Regulation of Peptide- π -Peptide Nanostructure Bundling: The Impact of “Cruciform” π -Electron Segments

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Abstract

The self-assembly and photophysical properties of peptide- π -peptide molecules with “cruciform” presentation of the π -electron core were studied. Derivatives of 1,4-bis(phenylethynyl)benzene were incorporated into a peptide backbone *via* the alkynylation of a central phenyl biscarboxamide core to yield peptide- π -peptide trimers wherein the π -conjugated unit was oblique to the peptide backbone. These molecules exhibited pH triggered self-assembly and yielded 1-D nanostructures with controlled diameters closely corresponding to their molecular lengths. We postulate that this size control is due to the oblique oligophenylyneethynylene (OPE) chromophores effectively blocking the hierarchical associations among the peptide stacks initially formed after self-assembly. Although the molecular design appeared to frustrate the higher order assembly, regardless of the peptide or OPE composition, significant variations in the photophysical properties were observed. The molecular design principles described here can be used to develop fundamental understanding of properties of self-assembled peptide based nanostructures of interest in biotechnological applications.

Keywords

Peptide- π -peptide conjugates, peptide self-assembly, 1-D nanostructures, nanostructure size control

1. Introduction

Short peptides that self-assemble into nanostructures are of enormous interest for biological, medical, and biotechnological applications. Peptide self-assembly is dictated by intramolecular as well as intermolecular hydrogen-bonding interactions among peptide chains, and the dihedral angles of the constituent amino acid residues that make up these chains. The differences in the nature of the aliphatic and aromatic residues in the amino acid substituents along the peptide backbone have a significant impact on the most favorable peptide conformations and on the subsequent higher-order interactions amongst adjacent peptide sheets, and hence result in vastly different nanomaterial morphologies.¹⁻⁷ A diverse set of assemblies having distinct secondary and tertiary structures has been designed by varying the peptide composition including vesicles from linear block co-peptides,^{8,9} porous cages from three-armed peptides,¹⁰ nano-belts from sequence-controlled short peptides,¹¹ nano-tubes from cyclic peptides¹² and nano-sheets from collagen mimic short peptides.¹³

Incorporation of semiconducting π -conjugated functionalities within peptide backbones enables organization of the π -conjugated subunits upon molecular self-assembly into 1-D nanomaterials.¹⁴⁻¹⁸ Being embedded in biologically compatible peptide backbones, peptide- π electron conjugated molecules become interesting candidates for bioelectronics applications. A variety of π -conjugated functionalities have been incorporated into short β -sheet forming peptide backbones to yield peptide- π -peptide ‘trimer’ materials which form 1-D nanostructures upon pH triggered self-assembly and exhibit interesting photophysical and electrical properties.¹⁷⁻²² The self-assembling properties of these peptide- π -peptide molecules can be directly influenced by the molecular composition of the peptide as well as the π -conjugated unit embedded within the peptide.

From a morphological perspective, the “linear” peptide- π -peptide molecules typically used for past studies, where the π -conjugation axis is coincident with the oligopeptide backbone, led to the formation of tape-like nanostructures as illustrated in Scheme 1a, where the amino acid residues project above and below the plane of the tape. These orthogonal residues are then responsible for driving higher order aggregation of the tape-like nanostructures into fibrillar networks, much like in standard amyloidogenic peptides. This hierarchical assembly has proven to be fairly uncontrollable, leading to nanomaterials with polydisperse diameter distributions.^{19,23} Seeking to learn how to minimize this hierarchical aggregation, we investigate here the effect of incorporating the π -conjugated chromophore oblique to the peptide backbone as a source of steric bulk to restrict the aggregation of peptide- π -peptide tape-like nanostructures into larger bundles (Scheme 1b). We hypothesized that such a “cruciform” design would frustrate the hierarchical associations among isolated peptidic assemblies and hence limit the higher order bundling effects. Cruciform molecules with cross-conjugated electronic conduits are interesting candidates for sensing and optoelectronic applications.²⁴⁻²⁷

Scheme 1. Schematic representation of peptide- π -peptide molecules with π -conjugated unit placed (a) along the axis of peptide backbone (linear) and (b) oblique to peptide backbone (cruciform).

Short oligophenyleneethynylene (OPE) derivatives were selected as the π -conjugated chromophores. These molecules are characterized by the efficient intramolecular electronic communication between the linearly conjugated phenyl rings, attributed in part to the cylindrical symmetry of the triple bond that maintains conjugation between these rings.²⁸ The self-assembling nature of OPEs has been evaluated extensively as these serve as sensitive reporters of their local molecular environment.²⁹⁻³⁶ This has led to these materials being utilized in sensing applications.^{27,37-39} In this manuscript, we describe the effect of incorporating differently substituted OPEs oblique to the peptide backbone on the self-assembling properties of the peptide- π -peptide molecules as well as the impact of varying the peptide sequence on the assembly and ensuing photophysical properties of the chromophore units. This design offers the ability to incorporate optoelectronic function within peptide nanomaterials while at the same time allowing for a finer degree of nanostructural control by helping to arrest the hierarchical fibrillation process often associated with amyloidogenic peptides.

2. Materials and Methods

We have previously demonstrated that incorporation of the 1,4-bis(phenylethynyl)benzene unit into a short peptide by way of flanking oligopeptide sequences like Asp-Phe-Ala-Gly (DFAG) results in a peptide- π -peptide hybrid molecule (DFAG-OPE-GAFD, Chart 1).²² We have found this tetrapeptide sequence to be sufficient to enable the supramolecular associations with a variety of embedded π -electron units. The photophysics of the supramolecular assemblies of this peptide- π -peptide hybrid molecule suggest the presence of typical *H*-like aggregation among the OPE units as characterized by a blue-shifted absorbance λ_{max} and a quenched photoluminescence intensity as compared to the unassembled, molecularly dissolved OPE peptide. In the present work, we explore a new orientation of the OPE unit with

respect to the peptide backbone realized by connecting the OPE unit through the central phenyl ring embedded within the peptide backbone as opposed to attaching the peptide to the terminal phenyl rings of the OPE chromophore, as in the earlier case. This should render the OPE unit oblique to the peptide backbone. The terminal phenyl rings were further substituted with methoxy groups at the 4- position (peptide **2**) and at the 3,4,5- positions (peptide **3**) as shown in Chart 1. The different methoxyphenyl groups were introduced to vary the propensity of π -stacking in the molecules. The peptide backbone was maintained the same as used in the previous work (DFAG).

DFAG-OPE-GAFD

- 1, $R^1, R^2, R^3 = -H$
 2, $R^1, R^3 = -H, R^2 = -OCH_3$
 3, $R^1, R^2, R^3 = -OCH_3$

Chart 1. Molecular structures of peptides DFAG-OPE-GAFD and **1-3**

To evaluate the effect of the peptide sequence on self-assembly properties of the cruciform peptide- π -peptide molecules, the peptide sequence was varied, as shown in Chart 2, while maintaining the OPE unit constant. Sequences with different amino acid residue presentation can encourage different degrees of interactions among the individual stacks that form after peptide assembly. For instance, glycine residues, the smallest among natural amino

acids, are known to induce flexibility within peptide chains,⁴⁰ while alanine, the next larger natural amino acid, is a weak β -sheet former with a higher preference for α -helices in solution.⁴¹⁻
⁴³ Amino acids with progressively larger residues, such as valine, isoleucine, and phenylalanine favor β -sheet formation, with valine having the strongest preference in forming such tape-like β -sheet rich motifs.^{42,43} Variation in the primary peptide sequence can therefore lead to variation in the hierarchical complexity of the 1-D supramolecular morphologies (stacked ribbons, tapes, or fibrils) much like other amyloid-like oligopeptides.^{40,44} Starting from the short peptide sequence, DFAG, subtle single amino acid substitutions at the internal locations, closest to the chromophore unit, along the peptide sequences were made. These substitutions were selected based on their relative size and hydrophobic nature, which could impact the intermolecular interactions and influence hierarchical fibrillization processes. Specifically, all four possible combinations of positioning the amino acids alanine and glycine, namely DFAG (**3**), DFGG (**4**), DFAA (**5**) and DFGA (**6**) were synthesized. The library of peptides thus generated was then evaluated to understand the self-assembly properties as well as the photophysical properties of the OPE units when embedded in the peptide backbone of these peptide- π -peptide molecules.

- 4, R¹, R² = -H
 5, R¹, R² = -CH₃
 6, R¹ = -H, R² = -CH₃

Chart 2. Structure of peptides **4-6**

The self-assembling peptides were obtained by first synthesizing the peptide sequence using standard solid phase peptide synthesis protocols starting with Wang resin loaded with Asp(OtBu)-NH-Fmoc which was alternately deprotected (using 20% (v/v) solution of piperidine in DMF) and coupled with the appropriate amino acid (using HBTU as the coupling reagent and DIPEA as the base). After completion of the required oligopeptide sequence, the terminal free amine was subjected to a double *N*-acylation protocol using 2,5-dibromoterephthalic acid and PyBOP as the coupling agent leading to “on-resin” dimerization of the peptide. The bromo-functionalized peptide thus obtained was then reacted, while still on the resin support, with the appropriate terminal alkyne using Sonogashira coupling conditions²² to yield the desired peptide- π -peptide trimer which was then cleaved off the solid support and purified using reverse phase HPLC. The peptide- π -peptide molecules were identified using ESI-MS and ¹H NMR spectroscopy.

3. Results and Discussion

The cruciform peptide- π -peptide molecules were designed to undergo a pH triggered self-assembly process, similar to those in our prior studies. Under alkaline conditions, the aspartic acid residues are deprotonated. The repulsive forces among like charges on the peptide backbones inhibit self-assembly and the peptide molecules remain in what we consider to be a “molecularly dissolved” state. Under acidic conditions, on the other hand, the aspartic acid residues are protonated leading to self-assembly of the peptide facilitated by the formal screening of the negative charges. The self-assembly process leads to the formation of intermolecular hydrogen-bonding among the amides (eg. β -sheets) and forces the π -electron systems into closer proximity, which helps intermolecular electronic delocalization (Scheme 1). The morphology of the nanostructures that form from the peptide- π -peptide self-assembly process was studied using

transmission electron microscopy (TEM) and the photophysics of the embedded chromophore units were evaluated using UV-vis absorption, photoluminescence (PL) and circular dichroism (CD) spectroscopies. These studies were carried out using self-assembled nanostructures generated in aqueous media where the pH was adjusted to 10 using 1M potassium hydroxide (KOH) solution or to 1 using 1M hydrochloric acid (HCl).

3.1 Effect of varying chromophore

3.1.1 Nanostructure morphology

Low level energy minimization calculations on these peptide- π -peptide molecules illustrate the formation of 1-D nanostructures with the terminal phenyl rings on the chromophore unit lying above and below the plane of the 1-D peptide stack. The formation of such stacks due to self-assembly of five molecules of peptides 1 and 3 is shown in Figure S1. Such assembly may lead to formation of nanostructures that are constrained from ‘*bundling*’ due to steric hindrance from the terminal phenyl rings preventing close inter-stack contacts. While the internal hydrogen-bonding networks within these stacks appeared to deviate from ideal β -sheet architectures, they still allowed for enthalpic stabilization of the assembly. Previous molecular dynamics simulations of peptide- π -peptide molecules found that the deviations from ideal β -sheet conformations can be attributed to entropic mixing within each assembled stack as well as to internal deformations caused by a combination of various stabilizing hydrogen-bonding interactions.²⁰ Also, the quadrupole associated with the central π -conjugated unit presents a distinctly non-natural intermolecular interaction deviating these peptide assemblies from ideal protein secondary structures.

The nanostructure morphologies of the peptide assemblies were observed using TEM imaging. A solution of the peptide (1 mg/mL) was acidified using 1M HCl solution to generate a

suspension of 1-D nanostructures in aqueous media. A formvar coated copper grid was then floated on a drop of this solution to adsorb the assemblies onto the grid, followed by staining using 2% uranyl acetate and air drying.

Figure 1. TEM images of (a) peptide **1**, (b) peptide **2** and (c) peptide **3**. Scale bar is 200 nm.

As shown in Figure 1, peptides **1-3** form 1-D nanostructures with controlled, uniform diameters and variable nanostructure lengths. The average nanostructure diameter calculated from at least 20 measurements was found to be 4.2 ± 0.3 nm in the case of peptide **1**, 4.7 ± 0.2 nm in the case of peptide **2** and 4.0 ± 0.3 nm in the case of peptide **3**. The molecular lengths of these peptides were calculated to be 3.06 nm, 3.08 nm and 3.19 nm for peptides **1-3**, respectively, using low level energy minimization calculations. This suggests that the nanostructures obtained from these peptides are predominantly comprised of isolated 1-D stacks of the peptide- π -peptide molecules, in agreement with the model (Scheme 1b). These stacks may resemble twisted tape-like stacks of individual molecules which have limited interactions among adjacent stacks due to the steric bulk of the oblique OPE units which project the terminal phenyl rings above and below the average peptide plane. This is in contrast to our previous work on “linear” peptide- π -peptide molecules that shows significant bundling in some cases leading to nanostructures with diameters ranging two to three times larger than expected based on the molecular length considerations.^{19,23}

While no significant variation in the morphology was observed based on the variation in the functionalization of the terminal phenyl groups, the observed diameters varied slightly. The deviations in absolute values of the observed diameters from the molecular dimensions may be attributed to effects of staining as well as possibility of some tertiary interactions among the stacks. Peptide **3**, while having the longest molecular length exhibits the smallest nanostructure diameters suggesting the greatest degree of segregation of individual 1-D stacks, likely arising from the steric bulk attributed to the three methoxy functionalities on the terminal phenyl rings.

3.1.2 Photophysical Properties

The photophysical properties of the peptide- π -peptide molecules were studied by recording UV-Vis absorbance spectra and photoluminescence (PL) spectra in aqueous media. As shown in Figure 2, in the case of the molecularly dissolved state at pH 10, the peptides exhibit a broad peak centered between 370-383 nm in the UV-vis absorption spectra (Table S1) and a broad PL peak centered around 506 nm for peptides **1** and **3** and at 535 nm for peptide **2**. The relatively large Stokes shift may be a result of the planarization of the OPE unit due to a combination of effects of intermolecular interactions and from the predominantly planar excited state structure of the chromophore as has been reported previously in the case of OPE molecules.⁴⁵⁻⁴⁷ Upon acidification using 1M HCl to pH 1, the absorption peak intensity decreased and red shifted slightly (by 7 nm in the case of peptides **2** and **3**, and 12 nm in the case of peptide **1**). This may indicate increased π - π interaction among chromophore molecules upon aggregation. Interestingly, all of these peptides show enhancement in photoluminescence intensity upon acidification. While the peak position remained unchanged in the case of peptide **1**, a red shift of 8 nm and 52 nm was observed in the case of peptides **2** and **3**, respectively. Poly(phenyleneethynylene)s have been previously shown to exhibit a red shift in the absorption

and emission spectra accompanied by enhanced fluorescence efficiency upon aggregation.⁴⁸ This has been attributed to kinetically controlled aggregate formation which may not show an organized structure.⁴⁹ In the case of peptides **1-3**, the aggregates may contain random molecular orientations arising from the rotational dynamics of the central phenyl ring about the peptide axis as well as due to the oblique arrangements of the OPE transition dipoles as shown in Scheme 2.

Figure 2. UV-vis (solid line) and photoluminescence (dashed line) spectra of (a) peptide **1** (λ_{ex} = 371 nm (pH 10); 383 nm (pH 1)), (b) peptide **2** (λ_{ex} = 383 nm (pH 10); 390 nm (pH 1)) and (c) peptide **3** (λ_{ex} = 382 nm (pH 10); 390 nm (pH 1))

Scheme 2. Representation of possible chromophore orientations within an isolated peptide nanostructure

Circular dichroism (CD) spectra were recorded for each of these peptides in both molecularly dissolved and assembled states (Figure 3). Exciton-coupled chromophores in chiral local environments are expected to show bisignate Cotton effects in CD spectra corresponding to the coupled π - π^* (or other relevant) transition dipoles. Such features are generally observed in

the spectral region of chromophore absorption with the cross-over near the absorption maximum of the chromophore. The signal arising from hydrogen-bonding interactions in the peptide backbone within a self-assembled nanostructure is observed around 190-220 nm, in the case of conventional β -sheets. These signals therefore, offer glimpses into the changes in the internal structure of the assemblies induced through variation in the chromophore unit or amino acid substitutions. However, it must be noted that signals originating from the amide bonds within peptide aggregates do not always provide definitive structural information.^{20,50,51}

Figure 3. CD spectra of (a) peptide **1**, (b) peptide **2** and (c) peptide **3**

As shown in Figure 3, while the molecularly dissolved peptides do not show any significant spectral features, the acidified solutions containing assembled peptides exhibit a strong minima at 232 nm (peptide **1**), 243 nm (peptide **2**) and 239 nm (peptide **3**). In the case of peptides **1** and **2**, no other prominent feature was observed in the scanned region between 190 - 550 nm. Spectral signatures present in the 350 – 400 nm window indicate the presence of some chirality possibly due to averaging over the entire ensemble population being analyzed, which may contain a combination of both possible handedness values leading to only a weak net signal being generated. In the case of peptide **3**, however, several bisignate features were observed with cross-overs at 387 nm, 352 nm, 263 nm and 252 nm with a local maxima at 258 nm. This suggests that the chromophore in this case may be held in a more uniform chiral environment,

possibly contributed to by the bulk of the three substituent groups on the terminal phenyl rings, but the exact nature of the exciton coupling is not clear.

FT-IR spectra of assembled peptides **1-3** support the formation of hydrogen-bonded nanostructures, as seen from the presence of amide I band ($1630\text{-}1640\text{ cm}^{-1}$) and amide II band ($1530\text{-}1550\text{ cm}^{-1}$) in the spectra as shown in Figure S2. These indicate that the nanostructures likely resemble β -sheet like structures.

3.2 Effect of varying peptide sequence

3.2.1 Nanostructure morphology

Figure 4. TEM images of (a) peptide **4**, (b) peptide **5** and (c) peptide **6**. Scale bar is 200 nm.

In order to vary the composition of peptide backbones close to the OPE chromophore, which may impact its rotational freedom, the amino acid sequence was varied from DFAG (peptide **3**) to DFGG (peptide **4**), DFAA (peptide **5**) and DFGA (peptide **6**). These permutations represent the simplest structural variations that may alter the self-assembly process. As shown in Figure 4, pH triggered self-assembly of the peptide- π -peptide molecules led to generation of 1-D nanostructures in all cases with surprisingly uniform diameters. The average nanostructure diameter calculated from at least 20 measurements was found to be $3.9 \pm 0.2\text{ nm}$ in the case of peptide **4**, $4.0 \pm 0.3\text{ nm}$ in the case of peptide **5** and $3.8 \pm 0.3\text{ nm}$ in the case of peptide **6**. This suggests that, as in peptides **1-3**, the nanostructures obtained from these peptides are predominantly comprised of isolated 1-D stacks of the peptide- π -peptide molecules, likely

resembling twisted tape-like geometry. In cases when the amino acid closest to the chromophore unit was alanine, bundling of nanostructures was observed but individual structures could be visualized within these bundles. Such structures were not observed when the flexible glycine residue was the closest to the chromophore, which yielded separated 1-D stacks intertwined into a random mesh like structure. This suggests that the increased interactions arising from alanine residues play a significant role in the assembly process, increasing the tendency for higher order interactions among individual stacks. While it is difficult to make a correlation between the molecular structure, down to a single residue, and macroscopic assembly structures, this observation is in agreement with the larger understanding of sequence dependence of supramolecular properties.⁵²⁻⁵⁴ For instance, variation of the amino acid adjacent to a terthiophene unit within a peptide amphiphile has been previously demonstrated to result in nanostructure morphologies ranging from flat spicules to nanotubes to flat sheets.⁵⁵ Remarkably, in the present case, minor perturbations still maintain the isolated 1-D nanostructure morphology without hierarchically complex geometrical changes being observed. It should be noted that, while the diameters of the nanostructures were fairly uniform, the variation in nanomaterial lengths was quite large. This remains an as-yet unmet supramolecular challenge.

3.2.2 Photophysical Properties

As shown in Figure 5, in the case of the molecularly dissolved state at pH 10, peptides **4-6** exhibit a broad peak centered at *ca.* 370 nm in absorption spectra, similar to peptide **3** (Figure 2c). However, the PL spectra vary significantly in peak position, with peptide **4** showing emission centered at 448 nm while peptides **5** and **6**, similar to peptide **3**, had PL spectra centered at 500 nm and 508 nm, respectively. As was noted in the case of peptides **1-3**, the red

shifted PL spectra may arise from planarization of the OPE chromophore in the excited state as well as some intermolecular interactions among the chromophore units.⁴⁵⁻⁴⁷

Figure 5. UV-vis (solid line) and photoluminescence (dashed line) spectra of (a) peptide **4** (λ_{ex} = 370 nm (pH 10); 374 nm (pH 1)), (b) peptide **5** (λ_{ex} = 370 nm (pH 10); 380 nm (pH 1)) and (c) peptide **6** (λ_{ex} = 370 nm (pH 10); 380 nm (pH 1))

The absorption spectra were red shifted by 5 – 10 nm upon pH triggered self-assembly at pH 1. PL behavior of the peptides appears to be dependent on the peptide sequence. In the case of peptide **4**, as with peptide **3**, the PL intensity increased about two-fold and red-shifted by 110 nm from 448 nm to 558 nm (Table S1). On the other hand, the PL intensity of peptides **5** and **6** was quenched significantly upon acidification. No significant shift in peak position accompanied these changes in intensity profiles. This suggests that the amino acid closest to the chromophore unit and the flexibility that it affords to the chromophore unit during self-assembly play critical roles in the photophysics of the nanomaterial ensembles. When the amino acid closest to the OPE unit was alanine (peptides **5** and **6**), its rotational freedom may be restricted leading to a more organized orientation in the assembly (Scheme 2) which exhibits PL quenching as is typically observed for OPE aggregates.³⁵⁻³⁸ Whereas, when glycine is closest to the OPE unit (peptides **3** and **4**), aggregates with random chromophore orientation may be formed leading to enhancement in the PL intensity.^{48,49} The higher order interactions among adjacent peptide stacks as seen from the TEM images also suggest increased aggregation of the OPE units, which may lead to the observed PL behavior. It must be noted, however, that the two measurements may not

be completely correlatable since the TEM images represent partially dry samples while the PL measurements were recorded for peptide assemblies suspended in solution. Advanced molecular dynamics simulations will be required to understand the effect of amino acid sequence on the chromophore orientation and subsequent electronic couplings within the supramolecular ensembles will need to be calculated to elucidate the observed behaviors.

Similar observations can be made from the CD spectra, as shown in Figure 6. The peptides **4-6** do not show any spectral features while in the molecularly dissolved state at pH 10. Upon assembly, a bisignate Cotton effect can be observed in all cases. However, the intensity and handedness of the assemblies observed in the CD spectra varied dramatically, suggesting that the local chiral environment of the chromophore varied significantly as the amino acid sequence was varied. Also, the position of the cross-over point varied from 342 nm in peptide **4**, 360 nm in peptide **5** to 385 nm in peptide **6**. This may reflect differences in the intermolecular interactions among the chromophore units within ensembles of each of the peptides. Higher energy peaks were observed at 235 nm, 212 nm and 203 nm in peptide **5** and 250 nm, 212 nm and 203 nm in peptide **6**. Also, as was observed in peptide **3**, multiple cross-over points were observed in the case of peptide **4** at 280 nm and 224 nm with a maxima at 208 nm. Peaks with inverted intensity in the amide region have been observed previously in natural systems and in short oligopeptides with aromatic residues and have been attributed to aromatic side-chain stacking within peptide nanotubes or β -turn conformations.^{56,57}

Figure 6. CD spectra of peptide **4-6**

4. Conclusions

This study elucidates the effect of primary amino acid sequence as well as the structure and relative orientations of chromophore and peptide on self-assembly of peptide- π -peptide molecules. Derivatives of 1,4-bis(phenylethynyl)benzene were incorporated oblique to the peptide backbone *via* a centrally embedded phenyl ring in order to frustrate the higher order interactions among peptidic stacks formed by hydrogen-bond driven self-assembly, with the desire to control the size distribution of the self-assembled 1-D nanostructures. Nanostructures with average diameters close to the molecular dimensions of these peptide- π -peptide molecules were obtained upon pH triggered self-assembly. The molecular design principle of inclusion of bulky groups above and below the plane of peptide organization may have led to hindrance of formation of higher, hierarchically complex nanostructures, allowing for the observation of individual isolated nanostructures. The unusual contributions of the embedded quadrupole also impacted the conformations of the peptide chains and their intermolecular hydrogen-bond organization. The kinetically trapped internal structures that are anticipated to form during the assembly process would provide significant degrees of disorder resulting in a range of photophysical behaviors observed in peptides **1-6**. Local environments experienced by the chromophores impact their association in the supramolecular assemblies which is reflected in,

for instance, the observation of aggregation induced PL enhancement in the case of peptides **1-4** and PL quenching in peptide **5** and **6**. The design principle developed here provides a roadmap for generation of size controlled 1-D nanostructures and can help in understanding the behavior of individual twisted tape-like nanostructures by restricting their aggregation into higher order assemblies. This will provide fundamental understanding of interactions of π -conjugated subunits embedded in peptide backbones, without interference from inter-structure π -electron communication, which is of prime importance in bioelectronics applications.

Acknowledgments

We thank Johns Hopkins University, and the Department of Energy Office of the Basic Energy Sciences (DE-SC0004857, peptide heterostructure synthesis) for generous support. We also wish to thank Prof. Gary Posner for his collegiality and several contributions to organic chemistry, and we congratulate him on the occasion of his retirement.

Supplementary Information

Supplementary data including synthetic procedures and characterization details (^1H NMR, ESI-MS and HPLC) related to this article can be found at

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