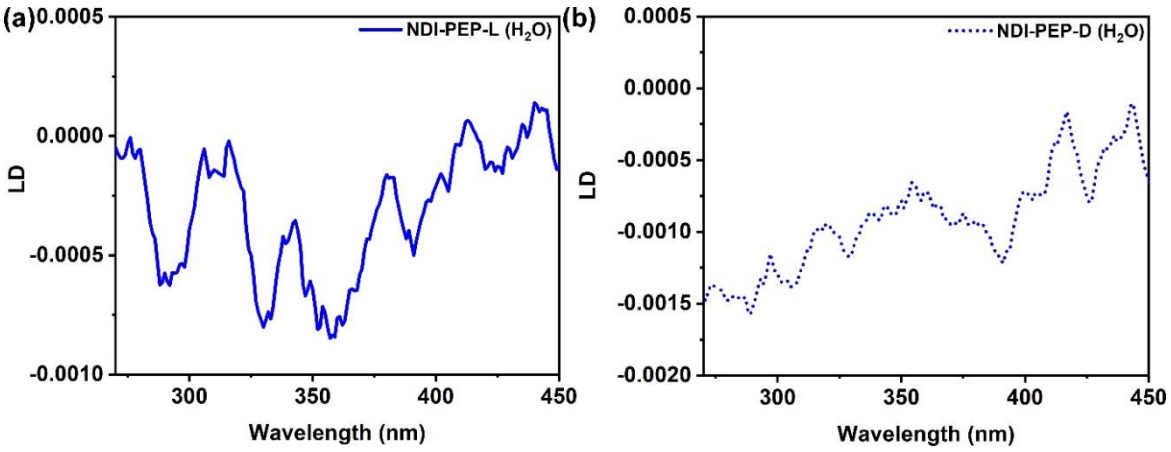


Figure S12. LD spectra of (a) **NDI-PEP-L** and (b) **NDI-PEP-D** in H_2O showing no signal indicating the CD signal produced from the macroscopic chirality of the systems.

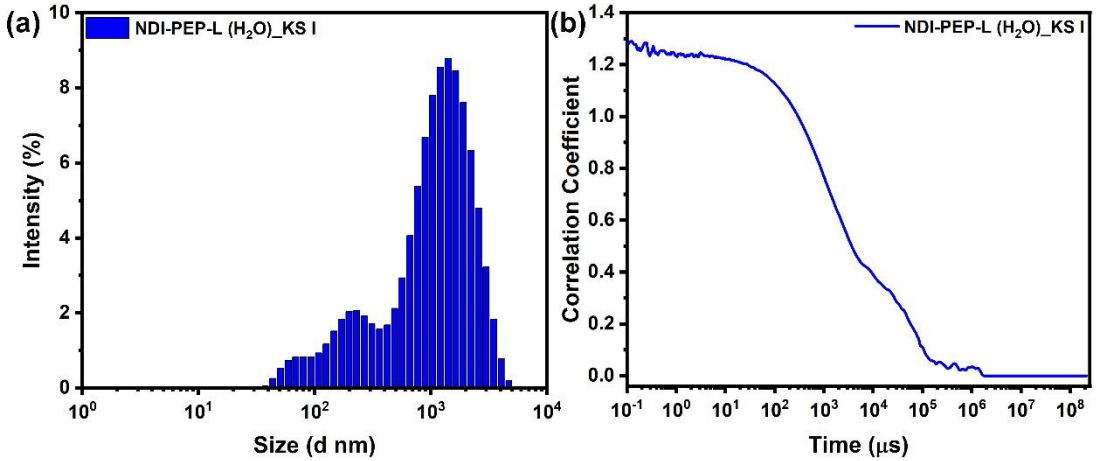


Figure S13. The DLS size distribution and corresponding correlation diagram of **NDI-PEP-L** in KS I showed a broader particle size distribution, indicating the formation of anisotropic nanostructures.

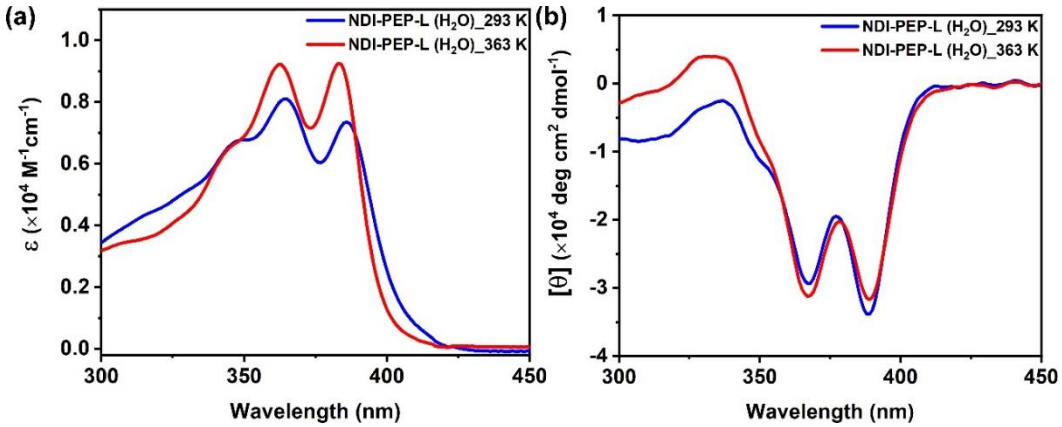


Figure S14. (a) UV-Vis spectra of **NDI-PEP-L** (*C* = 0.01 mM) in H_2O at 293 K and 363 K indicating stable assembly. (b) Corresponding CD spectra (*C* = 0.01 mM) in same condition suggests stable aggregation. No significant change in the CD spectra suggested formation of stable aggregated state in H_2O which also doesn't disassemble under high temperature.

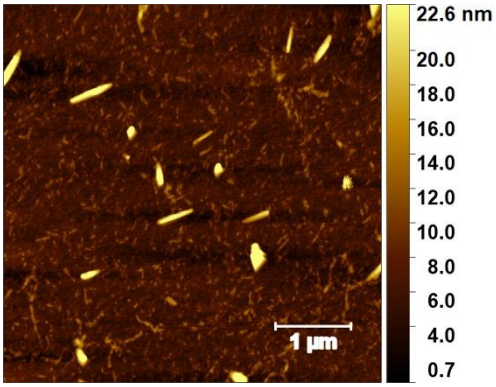


Figure S15. AFM image of **NDI-PEP-L** in H_2O after heating at 363 K showing stable rod like nanostructures.

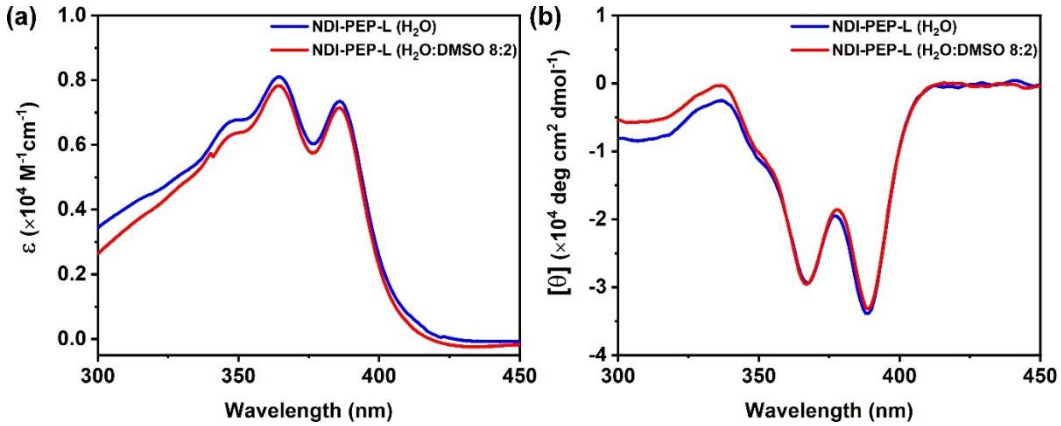


Figure S16. (a) Similar UV-Vis spectra obtained for **NDI-PEP-L** (*C* = 0.01 mM) in H_2O and H_2O:DMSO (8:2) indicates similar internal order of the chromophore. (b) Corresponding CD spectra (*C* = 0.01 mM) of the systems suggest the identical molecular order.

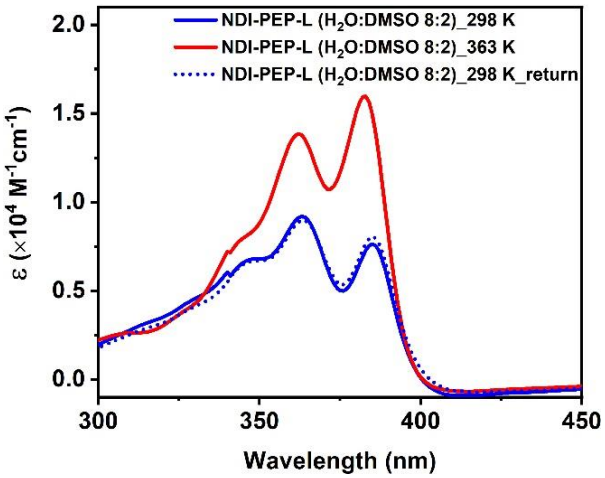


Figure S17. (a) UV-Vis spectra of **NDI-PEP-L** (*C* = 0.01 mM) in H_2O:DMSO (8:2) at 298 K and 363 K indicates the disassembly of the aggregated structure upon heating.

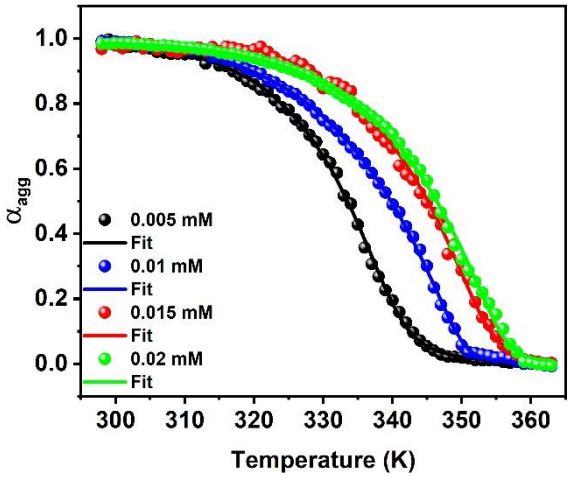


Figure S18. Cooling curves of **NDI-PEP-L** at different concentration monitored at 383 nm and fitted to cooperative model [cooling rate = 1 K min^{-1}].

Sl No.	Concentration	Elongation temperature
1.	0.005 mM	339.25 K
2.	0.01 mM	349.14K
3.	0.015 mM	352.44 K
4.	0.02 mM	354.97 K

Figure T1. Table of elongation temperatures at different concentrations of **NDI-PEP-L**, obtained from fitting the cooling curve.

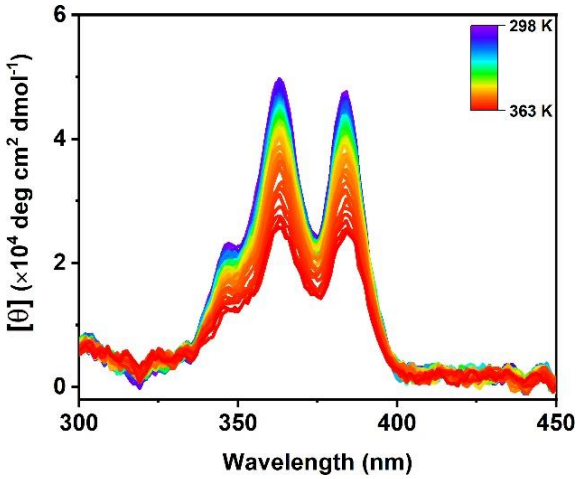


Figure S19. VT-CD spectra of **NDI-PEP-L** (*C* = 0.01 mM) after reaching a thermodynamic state (TS) via the slow cooling method, showed no disassembly of the TS during further heating process.

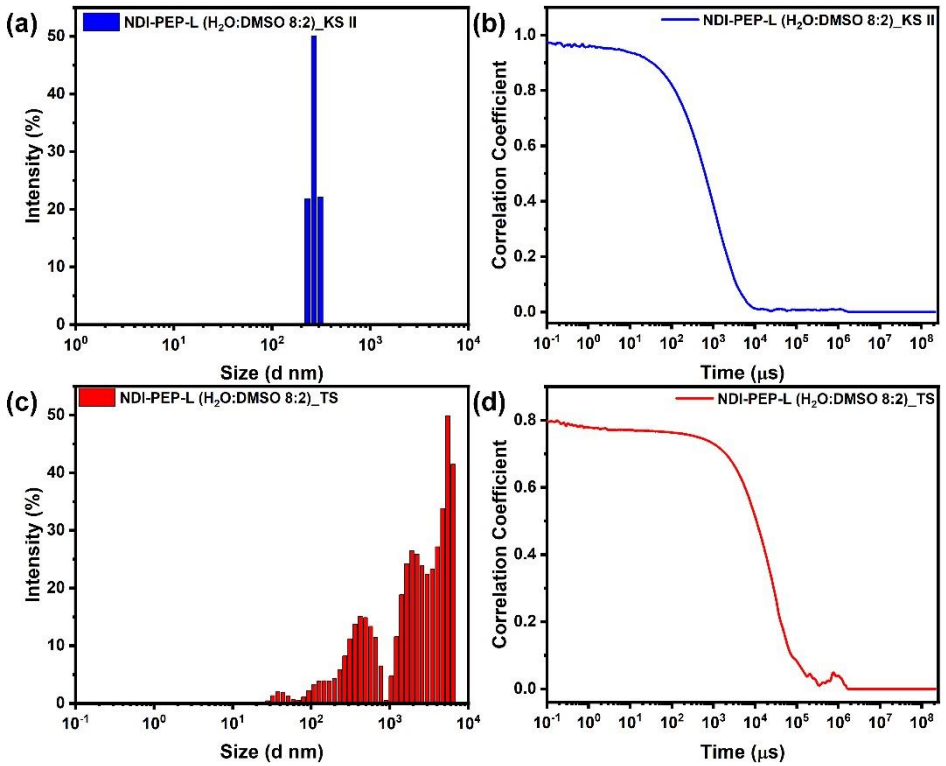


Figure S20. The DLS size distribution and corresponding correlation diagrams of **NDI-PEP-L** in (a, b) KS II demonstrates smaller isotropic nanostructures, while those in (c, d) TS indicates a larger hydrodynamic size of the system.

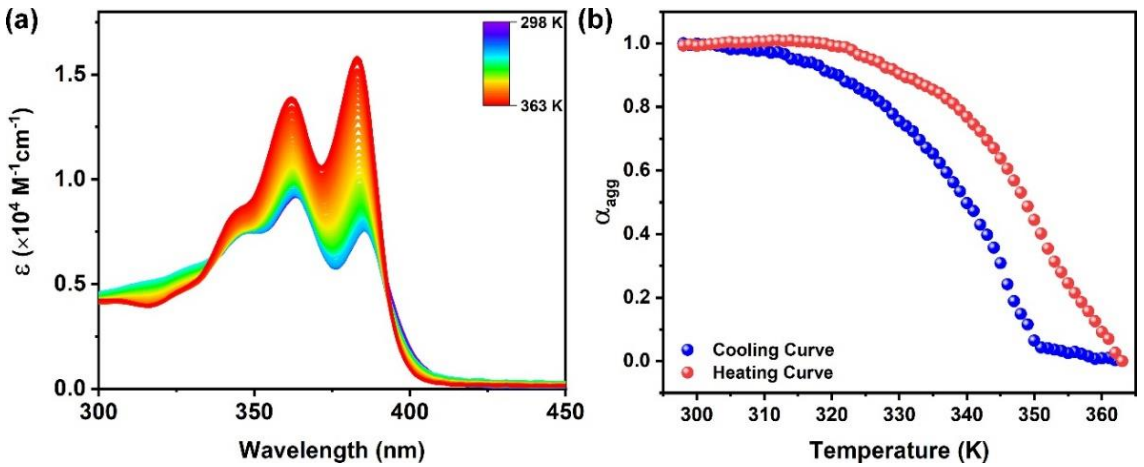


Figure S21. (a) VT UV-Vis spectra of **NDI-PEP-L** during heating from 298 K to 363 K in H_2O:DMSO (8:2) at 0.01 mM concentration. (b) Comparison of secondary curves during cooling and heating showing a hysteresis.

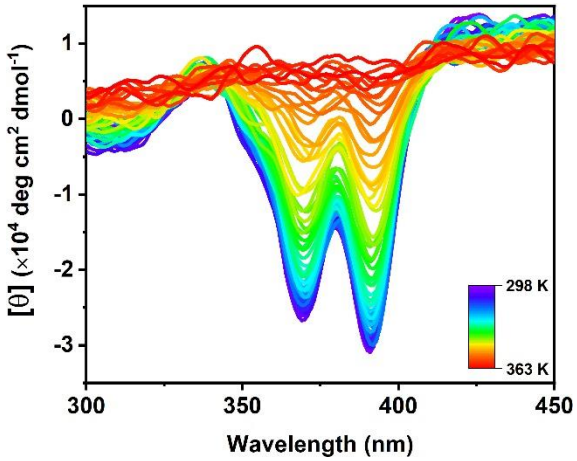


Figure S22. (a) VT CD spectra of **NDI-PEP-L** during heating from 298 K to 363 K in H_2O:DMSO (8:2) at 0.01 mM concentration.

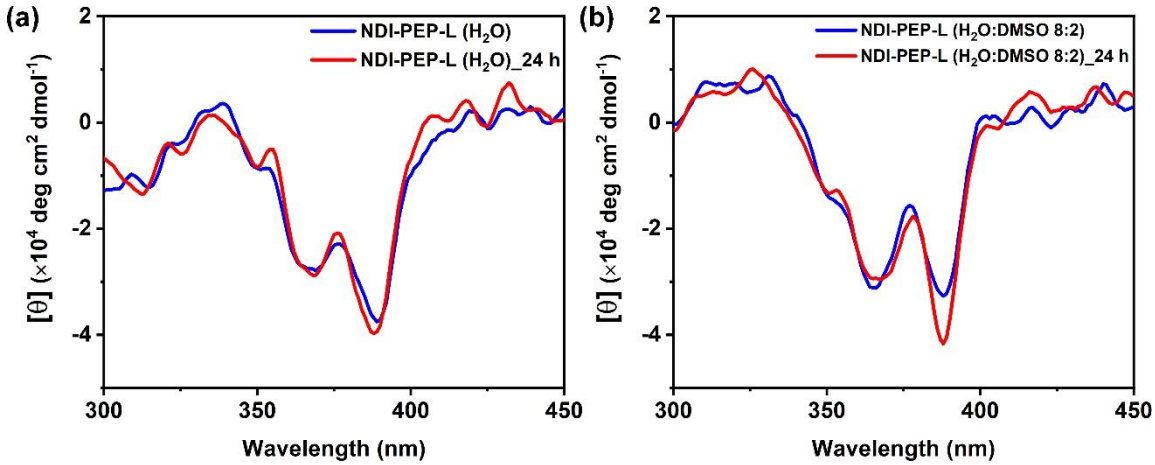


Figure S23. (a) Time dependent CD spectra of **NDI-PEP-L** (*C* = 0.01 mM) in H_2O (b) and 20% DMSO in H_2O, indicating no changes over the time period and stability of the kinetic state.

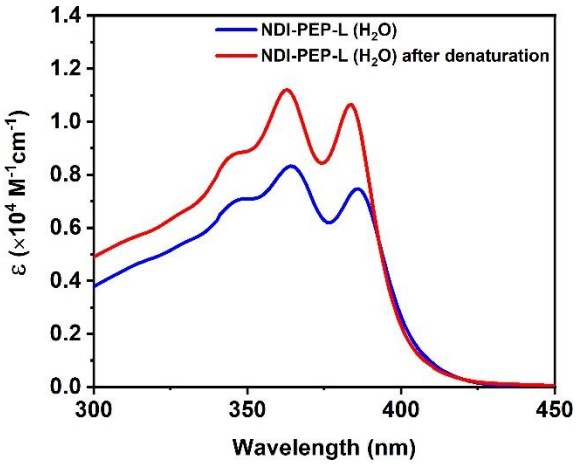


Figure S24. UV-Vis spectra of **NDI-PEP-L** (*C* = 0.01 mM) after completion of the denaturation process showing aggregated nature of the peptide.

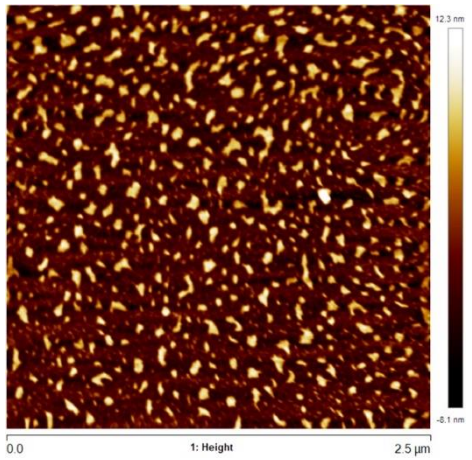


Figure S25. AFM image of **NDI-PEP-L** at H_2O:DMSO (1:1) solvent condition during the denaturation process.

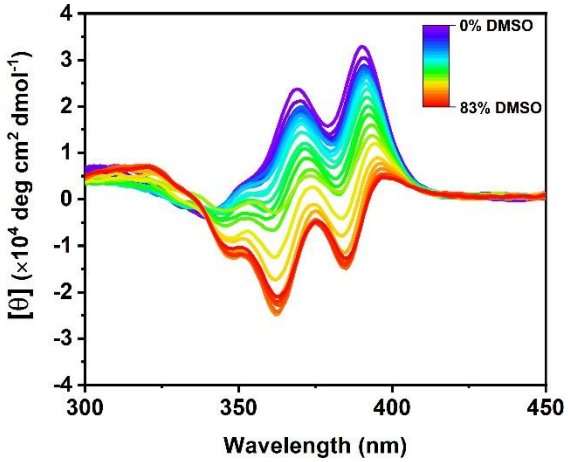


Figure S26. Denaturation induced supramolecular chirality inversion in **NDI-PEP-D** at 0.01 mM concentration showing the positive signal corresponding to the P-helix get reversed to M-helix with negative CD signal.

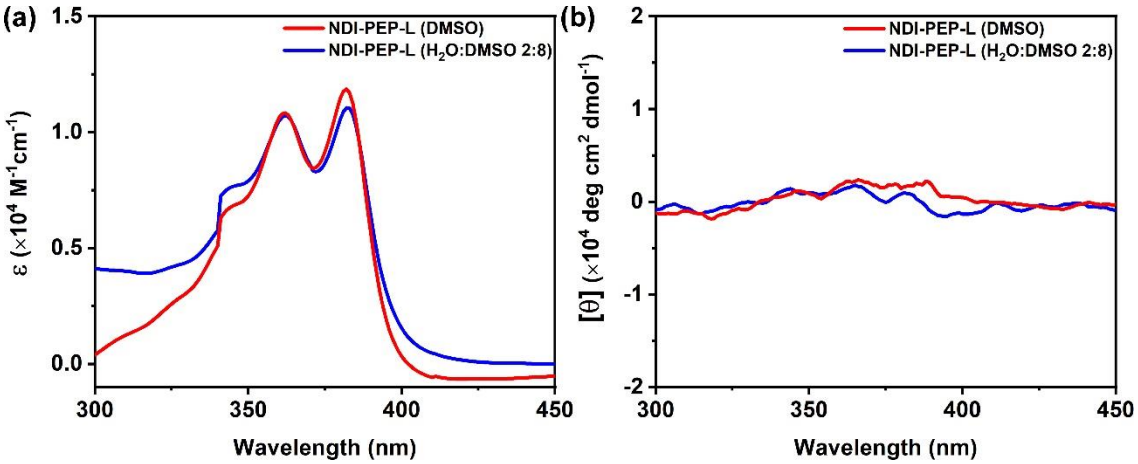


Figure S27. (a) UV-Vis comparison spectra of **NDI-PEP-L** (*C* = 0.01 mM) in DMSO and H_2O:DMSO (2:8) (direct preparation method) showing monomeric characteristic of the band of the NDI chromophore. (b) CD comparison spectra of **NDI-PEP-L** (*C* = 0.01 mM) in DMSO and H_2O:DMSO (2:8) (direct preparation method) showing monomeric characteristic features.

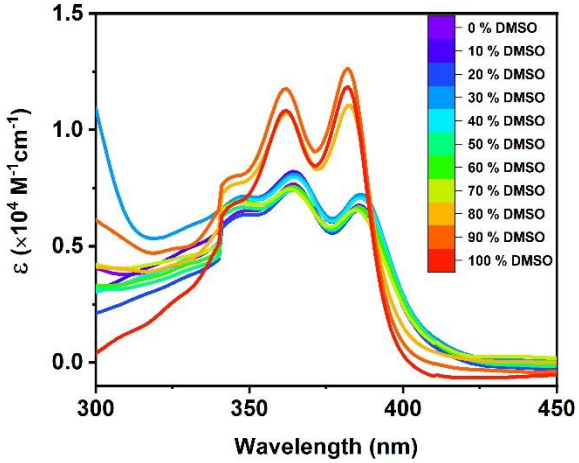


Figure S28. Percentage profile UV-Vis spectra of **NDI-PEP-L** (*C* = 0.01 mM) at different DMSO/H_2O condition.

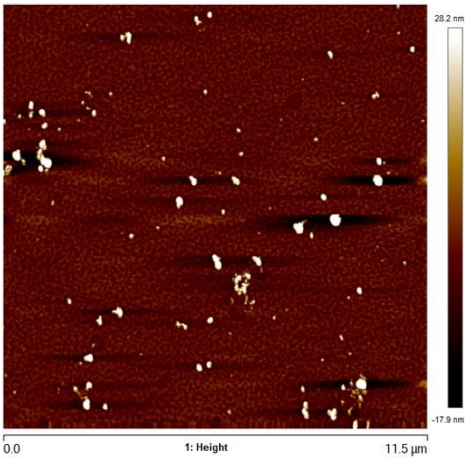


Figure S29. AFM image of **NDI-PEP-L** at H_2O:DMSO (1:1) solvent condition showing formation of ill-defined aggregated nanostructures.

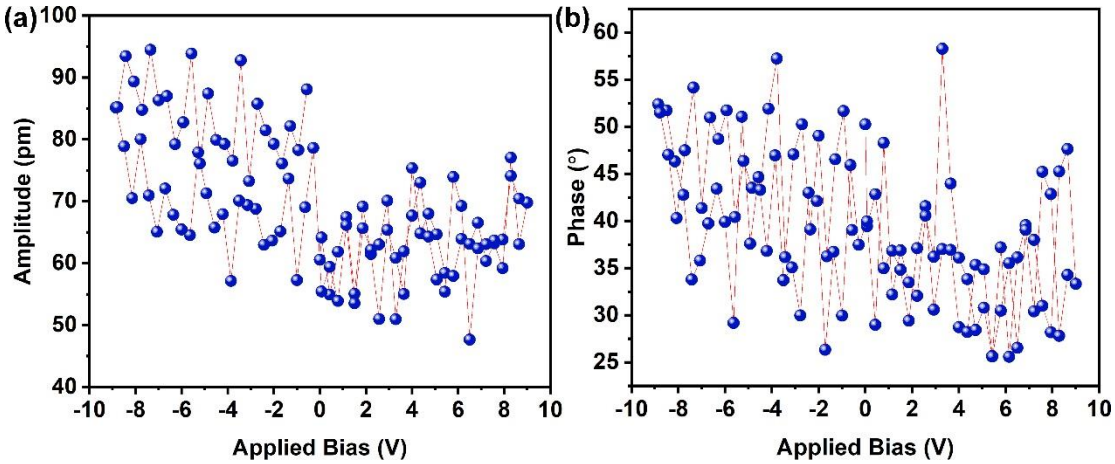


Figure S30. PFM (a) amplitude voltage butterfly loop and (b) phase voltage hysteresis loop obtained for **NDI-PEP-L** in kinetic state (KS-I) state in H_2O.

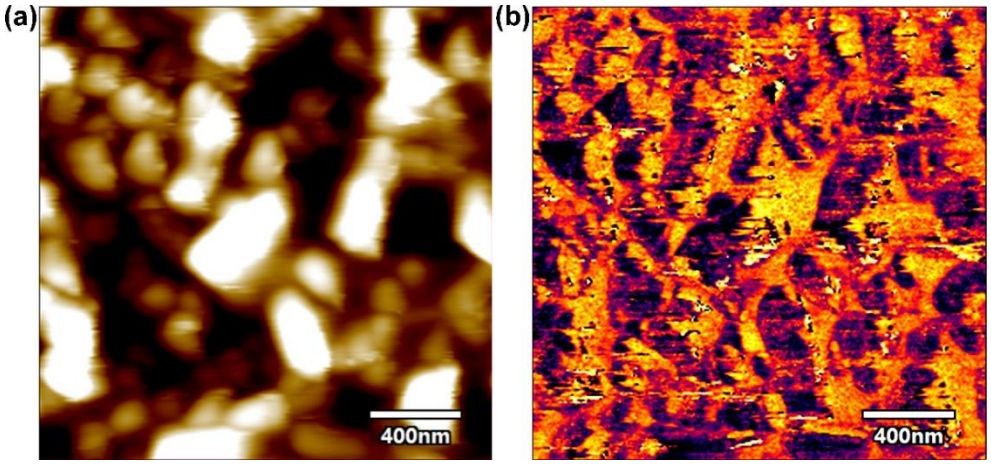


Figure S31. PFM (a) topography image and (b) phase image of **NDI-PEP-L** in thermodynamically aggregated state.

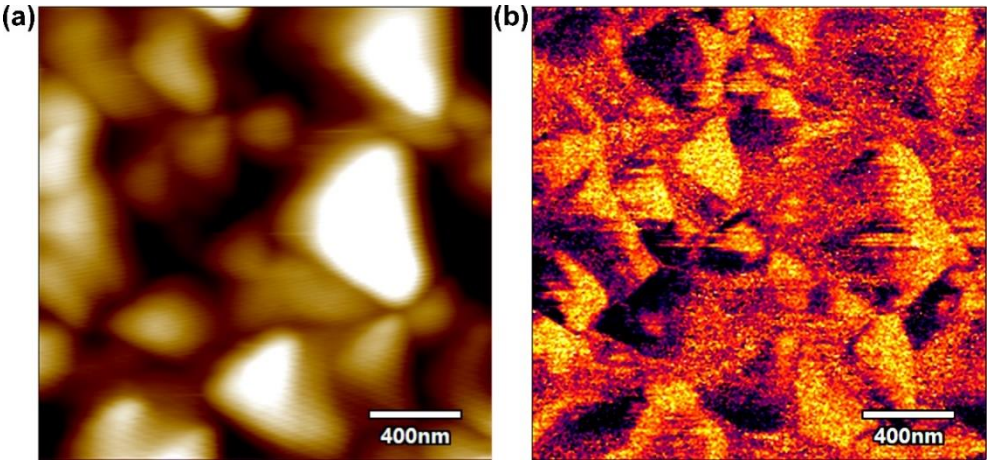


Figure S32. PFM (a) topography image and (b) phase image of **NDI-PEP-D** in thermodynamically aggregated state.

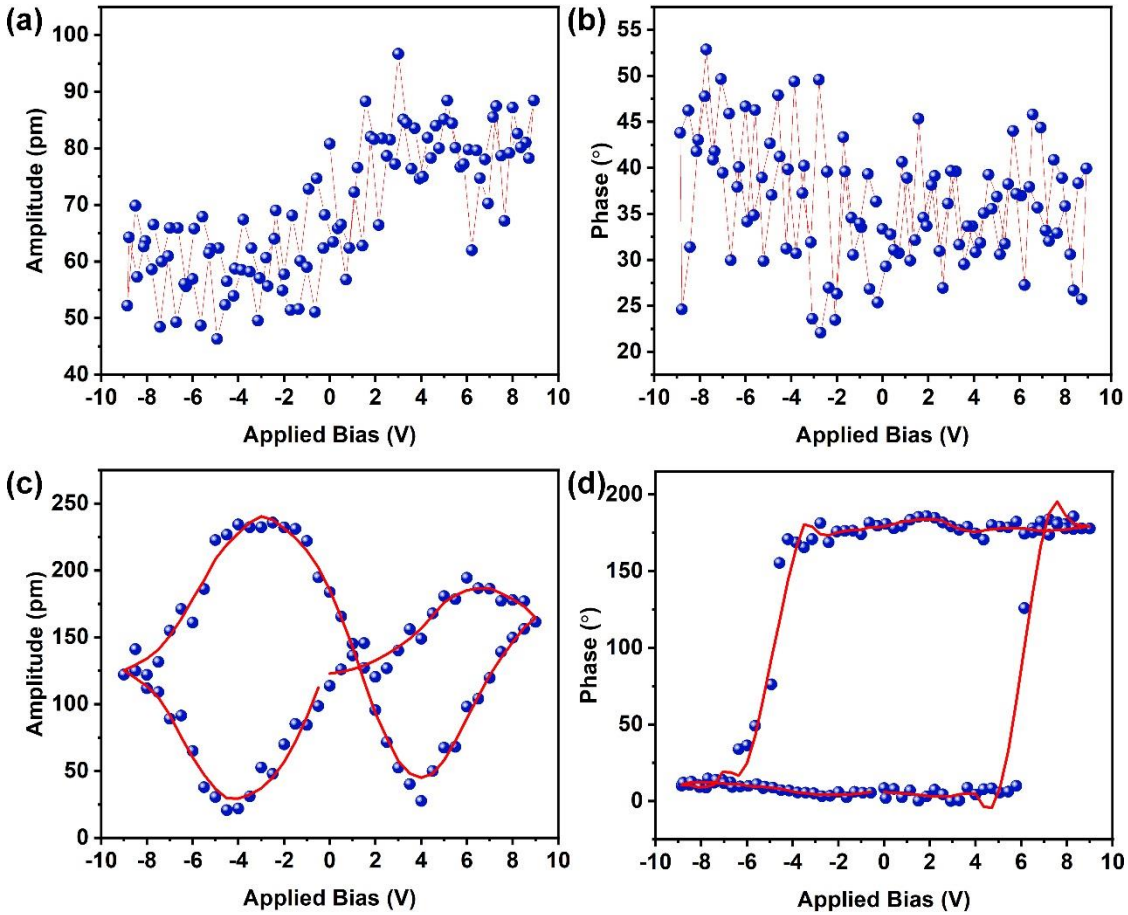


Figure S33. PFM (a) amplitude voltage butterfly loop and (b) phase voltage hysteresis loop obtained for **NDI-PEP-D** in kinetically aggregated state (KS II). PFM (c) amplitude voltage butterfly loop (off state) and (d) phase voltage hysteresis loop (off state) obtained for **NDI-PEP-D** in thermodynamically aggregated state.

7. **References**

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

[2] H. M. M. ten Eikelder, A. J. Markvoort, T. F. A. de Greef and P. A. J. Hilbers, *J. Phys. Chem. B*, 2012, **116**, 5291.

[3] A. J. Markvoort, H. M. M. ten Eikelder, P. A. J. Hilbers, T. F. A. de Greef and E. W. Meijer, *Nat. Commun.*, 2011, **2**, 509.

[4] G. Ghosh, R. Barman, J. Sarkar and S. Ghosh, *J. Phys. Chem. B*, 2019, **123**, 5909.

[5] H. Shao, T. Nguyen, N. C. Romano, D. A. Modarelli, J. R. Parquette, Self-Assembly of 1-D n-Type Nanostructures Based on Naphthalene Diimide-Appended Dipeptides, *J. Am. Chem. Soc.* 2009, **131**, 16374–16376.