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Tuning the mechanistic pathways of peptide self-assembly by aromatic interactions†

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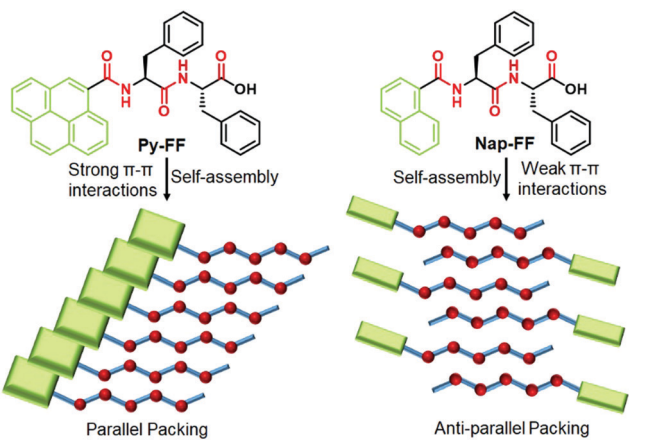
Herein, we have unravelled the key influence of aromatic interactions on the mechanistic pathways of peptide self-assembly by introducing suitable chromophores (pyrene *vs.* naphthalene). Although both self-assembled peptides are indistinguishable in their morphologies, this minor structural difference strongly affects the packing modes (parallel *vs.* antiparallel) and the corresponding self-assembly mechanism (cooperative *vs.* isodemsic).

Self-assembly is a ubiquitous phenomenon that regulates key processes and functions in living organisms.[1] Inspired by these sophisticated architectures and with the aim of addressing crucial issues in biology and materials science, a wide variety of artificial building blocks have been explored for constructing programmable self-assembled nanostructures.[2] In this regard, peptides are archetypal building blocks because of their biocompatibility, biodegradability and tunable self-assembly process and corresponding nanostructures.[3] Additionally, experimental variables such as pH, temperature, light, ionic strength, solvent interactions and salt concentration are known to strongly affect the aggregation pathways, mechanism and morphology of peptide assemblies.[4] More importantly, the self-assembly of peptides is largely dominated by the subtle interplay between different attractive non-covalent interactions (such as aromatic, van der Waals, hydrogen bonding (H-bonding) and electrostatic interactions) and repulsive interactions (such as electrostatic interactions among similar charges and steric effects).[5] Among all non-covalent interactions, aromatic interactions not only play a significant role in self-assembly and gelation processes of peptides, but they can also balance the hydrophobicity of the systems.[6] Recently, peptide amphiphiles bearing chromophores such as pyrene,[7a] naphthalene,[7b,c] anthracene,[7d] perylene diimide[7e] and naphthalene diimide[7f] have received considerable attention for the bottom-up construction of tunable luminescent nanomaterials. However, understanding the relationship between aromatic interactions and mechanistic pathways of peptide self-assembly remains elusive.[8]

To address this issue, we herein unravel the role of aromatic interactions on tuning the self-assembly mechanism of peptide assemblies in aqueous media. To demonstrate this approach, we have rationally designed a well-known dipeptide sequence "Phe-Phe" (**FF**)[9] and conjugated it with different π-chromophores such as pyrene (**Py-FF**) and naphthalene (**Nap-FF**) (Scheme 1). Furthermore, a control molecule without chromophore (**Ac-FF**) has also been prepared (Scheme S3 in ESI†). Detailed experimental studies of both peptides unveiled a dramatic change in their mechanistic pathways of supramolecular polymerization (SP) depending on the extent of aromatic interactions (cooperative SP for **Py-FF** *vs.* isodesmic SP for **Nap-FF**). Intriguingly, despite the different assembly mechanism, both molecules self-assemble into the same aggregate morphology (1D nanofibers), which is a rare phenomenon in self-assembly.[10]



Scheme 1 Chemical structures of **Py-FF** and **Nap-FF** (top) and schematic representation of their packing modes upon aqueous self-assembly (bottom).

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The comparative self-assembly studies of **Py-FF** and **Nap-FF** were investigated by different spectroscopic and microscopic techniques such as UV-vis, fluorescence, circular dichroism (CD), Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) and atomic force microscopy (AFM). The UV-vis spectra of **Py-FF** at 1×10^{-4} M and room temperature (RT) in a 'good' solvent such as tetrahydrofuran (THF) shows three sharp bands at 341, 276 and 243 nm (Fig. 1a), indicating a monomeric state.[11] On the other hand, a broad UV-vis spectrum with significant reduction of absorption is observed for **Py-FF** in aqueous media (H_2O/THF = 9/1), suggestive of a plausible self-assembly through strong π–π interactions (Fig. 1a). By contrast, the UV-vis spectra of **Nap-FF** in THF and H_2O (or in H_2O/THF = 9/1) at 1×10^{-4} M and RT exhibit no significant changes, suggesting weak aromatic interactions (Fig. 1e). Fluorescence studies of monomeric **Py-FF** in THF reveal two strong emission bands at 385 and 404 nm that are considerably reduced upon aggregation in H_2O/THF (9/1) (Fig. 1b), suggesting aggregation-caused quenching (ACQ) due to strong π-stacking.[12] This behaviour strongly differs for **Nap-FF** in H_2O, where an enhanced emission intensity was observed compared to THF (Fig. 1f). This behaviour may possibly result from aggregation-induced emission (AIE)[12] owing to weak aromatic interactions between the chromophores, which further corroborates the findings noticed by UV-vis spectroscopy. The significant differences in the strength of aromatic interactions for both peptides are further supported by the observation of up field shifts for the aromatic protons of **Py-FF** in solvent-dependent ^{1}H NMR studies (Fig. S7, ESI†), whereas no such shifts were noticed for **Nap-FF** (Fig. S8, ESI†). FT-IR spectra of both **Py-FF** and **Nap-FF** in THF reveal two sharp peaks at 3569 and 3511 cm^{-1}, corresponding to two different non-hydrogen bonded NH protons (Fig. 1c and g). Notably, these two bands merge into a single band at lower frequency (3410 cm^{-1}) in D_2O, demonstrating the involvement of strong H-bonding. The self-assembly of **Py-FF** and **Nap-FF** was also investigated by CD spectroscopy (Fig. 1d and h). While the solution of monomeric **Py-FF** in THF is nearly CD silent, strong negative CD signals at 340, 285, 242 and 216 nm were observed upon aggregation in H_2O/THF (9/1). The bands at 340, 285 and 242 nm correspond to absorption bands noticed in UV-vis spectroscopy, indicating strong exciton coupling among the pyrene chromophores. The additional intense negative band at 216 nm is suggestive of the formation of β-sheet rich secondary structures. This is further proven by the appearance of two intense peaks at 1634 and 1669 cm^{-1} in the amide I region in FT-IR studies (Fig. S9, ESI†).[13,14] Additionally, a thioflavin T (ThT) assay was also performed to probe β-sheet formation (Fig. S10, ESI†). On the other hand, the CD spectrum of **Nap-FF** in H_2O exhibits an intense single positive band at 222 nm, whereas only residual bands are observed in the region where the naphthalene dye absorbs (250–300 nm; Fig. 1h). These results suggest negligible exciton coupling of the naphthalene chromophores during aqueous self-assembly. The CD signal of **Nap-FF** originates from the **Ac-FF** motif, as a similar positive band at 221 nm arises for the latter in water (Fig. S11, ESI†), suggesting a similar type of orientation. As expected, the nearly CD silent spectrum observed for **Nap-FF** in THF indicates a lack of aggregation in this media (Fig. 1h). Moreover, the CD spectra and ThT assay (Fig. S12, ESI†) of **Nap-FF** rule out the possibility of β-sheet formation, which is further supported by the position of the amide I bands obtained in FT-IR spectra (Fig. S13, ESI†).[14,15] The FT-IR bands of **Ac-FF** (Fig. S14, ESI†) exhibit similar trends as those found for **Nap-FF**, further indicating a similar type of packing. Comparison of the CD spectra and ThT assay of **Py-FF** and **Nap-FF** discloses clear differences in the

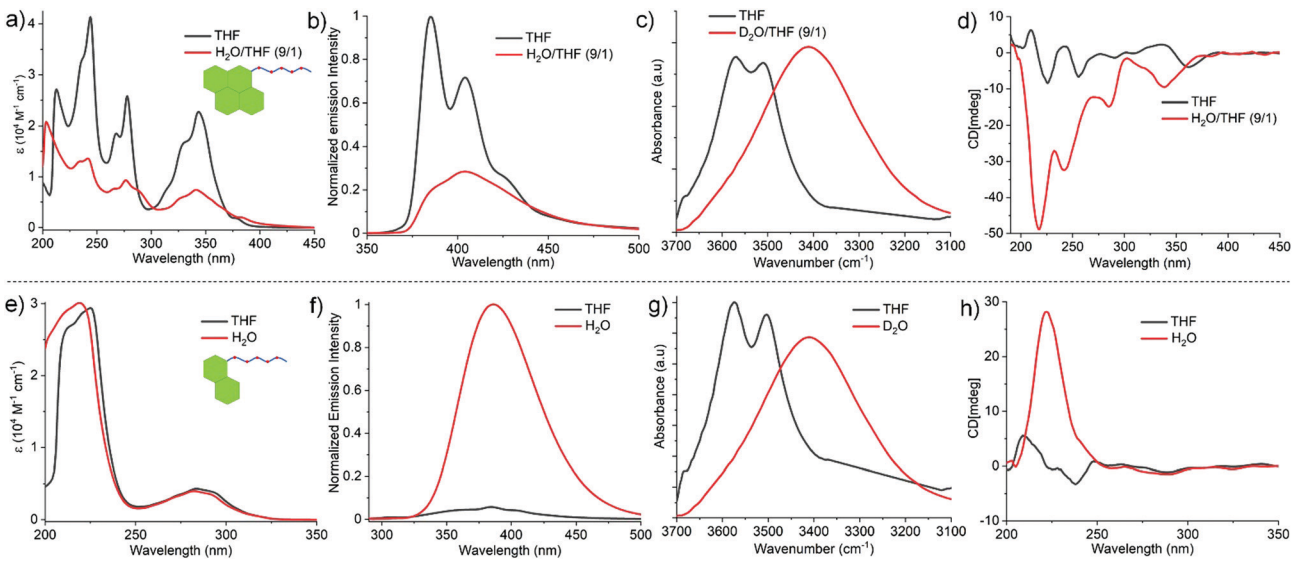

Fig. 1 Solvent-dependent UV-vis spectra of (a) **Py-FF** and (e) **Nap-FF** (the peak below 250 nm corresponds to n–π* band of amides of FF motif); fluorescence spectra of (b) **Py-FF** (excitation wavelength = 340 nm) and (f) **Nap-FF** (excitation wavelength = 290 nm); FTIR spectra of (c) **Py-FF** and (g) **Nap-FF**; CD spectra of (d) **Py-FF** and (h) **Nap-FF** [$C = 1 \times 10^{-4}$ M; T = 298 K].

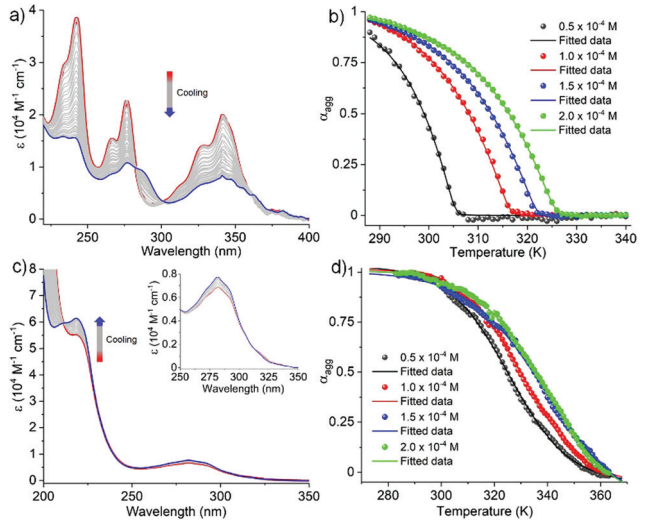

Fig. 2 (a) VT UV-vis spectra of **Py-FF** in H_2O/THF (9/1); (b) cooling curves of **Py-FF** monitored at 243 nm and fitted to the cooperative model; (c) VT UV-vis spectra of **Nap-FF** in H_2O (inset image corresponds to zoomed area of the absorption of naphthalene moiety); (d) cooling curves of **Nap-FF** monitored at 219 nm and fitted to the isodesmic model [cooling rate = 1 K min^{-1}; $C = 1 \times 10^{-4}$ M].

supramolecular organization for both systems,[16] which agrees well with the observations described earlier.

From these observations, we conclude that the self-assembly of **Py-FF** occurs by the synergistic effect of strong π–π interactions between the pyrene motifs and H-bonding involving the peptide groups *via* a parallel molecular organization (Scheme 1). On the other hand, the self-assembly of **Nap-FF** is largely stabilized by H-bonding between the peptide moieties, which have to arrange in a way that no effective aromatic interactions occur between the naphthalene moieties. The arrangement of the **Nap-FF** monomers in an antiparallel fashion, as shown in Scheme 1, might be one plausible possibility that accounts for the experimental observations.

In order to investigate the mechanistic pathways of self-assembly for both **Py-FF** and **Nap-FF** in aqueous media, variable temperature (VT) experiments were carried out by monitoring the UV-vis, fluorescence and CD spectral changes. For **Py-FF**, cooling the solution from 343 to 288 K at a rate of 1 K min^{-1} leads to similar spectral changes to those observed when comparing the UV-vis spectra in THF and aqueous solution (see Fig. 1a), indicating a transition from monomeric to aggregated state (Fig. 2a). The obtained non-sigmoidal cooling curves at different concentrations (Fig. 2b) clearly suggest a cooperative self-assembly pathway driven by synergistic aromatic and intermolecular H-bonding interactions. Global fitting of the cooling curves to the nucleation–elongation (cooperative) model[17] (Fig. 2b) yields the following parameters: $\Delta H^\circ = -64.45$ kJ mol^{-1}, $\Delta S^\circ = -127.31$ J mol^{-1} K^{-1}, $\Delta G^\circ = -26.49$ kJ mol^{-1} and degree of cooperativity (σ) = 4.5×10^{-5} (Table 1). The cooperative mechanism was also supported by the non-sigmoidal cooling curves extracted from VT-CD (Fig. S16, ESI†) and VT-fluorescence studies (Fig. S17, ESI†).

In contrast to **Py-FF**, VT-UV-vis cooling experiments for **Nap-FF** (from 363 to 283 K; 1 K min^{-1}) disclose clear sigmoidal curves at different concentrations (Fig. 2d), indicating an isodesmic supramolecular polymerization.[18] This switch in mechanism from cooperative (for **Py-FF**) to isodesmic (for **Nap-FF**) can be rationalized by the lack of synergistic non-covalent interactions for the latter, *i.e.* largely driven by intermolecular H-bonding.[19] Application of the isodesmic model to the experimental data allows the derivation of the corresponding thermodynamic parameters (Fig. S19, ESI†): $\Delta H = -74.19$ kJ mol^{-1}, $\Delta S = -151.31$ J mol^{-1} K^{-1}, $\Delta G^\circ = -29.1$ kJ mol^{-1} (Table 1). In analogy to **Py-FF**, the isodesmic mechanism for **Nap-FF** was further supported by VT-CD (Fig. S20, ESI†) and VT-fluorescence studies (Fig. S21, ESI†).

Interestingly, in stark contrast to most examples of supramolecular assemblies reported to date, the different assembly mechanism for **Py-FF** and **Nap-FF** is not accompanied by a significant change in aggregate morphology. Microscopic studies by AFM on mica disclose similar type of fiber-like 1D morphologies for both compounds. Whereas **Py-FF** self-assembles into 1D nanofibers (height 2.5 ± 0.3 nm and width 60-70 nm) with lengths of several micrometers (Fig. 3a and Fig. S22, ESI†), bundled 1D nanofibers (Fig. 3b; height 3.0 ± 0.5 nm and width 35 nm of single fiber, Fig. S23, ESI†) are formed by **Nap-FF**. The higher tendency of **Nap-FF** to bundle might be explained by the difficulty in shielding the hydrophobic naphthalene core from the polar solvent molecules in the proposed antiparallel arrangement (see Scheme 1), leading to a more pronounced lateral growth.

In summary, we have rationally designed two peptide amphiphiles with different chromophores (pyrene: **Py-FF** and naphthalene: **Nap-FF**) and studied their comparative aqueous self-assembly by various techniques. Interestingly, the different extent of aromatic interactions of the pyrene and naphthalene groups governs the packing modes (parallel for **Py-FF** *vs.* antiparallel for **Nap-FF**) and the resulting self-assembly mechanism (cooperative for **Py-FF** *vs.* isodesmic for **Nap-FF**). Despite these marked differences, both molecules form the same type of aggregate morphology (1D nanofibers), a phenomenon that is

Table 1 Thermodynamic parameters obtained from temperature-dependent UV-vis experiments of **Py-FF** and **Nap-FF**

Compound	ΔH° [kJ mol^{-1}]	ΔS° [J mol^{-1} K^{-1}]	ΔH°_{nucl} [kJ mol^{-1}]	T_e/K	ΔG° [kJ mol^{-1}]	K_{el}/M^{-1} [298 K]	K_{nucl}/M^{-1} [298 K]	σ
Py-FF	−64.45	−127.31	−24.81	316.1	−26.49	4.4×10^4	1.98	4.5×10^{-5}
	ΔH [kJ mol^{-1}]		ΔS [J mol^{-1} K^{-1}]		ΔG° [kJ mol^{-1}]		K_a/M^{-1} [298 K]	
Nap-FF	−74.19		−151.31		−29.10		1.7×10^5	

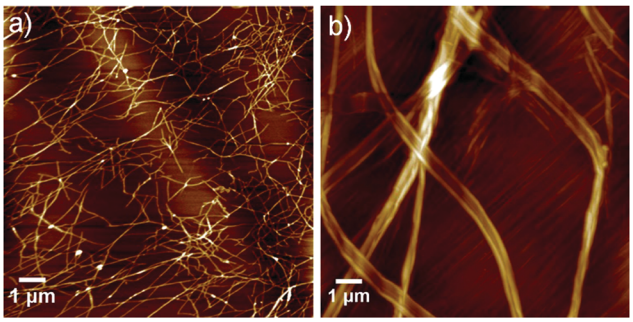

Fig. 3 AFM images of (a) **Py-FF** and (b) **Nap-FF** [$C = 1 \times 10^{-4}$ M].

rare in self-assembly. Our experimental findings highlight the importance of aromatic interactions in controlling the mechanism of peptide self-assembly and, consequently, the properties of the secondary structures, which may have important implications in the biomedical field.

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Conflicts of interest

There are no conflicts to declare.

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