Supramolecular Polymorphism: Tunable Electronic Interactions within π-Conjugated Peptide Nanostructures Dictated by Primary Amino Acid Sequence

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Supporting Information

ABSTRACT: We present a systematic study of the photophysical properties of one-dimensional electronically delocalized nanostructures assembled from π-conjugated subunits embedded within oligopeptide backbones. The nature of the excited states within these nanostructures is studied as a function of primary amino acid sequence utilizing steady-state and time-resolved spectroscopies, and their atomistic structure is probed by molecular simulation. Variations introduced into the amino acid side chains at specific residue locations along the molecular peptide backbone lead to pronounced changes in the observed photophysical behavior of the fibrillar structures (spanning H-like excitonic coupling and disordered excimeric coupling) that arise from subtle changes in the π-stacking within them. These results indicate that residue modification—in terms of relative size, solvation properties, and with respect to the distance from the central π-electron core—enables the ability to tune chromophore packing and the resulting photophysics of supramolecular assemblies of π-conjugated bioelectronic materials in a rational and systematic manner.

INTRODUCTION

The electronic properties of organic π-conjugated polymers and oligomers are greatly influenced by a wide variety of intra- and intermolecular interactions. Planarization of a π-electron segment, for example, is a well-established strategy to lower the energy of key electronic transitions. When considering electronic applications where feature sizes are greater than typical intramolecular exciton diffusion lengths, intermolecular interactions become increasingly important. Kash and Davydov developed exciton models that describe how spatial arrangements of transition dipoles within interacting chromophores (such as H- and J-aggregates) impact optical responses from purely electronic considerations.1–3 More recent theoretical work led by Brédas4 and Spano5 has furthered the understanding of energy and charge transport in π-conjugated materials by evaluating intermolecular interactions along with electronic–vibrational coupling and taking disorder into account. These models have been invoked for numerous interacting π-electron systems.

Single crystals of π-electron molecules are used as active layers in high performance organic field-effect transistors and could be viewed as “ideal” aggregates.6 Anthony and Marks employed judicious monomer design to induce different crystal polymorphs, thus altering electrical properties.7,8 However, natural crystal packings are difficult to predict and tune by design, and these materials are limited in their domain size scalability. Polymeric materials offer another opportunity, but their performance and characterization are dependent on sample processing history and their complex morphologies.9,10 The need for structural control is evident: the high charge carrier mobilities found in regioregular poly(3-hexylthiophene) can be attributed to the lamellar order and favorable interchain interactions between polymer backbones, but engineering a wider range of new polymers that exhibit this type of

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organization requires sophisticated molecular-level structural design and synthesis. An alternative to these two approaches lies in the fabrication of electronically delocalized supramolecular materials leading to ordered assemblies of π-conjugated materials with key feature sizes in the 10–1000 nm regime. Electronic properties in these materials are dictated by the precise control of the monomer organization driven by engineered supramolecular interactions, which has been achieved with great success for a variety of prototypical π-electron motifs such as oligo-phenylenevinylene and perylene diimides. In all three cases, however, the very poor water solubilities of the chromophore units have limited their study and processing to organic media.

The emerging field of bionanotechnology has inspired the design of aqueous self-assembling π-conjugated subunits with fluorescent or electroactive properties. Short oligopeptides offer one type of water-solubilizing group with vast potential for tunability, and their self-assembling propensities have been used for a variety of materials applications. These approaches benefit from the potential to modify the underlying peptidic molecular precursor without severely disrupting the natural assembly mechanism. Rational design of these π-conjugated peptide molecules seeks to balance the competing forces of peptide hydrogen bonding/aggregation and quadrupole interactions among stacked π-conjugated segments to favor extended assemblies with defined hydrogen bonding networks and intimate π–π interactions. In principle, electronic materials derived from peptides could be tailored for biocompatibility and employed in bioelectronic applications.

Peptide moieties offer a synthetically tractable approach to achieve rapid variation of steric and/or electrostatic properties, but changes in amino acid composition within π-conjugated peptide molecules have profound impacts on the morphologies of the resulting peptide nanostructures (tapes, tubes, sheets, etc.) and thus on the nature of the resulting intermolecular electronic interactions. Optoelectronic properties are also dictated by chiral supramolecular spaces found in nucleic acid secondary structure and in proteins. Differences in the nature of the aliphatic and aromatic amino acid substituents presented on oligopeptides can have a dramatic impact on the peptide conformations and their subsequent higher-order tape–tape interactions and resulting nanomaterial morphologies. Decoupling specific alterations in intermolecular π-electron interactions driven by a molecular change from changes in local environmental factors and global nanostructure morphologies is a complex problem. The development of a robust predictive framework through which to engineer specific optoelectronic properties originating from exciton coupling phenomena inherent to the self-assembled supramolecular nanostructures offers an intriguing field of study due to the multiple levels of hierarchical structure that can be tuned by molecular modifications. A systematic study of residue/structure correlations is needed to develop fundamental understanding and design rules for nanostructures with desired optoelectronic properties.

We describe here the preparation and investigation of three families of π-conjugated peptide nanomaterials with primary amino acid sequences that differ in their size and their “hydrophobicity” and which are therefore expected to perturb the intermolecular electronic properties of the resulting supramolecular nanostructures. We show that these modifications directly influence the nature of the excitonic or excimer-like excited states within the nanostructures with concomitant attenuation of excited-state lifetimes: exciton coupling is associated with more delocalized electronic states that might be viewed as useful for encouraging exciton migration during the lifetime of the excited state, while excimer states are more often viewed as exciton traps and could be useful when seeking light-emissive nanomaterial constructs. While these regimes are spectroscopically well-characterized for a variety of chromophore systems, their realization is more often guided by trial-and-error approaches rather than specific molecular design. A complete quantitative predictive structure/function relationship between primary amino acid sequence and photophysical properties remains challenging, but this study provides a better understanding of interchain interactions and how to engineer them in peptide-based nanostructures en route to new aqueous self-assembled electronic materials.

## EXPERIMENTAL SECTION

**Peptide Synthesis.** Peptides were prepared according to our previous reports. Details can be found in the Supporting Information.

**UV-vis and Photoluminescence (PL).** UV–vis spectra were collected using a Varian Cary 50 Bio UV–vis spectrophotometer. PL data were collected using a PTI Photon Technology International Fluorometer with an Ushio Xenon short arc lamp. Micromolar concentration samples were prepared using Millipore water, and the pH was adjusted by the addition of 10 μL of 1 M HCl or 1 M KOH. PL spectra were obtained using an excitation wavelength corresponding to the λ_{max} of absorption. These data were recorded under conditions dilute enough to avoid complications due to scattering.

**Circular Dichroism (CD).** CD spectra were acquired using a Jasco J-810 spectropolarimeter. Acidic and basic samples were prepared by adding 10 μL of 1 M HCl or 1 M KOH, respectively, to a stock solution (1.0–10.0 μM) of the peptide in Millipore water.

**Transmission Electron Microscopy (TEM).** TEM images were acquired on a Philips EM 420 TEM equipped with an SIS Megaview III CCD digital camera with an operating voltage of 100 kV. Formvar carbon-coated copper grids (200 mesh) were purchased from Electron Microscopy Sciences. Grids were prepared as follows: A stock solution of 0.1 mg/mL peptide in Millipore water was exposed to concentrated HCl vapor for 1 min. 10 μL of this solution was pipetted onto a grid. The grid was incubated for 8 min. The grid was then dipped sequentially into water then into a solution of 2% uranyl acetate stain and allowed to dry in air. For DFAV, a 0.09 mg/mL stock solution in 99:1 H₂O:MeOH was used and acidified and stained as previously described. A 0.05 mg/mL stock solution was used for DVAG and DFGV peptides and acidified and stained as previously described.

**Molecular Simulations.** Molecular dynamics simulations were conducted using the GROMACS 4.0.5 simulation suite. Each DXX monomer was initialized in an elongated conformation, and molecular topology files employing the GROMOS96 43a2 force field were generated using PRODRG2. The initial coordinates of each peptide ribbon were generated by stacking 35 DXX monomers with a relative twist angle of −10° to mimic the approximate twist angle found in standard parallel β-sheets. Periodic boundary conditions were implemented to simulate an infinite ribbon. Solvent was modeled explicitly using the SPC/E water model, and Na⁺ counterions added to neutralize the −4 charge carried by each DFAX monomer under weakly acidic conditions. Relaxation of the ribbon to a stable structure was monitored by following the twist angles and spacing between neighboring monomers during a 20 ns NPT equilibration run at 298 K and 1 bar. Conformational statistics of the equilibrated structure were harvested during a subsequent 10 ns production run. Full details of our simulation protocols are provided in the Supporting Information.


RESULTS AND DISCUSSION

We previously demonstrated that peptide-π-peptide triblock molecules, under appropriate experimental conditions, assembled into 1-D nanostructures with intimate π−π interactions observable via the resulting photophysical changes induced upon assembly.35−38 Representative trends from the triggered assembly of several chromophore systems led to photophysical outcomes characteristic of “H-like” aggregates.39 At basic pH, deprotonated carboxylate residues provide Coulombic repulsion between peptide molecules, thereby hindering them from assembling. Lowering the pH screens these charges through protonation, thereby minimizing the repulsion and triggering the peptide assembly process. This assembly results in an overall hypsochromic shift in the absorption maxima and typically results in a quenched bathochromic photoluminescence (PL) profile with respect to the monomer. An induced circular dichroism (CD) signal for the π-conjugated subunit is observed after the assembly, indicating exciton coupling between the segments held within a chiral environment (Figure 1b,c). These outcomes are consistent with a one-dimensional stacked model such as the idealized depiction shown in Figure 1 with highly ordered amyloid-like β-sheet structure, but such ordered internal structures are not necessarily needed for the creation of delocalized electronic states. Transmission electron microscopy (TEM) of these materials also shows the formation of 1-D aggregates typically on the order of a micrometer in length and 10 nm in diameter. In this work, we will describe the spectral dynamics of these nanomaterials keeping the chromophore constant while systematically varying the flanking peptide sequences with different steric and hydrophobic demands.

Molecular Design: OPV Chromophore. The π-conjugated core segment used for this study is 1,4-distyrylbenzene, an OPV with well-characterized spectroscopic behavior in solution-based aggregates and in thin films. This and longer OPVs have been the subject of considerable experimental and theoretical interest due to the applications of arylene vinylene polymers in organic electronics.40−43 OPV “dimers” with well-defined intermolecular orientations have been studied extensively through attachment to a common [2.2]paracyclophane core showing a critical relationship between the substitution geometry and the observed electronic structure.44,45 OPVs were also part of Schenning and Meijer’s seminal supramolecular electronic construct consisting of self-complementary hydrogen-bonding OPV units that dimerize and under appropriate organic solution conditions self-assemble into one-dimensional chiral stacks,13 in addition to our own aqueous peptide nanomaterials.36

Molecular Design: Peptide Sequences. The tetrapeptide sequence DFAG was chosen as a starting point based on the assembly behavior this sequence displayed in our previous work. Variations of this parent sequence led to a series of peptides with single amino acid substitutions at different locations along the tetrapeptide flanking sequences. These substitutions vary both in their relative size and in their hydrophobic nature, which could impact the intermolecular interactions and influence hierarchical fibrillation processes. Figure 2 shows the three different series of peptides referred to as DFXAX, DFXG, and DXAG, where the aspartic acid residue (D) remains unchanged and serves as a pH trigger for assembly, X denotes the location and identity of the amino acid substitution (Gly/G, Ala/A, etc.), and R indicates the specific side chain variation (H, Me, Ph, etc.). We constructed sequence variants with glycine (G, R = H), alanine (A, R = CH3), valine (V, R = CH(CH3)2), isoleucine (I, R = CH(CH3)(CH2CH3)), and phenylalanine (F, R = CH2Ph) to perturb the internal structure of the assemblies and nature of the associated electronic delocalization.

Systematic increases in the size of the residue could lead to progressively less π-electron overlap due to increased OPV spacing resulting from steric crowding imposed within 1-D tapelike assemblies. The progressively more hydrophobic residues could also encourage more intermolecular van der Waals-type attractions among the larger hydrophobic groups in lieu of aqueous solvation, a well-established factor in the formation and stabilization of amyloid-based materials, leading to decreased OPV spacing and strengthened electronic coupling. In the three families of peptide variations that we systematically study here, we vary the placement of the residues with respect to the OPV core and the large and aromatic phenylalanine (F) residue of the parent DFAG structure. This allows for a survey of the optoelectronic influences of situating demanding residues on the same face of the formed nanotapes as the constant phenylalanine (DFAX series) and on the opposite face (DFXG series). A third class (DXAG series) is presented that assesses aliphatic steric bulk well removed from the chromophore unit. With these three families, we demonstrate the complicated interplay of steric and hydrophobic influences that elicits dramatic variation in the electronic coupling of the OPV cores within the peptidic nanostructures.

Figure 1. Energy-minimized model of a 1-D nanostructure expected after self-assembly (a) and representative UV−vis/PL (b) and CD spectra (c) of an OPV peptide in basic, molecularly dissolved (···) and acidic, assembled solutions (—). Data taken from ref 36.

Figure 2. Primary amino acid sequence variations prepared and studied in this work.
Nanostructure Morphology. We explored the morphology of the 1-D assemblies because primary amino acid sequence variations of related materials lead to dramatic changes in assembly motifs. For example, variation of the amino acid adjacent to a π-conjugated terthiophene within a peptide amphiphile resulted in nanostructure morphologies ranging from flat spicules to nanotubes to flat sheets. It will be difficult to deconvolute specific impacts of molecular structure variations on intermolecular π-electron interactions if these variations also lead to different supramolecular morphologies that may themselves impose geometric constraints to the chromophore delocalization (such as different curvatures splaying chromophores to different extents).

The peptide-π-peptide systems studied here uniformly form one-dimensional structures, although the morphologies observed with TEM need not correlate to those of the assemblies prepared from more dilute spectroscopic solutions discussed below. TEM (Figure 3) revealed that the main variations to the 1-D assemblies were the apparent nanostructure length and the presence of bundled peptide fibrils (∼10 nm diameters) alongside standard tapes and ribbons that commonly coexist in related peptide assemblies, in accord with our past visualization of related structures. These peptide nanomaterials provide a morphological normalization that enables a more direct molecular structure–electronic function correlation among the systematic variations discussed here without potentially complicating issues such as variance in curvature (and subsequent variance in local chromophore arrangements) that may arise among dramatically different morphologies often encountered in other peptide assembly platforms.

Different sequences with different residue presentation might encourage different degrees of lateral association or otherwise progress toward more hierarchically complex but still 1-D supramolecular morphologies (stacked ribbons, tapes, or fibrils) much like other amyloid-like oligopeptides. Lateral electrostatic and solvation preferences arising from the composition of the residues presented on the ribbon or tape surfaces can influence these hierarchical associations, especially when multiple aromatic amino acid residues are involved. The empirical nature of our study necessarily requires us to consider the materials “as-is” whereby molecular variation leads to different degrees of intermolecular (and intratape) interactions while at the same time subjecting the resulting tapes to uncontrollable and unpredictable degrees of intertape fibrillation processes. Decoupling these two phenomena is challenging from a molecular design standpoint and will be the subject of future investigations.

General Peptide Photophysics. The photophysical properties induced upon assembly of the three peptide families gave insight into the differences in electronic coupling between the molecularly dissolved samples (taken at pH 8) and their assembled counterparts (taken at pH 2). As expected, each molecularly dissolved peptide solution showed nearly identical steady-state and time-resolved spectral behavior, indicating that the specific amino acid sequence did not noticeably affect the photophysics of the isolated chromophore. Upon self-assembly, these peptides showed trends uniformly consistent with offset

Figure 3. Representative TEM images of peptide nanostructures composed of DFAG (a), DFGG (b), DGAG (c), DFAV (d), DFVG (e), and DVAG (f). Samples were imaged with a 100 kV operating voltage on 200 or 300 mesh Formvar carbon-coated copper grids and stained with 2% uranyl acetate.

Figure 4. CD spectra for solution-based assemblies of DFAH (a), DFXG (b), and DXAG (c) recorded under aqueous acidic conditions.
H-type aggregates arising from assembly models consisting of extended intermolecular peptide-based hydrogen-bonding networks that enforce cofacial $\pi$-electron interactions. The spectroscopic samples should be viewed as ensembles of different persistence lengths if not even having finite contributions from molecular peptides, much like conjugated polymers have polydisperse length distributions. The trends found among these ensembles are not limited to spectroscopically diluted conditions: they are also apparent in materials formed in macroscale hydrogels and in mesoscale structures formed using fluidic-directed assembly procedures.48

Exciton-coupled chromophores in chiral local environments should show pronounced bisignate Cotton effects in circular dichroism spectra (CD) corresponding to the coupled $\pi-\pi^*$ (or other relevant) transition dipoles. The molecularly dissolved samples studied here showed no significant low-energy absorptions in the CD spectra, but induced bisignate CD signatures were observed in the 250–450 nm region upon triggered assembly (Figure 4). Most of the peptides exhibited a crossover near the absorption maximum for the OPV subunit as expected for excitonically coupled chromophores; however, the intensity, handedness, and features of the CD spectra varied dramatically, suggesting that the local chiral environment encompassing the OPV was different for the different sequences. These signals offer glimpses into the changes in aggregate internal structure that can be induced through amino acid substitutions.

Our primary interests were to characterize the electronic processes within these aggregates specifically in terms of $\pi-\pi^*$ electronic transitions, but CD and IR spectroscopy were also used to interrogate the hydrogen-bonding networks among amide-bond regions of the molecules. In the three peptide families presented here, CD spectra generally revealed a high-energy negative feature suggestive of $\beta$-sheet arrangements among the associated molecules (Figure 4, <250 nm). The ATR-IR spectra recorded on solid samples of lyophilized peptides (Figures S12–S22) revealed fairly consistent amide I signatures associated with parallel $\beta$-sheets (or perhaps even unordered structures) at ca. 1630–1640 cm$^{-1}$ along with presumed amide II signatures at ca. 1525–1545 cm$^{-1}$, although there were a couple notable deviations from these ranges (e.g., DFAF, DFAI, DFFG, DIAG). Furthermore, the IR spectra in this region were significantly broadened in a reflection of the anticipated polydispersities of intermolecular interactions present in these peptides as solid lyophilized samples.

The CD (and IR) signals originating from the amide bonds within peptide aggregates do not always provide definitive structural information. For example, the handedness of insulin fibrils can be a function of the kinetically controlled assemblies of different monomer conformations early in the assembly process as well as of the physical sample manipulations.49 Although $\beta$-sheet content in oligopeptide gellers may correlate to alignment and macroscale mechanical properties,50 it is not necessarily a requisite spectral signature for peptide-derived 1-D fibrillar structures. Recently, signatures associated with classic antiparallel $\beta$-sheet motifs in Fmoc-containing dipeptide assemblies were actually shown to arise from “unnatural” carbonyl functionalities (i.e., the carbamate group at the site of Fmoc attachment).51 The hydrophobic peptide $\pi$-conjugated hybrid structures studied here should be even more kinetically driven compared to natural systems and as such may not present the ideal intermolecular peptide hydrogen-bonding networks expected for the well-behaved natural peptide sequences associated with soluble proteins. The carboxamide chromophores at the sites of peptide attachment to the OPV3 core unit might also artificially indicate $\beta$-sheet assignments (related molecules show an amide I signature at 1629 cm$^{-1}$).52 Regardless of the actual composition of the peptide secondary structure(s) and conformation(s), the solution aggregate ensembles with which the spectroscopic measurements were obtained indicate a predominance of one twist sense (either positive or negative exciton couplets), thus supporting the idea that the embedded chromophores maintain relatively consistent electronic ordering in a global sense. They also indicate that the peptide nanostructures provide environments conducive to intermolecular chromophore interactions leading to explicit exciton coupling or excimer-like formation.

**DFAX Photophysics.** The DFAX family places the site of residue variation directly adjacent to the OPV chromophores and results in it being projected on the same face of the resulting peptide nanotape as the phenylalanine (F) residue three positions removed from the OPV unit. Following trends typically associated with H-like aggregation, each peptide analogue upon assembly showed an overall shift in absorption $\lambda_{\text{max}}$ to higher energy compared to the molecularly dissolved sample, with each also exhibiting an appearance of a weak lower energy onset feature (Figure 5a). The magnitude of the blue-shifts decreased in a manner that tracked with the relative size of the residue: peptides with the smaller glycine (DFAG) and alanine (DFAA) substituents had the largest blue-shifts while those with bulkier isoleucine (DFAI) and valine (DFAV) had much smaller shifts. DFAF presented a different steric and hydrophobic contributor as a planar aromatic residue rather than a branched alky group but nevertheless fit in with the other “large” substituents.

In all cases, the PL of the assembled nanostructures was quenched (i.e., was weaker) with respect to the monomer, but the resulting spectral features revealed significant differences when compared to the molecularly dissolved peptides (Figure 5b). Although the PL intensities of the assembled materials are weaker than their monomeric counterparts, in general the hologrins are bright emitters with quantum yields of 5% or greater. DFAG nanostructures revealed a quenched and featureless red-shifted PL typical for an excimer or charge-transfer band. The PL of DFAA nanostructures seemed to fall under the spectral footprint for the molecular PL spectrum but with the progression in vibronic fine structure and intensity summarized by Spano for related electronically coupled...
The emission properties observed in these well-defined model systems could be attributed to differences in ground state geometries and enforced predispositions to form excited state excimeric structures. These structural features are quite readily tunable within the peptide platform described here, both directly adjacent to the OPV core (vide supra) and more remotely (vide infra).

Molecular Dynamics Simulations. To aid in our understanding of the nanoscale internal structure and the alterations that are caused by changing the amino acid sequences, we conducted molecular dynamics simulations of single 1-D ribbons composed of each peptide reported herein. These particular simulations do not take any differences in solvation within or among the peptide families into account. CD and TEM collectively indicate that the assemblies exist as ribbon-like nanostructures that typically maintain left-handed helical twist sense (as do natural β-sheet aggregates) among the embedded chromophores. This motivated us to initialize all molecular simulations with an intermolecular spacing and

<table>
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<th>Peptide Sequence</th>
<th>(\lambda_{\text{max,obs}}/\text{nm}^a)</th>
<th>(\lambda_{\text{max,fit}}/\text{nm}^a)</th>
<th>QY (^b)</th>
<th>(t/\text{ns}^c)</th>
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<td>0.38 0.12</td>
<td>1.09 (100)</td>
<td>7.52 (100)</td>
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<td>N/A (^d)</td>
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<tr>
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<td>0.84 (92); 3.9 (8)</td>
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<td>0.42 0.04</td>
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<td>5.65 (49); 1.41 (51)</td>
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<td>1.07 (100)</td>
<td>1.30 (64); 8.54 (36)</td>
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\(^a\)Conditions as described in Figure 5. \(^b\)Absorption maximum was used for excitation wavelength. \(^c\)Relative to quinine sulfate in 0.1 N H_2SO_4 using 350 nm excitation. \(^d\)Fitted percent contributions for the multiexponential decays in parentheses. \(^e\)Insufficient intensities observed for meaningful measurements.

The excited-state lifetimes of the DFAF peptides were identical in the molecularly dissolved samples at 1.09 ns (Table 1). The lifetimes for the assembled peptides suggest differing fates of the resulting excited states. The decay of DFAF aggregates, with the most red-shifted and featureless PL profile, had a single-exponential lifetime of 7.50 ns. The other sequences in the series gave rise to multiexponential decays, where assemblies showing greater contributions from the lower-energy PL feature had longer lifetimes. For example, DFAA, which showed steady-state PL features suggestive of contributions of both exciconic and excimeric states, had a greater contribution (32%) of a significantly longer component (4.02 ns) leading to a \(\tau_{\text{avg}}\) of 2.97 ns. DFAF and DFAV nanostructures, both of which showed more structured higher-energy excimeric emission and less contribution of a low-energy excimeric tail, were dominated by shorter-lived components that resulted in much shorter average lifetimes (\(\tau_{\text{avg}} = 1.08\) and 0.51 ns, respectively). The shorter components within the observed multiexponential lifetime decays in the assembled states are not sufficiently close to those for the molecular samples to be suggestive of extensive monomeric contributions within the spectroscopic ensembles.
relative twist angle of 0.46 nm and $-10^{3}$ between successive monomers, respectively, and monitor the relaxation of these variables over time scales of tens of nanoseconds (cf. Molecular Simulations section). The results of the current simulation work clearly demonstrate that single amino acid substitutions significantly alter the intermolecular spacing (Table 2) as well as the distribution of twist angles within the ribbon (Figure 6, Figures S45–S47 and Tables S1–S3).

<table>
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<tr>
<th>peptide</th>
<th>$\Delta r$/nm</th>
<th>peptide</th>
<th>$\Delta r$/nm</th>
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<th>$\Delta r$/nm</th>
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<td>0.61 ± 0.15</td>
<td>DFIG</td>
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<td>0.52 ± 0.17</td>
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<td>0.56 ± 0.13</td>
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<td>DFFG</td>
<td>0.55 ± 0.14</td>
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<td>0.59 ± 0.18</td>
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Table 2. Mean and Standard Deviation in the Interplanar Spacings ($\Delta r$) between Neighboring Monomers in the 1-D Ribbons for the DFAX, DFXG, and DXAG Peptide Families Computed by MD Simulations.

The mean interplanar spacing between successive peptides in the terminal structure was ca. 0.60 nm for the DFAG, DFAA, and DFIA peptides, compared to 0.52 nm for DFAV and DFAF. These distances reflect the spacings of the OPV centers of mass among nearest neighbors within an aggregate while still maintaining interpeptide hydrogen bonding. The larger substituents (F, V) result in closer packing of the peptides relative to the smaller substituents (G, A), presumably due to enhanced dispersion interactions between neighboring chains in the former cases. The larger isoleucine residue does not fit into this trend: we speculate that the similarities of the vibronic peak placement in the assembled DFAI and DFFA peptides coupled to the similarities in the mean interplanar spacings suggest a steric repulsion rather than a dispersive attraction is operative in DFFA nanomaterials. These simulations corroborate the photophysical findings presented above whereby the larger residues offered expected exciton coupling facilitated by these closer enforced distances whereby the smaller residues led to further removed chromophores not expected to exhibit as strong of an exciton coupling of the OPV transition dipoles. This is consistent with the collective influences of dispersive attractions among the larger aliphatic and aromatic residues seeking a more stabilizing solvation environment than that afforded by the aqueous ionic medium.

Figure 6a shows the starting point for our simulations: the idealized twisted ribbon $\beta$-sheet identified from energy-minimized models. During the course of the simulations, the nanostructures progress to states of much less internal order (Figure 6b), and we were unable to draw any global conclusions that pertain to the observed photophysical trends within the three studied peptide families. In some cases, narrow distributions are found (e.g., DFAG) while others have polymodal segregations (e.g., DFAA, DFIG) or substantial contributions from largely positive $\Delta \theta$ local configurations (e.g., DFIG): a compendium of these histograms can be found in the Supporting Information (Figures S45–S47). Although the interpeptide spacing increases from a value of 0.46 nm in the initial ribbon (Figure 6a) to 0.59 ± 0.18 nm in the terminal configuration of DFAG stacks (Figure 6b) (cf. Table 2), the overall length of the stack decreases. Entropic mixing within the stack and internal deformations of the constituent peptides causes the director between adjacent peptides to deviate from the vertical, such that spacings between successive peptides do not add linearly in the z-direction, resulting in a net overall contraction of the stack height. This disorder would be expected to result in compromised contributions of pure-$\beta$-sheet conformations driving the stabilities of the resulting nanostructures, and we stress that these assemblies need not rely exclusively on the $\beta$-sheet paradigm so long as other stabilizing hydrogen-bonding interactions can evolve. Indeed, even polyproline II type conformations have been invoked within Fmoc-based oligopeptide assemblies.47

A detailed program of future simulation studies is planned to systematically probe the influence of initial geometry, temperature, pressure, pH, ribbon size, and oligopeptide and conjugated subunit chemistry upon the structure of the 1-D objects using implicit solvent simulations to access microsecond time scales. We also propose to obtain better X-ray diffraction data to further corroborate these simulations and the experimental electronic properties.46 More exacting structural metrics would allow for a future correlation of exciton coupling geometries and the anticipated electronic responses from a computational perspective. Regardless, our present simulation data corroborate the experimental finding that single amino acid substitutions can substantially impact nanostructure internal geometry, and we can make some qualitative rationales of peptide family specific trends.

**DFXG Photophysics.** The DFXG series contains sequences that vary the sterically bulk two amino acids removed from the OPV core while maintaining the glycine residue, directly next to the OPV core and the aspartic acid residue as a pH trigger for assembly. In contrast to DFAX, this family presents residue variations that project on the opposite face of the constant phenylalanine residue assuming an ideal $\beta$-sheet molecular
motif within the resulting nanostructures. The steady-state spectroscopy for the DFXG series did not follow as progressive of a trend with residue size as did the DFAX series, but there were clear differences in the assembled DFXG nanostructures photophysics nevertheless (Figure 7). The absorption spectra of the assembled peptides with varied aliphatic residues showed similar trends with a shift in the absorption maxima to higher energy upon assembly. The CD spectra of the DFXG assemblies revealed negative minima in the amide region expected for $\alpha$-sheet-like conformations as well as split Cotton signals for the OPV units upon aggregation (Figure 4b). DFFG and DFVG showed two local minima in the amide region commonly associated with $\alpha$-helices. As was seen previously, the handedness of the OPV subunit was different and DFFG and DFVG showing a right-handed Cotton effect, indicative of a right-handed twist among the aggregated OPV segments.

The DFFG nanostructures showed the vibronically structured emission fine structure found in the DFAX series above, but all of the other aliphatic DFXG peptides resulted in excimer-like emission bands. Although the larger amino acid side chains directly adjacent to the OPV core in the DFAX series promoted excitonic-like emission behavior, in this case being two removed, the opposite trend is found whereby larger aliphatic residues enforce excimer-like responses. DFFG had the largest shift to higher energy in the absorption spectra and interestingly exhibited a vibronically resolved emission spectra resembling DFAG and DFFG (although slightly red-shifted overall), while the other peptides in this series showed featureless lower energy emissions comparable to DFAG. The fact that even the simple glycine substituent offers such a dichotomy in excited state outcomes of the corresponding DFAX and DFXG peptides reinforces the idea that a “one size fits all” story is unattainable, but rather that the underlying associations governing the assembly of each peptide family in terms of residue placement must be considered on a case-by-case basis. The excited-state lifetimes for the assembled DFXG series of peptides displayed complex multieponential decays with lifetime averages between 3.0 and 4.5 ns, chiefly composed of comparably weighted long-lived (5–12 ns) and short-lived (ca. 1 ns) components.

DFFG nanostructures, the only example with an aromatic residue placed in the second position, exhibited a lower energy absorption component, but the absorption maxima did not shift compared to the molecular case. The TEM images for DFFG nanostructures revealed shorter one-dimensional aggregates (Figure S48). This could explain the nonapparent UV−vis blue-shift upon assembly because more truncated aggregates would be presumed to exhibit more disordered “end” effects such as being more prone to conformational deformities as well as a less coupled excited state. The DFFG nanostructure emission had similar vibronic fine structure as found for DFFG nanostructures. Phenylalanine dipeptides (Phe-Phe) and other phenylalanine-rich oligopeptide assemblies are known to exhibit unique nanotubular assemblies, so the DFFG outlier—with two adjacent phenylalanine residues—could be attributed to complicated yet favorable phenylalanine aromatic side-chain interactions formed upon assembly that the other sequences do not experience.

This series would not be expected to have as large of a large steric component altering the assembly with respect to $\pi$-electron interactions because the steric demand is further away from the embedded chromophores and thus leading to differential photophysical responses than those observed in the DFAX series. Furthermore, there is increasing hydrophobic character on the amino acid adjacent to the constant phenylalanine. In an organized $\beta$-sheet tape, the DFXG peptides would present the F and the X residues on opposite surfaces, perhaps altering the kinetics of the hierarchical assembly or otherwise altering the solvation of the molecular entity that subsequently affects the enthalpic stabilization upon self-assembly (i.e., the internal solvation). It has been shown that altering hydrophobic and hydrophilic residues increases the amyloidogenic behavior of oligopeptides due to the presentation of one uniformly hydrophobic surface.

**DXAX Photophysics.** The DXAX family was prepared in an effort to better probe the role of peptide sequence hydrophobicity further removed from the OPV core. The DFAX series was clearly perturbed by the adjacent amino acid, where the smaller residues led to more excimer-like photophysics while the larger residues provided more excitonic interactions. The DFAX series, having peptides of comparable composition but lacking the same magnitude of steric influence directly at the chromophore, seemed to be dominated by excimeric interactions regardless of size. We hypothesized that the larger aliphatic members of the DFAX family were too hydrophobic to properly balance the OPV core electrostatics leading to rapid formation of kinetically trapped assemblies and perhaps increased higher order lateral aggregation/assembly due to the enhanced hydrophobic surface presentation on both sides of an idealized $\beta$-sheet tape. The DXAX substitutions allow us to vary relative hydrophobicities while having much less effect on the steric environment locally around the OPV core. With the aromatic phenylalanine removed, these substitutions will result in entirely aliphatic sequences whose assembled structures will not present aromatic surfaces above and below the self-assembled ribbons. It also allows for an examination of aliphatic size trends that eliminate the influences of the quadrupolar character of DFAG’s phenylalanine residue.

Compared to the DFAX series, the DXAX peptides had much more dramatically blue-shifted absorption $\lambda_{\text{max}}$s, suggesting that the steric bulk provided by the amino acid adjacent to the chromophore in the DFAX series seemed to play a role in the extent of neighboring interchain interactions. For the DXAX peptides, the sequences with the larger residues (DFAG and DIAx) had the smallest absorption shifts, where the other amino acids were perturbed much more significantly (Figure 8). The lower energy absorption onset peak at ca. 410 nm was only apparent for the DFAG and DIAx peptides, all the remaining peptides of this series showed weaker low-energy absorption indicating more uniform H-aggregate spectral
signatures. The amide region of the DXAG peptides all showed CD spectra with a negative peak between 200 and 220 nm (Figure 4c); however, even with these less hydrophobic sequences the excitonic couplet still varied in overall handedness depending on the sequence variations.

The emission spectra from the DXAG series of peptides were all significantly more red-shifted compared to the DFAX peptides (with substitutions larger than glycine) and in fact seemed to resemble the broad excimeric emission profile of DFAG. The peptides in this series showed varied extents of vibronic features but were much more dramatically shifted to lower energy compared to the DFAX series. The simulations revealed that all DXAG peptide nanostructures presented intermolecular spacings of ca. 0.60 nm (Table 2), similar to the DFAX peptides showing excimeric emission (DFAG, DFAA, and DFAI, Table 2). Furthermore, DFAV presents an inverted excitonic couplet, and this seems to be corroborated in the unusually high contribution of positive \( \Delta \theta \) values after completion of the nanostructure simulation run (ca. 32% compared to 10–20% for the other DXAG peptides, Figures S45–S47). These peptides displayed multieponential excited-state lifetimes, with the average lifetime being longer for the peptides with the most red-shifted emission spectra, and the peptides with more pronounced vibronic spectra had larger contributions of shorter-lived components compared to the unassembled peptides (Table 1). These results suggest that, aside from the steric perturbations caused by an adjacent amino acid side chain, the overall hydrophobicity of the sequence and how it presents the hydrophobic side chains influence the aggregate behavior and the resulting photophysical response from the OPV core.

## CONCLUSIONS

A series of peptide-\( \pi \)-peptide constructs were synthesized from a common OPV core structure that results in a broad range of absorption, emission, and chiroptical properties that arise from modest systematic changes in primary amino acid sequence. These properties range from varied H-like aggregates that have dramatic blue-shifted absorption spectra to ones that were only weakly perturbed from the monomer. Photoluminescence from the assemblies spanned from structured excitonic-like emission expected for H-aggregates to featureless red-shifted charge-transfer-like excimer emission, indicating a range of peptide nanomaterials that had spectral properties consistent with varying degrees of electronic coupling. These influences arise from structural considerations including differences in steric bulk of primary amino acid sequences coupled with the dispersive roles that these larger groups play to enforce specific intermolecular configurations. We were able to alter interchromophore/interchain interactions and perturb the photophysical properties within nanostructures, but the complete correlation of how these interactions experimentally impact electronic coupling in the final aggregate structures remains a complex problem sensitive to the precise nature and location of the physicochemical modification.

The simple assembly model of a one-dimensional peptide aggregate leads us to reasonably predict the overall spectral trends observed within a given peptide family, but it does not yet explain the some of the subtleties needed for complete and quantitative rationalization. However, we have created a controlled system where these types of peptide aggregates can be studied in depth and with minimal perturbation of nanostructural morphology. The spectroscopic handle allowing us to interrogate the photophysical perturbation of the assemblies will aid in understanding important intermolecular interactions of \( \pi \)-conjugated materials and hierarchal self-assembly prevalent in complex natural systems. The TEM and CD results are telling of the overall complexities of peptide nanomaterials systems. We observe different levels of assembly manifested in different lengths of the one-dimensional fibrils. The subtle differences were likely a result of the early aggregate structures that were influenced by the surface presentation of the amino acid side chains as well as the overall hydrophobicity and resulting propensity to aggregate or form higher order structures (bundles, etc.). The more hydrophobic sequences might have a higher propensity to assemble and, just as in the case with native peptides, could assemble based on monomer conformations that are not necessarily the most thermodynamically stable. The fact that the aggregates were consistently one-dimensional (without indications of large plates, tubes, or vesicles) indicates that our design for placing the \( \pi \)-conjugated segment within the peptide backbone allows for the peptide to dictate the assembly behavior and the local intermolecular chromophore interactions without complications from higher order morphological structure variations. This study shows how peptide sequence variation can access two very important and different electronic coupling regimes—from the disordered excimeric structures to the electronically ordered excitonically coupled states. The principles set forth in this work will give a good starting roadmap for controlling the fate of the electronic excited states in related systems. The systematic explication of how primary sequence structure impacts electronic properties will be of great interest to further explore the bioelectronic potential of these and related supramolecular nanomaterials for energy-transporting and/or light-emitting needs.

## ASSOCIATED CONTENT

Supporting information
Experimental procedures and characterization data for the peptides, full details of the molecular simulation protocols and structural observations, Figures S1–S48 and Tables S1–S3. This material is available free of charge via the Internet at dx.doi.org/10.1021/la500222y.

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REFERENCES

(31) We use the term “hydrophobicity” very loosely here and in accord with current convention common in contemporary research reports. We recognize that these functional groups might experience different solvation preferences as molecular entities or in the resulting assemblies. One resulting outcome of varying “hydrophobicity” (or varied solvation preferences) would be attenuated induction of enthalpically stabilizing intermolecular interactions once brought together in a peptide aggregate.
(39) The term “H-like” is used to describe these systems because an ideal H-aggregate would not show the lower energy absorption feature.
We can attribute this feature to contributions from intramolecular chromophore planarization and/or local intermolecular electronic disorder.


(46) We have been unsuccessful in measuring specific intermolecular distances/spacings despite attempts with multiple diffraction sources. The data are noisy at best, yet in some instances indicate intermolecular spacings of ca. 4.6–4.8 Å. We have recently developed techniques to globally align these nanostructures within a macroscopic volume and will apply these techniques in the future for the preparation of thicker diaphragms.


