Tuning the Mechanistic Pathways of Peptide Self-assembly by Aromatic Interactions

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Materials and methods

Chemicals and reagents: All the required chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), TCI Europe N.V. (Japan) or Alfa Aesar (USA) and used without further purification unless otherwise stated. All the solvents were purified and dried following standard protocols before use.[1]

NMR spectroscopy: Avance II 300 and Avance II 400 from Bruker were used to record ^{1}H and ^{13}C NMR spectra at 298 K by using tetramethylsilane (TMS) as internal reference, and for characterization purposes, DD2 500 and DD2 600 from Agilent were used. Multiplicities for proton signals are abbreviated as s, d, t, q and m for singlet, doublet, triplet, quadruplet and multiplet, respectively.

Mass spectrometry: ESI mass spectra were measured on a Bruker MicrOToF system.

UV-Vis spectroscopy: UV/Vis absorption spectra were recorded on a JASCO V-750 or a JASCO V-770 spectrophotometers with a spectral bandwidth of 1.0 nm and a scan rate of 500 nm min^{-1}. Instruments are equipped with peltier cells and Julabo F250 water circulation units. Variable temperature measurements were performed with a ramp rate of 1K min^{-1} unless otherwise specified. For all measurements, spectroscopic grade solvents were used. All experiments were carried out using quartz cuvettes with optical paths of 1 cm or 1 mm.

Fluorescence spectroscopy: Fluorescence spectra were recorded on a JASCO FP-8500 spectrofluorimeter equipped with the same water circulation unit. Sample preparation and variable temperature experiments for fluorescence spectroscopy were same as for UV-Vis spectroscopy. The protocol for thioflavin T (ThT) assay was followed from recent reports by Ghosh et. al.[2]

FT-IR spectroscopy: FT-IR studies were carried out using a JASCO-FT-IR-6800 with a CaF_2 cell of path length of 0.5 mm in solution phase. Deuterated solvents were used for FT-IR measurements. Solid-state FT-IR was performed by drop-casting the concentrated aggregate solutions followed by slow evaporation overnight under air. The obtained dried solid sample was then investigated by FTIR.

Atomic Force Microscopy: AFM images were recorded on a Multimode® 8 SPM System (AXS Bruker). Silicon cantilevers with a nominal spring constant of 9 Nm^{-1} and with resonant frequency of ~150 kHz and a typical tip radius of 7 nm (OMCL-AC200TS, Olympus) were employed. To prepare the AFM samples, 10 μL of corresponding aggregated solution were drop-casted followed

by spin coating at 1000 rpm onto mica surface. After that, the samples were kept for 24 h to air dry.

Analysis of temperature dependent spectroscopic data

Nucleation-Elongation model for Cooperative Supramolecular Polymerizations

The equilibrium between the monomeric and supramolecular species can be described in a cooperative process with the Nucleation-Elongation model which is developed by Ten Eikelder, Markvoort and Meijer.[3,4] This model is used to describe the self-assembly of **Py-FF** which exhibits a non-sigmoidal cooling curve as shown in temperature-dependent UV-Vis, fluorescence and CD experiments. The values T_e, $\Delta H°_{nucl}$, $\Delta H°$ and $\Delta S°$ can be determined by a non-linear least-square analysis of the experimental melting curves. The equilibrium constants associated with the nucleation and elongation phases can be calculated using equations 1 and 2:

Nucleation step: $K_{nucl} = e^{\left(\frac{-(\Delta H^o - \Delta H^o_{nucl}) - T\Delta S^o}{RT}\right)}$ ---------- (1)

Elongation step: $K_{el} = e^{\left(\frac{-(\Delta H^o - T\Delta S^o)}{RT}\right)}$ ---------- (2)

Cooperativity factor: $\sigma = \frac{K_{nucl}}{K_{el}} = e^{\left(\frac{\Delta H^o_{nucl}}{RT}\right)}$ ---------- (3)

Gibbs free energy: $\Delta G^o = \Delta H^o - T\Delta S^o$ ----------(4)

Where ΔG^o = standard Gibbs free energy; ΔH^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.

Isodesmic Model

The sigmoidal cooling curve for **Nap-FF** was fitted to the isodesmic model.[5] In this model, the fraction of aggregated species (α_{agg}) and the equilibrium constant (K_a) were calculated by equation 4 and 5, respectively. Where A_T, A_M and A_{agg} represent absorption intensity at a given temperature, at the monomer*ic* state (highest temperature) and in the aggregated state (lowest temperature), respectively. C_T (the total concentration) and DP_N (number-averaged degree of polymerization)

$$\alpha_{agg} = \frac{A_T - A_M}{A_{agg} - A_M} \text{----------} (4)$$

$$K_a(T) = \frac{[2DP_N(T) - 1]^2 - 1}{4C_T} \text{----------} (5)$$

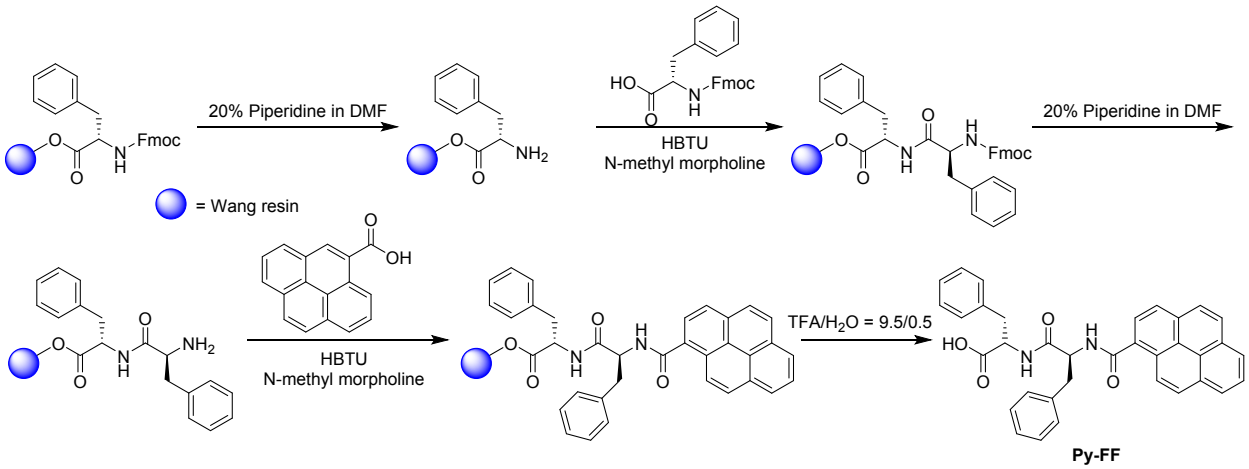
$$DP_N(T) = \frac{1}{\sqrt{1-\alpha(T)}} \text{----------} (6)$$

$$\ln K_a = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \text{----------} (7)$$

can be calculated by using equation 6. Furthermore, change of enthalpy (ΔH) and entropy (ΔS) can be calculated from equation 7.

Synthetic procedure

Synthesis of Py-FF: Py-FF peptide was synthesized on solid phase employing Fmoc chemistry manually by using homemade special apparatus under nitrogen atmosphere. The standard protocols were followed from a recent report by Ghosh et. al.[2] Wang resin attached with fmoc-protected phenylalanine (0.5 mM, 0.78 g) was soaked in 10 ml of DMF for two hours and then transferred to the special apparatus. After deprotection of Fmoc- group by 20% piperidine in DMF, coupling of Fmoc-protected phenylalanine (2 mM 0.77 g) was performed by using HBTU (2 mM, 0.76 g) as coupling agent and N-methylmorpholine (4 mM) as base. The same procedures were repeated until the desired peptide was obtained. Cleavage of the final peptide was performed by applying the cocktail (TFA/H_2O = 9.5/0.5). The solution was drained off and concentrated to dryness in a round-bottom flask. The peptide was washed several times with cold ether. The crude peptide was purified by column chromatography and obtained the pure white solid by using 5% CH_3OH in CH_2Cl_2 as eluent. Yield = 80 mg (41%).



Scheme S1. Synthetic scheme of **Py-FF**.

^{1}H NMR (400 MHz, DMSO-d_6): δ (in ppm) = 8.89 (d, J = 8.7 Hz, 1H), 8.38 (d, J = 7.6 Hz, 1H), 8.36 – 8.18 (m, 5H), 8.15 – 8.05 (m, 2H), 7.98 (d, J = 9.3 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.56 – 7.14 (m, 10H), 4.99 (m, 1H), 4.56 (m, 1H), 3.20 (m, 1H), 3.05 (dd, J = 13.9, 8.4 Hz, 1H), 2.91 (dd, J = 13.9, 11.4 Hz, 1H).

^{13}C NMR (100 MHz, DMSO-d_6): δ (in ppm) = 173.0, 171.2, 168.66,138.3, 137.6, 131.7, 131.5, 130.69, 130.1, 129.3, 128.2, 128.1, 127.8, 127.6, 127.1, 126.5, 126.4, 126.3, 125.7, 125.5, 125.1, 124.8, 124.2, 123.6, 123.5, 54.7, 53.8, 37.3, 36.8.

ESI-MS (TOF): m/z 563.1932 [M+Na]+, calculated for $C_{35}H_{28}N_2O_4Na$: 563.1947.

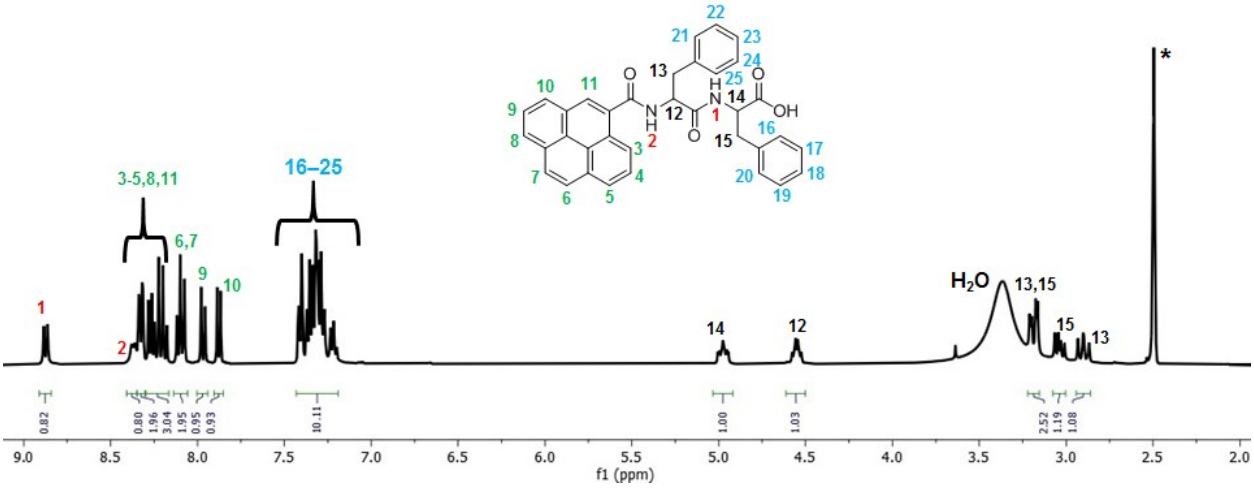


Figure S1. ^{1}H NMR (400 MHz, DMSO-d_6) of **Py-FF**.

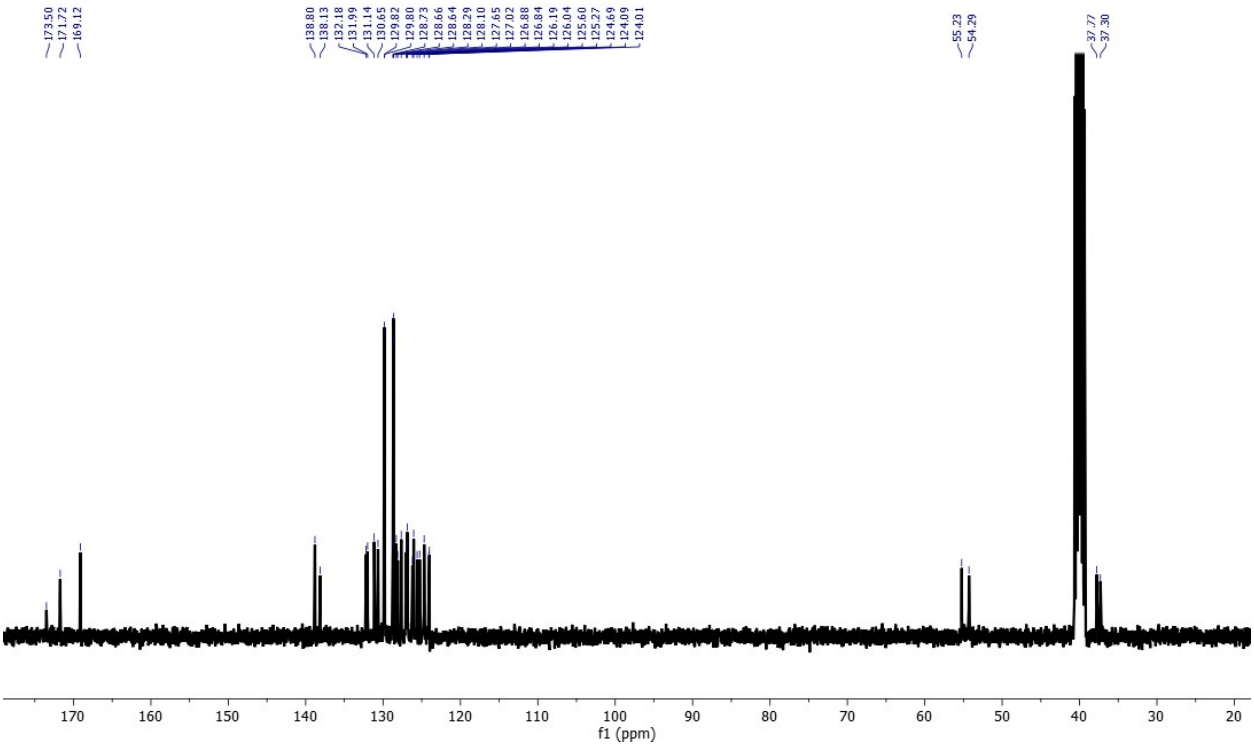
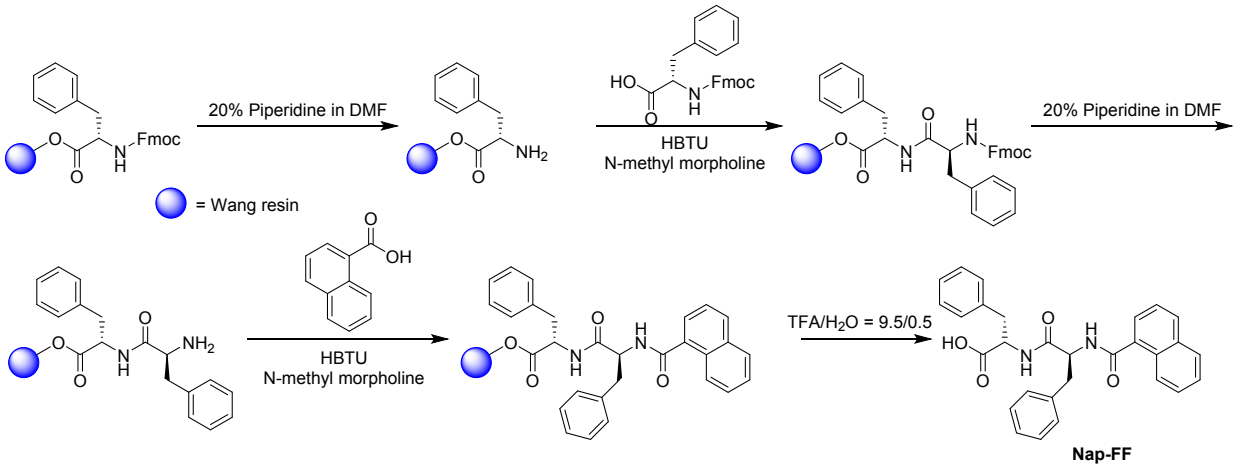


Figure S2. ^{13}C NMR (100 MHz, DMSO-d_6) of **Py-FF**.

Synthesis of Nap-FF: The same synthetic procedure was followed for Nap-FF. Yield = 120 mg (61.5%).



Scheme S2. Synthetic scheme of **Nap-FF**.

^{1}H NMR (400 MHz, DMSO-d_6): δ (in ppm) = 8.69 (d, J = 8.8 Hz, 1H), 8.28 (d, J = 7.7 Hz, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.93 (d, J = 8.2 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.44 – 7.16 (m, 12H), 4.88 (m, 1H), 4.52 (m, 1H), 3.15 (m, 2H), 3.01 (dd, J = 13.9, 8.5 Hz, 1H), 2.85 (dd, J = 13.8, 11.4 Hz, 1H).

^{13}C NMR (100 MHz, DMSO-d_6): δ (in ppm) = 173.4, 171.6, 168.7, 138.7, 138.0, 135.0, 133.4, 130.1, 130.0, 129.7, 128.6, 128.5, 126.9, 126.8, 126.7, 126.6, 126.0, 125.4, 125.2, 54.9, 54.2, 37.6, 37.2.

ESI-MS (TOF): m/z 489.1786 [M+Na]+, calculated for $C_{29}H_{26}N_2O_4Na$: 489.1791.

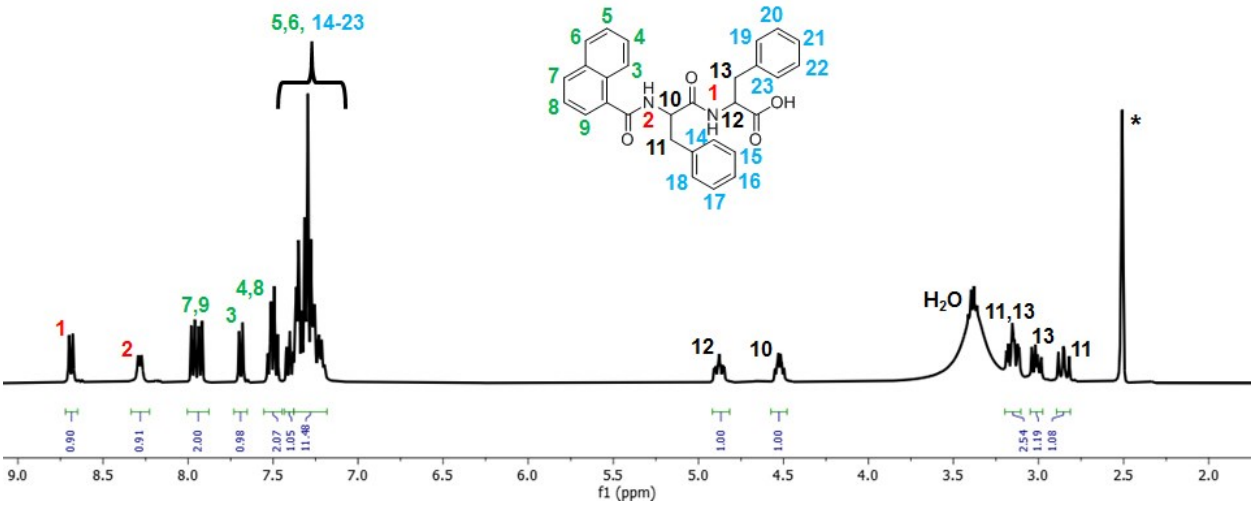


Figure S3. ^{1}H NMR (400 MHz, DMSO-d_6) of **Nap-FF**.

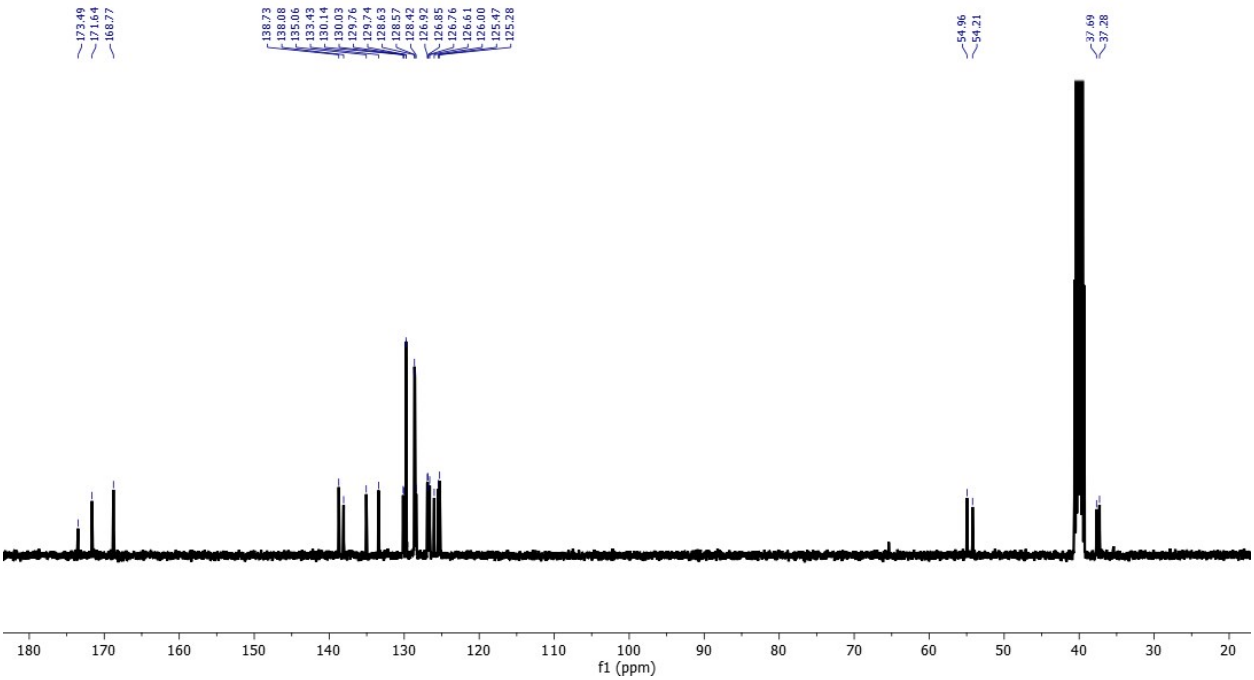
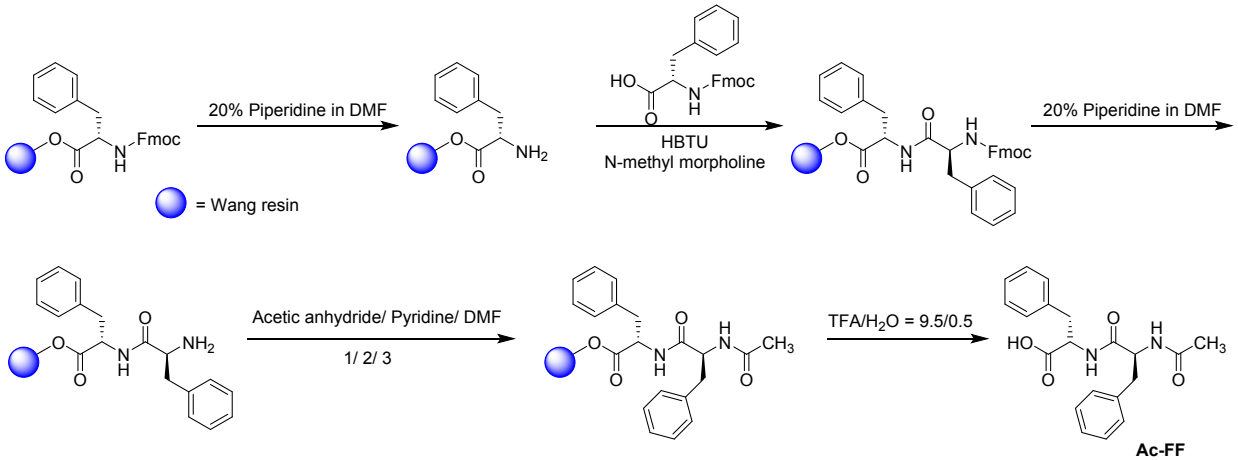


Figure S4. ^{13}C NMR (100 MHz, DMSO-d_6) of **Nap-FF**.

Synthesis of Ac-FF: The same synthetic procedure was followed for Ac-FF. Instead of chromophore coupling, acetylation was performed by using a mixture of acetic anhydride/ Pyridine/ DMF (1/ 2/ 3) in the last step. Yield = 132 mg (64.7%).



Scheme S3. Synthetic scheme of **Ac-FF**.

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ (in ppm) = δ 8.27 (d, J = 7.8 Hz, 1H), 8.04 (d, J = 8.6 Hz, 1H), 7.47 – 7.00 (m, 10H), 4.51 (m, 1H), 4.43 (m, 1H), 3.07 (dd, J = 13.9, 5.2 Hz, 1H), 3.01 – 2.85 (m, 2H), 2.65 (dd, J = 13.9, 10.3 Hz, 1H), 1.70 (s, 3H).

^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ (in ppm) = 173.2, 171.9, 169.5, 138.4, 137.8, 129.6, 128.6, 128.4, 126.9, 126.6, 54.0, 53.9, 37.9, 37.0, 22.8.

ESI-MS (TOF): m/z 353.1503 [M-H]+, calculated for $C_{20}H_{21}N_2O_4$: 353.1580.

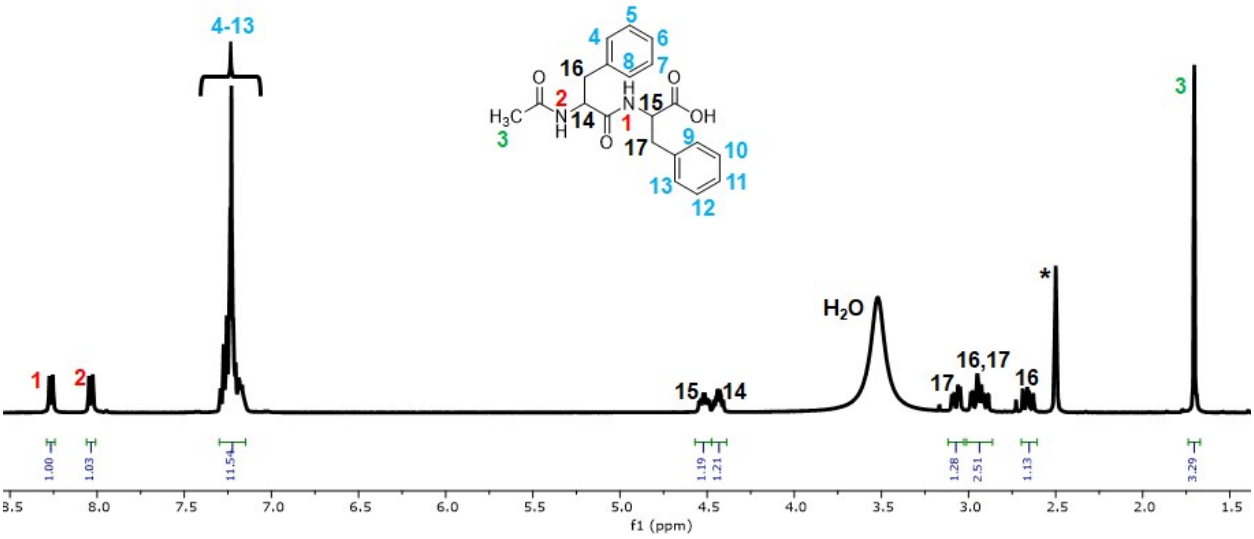


Figure S5. ^{1}H NMR (400 MHz, DMSO-d_6) of **Ac-FF**.

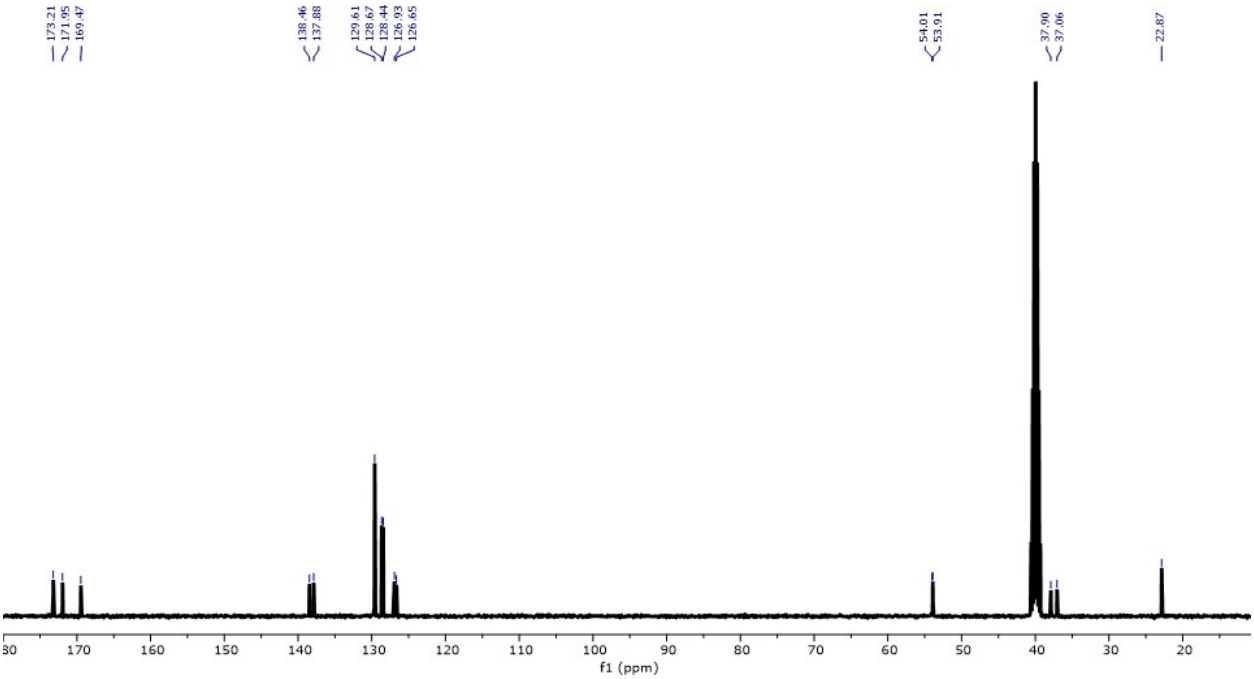


Figure S6. ^{13}C NMR (100 MHz, DMSO-d_6) of **Ac-FF**.

Additional Figures

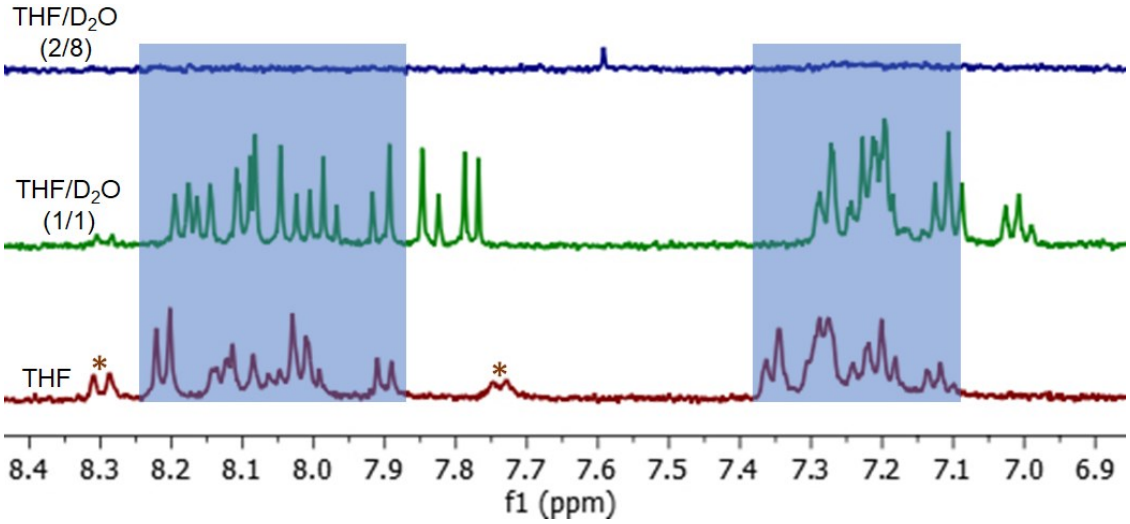


Figure S7. Solvent-dependent ^{1}H-NMR studies of **Py-FF**. Asterisks represent the NH protons which disappear upon addition of D_2O. [c = 1 x 10^{-3} M; T = 298K]

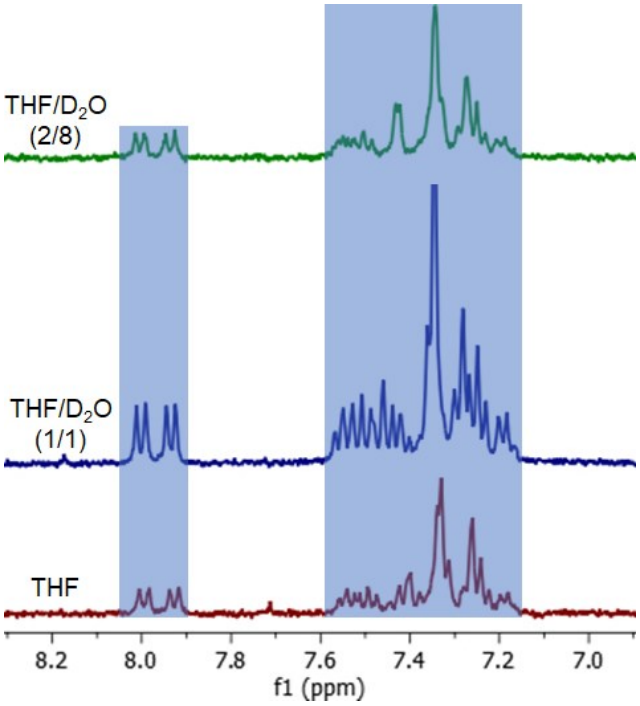


Figure S8. Solvent-dependent ^{1}H-NMR studies of **Nap-FF**. [c = 1 x 10^{-3} M; T = 298K]

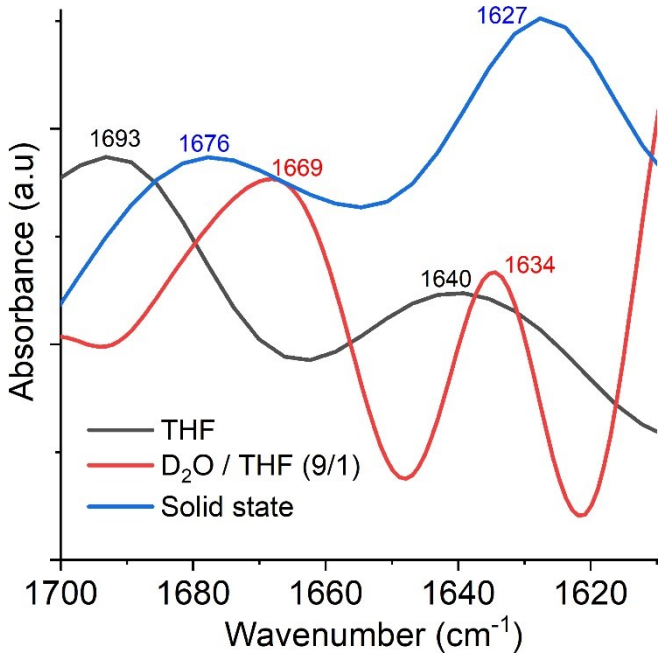


Figure S9. FT-IR spectrum of **Py-FF** of the amide I region suggesting the formation of β-sheet secondary structures. [c = 5 x 10^{-4} M, T = 298K]

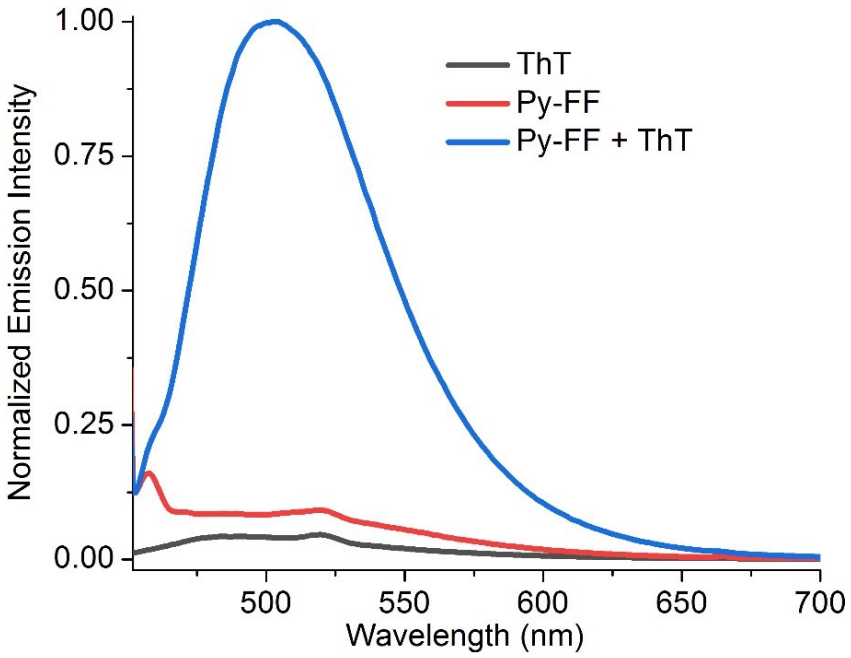


Figure S10. ThT fluorescence assay of **Py-FF** in H_2O/THF (9/1). ThT is a well-known amyloid-specific fluorescent dye that can bind specifically to multi-stranded β-sheets.[6] Upon excitation at 440 nm, the fluorescence intensity of the mixture of **Py-FF**+ThT is significantly enhanced compared to the individual components (**Py-FF** and ThT), which is in accordance with the formation of β-sheet structures. [c = 1 x 10^{-4} M, T = 298K]

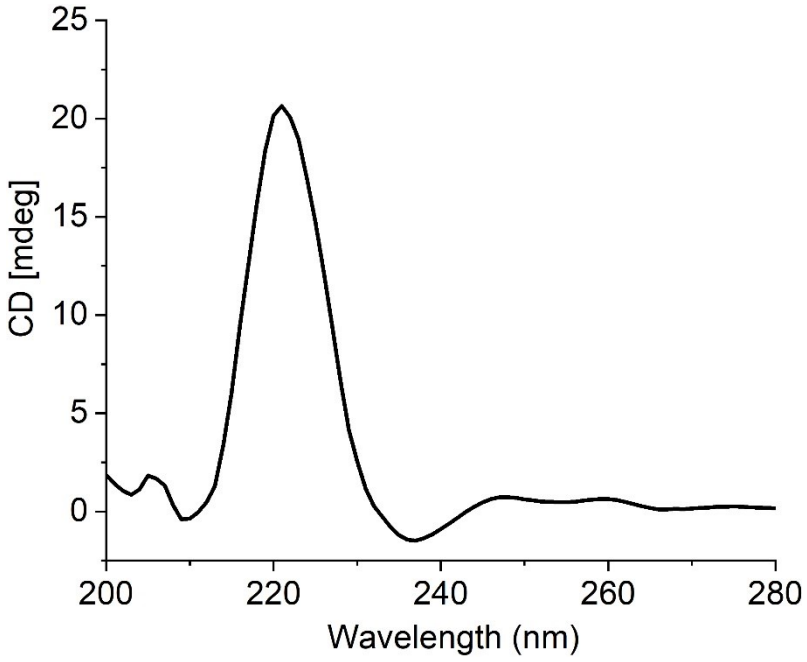


Figure S11. CD spectrum of **Ac-FF** in water. [c = 1 x 10^{-4} M, T = 298K]

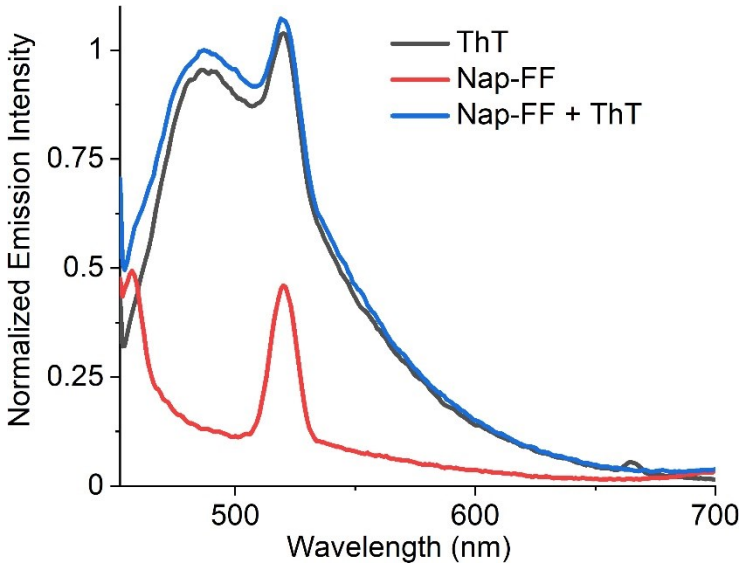


Figure S12. ThT fluorescence assay of **Nap-FF** in H_2O. No increase in emission intensity of ThT after mixing suggested that **Nap-FF** is unable to form β-sheet structure. [c = 1 x 10^{-4} M, T = 298K].

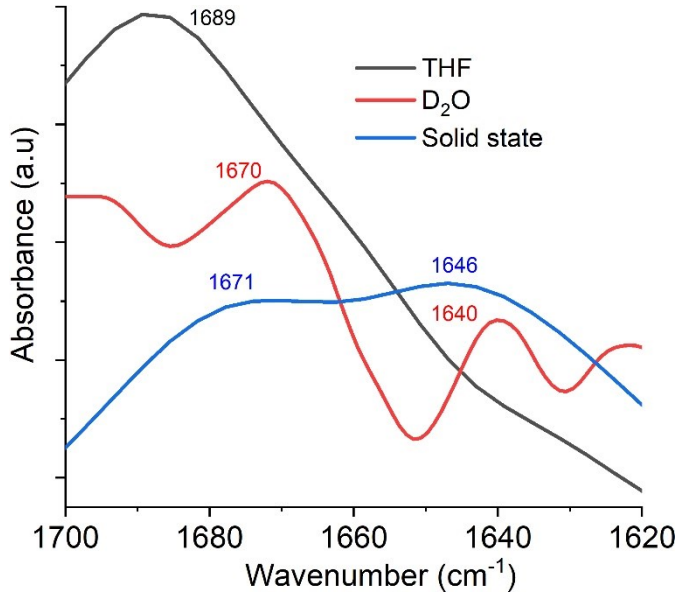


Figure S13. FT-IR spectrum of **Nap-FF** in the amide I region. [c = 5 x 10^{-4} M, T = 298K]

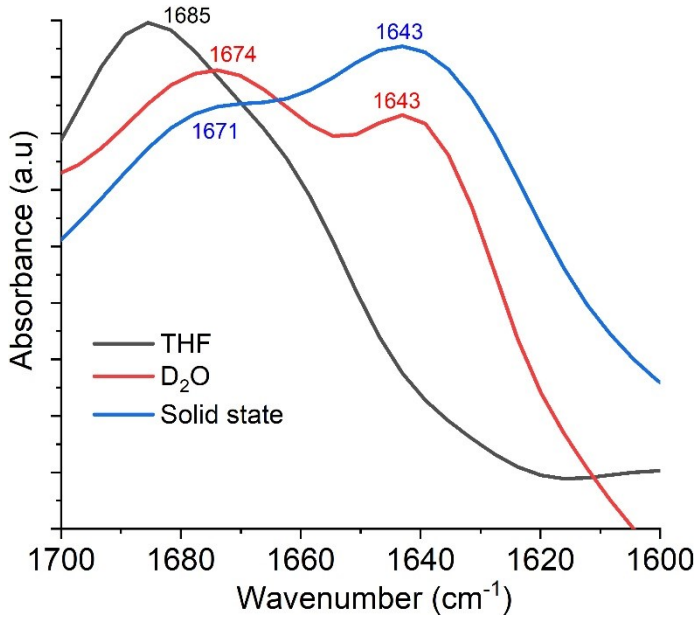


Figure S14. FT-IR spectrum of **Ac-FF** in the amide I region. [c = 5 x 10^{-4} M, T = 298K]

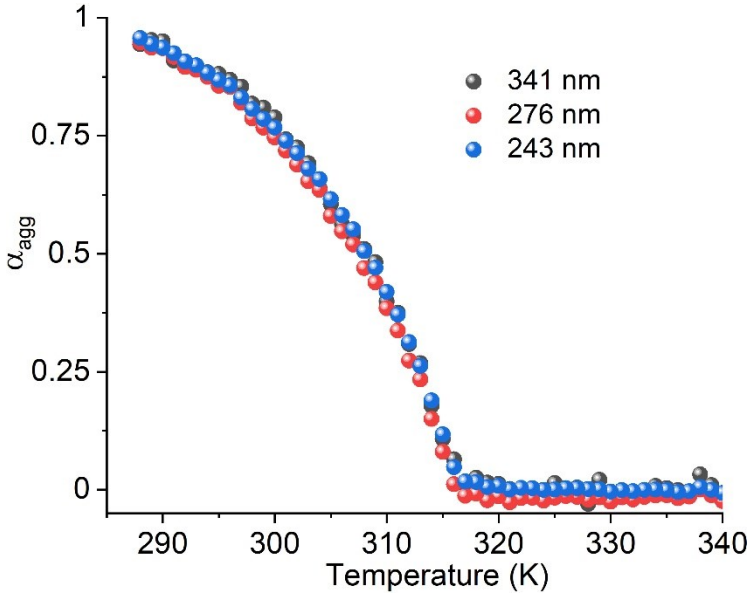


Figure S15. Cooling curves of **Py-FF** at different wavelengths obtained from UV-Vis spectroscopy.

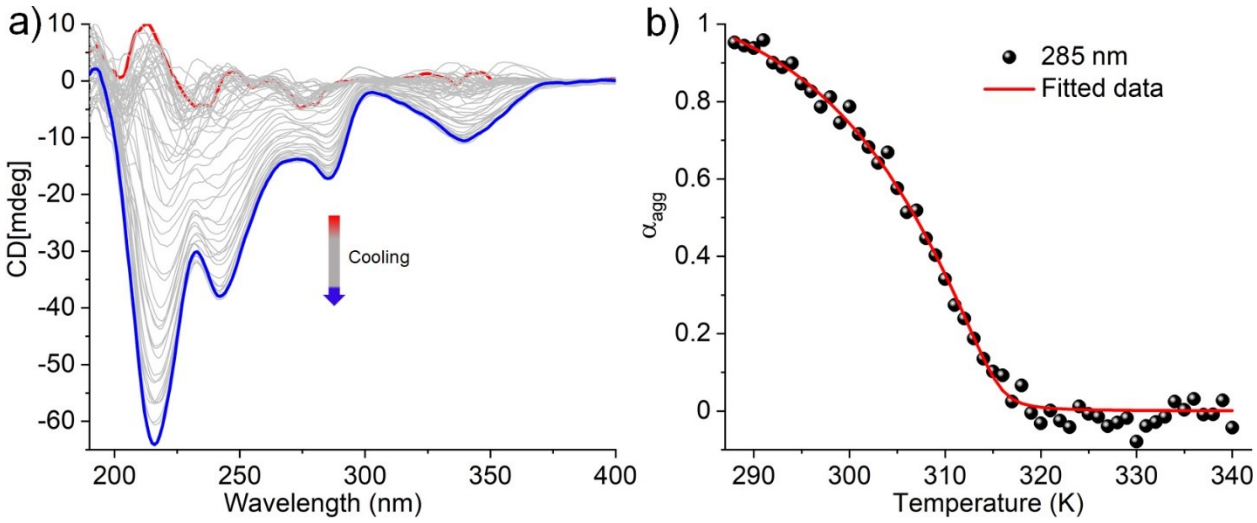


Figure S16. a) Temperature-dependent CD spectra of **Py-FF** (cooling rate = 1 K/min) and b) cooling curve monitored at 285 nm and fitted to the cooperative model. [c = 1 x 10^{-4} M]

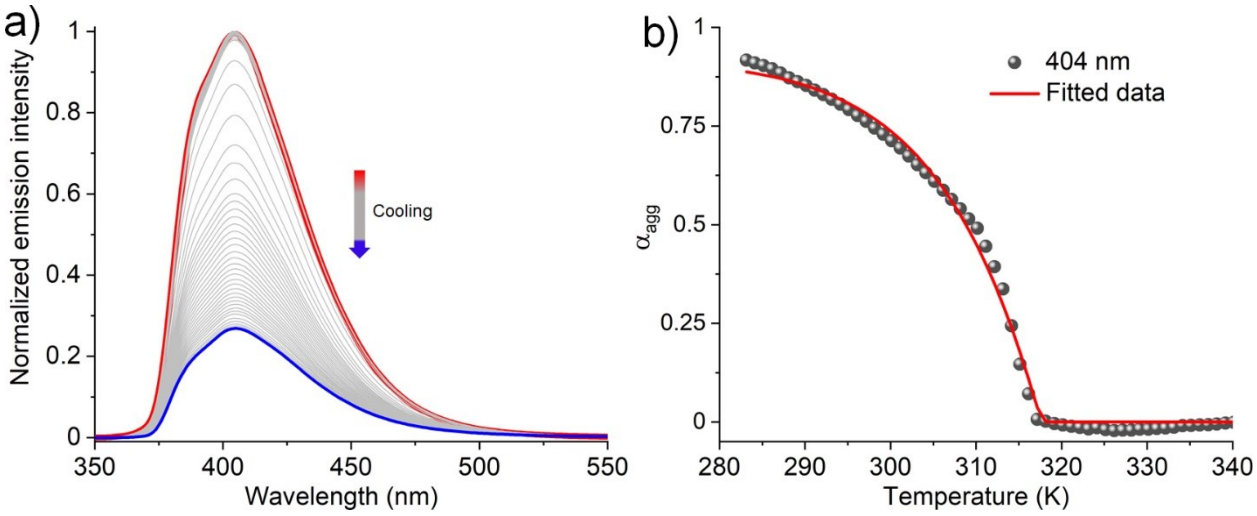


Figure S17. a) Temperature-dependent fluorescence spectra of **Py-FF** (cooling rate = 1 K/min) and b) cooling curve monitored at 404 nm and fitted to the cooperative model. [c = 1 x 10^{-4} M]

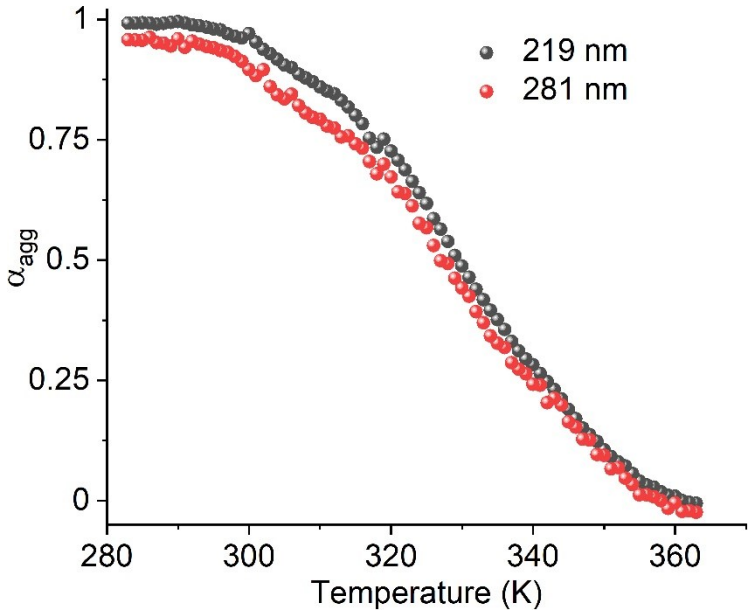


Figure S18. Cooling curves of **Nap-FF** at different wavelengths obtained from UV-Vis spectroscopy in H_2O. [c = 1 x 10^{-4} M]

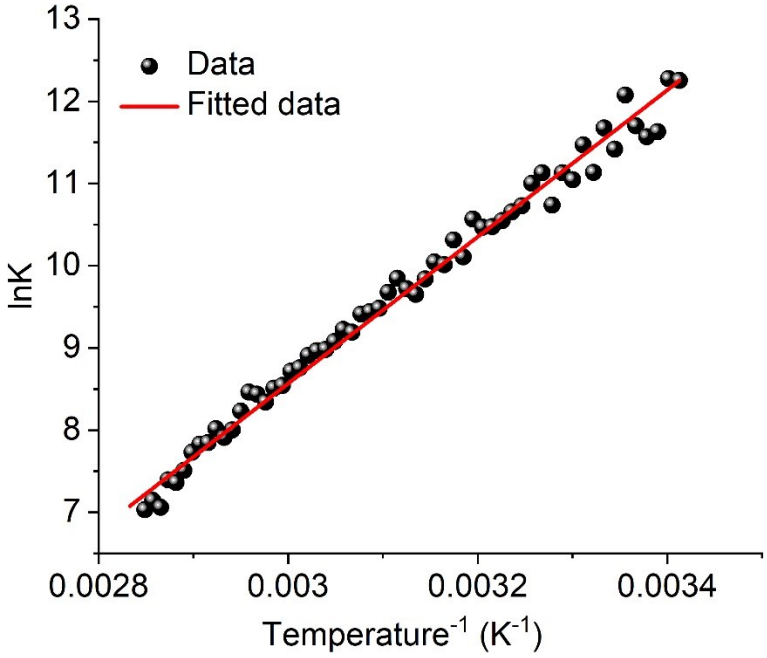


Figure S19. Van't Hoff plot derived from cooling experiment of **Nap-FF** in UV-Vis spectroscopy.

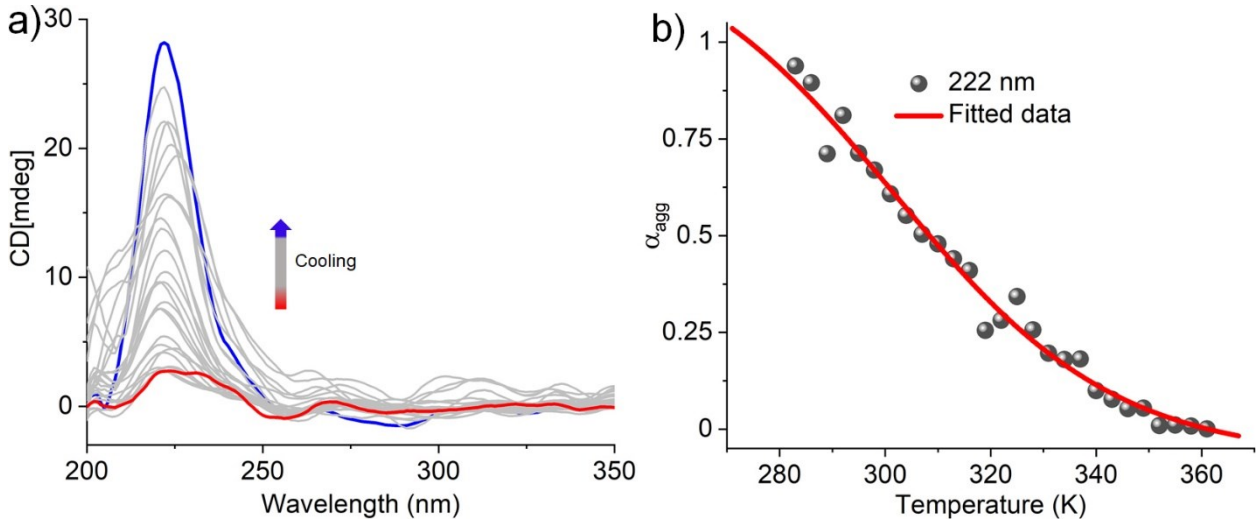


Figure S20. a) Temperature-dependent CD spectra of **Nap-FF** in H_2O (cooling rate = 1 K/min) and b) cooling curve monitored at 222 nm and fitted to the isodesmic model. [c = 1 x 10^{-4} M]

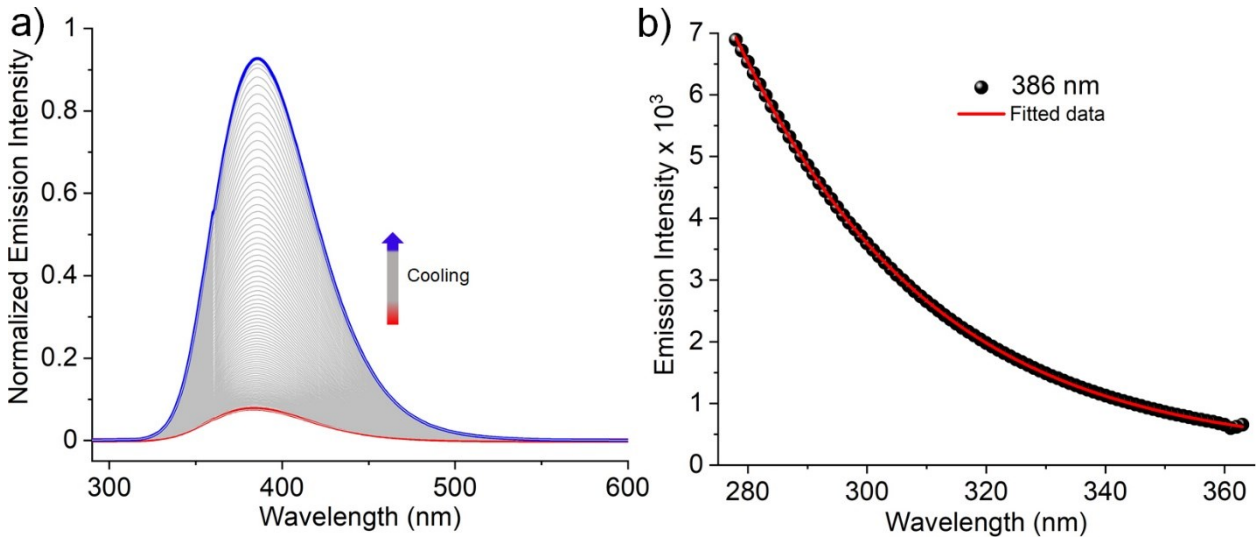


Figure S21. a) Temperature-dependent fluorescence spectra of **Nap-FF** in H_2O (cooling rate = 1 K/min) and b) cooling curve monitored at 386 nm and fitted to the isodesmic model. Although there is no such saturation at low temperature, the investigated region of the cooling curve is reproduced by the isodesmic model. [c = 1 x 10^{-4} M]

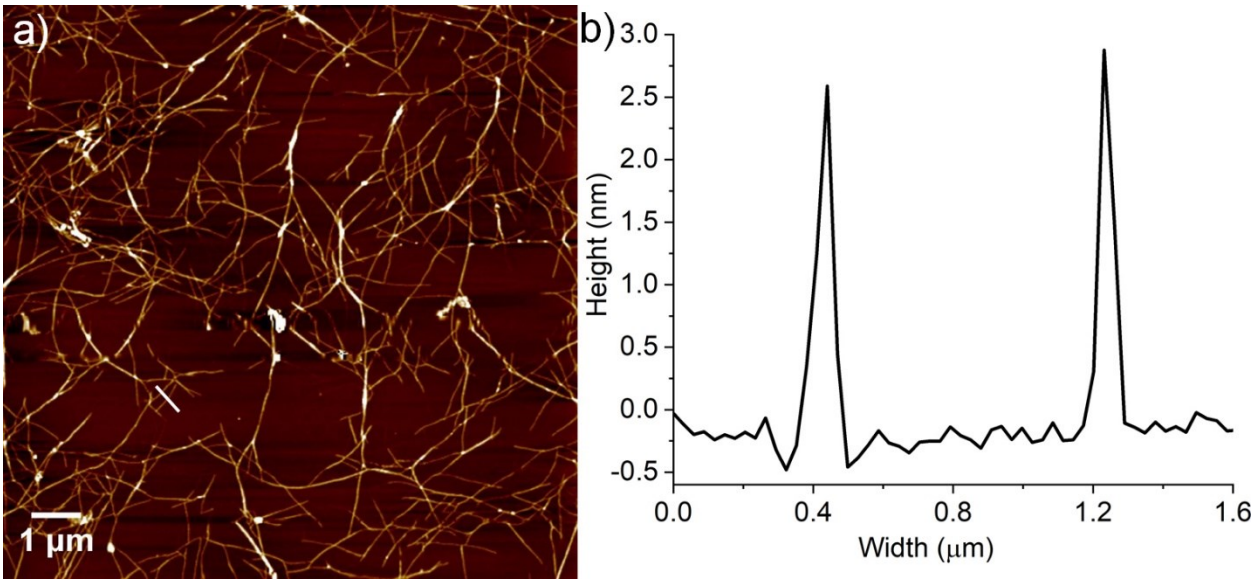


Figure S22. a) AFM image of **Py-FF** in H_2O/THF (9/1) captured after drop-casting the solution onto mica surface followed by spin coating at 500 rpm and b) corresponding height profile. [c = 1 x 10^{-4} M]

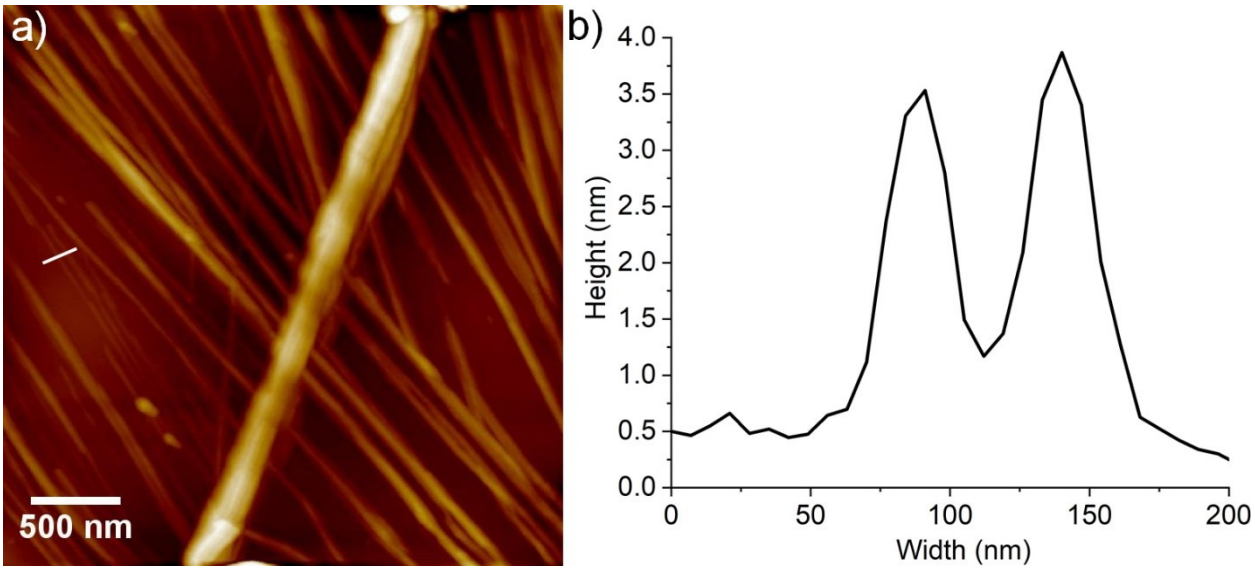


Figure S23. a) AFM image of **Nap-FF** in H_2O captured after drop-casting the solution onto mica surface followed by spin coating at 500 rpm and b) corresponding height profile. [c = 1 x 10^{-4} M]

References

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3 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.

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6. a) M. J. Groenning, *Chem. Biol.,* 2010, **3**, 1; b) R. Khurana, C. Coleman, C. Ionescu-Zanetti, S. A. Carter, V. Krishna, R. K. Grover, R. Roy and S. Singh, *J. Struct. Biol.,* 2005, **151**, 229.