Controlling supramolecular chirality in peptide-π-peptide networks by variation of alkyl spacer length

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Abstract:

Self-assembled supramolecular organic materials with π-functionalities are of great interest due to their applications as biocompatible nanoelectronics. Detailed understanding of molecular parameters to modulate the formation of hierarchical structures can inform design principles for materials with engineered optical and electronic properties. In this work, we combine molecular-level characterization techniques with all-atom molecular simulations to investigate the subtle relationship between the chemical structure of peptide-π-peptide molecules and the emergent supramolecular chirality of their spontaneously self-assembled nanoaggregates. We demonstrate through circular dichroism measurements that we can modulate the chirality by incorporating alkyl spacers of various lengths in between the peptides and thienylene-phenylene π-system chromophores: even numbers of alkyl carbons in the spacer units (0, 2) induce M-type helical character whereas odd numbers (1, 3) induce P-type. Corroborating molecular dynamics simulations and explicating machine learning analysis techniques identify hydrogen-bonding and hydrophobic packing to be the principal discriminants of the observed chirality switches. Our results present a molecular-level design rule to engineer chirality into optically and electronically active nanoaggregates of these peptidic building blocks by exploiting systematic variations in alkyl spacer length.
Introduction:

The self-assembly of small molecules into well-defined supramolecular aggregates has fascinated synthetic chemists over the past few decades due to the immense potential for control of bionanomaterials at the molecular scale.\(^1\)-\(^7\) In this regard, peptide-based supramolecular aggregates received significant attention due to their ability to form well-defined hierarchical structures based on collective intermolecular interactions (hydrogen-bonding, electrostatics, van der Waals forces etc.).\(^3\),\(^8\)-\(^11\) Peptides have a rich structural diversity with multifarious secondary structures such as α-helices, β-sheets and β-turns giving rise to diverse nanostructures that are difficult to achieve through synthetic chemistry or lithographic techniques. Peptides functionalized with π-electron conjugates can take advantage of this programmed assembly to form biologically-inspired π-stacked conduits relevant for electronic applications such as organic solar cells, field-effect transistors and chemical sensors.\(^12\)-\(^23\) In general, a delicate balance of π-π interaction and other non-covalent interactions determines the charge percolation pathways through the networks. For all but the simplest molecular building blocks, a complete understanding of bottom-up principles by which individual molecules may be designed to produce supramolecular aggregates with engineered structure and function remains lacking, precluding the rational design of molecular aggregation and associated electron couplings.

Chirality is a pervasive subject in chemical science, from the molecular stereocenters whose inversions can impact drug efficacy to the polymeric tacticity considerations that influence chain crystallization. Supramolecularly derived materials also express chirality as mediated through the presence of molecular stereocenters or through regular patterns of intermolecular associations that impart a local chiral environment (such as helicity).\(^24\)-\(^26\) In the case of chromophore-bearing molecules, the local chirality can influence chiroptical properties as well.
Although the control of supramolecular chirality has implications in applications such as optics (spin-selected transport), catalysis (enhancement of enantioselectivity of asymmetric reactions), biomedicine (increased cell proliferation of due to stereospecific interaction) and purification (separation of enantiomers),\textsuperscript{27-34} the specific supramolecular building blocks with respect to electronic function tend to be designed in isolation based on empirical guidance due to the difficulty in predicting the chiroptical properties of supramolecular architectures via molecular dynamics simulations.\textsuperscript{35-40} Henze \textit{et al.} observed odd-even effects of chiral aggregation using CD spectroscopy in a series of oligothiophene molecules substituted with oligo(ethylene oxide) chains going from quinqueothiophene to septithiophene.\textsuperscript{35} Chirality driven self-sorting processes were also investigated in naphthalenediimide and perylenediimide based systems using CD spectroscopy.\textsuperscript{41-43} Chiral dopants have also been shown to influence helicity from achiral starting materials.\textsuperscript{44, 45} Many groups investigated the formation of one-dimensional nanostructures from chromophores bearing oligopeptides,\textsuperscript{46-57} which present obvious points of chirality arising from the constituent amino acids.\textsuperscript{58-60} Indeed, supramolecular chirality of oligothiophene networks could also be tuned with the change in chirality of a single amino acid attached to it.\textsuperscript{59} Chiral self-assembly of amyloid-like oligopeptides was investigated by Marty \textit{et al.} demonstrating a ‘two-fold’ odd-even effect as observed in circular dichroism (CD) spectroscopy when oligopeptide-polymer conjugates coupled to perylenediimide chromophores varied with the number of L-alanine units and the spacer length between the chromophore and the oligopeptide.\textsuperscript{58} Despite these advances over the past decade, a detailed understanding and predictive control of chirality within peptide-\(\pi\)-peptide nanostructures has yet to be established.

In this report, we describe an expansion of our established synthesis methodology to incorporate \(\pi\)-systems into peptidic segments where aliphatic spacers are placed between the
peptidic segments and the conjugated $\pi$-electron regions in order to decouple the electronic effect of the attaching electron-withdrawing carboxamide peptidic segments to the central $\pi$-conjugated chromophores. Specifically, Pd-mediated cross-coupling reactions were used to install the necessary $\pi$-electron functionality within peptide conjugates by promoting aryl-aryl bond formation.\textsuperscript{50, 70, 71} In principle, the carboxamide groups directly attached to the $\pi$-system should decrease the efficacy of hole transport by destabilizing the formal cationic structure formed during field-effect gating. We envisioned that introducing an “insulating” spacer would better facilitate semiconductive properties. As representative $\pi$-core examples, we included different thienylene-phenylene mixed oligomers and a thienoacene linkage as a “high performance” organic semiconductor\textsuperscript{72} that could facilitate aggregation due to stronger $\pi$-$\pi$ stacking and eventually facilitate energy transport.

We present a comprehensive investigation into how alkyl spacers between $\pi$-cores and flanking peptides influence the internal arrangement of the $\pi$-stacks within self-assembled nanostructures. Incorporation of the spacer groups imparts different conformational preferences around the linkages thus different molecular geometry starting points from which to form supramolecular aggregates with different electronic couplings. In general, the dependence of carrier transport properties as a function of the intermolecular geometric interaction of wavefunctions of molecular organic semiconductors can be treated computationally,\textsuperscript{73, 74} but waging predictive control is difficult to realize experimentally. The peptide self-assembly processes were monitored using standard spectroscopic techniques (UV-Vis and PL spectroscopy) and the resulting assemblies were visualized using electron microscopy. We show a remarkable serendipity of systematically tuning the supramolecular chirality as observed by CD based on the peptide-chromophore spacer length, regardless of chromophore composition. This shows how a
subtle modification in the molecular structure imparts a substantial change in the supramolecular aggregates formed from these otherwise well-understood systems. Accompanying molecular dynamics simulations precisely recapitulate the experimentally-observed trends in chirality, and interrogative "white box" machine learning exposes the particular atoms and inter-molecular interactions most discriminative of these trends.

**Experimental:**

**General information**

THF was acquired from an Innovative Technologies Pure Solv solvent purification system and dried over 4Å molecular sieves. DMF and triethylamine were purchased from Sigma-Aldrich and dried over 4Å molecular sieves. N-methyl-2-pyrrolidone (NMP) was obtained from Advanced ChemTech and dried over 4Å molecular sieves. DCM and n-hexane were freshly distilled and dried over 4Å molecular sieves. All solvents were degassed by sparging with nitrogen gas at least 30 min prior to use. \(O\)-(Benzotriazol-1-yl)-\(N,N,N',N'\)-tetramethyluronium hexafluorophosphate (HBTU), trimethylsilylacetylene, 3-(4-bromophenyl)propionic acid, 4-bromophenylacetic acid, 4-(4-bromophenyl)butanoic acid, trimethylsilylacetylene and phenylboronic acid were purchased from Oakwood Products Inc. Tetrakis(triphenylphosphine)palladium and trans-dichlorobis(triphenylphosphine)palladium(II) were obtained from Strem Chemicals. 1,4-Diiodo-2,5-dibromobenzene was purchased from TCI America. Wang resin (pre-loaded with amino acid) and Fmoc-protected amino acids were obtained from Advanced Chem Tech. Ethanol was purchased from Pharmco-AAPER. 5-Bromo-2-thiophenecarboxylic acid was obtained from Accela ChemBio Co. Ltd. 4-iodobenzoic acid was purchased from Alfa Aesar. ((2,5-dibromo-1,4-phenylene)bis(ethyne-2,1-diyl))bis(trimethylsilane),\(^{75}\) Benzo[1,2-b:4,5-b']dithiophene\(^{76}\) and 2,6-bis(trimethylstanny1)benzo[1,2-b:4,5-b']dithiophene\(^{76}\) were prepared following the literature protocol and \(^1\)H NMR matched literature data. Biotech grade cellulose ester dialysis tubing
(MWCO 500-1000) was purchased from Spectrum Labs. All other reagents and starting materials were obtained from Sigma-Aldrich and were used as received.

**NMR Spectroscopy:** ¹H-NMR spectra were obtained using a Bruker Advance 400 MHz FT-NMR spectrometer and processed with Bruker Topspin 1.3. Peptide ¹H NMR spectra were acquired using a presaturation pulse to suppress water. Chemical shifts are reported in parts per million relative to the residual protio solvent [HOD δ: 4.79, CHCl₃ δ: 7.26].

**Electrospray Ionization Mass Spectrometry (ESI-MS):** ESI samples were collected using a Thermo Finnigan LCQ Deca Ion Trap Mass Spectrometer in negative mode. Samples were prepared in a 1:1 MeOH:water solution with 1% ammonium hydroxide.

**Reverse-Phase HPLC:** HPLC purification was performed on an Agilent 1100 series (semi-preparative/analytical) and a Varian PrepStar SD-1 (preparative) instruments using Luna 5 μm particle diameter C8 with TMS endcapping columns with silica solid support. An ammonium formate aqueous buffer (pH 8) and acetonitrile was used as the mobile phase.

**UV-Vis and Photoluminescence:** UV-Vis spectra were obtained using a Varian Cary 50 Bio UV-Vis spectrophotometer. Photoluminescence spectra were obtained using a PTi Photon Technology International Fluorometer (QuantaMaster 40) with a 75-W Ushio Xenon short arc lamp and operated with Felix32 Version 1.2 software. Spectroscopic samples were prepared by diluting the peptide solution to the appropriate concentration (exact concentrations given in spectra captions) in Millipore water to achieve an optical density near 0.1. The pH was then adjusted by adding 30 μL of 2M KOH (basic) followed by addition of 80 μL of 2M HCl (acidic).

**Circular Dichroism (CD):** CD spectra were obtained using a Jasco J-810 spectropolarimeter. Spectroscopic samples were prepared by diluting the peptide solution to the appropriate
concentration (exact concentrations given in spectra captions) in Millipore water. The pH was then adjusted by adding 30 μL of 2M KOH (basic) followed by addition of 80 μL of 2M HCl (acidic).

**Dynamic Light Scattering (DLS):** CD spectra were obtained using a Zetasizer Nano-ZS90 (Malvern Instruments). Spectroscopic samples were prepared by diluting the peptide solution to the appropriate concentration (exact concentrations given in spectra captions) in Millipore water. The pH was then adjusted by adding 30 μL of 2M KOH (basic) followed by addition of 80 μL of 2M HCl (acidic).

**Attenuated total reflection Fourier transform infrared (ATR-FTIR):** ATR-FTIR spectra were obtained on lyophilized acidic peptide solutions using a Thermo Scientific Nicolet iD5 ATR-IR.

**Transmission Electron Microscopy (TEM):** Imaging was performed on a Philips EM 420 transmission electron microscope equipped with an SIS Megaview III CCD digital camera at an accelerating voltage of 100 kV. Acidic solutions (0.1%) of each peptide were prepared by placing the samples in a closed container with a vial of conc. HCl opened within, which initiated the assembly process by diffusion of HCl vapor to the sample. The assembled peptides in water were pipetted (drop of 1 mg/mL solution) onto 200 mesh copper grids coated with carbon and incubated for 5 minutes at 25°C. Excess solution was wicked off by touching the side of the grid to filter paper. The samples were then stained with a 2% uranyl acetate solution and excess moisture was wicked off. The grid was allowed to dry in air before imaging.

**General solid phase peptide synthesis (SPPS)**

All peptides were synthesized using the standard Fmoc solid phase technique with Wang resin pre-loaded with the terminal amino acid (Wang-Val, 0.700 mmol/g). To the resin in a peptide chamber, Fmoc-deprotection was accomplished by adding a (1:4) piperidine/DMF solution twice
(successive 5-and 10-minute treatments) followed by washing with NMPx3, methanolx3 and DCMx3. For the amino acid couplings, 3.0 eq. of the Fmoc-protected amino acid was added along with 2.9 eq. of HBTU and 10 eq. diisopropylethylamine (DIPEA). The reaction mixture was allowed to mix for 45–90 minutes, after which was rinsed with NMP, methanol and DCM (3 times each). The completion of all couplings was monitored using a Kaiser test on a few dry resin beads, repeating same amino acid coupling as needed. The general procedure for amino acid coupling was repeated until the desired peptide sequence was obtained.

**General N-acylation procedure for peptides**

Following our previous procedure, a solution containing 3 eq. of aryl halide carboxylic acid, HBTU (2.9 eq.) and DIPEA (10 eq.) was mixed for 3 h with the resin forming an N-acylated peptide capped with desired halide. The completion of the couplings was assessed using a Kaiser test on a few dry resin beads. The resin was washed with NMP, methanol and DCM (3 times each).

**General On-Resin Stille Coupling Procedure**

The solid supported peptide capped with an aryl halide was made following the general SPPS and N-acylation procedures. The resin (1 eq.) was transferred to a Schlenk flask equipped with a reflux condenser. The resin was dried under vacuum. Pd(PPh₃)₄ (4 mol%, relative to resin loading) was added to the reaction vessel. An approximately 15 mM solution of the bis-stannylated aryl reagent (0.50 eq) was prepared in DMF. The solution was added to the reaction flask via syringe. The mixture was heated to 80°C for 16-21 h and was agitated constantly by bubbling nitrogen through the solution. The mixture was allowed to cool to room temperature. The peptide was subjected to the general cleavage and work-up procedure to yield the crude product, then further purified by HPLC.
General On-Resin Suzuki Coupling Procedure

The solid supported peptide capped with an aryl halide was made following the general SPPS and N-acylation procedures. The resin (1 eq.) was transferred to a Schlenk flask equipped with a reflux condenser. The resin was dried under vacuum. Pd(PPh₃)₄ (4 mol% relative to resin loading) and benzene-1,4-diboronic acid (0.55 eq.) were added to the reaction vessel. K₂CO₃ (8 eq.) was dissolved in 0.50 mL of water and was added to the reaction flask along with 5-10 mL DMF via syringe. The mixture was heated to 80°C for 20-27 h and was agitated constantly by bubbling nitrogen through the solution. The mixture was allowed to cool to room temperature. The resin was washed with water and then subjected to the general cleavage and work-up procedure to yield the crude product, then further purified by HPLC.

General cleavage, work-up procedure of peptides

Following solid-phase cross-coupling, the resin was returned to the peptide chamber and again subjected to a standard wash cycle: 3x NMP, 3x DMF, 3x methanol and 3x DCM. The resin was treated with 9.50 mL of trifluoroacetic acid, 250 μL water, and 250 μL of triisopropylsilane for 3 h. The peptide solution was filtered from the resin beads, washed 3x with DCM, and was concentrated by evaporation under reduced pressure. The crude peptide was then precipitated from solution with 60-80 mL of diethyl ether and isolated through centrifugation. The resulting pellet was triturated with diethyl ether to yield crude product, which was dissolved in approximately 10-15 mL of water and 50 μL potassium hydroxide (1M) and lyophilized. The solution was placed inside dialysis tubing of the appropriate length. The tubing was stirred in 1L of water for 1 h, the water was exchanged, and the tubing was allowed to stir for another 1 h. This process was repeated twice and then the tubing stirred overnight (approx. 15 h). The tubing was removed from water, and the peptide solution transferred to a separate container and lyophilized.
Molecular simulations

All simulations were conducted using the Gromacs 2018.6 simulation suite patched with the PLUMED version 2.2 enhanced sampling libraries. The Automated Topology Building (ATB) server was used to generate topologies and initial configurations of oligopeptides modeled using the GROMOS 54a7 force field. Dimers of peptides 1a, 1b, 1c, and 1d (Figure 1) containing no (n = 0), methylene (n = 1), ethylene (n=2), and propylene (n = 3) spacers were initialized with a center of mass separation $|d| = 0.45$ nm and a relative twist angle of $\theta = 10^\circ$, placed in a cubic simulation box with 1.5 nm spacing from the box edges to the molecule and employing three-dimensional periodic boundary conditions, and solvated in water with simple point charge (SPC) model. Simulations were conducted in the NPT ensemble at 1 bar and 300 K employing a Parrinello-Rahman barostat and velocity-rescaling thermostat. Initial dimer configurations were equilibrated using steepest descent energy minimization to remove forces larger than 50 kJ/mol.nm followed by 100 ps NVT and NPT equilibration runs. Initial molecular velocities were drawn from a Maxwell–Boltzmann distribution at 300 K. Equations of motion were numerically integrated using the leap-frog algorithm with a 2 fs time step. Lennard-Jones interactions were smoothly shifted to zero at 1.0 nm. Particle mesh Ewald was used to treat electrostatic interactions with a 1.0 nm real-space cutoff and 0.16 nm Fourier grid spacing, both of which were optimized for performance during runtime. Covalent bonds involving hydrogen were held fixed using the LINCS algorithm. Well-tempered metadynamics simulations employing the signed twist angle $\theta$ between the dimers as a collective variable were performed for each spacer length with a Gaussian deposition pace of 1 ps, width of 0.2 radians, initial height of 1.2 kJ/mol, and bias factor of $\gamma = 5.0$. Harmonic restraining potentials with a force constant of 500 kJ/mol.rad are applied if the twist angle exits the window $\theta = [-1.25,1.25]$ radians. Harmonic restraining potentials with a
force constant of 500 kJ/mol.nm are applied at center of mass separations of $|d| > 0.6$ nm. Estimates of the unbiased potential of mean force (PMF) as a function of the twist angle $F(\theta)$ were extracted from the converged well-tempered metadynamics runs by reweighing contributions to negate the effects from all sources of imposed bias. By tracking the height of the deposited Gaussians, the frequency of transitions in $\theta$, and stabilization of the PMF profiles over the course of the simulation, 2 $\mu$s were found sufficient to produce well-converged PMF estimates for all spacer lengths considered with data from the terminal 1 $\mu$s employed for analysis.

**Machine learning**

We consider intermolecular distances between the $m = 46$ peptide wing atoms in each molecule to furnish $2m^2$ pairwise distances – $m^2$ for each of the two sets of proximate peptide wings – and define a featurization $x_i \in \mathbb{R}^{m^2} = \mathbb{R}^{2,116}$ for each snapshot $i$ of the simulation trajectory. The snapshot is additionally assigned a label $y_i \in \{+1, -1\}$ defining the instantaneous supramolecular chirality: (+1) if it is M-type (left-handed) with $\theta = [0, \pi]$ and (-1) if it is P-type (right-handed) with $\theta = [-\pi, 0]$. Given training data $X = \{(x_1, y_1), (x_2, y_2), ..., (x_N, y_N)\}$ a linear SVM attempts to learn a maximal margin separating hyperplane between the two classes within the feature space under the decision rule,

$$f(x) = sgn(w \cdot x + b),$$  \hspace{1cm} \text{Eqn. 1}$$

where $w$ and $b$ are parameters to be learned. The vector $w$ corresponds to weights given to different features in $x$ discriminating between the two classes and can be interpreted as the orientation of the SVM hyperplane in the feature space. The scalar $b$ is a bias term that can be interpreted as the offset of the hyperplane from the origin. In practice, the data is typically not
linearly separable, and we instead adopt a soft margin SVM corresponding to the following convex optimization for the parameters $w$ and $b^{89}$,

$$
\min_{w, b, \xi} \frac{1}{2} \|w\|^p + C \sum_{i=1}^N \xi_i \quad \text{Eqn. 2}
$$

subject to: $y_i(w \cdot x_i + b) \geq 1 - \xi_i; \xi_i \geq 0 \forall i = 1, ..., N$,

where the slack variables $\xi_i$ measure the distance from the corresponding classification boundary of misclassified samples, the penalty parameter $C$ controls the relative weighting within the objective function between misclassification of points to maximization of the margin, and $p$ defines the norm used for the weight decay term. To train this soft margin SVM for chirality classification each feature over the extracted $N$ samples is standardized to zero mean and unit variance with 80% partitioned as training data and the remaining 20% held-out to evaluate classification accuracy. Conventionally, SVMs use the L2 norm ($p = 2$) where all features in $x$ contribute to the decision through non-zero weights in $w$. Fitting an L2 norm SVM achieved reasonable classification accuracies of 65-75% (Table S1).

If instead we employ the L1 norm ($p = 1$), this has the property of generating trained SVMs with sparse solutions wherein some of the weights $w$ are set exactly to zero. Varying $C$ under the L1 norm may be conceived as a means of performing feature selection to identify those features that are most important in discriminating supramolecular chirality. At $C=0$ the objective function in Eqn. 2 contains no contribution from classification accuracy and the L1 norm returns the null model ($w = 0$). As $C$ increases from zero the trained model accumulates progressively more non-zero elements within $w$ corresponding to the participation of more features (i.e., inter-atomic pairwise distances) into the classification model. As more features are included in the model the classification accuracy improves, but we observe a knee in the accuracy at a particular number of
features. Beyond this point there are diminishing marginal improvements in classification accuracy upon incorporating more features (Figure S1). The L1 norm SVM performs automated feature selection by picking out those intermolecular pairwise distances that are most discriminatory of the dimer chirality. To identify those atoms in the peptide wings most important in distinguishing chirality we analyze the SVMs trained up to the knee and assign each atom a rank. The rank is defined as the sparsity (i.e., number of non-zero coefficients) in the L1 norm SVM model within which it first appeared. For example, in the case of methylene spacer (n = 1) the C18 atom first appeared within the C18-C14 pairwise distance in the 5th non-zero coefficient model, meaning it was assigned a rank of 5. We average the ranks of each atom over the four molecules (n = 0, 1, 2, 3). Atoms with high ranks (i.e., low numerical values) are those that play important roles in discriminating chirality across the four molecules.

Results:

Scheme 1: Peptide synthesis protocol using Pd-catalyzed on-resin coupling method. X = Br, I; Y = SnMe3, B(OH)2.

Design consideration and synthetic strategy: Syntheses of the peptide-spacer-π conjugates were achieved via Pd-mediated cross coupling. We previously showed that pre-formed di-acids with alkyl spacers separating the π-system were difficult to synthesize and were poorly soluble.90 We were also worried that the increased acidity of the methylene protons adjacent to the π-system might also lead to undesired reactivity in longer conjugated structures. Herein, Fmoc-based solid-
phase peptide synthesis was used to synthesize the peptidic regions followed by N-acylation of the oligopeptides to install a portion of the chromophore (Ar\(^1\)) onto the peptidic backbone. These acylation partners were functionalized with bromine/iodine making them suitable for palladium catalyzed on-resin couplings with another arene (Ar\(^2\)) to synthesize peptide-\(\pi\)-peptide compounds (Scheme 1). The generality of the method was validated by using either distannylated (\(Y = \text{SnMe}_3\)) cores for Stille coupling and diboronated (\(Y = \text{B(OH)}_2\)) cores for Suzuki coupling. In contrast to our prior synthetic strategies, we show here how N-acylating agents with varied alkyl spacers are suitable for this chemistry.

Using this method, all peptides were synthesized in moderate yield (Figure 1). Following our prior molecular design,\(^7\) 4-iodobenzoic acid was used as zero-carbon spacer N-acylating agent which was then coupled with stannyl or boronic acid reagents leading to 1a, 2a, 3a or 4a respectively (\(n = 0\), Figure 1). Peptides with methylene spacers (1b, 3b, 4b) were synthesized using 4-bromophenylacetic acid as the N-acylating Ar\(_1\) group with different Ar\(_2\) groups under Stille conditions, whereas 2b was synthesized using Suzuki coupling conditions. Ethylene spacer peptides (1c, 2c, 3c, 4c) were synthesized using 3-(4-bromophenyl)propionic acid as the Ar\(_1\) group, and propylene spacer peptides (1d, 2d, 3d, 4d) were synthesized similarly with 4-(4-bromophenyl)butanoic acid as the Ar\(_1\) group. Attempts to use a thiophene based Ar\(_1\) group alkyl spacer (e.g. 2-(5-bromothiophen-2-yl)acetic acid) were unsuccessful: a significant color change was observed during activation, and the corresponding peptides were isolated in extremely low yield. It is possible that during activation of the carboxylic acid to promote solid-phase coupling, the acidic \(\alpha\)-carbon protons (between the thiophene and the carboxylic acid) might be deprotonated competitively. The peptide sequences were mainly chosen to maintain molecular solubility in aqueous conditions. We found previously that VEVAG-sequences led to soluble peptide-\(\pi\)-peptide
conjugates at basic pH and hence they were used again here. At basic pH, the carboxylic acid groups (C-terminal and glutamic acid side chains) remain deprotonated and impart intramolecular repulsion thus preventing aggregation. The pentapeptide sequence was kept constant for all the peptides whereas spacer length and chromophore structure were varied to identify the effect of spacer and chromophores in the self-assembly processes which were visualized using electron microscopy and monitored through solution phase spectroscopic techniques.

**Figure 1**: Peptides synthesized with different π-chromophores and with varying spacer length.

**Electron microscopy**: The nanostructures were prepared by acidifying the aqueous ‘dissolved’ peptide solutions with HCl vapor in a closed chamber (assembled peptides were pipetted onto copper grids coated with carbon followed by staining with 2% uranyl acetate solution) and were observed by TEM. TEM imaging revealed that the introduction of a spacer group did not have a dramatic or predictable influence on the adopted supramolecular morphology. All the nanostructures form 1-D tape like structures with high aspect ratios that are on the order of micrometers in length which are comparable to other peptide-π-peptide conjugates reported previously. The nanostructures showed different widths and persistence lengths as expected from these highly disperse supramolecular networks. For example, peptide 1a formed nanostructures that were generally over microns in length whereas 1b-1d all formed relatively shorter nanostructures that were only few hundreds of nanometers in length (Figure 2). Nanostructures formed from 1a and 1d were around 10.2 ± 0.6 nm and 10.4 ± 0.6 nm respectively in diameter while those from 1b and 1c had relatively smaller widths (ca. 5.2 ± 0.5 nm and 6.7 ±
0.7 nm respectively). Other TEM images may be found in the Supporting Information (Figures S50-S53). Although the sizes of the nanostructures do not follow any specific trends, these observed bundling characteristics indicate different extents of lateral interactions of one or more filaments are impacted by the spacer length and the associated changes in electrostatic interactions and solvation of the molecules. We did not observe any finer superstructure details of these nanostructures using AFM or TEM (e.g. twisting or coiling) that could be related in any way to the chirality of molecular precursors or the overall handedness of the resulting supramolecular assemblies.

![Representative TEM images of peptides](image)

**Figure 2:** Representative TEM images of peptide **1a** (1st row, left), **1b** (1st row, right), **1c** (2nd row, right), **1d** (2nd row, left).

**UV-Vis absorption and photoluminescence:** Photophysical responses of different peptides in acidic (pH 2) and basic (pH 8) conditions provide information about the extent of electronic coupling within peptide-π-peptide nanostructures. In these types of triblock molecules, the acidic glutamic acid side-chains are deprotonated at basic pH which renders the peptides essentially “dissolved” while acidic pH screens the carboxylate charges via protonation and allows for self-assembly. The photophysical responses recorded here should be treated as ensemble averages of different local chromophore environments because the materials tend to form highly disperse
networks with varying widths and persistence lengths as evident from TEM images. Four different chromophore segments were studied, where each segment was subject to different spacer lengths. In line with established studies, exciton coupling (i.e. interaction of the transition dipoles associated with interacting chromophores that leads to new electronic states) in these tri-block molecules is expected to result from the hydrogen-bonding between amino acids in the peptidic regions that forces cofacial interactions among the chromophores. Absorption spectra of all the peptides in the assembled state showed significant blue-shifts with respect to the spectra recorded in molecularly dissolved solutions, which indicates classic H-type aggregation (i.e. transition dipoles of the chromophores are oriented in parallel-like fashion shifting the absorption maxima to higher energies side). Generally, stronger co-facial interaction should be responsible for larger blue-shifts in the absorption maxima, when moving from the molecularly dissolved to the assembled states. However, quadrupole repulsion between the chromophores might compete with cofacial stacking, potentially moving to more of a twisted slip-stack type configuration. Incorporation of a spacer should enable conformational freedom which was not accessible in peptides with direct conjugation of carboxamide groups and internal \( \pi \)-systems.

The absorption profiles of the chromophores in molecularly dissolved (basic) conditions show that carboxamide groups are decoupled from the chromophore regions when the spacer groups are inserted. As a representative example, the direct carboxamide attachment of 3a in basic solution shows a \( \lambda_{\text{max}} \) of 397 nm vs a substantially blue-shifted 372 nm in methylene-spaced 3b (Figure 3a). Furthermore, the methylene, ethylene and propylene spaced compounds (3b, 3c and 3d, respectively) had near-identical \( \lambda_{\text{max}} \) values in basic conditions which indicates a lack of \( \pi \)-character in the intervening alkyl spacers regardless of the length (Figure S62c). These trends were also apparent in the molecularly dissolved spectra of 1a-d and 2a-d (Figure S62a-b). Upon
assembly in acidic media, the classic signatures associated with H-like aggregation were observed: 

3a showed a blue shift of about 40 nm in assembled state whereas 3b, 3c and 3d showed blue shifts of about 25-30 nm (Figure 3a, S62c). More conformational freedom around the peptide-π chromophore connection region for spacer-peptides may lead to geometric changes in the excited states resulting in low oscillator strengths which are also possible due to breaks in conjugation between carboxamide group and the π-cores. Similar results were found for assemblies derived from 1a-d and 2a-d (Figures S62a-b).

UV-Vis spectroscopy of molecularly-dissolved peptides 4a-4d showed similar features at ca. 240 nm and 290 nm. The lower-energy features revealed the spacer-dependent differences, whereby 4a had a peak absorption at 354 nm with a shoulder at 402 nm, while 4b had a blue shifted peak at 338 nm with a shoulder at 387 nm (Figure 3b). Ethylene and propylene spaced variants 4c and 4d were essentially identical to the methylene spacer system (Figure S62d). Upon going from molecularly dissolved (basic pH) to aggregated (acidic pH) state, the low-energy absorption maxima were blue shifted for all the chromophores (4a-4d) indicating H-type aggregation that would suggest more co-facial interaction of the chromophores. 4a showed blue shifts of ~10 nm whereas 4b-d showed blue shifts of ~5 nm.

Figure 3: UV-Vis spectroscopy of (a) 3a,3b and (b) 4a,4b at basic (solid lines) and acidic (dashed lines) condition acquired at a concentration of 20 µM. Basic solutions were made by adding 30 µL of KOH (2M) and acidified by adding 50 µL of HCl (2M) to the basic solution.
Monomeric peptides with no inserted spacers show relatively unstructured emission profiles whereas those with alkyl spacer units show more structured peaks with clear vibronic progressions (Figures 4a, 4b, S63, S64, S65). The spectral signatures also vary significantly between the unassembled (basic pH) and aggregated (acidic pH) state, again suggestive of the formation of H-type aggregates as evident from the pronounced quenching of the signal intensity. Subtle variations in the component peptide residues can tune excimer-like (weakly coupled chromophores) to exciton-like (tightly coupled chromophores) structure formation which was primarily justified based on the hydrophobicity of the component residues and associated aqueous solvent influences. In general, no-spacer molecules show broad, featureless emission peaks resembling excimer type-structures in their aggregated states whereas more vibronic features were apparent for peptides with alkyl spacer units. Representative spectra are shown in Figure 4 for the phenyl-benzodithiophene-phenyl series (4a-4d). This shows that peptides with spacer units (4b, 4c, 4d) show structured spectra with pronounced higher-energy vibronic features whereas the emission from the direct peptide-attached 4a shows a broad featureless spectral signature.

Figure 4: PL spectroscopy of (a) 4a-4d in basic (ca. pH 10) solution (excitation λ: 355 nm (4a), 344 nm (4b-4d)) and (b) 4a-4d in acidic (ca. pH 2) solution (excitation λ: 345 nm (4a), 338 nm (4b-4d)) acquired at a concentration of 5 µM. Basic solutions were made by adding 30 µL of KOH (2M) and acidified by adding 50 µL of HCl (2M) to the basic solution.

Circular dichroism: Circular dichroism (CD) was also employed to examine the locally chiral chromophore environments imposed during molecular self-assembly. Peptide hydrogen bonding
networks are routinely analyzed by CD spectroscopy based on the collective response of the amide n-π* transitions. The assembled peptides here typically showed a clear minimum around 220-230 nm region which is indicative of a twisted β-sheet formation that is red-shifted from the ideal β-sheet signature of 216 nm (Figure S66). FT-IR spectra from lyophilized samples also showed amide I bands (ca. 1640-1620 cm⁻¹) associated with typical β-sheet type signature (Figure S54-S57). More importantly, all the peptides showed exciton-coupled bisignate Cotton effects (i.e. the sign change of the CD signal around absorption maxima due to the chiral interaction of transition dipoles of adjacent chromophores) for the π-π* transitions associated with the π-conjugated chromophores, where the CD signatures undergo a change in sign near the chromophore’s absorption maximum. The influences of subtle changes in the molecular structure of the local arrangement of the chromophores were clearly visible from the CD spectra. Longer chromophores usually show more intense signatures, as expected from their greater UV-Vis extinction coefficients. A pronounced “odd-even” effect depending on the spacer length in between the π-core and the peptidic region was visible which resulted in variation of the sign of the CD signal. We found negative Cotton effects for peptides with no spacer (n = 0) and ethylene spacer (n = 2) that correspond to M-type helical supramolecular aggregates (akin to the left-handed supramolecular twist in natural β-sheet peptides) whereas peptides with methylene (n =1) and propylene (n = 3) spacers show positive Cotton effects corresponding to P-type helical bias (Figure 5a and 5b). Idealized illustrations are shown in Scheme S1.

Another obvious CD feature was the “odd-even” change in intensity. The intensity of the Cotton effect varied from one spacer length to another which gives an idea about the extent of π-π interaction and the strength of the subsequent exciton coupling. For example, 3a (no-spacer) and 3c (2-carbon spacers) showed higher molar ellipticities whereas 3b (1-carbon spacer) and 3d (3-
carbon spacers) showed relatively weaker signatures (Figure 5c). These trends were also apparent in the CD signature of 1a-d and 4a-d (Figure 5a, 5b). In principle, a more organized nanostructure with a stronger helical response should result in higher molar ellipticity, however, the possibility of having both left and right-handed helical orientations and highly dynamic helical segments within a more disordered assembly can also contribute to relatively lower molar ellipticities.

Peptides with embedded benzodithiophene units (4a-4d) showed interestingly different behavior as compared to the other series (Figure 5d). 4a without a spacer displayed a small degree of exciton coupling whereas peptides (4b, 4c and 4d) with spacers did not show the split Cotton band but rather a non-zero response with peaks that mirrored those in the absorption spectrum. This would indicate that these chromophores are not undergoing exciton coupling but rather are nevertheless held in the chiral environments dictated by the peptide helical assemblies. We speculate that the larger quadrupolar surfaces of the phenyl-appended benzodithiophene cores are frustrated from significant exciton coupling in the assembled state due to the additional freedom imparted by the intervening alkyl spacers.

Odd-even effects are commonly observed for n-alkanes and their derivatives, impacting for example their melting point and glass-transition temperature. Pronounced odd-even effects are also observed for assembly of n-alkanes on monolayer surfaces resulting from different conformation of chains and also impacting their charge conduction properties. In liquid crystals, odd-even effects lead to notable changes in thermal properties, and the movement of an asymmetric center along the terminal alkyl spacer led to alternating ferroelectric properties. Field-effect mobilities were also tuned periodically with odd and even number of carbons in alkyl side chains of a diketopyrrolopyrrole-vinylene thiophene conjugated polymer.
To the best of our knowledge, chiroptical impacts of odd-even alternation and their impacts in peptide-\(\pi\) conjugated systems have not been widely investigated\(^{38}\).

**Figure 5:** CD spectroscopy of peptides 1a-1d (a), 2a-2d (b), 3a-3d (c) and 4a-4d (d) in their assembled states (acidic pH). Spectroscopic data were collected at 20 \(\mu\)M concentration. Solutions were prepared adding 30 \(\mu\)L of KOH (2M) and then made acidic using 50 \(\mu\)L of HCl (2M).

**Computational Studies:**

To complement the experimental studies and resolve the molecular origin of the observed preferences for supramolecular chirality as a function of spacer length, we conducted all-atom molecular dynamics simulations of dimerized pairs of peptides 1a-1d. We previously employed molecular dynamics simulation and free energy calculations to quantify the thermodynamic driving forces underpinning the assembly into nanoaggregates of a closely related family of
oligopeptides containing oligophenylvinylene π-systems\textsuperscript{104}. These calculations revealed (i) a favorable free energy for the addition of each subsequent peptide monomer to the growing nanoaggregate of $\sim 25 k_BT$, (ii) a favorable peptide-peptide driving free energy of $\sim 100 k_BT$ that is counterbalanced by a large unfavorable solvent contribution of $\sim 80 k_BT$ and a small unfavorable intrapeptide contribution of $\sim 10 k_BT$, and (iii) the interaction between the π-cores to be responsible for $\sim 20\%$ of the peptide-peptide interaction with the remainder made up by interactions between the π-cores and the peptide wings and between the peptide wings. In the present work we conduct molecular simulations to quantify the chiral preferences of dimers as a function of spacer length. The chiral preferences resolved by our molecular simulations of the dimer are in excellent agreement with those observed in experiment, suggesting that our reductionist focus on the dimer as the fundamental unit of a self-assembled nanoaggregate stack is well founded. We employ well-tempered metadynamics\textsuperscript{87} as an enhanced sampling technique to estimate the potential of mean force (PMF) as a function of the twist angle $\theta$ between the dimer pair. A similar approach was previously employed by Kang et al. to identify metastable configurations of camptothecin dimers.\textsuperscript{67} The twist angle is defined as the signed angle between the cores of two dimerized oligopeptides arbitrarily indexed as molecule 1 and 2,

$$\theta = \cos^{-1}(\mathbf{m}_1 \cdot \mathbf{m}_2) \cdot \text{sgn}((\mathbf{m}_2 \times \mathbf{m}_1) \cdot \mathbf{d})$$

\textbf{Eqn. 3}

where $\mathbf{m}_1$ and $\mathbf{m}_2$ are unit vectors connecting the terminal aromatic core carbon atoms in molecules 1 and 2, respectively, and $\mathbf{d}$ is the unit vector directed from the center of mass of molecule 1 to molecule 2. The sense of the vectors $\mathbf{m}_1$ and $\mathbf{m}_2$ and identity of molecules 1 and 2 is immaterial provided that they are consistently defined. The definition of $\theta$ in \textbf{Eqn. 3} can be understood as follows. The first term, $\cos^{-1}(\mathbf{m}_1 \cdot \mathbf{m}_2)$, computes the unsigned angle between the two vectors $\mathbf{m}_1$ and $\mathbf{m}_2$ and is restricted to the range $[0-\pi]$ radians. The second term,
\( sgn((m_2 \times m_1) \cdot d) \), endows \( \theta \) with a sign by computing the handedness of the twist through the projection of cross product \( m_2 \times m_1 \) onto \( d \) and taking the sign. So defined, the angle lies in the range \( \theta = [-\pi, \pi] \), where positive values of \( \theta \) correspond to left-handed twist angles (M-type supramolecular stacking) and negative values to right-handed (P-type). Inverting the identity of the molecules leaves the twist angle unchanged. Inverting the sense of one or other of \( m_1 \) and \( m_2 \) replaces the twist angle with its negative supplement \( \theta \rightarrow (\pi - \theta) = (\theta - \pi) \). By the symmetry of the oligopeptides, twist angles of \( \theta \) and \( (\theta - \pi) \) define equivalent configurations of the dimer and the PMF need only be estimated over the range \( \theta = [-\pi/2, \pi/2] \). In fact, we resolve all the interesting features of the PMF and markedly improve our sampling efficiency by restricting sampling to the range \( \theta = [-1.25, 1.25] \) wherein we apply harmonic restraining potentials if the twist angle moves outside of this window. We elected not to perform sampling over the full range \( \theta = [-\pi/2, \pi/2] \) as tests showed that this frequently led to the formation of interlocked configurations in the vicinity of \( \pm \pi/2 \) where each peptide would curl around the other in a configuration resembling a pair of interlocking “U” characters or a link in a chain. These configurations are artifacts associated with the dimer at large twist angles and are not representative of configurations observed in multimeric peptide stacks. These kinetically trapped configurations led to hindered sampling in \( \theta \) and required prohibitively long simulations to reach convergence. We also applied harmonic restraining potentials to the center of mass separation between the oligopeptides for \( |d| > 0.6 \text{ nm} \) in order to maintain the oligopeptides within a contact dimer and better mimic the conditions within a multimeric peptide stack. Full details of our molecular simulation and enhanced sampling protocols are provided in the Experimental Methods.

The unbiased PMFs as a function of the twist angle \( F(\theta) \) estimated from well-tempered metadynamics simulations at each of the four spacer lengths are presented in Figure 6. In excellent
agreement with experimental observations, the global minimum of the PMF for no and ethylene spacers (n = 0, 2) lies at positive values of $\theta$ corresponding to a thermodynamic preference for a M-type (left-handed) supramolecular stacking, whereas for ethylene and propylene spacers (n = 1, 3) it lies at negative values of $\theta$ corresponding to P-type (right-handed) stacking. This agreement between experiment and computation lends strong support to our hypothesis that pairwise intermolecular interactions govern supramolecular chirality. We now proceed to interrogate our simulation data to identify the molecular-level root of this effect.

**Figure 6.** Potentials of mean force (PMF) as a function of the signed twist angle $\theta$ within the oligopeptide dimer for each of the four spacer lengths. Positive twist angles $\theta = [0, \pi]$ correspond to M-type (left-handed) supramolecular stacking whereas negative twist angles $\theta = [-\pi, 0]$ correspond to P-type (right-handed) stacking. Harmonic restraining potentials with a force constant of 500 kJ/mol.rad is applied if the twist angle exits the window $\theta = [-1.25, 1.25]$ radians and of 500 kJ/mol.nm for center of mass separations $|d| > 0.6$ nm. The PMFs $F(\theta)$ are computed over the terminal 1 $\mu$s of the metadynamics simulation runs. Uncertainties are estimated from block averages over PMFs computed over five 200 ns contiguous blocks where each PMF is shifted to be mean-free and error bars calculated pointwise. In agreement with experimental observations the oligopeptides with no and ethylene spacers (n = 0, 2) show a thermodynamic preference for M-type supramolecular chirality ($\theta > 0$) whereas for methylene and propylene spacers (n = 1, 3) impose a preference for P-type chirality ($\theta < 0$). Insets show representative snapshots of the dimer system near the respective global minima where water is removed for clarity. Molecular renderings are constructed using VMD\textsuperscript{105}. 
A number of prior experimental and computational studies have explored chirality transfer from molecular chiral centers to supramolecular assemblies\textsuperscript{24, 39, 67-69}, with a subset of these focused on chirality inversion mediated by molecular spacers\textsuperscript{43, 58, 106, 107}. Proposed molecular mechanisms underpinning supramolecular chirality transfer include hydrogen bonding, hydrophobicity, electrostatics, host-guest interaction, metal-ligand coordination, and solvent mediation\textsuperscript{24}. To date, however, it has remained a challenge to identify from molecular simulations the molecular mechanism underpinning the observed chirality inversion. This failure can be largely attributed to the high-dimensional nature of the molecular simulation trajectories and challenges in comprehensively sampling the thermally-accessible phase space. In this work, we surmount both of these challenges by performing metadynamics enhanced sampling to comprehensively explore the relevant oligopeptide configurations and by training simple "white box" machine learning models to identify the molecular variables that are most responsible in discriminating the supramolecular chirality. Specifically, we take each frame of our metadynamics molecular simulations where $|d| < 0.6$ nm and featurize it with the intermolecular pairwise distances between all atoms in the peptide wings (i.e., excluding atoms in the core or spacer regions). Considering the $m = 46$ peptide wing atoms in each molecule furnishes $2m^2$ pairwise distances $- m^2$ for each of the two sets of proximate peptide wings – defining a featurization $x_i \in \mathbb{R}^{m^2} = \mathbb{R}^{2,116}$ for snapshot $i$ of the simulation trajectory. The simulation snapshot is assigned a label $y_i \in \{+1, -1\}$ defining the instantaneous supramolecular chirality: (+1) if it is M-type (left-handed) with $\theta = [0, \pi]$ and (-1) if it is P-type (right-handed) with $\theta = [-\pi, 0]$. It is our hypothesis that training L1-regularized linear support vector machine (SVM) classifiers to predict supramolecular chirality as a function of intermolecular pairwise distances through a linear relation $\hat{y}_i = f(x_i)$ we can distill from our model those atoms that are the most important discriminants of chirality. This
approach can be conceived of as performing simultaneous model training and feature selection to identify a sparse model – one that uses a small number of intermolecular pairwise distances – to make a predictive classification of chirality. Those intermolecular distances that are retained by the model identify those atoms with the greatest discriminatory power in correctly identifying whether the molecules have a thermodynamic preference for M-type or P-type stacking. We present full mathematical details of our approach in the Experimental Methods.

We present in Figure 7A a rank ordered list of the atoms identified by our SVMs to possess the highest discriminatory power in distinguishing molecular chirality. A chemical structure providing a key to the atom labels is provided in Figure 7B. The highest-ranked atoms are those involved in hydrogen bonding (H11, O9, H6, O8) and the hydrophobic alanine and valine side-chain atoms (C18, C14, C15). This indicates that the formation of hydrogen bonds and packing of hydrophobic side-chain atoms are critical discriminants of supramolecular chirality. The high ranks of hydrophobic side-chain atoms suggest that unfavorable handedness may lead to conformational changes along the backbone which discourage good packing of these sidechains into hydrophobic cavities created by the neighboring molecule (Figure 7C). The consistently highly ranked backbone carbonyl oxygens (O9, O8, O7, O6) and amide hydrogens (H11, H6, H10) further reveal that hydrogen bonding along the backbone plays an important role with hydrogen-bonding participants nearer to the core generally selected as more impactful in discriminating chirality than those farther along the peptide wing.
Figure 7. Atoms identified by the L1 norm SVM as those most discriminatory of supramolecular chirality. (A) Atomic ranks averaged over all four molecules with spacers n = 0, 1, 2, and 3. The rank is defined as the sparsity of the L1 norm SVM model in which the atom first appears. High ranks (i.e., 1, 2, 3, …) imply that the atom is an important discriminant of chirality. (B) Chemical structure indicating the locations of the atoms within the peptide wings. The color coding of the chemical structure matches that in (A). (C) Selected snapshot from the molecular simulation of the methylene spacer (n = 1) dimer. The thermodynamically preferred right-handed configuration exhibits better hydrophobic packing of the Ala sidechain united-atom carbon (C18, circled) against the other chain, whereas in the thermodynamically disfavored left-handed configuration it is solvent exposed.

Finally, we performed a decomposition of the intermolecular interaction energy into the Coulombic, π-π interactions, and hydrogen bonding interactions as a function of spacer length averaged over our molecular simulation trajectories (Table 1). We observe that interactions between the π-cores constitutes ~15% of the total intermolecular interaction energy and is
approximately constant as a function of spacer length. The magnitude of this contribution is similar to the value of ~20% we previously determined for oligopeptides containing oligophenylvinylene π-cores.\textsuperscript{104} The hydrogen bonding interactions are responsible for ~20% of the interaction energy for no spacer (n=0), but this falls to ~10% for n=1,2,3. A similar trend is observed in the Coulombic contribution, which decreases monotonically from ~25% at n=0 to ~15% at n=3. These trends reflect our observations from simulation wherein hydrogen bonds – particularly those nearest to the aromatic cores – are less frequently made for the molecules containing spacers than those without, likely due to the larger configurational flexibility provided to the peptide wings by the spacers. Our results also show the Coulombic contribution to the intermolecular interaction energy is dominated 3:1 or more by the Lennard-Jones contribution at all spacer lengths.

Table 1. Decomposition of the intermolecular interaction energy within the peptide dimer as a function of spacer length. The total interaction energy is the sum of the Coulombic and Lennard-Jones interactions between all atoms in one molecule and all of those in the other. The π-core – π-core interaction comprises the intermolecular Coulomb and Lennard-Jones interactions restricted to those atoms within the phenyl-thiophene-phenyl core. The hydrogen bonding interaction comprises the intermolecular Coulomb and Lennard-Jones interactions between the C=O and N-H groups within the peptide wings.

<table>
<thead>
<tr>
<th></th>
<th>no spacer (n=0)</th>
<th>methylene spacer (n=1)</th>
<th>ethylene spacer (n=2)</th>
<th>propylene spacer (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coulombic</td>
<td>(25.5 ± 5.1) %</td>
<td>(19.7 ± 0.8) %</td>
<td>(18.7 ± 4.7) %</td>
<td>(15.9 ± 1.0) %</td>
</tr>
<tr>
<td>π-core – π-core</td>
<td>(13.0 ± 0.5) %</td>
<td>(14.8 ± 0.3) %</td>
<td>(15.3 ± 0.6) %</td>
<td>(14.1 ± 0.5) %</td>
</tr>
<tr>
<td>hydrogen bonding</td>
<td>(19.7 ± 1.7) %</td>
<td>(11.8 ± 0.3) %</td>
<td>(10.6 ± 1.4) %</td>
<td>(11.3 ± 0.6) %</td>
</tr>
</tbody>
</table>
Conclusions:

We have developed a synthetic methodology to incorporate alkyl spacers in between peptides and π-electron groups as a way to mitigate the deactivating/electron withdrawing influence of the directly conjugated peptide carboxamide groups. UV-Vis and CD spectroscopy of the supramolecular aggregates indicated that subtle changes in molecular structure correspond to different arrangements of π-π stacking in the supramolecular aggregates. This was especially pronounced in the CD spectra as the changes in spacer length resulted in changes of supramolecular chirality of the nanostructures. TEM showed a variety of nanostructure bundling characteristics. Accompanying all-atom molecular dynamics simulations and interrogative machine learning revealed a thermodynamic origin for the experimentally-observed chiral preferences and revealed the formation of hydrogen bonds and packing of hydrophobic side-chain atoms to be primary discriminants of the supramolecular chirality. The observed formation of well-defined nanostructures with substantially different chiroptical properties provides an essential guideline for the synthetic chemist to design hierarchical structures from different molecular starting points. It is thus necessary to not only understand the molecular origins of a given self-assembly but to also develop reliable and predictive design rules for self-assembled nanowires with hierarchical order that can be optimized for energy migration as we have done here for supramolecular helicity and chromophore coupling. Future studies will probe the chiroptical implications of these nanostructures in the context of energy transport and biological recognition.

Notes:

The authors declare no financial conflicts.

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References:


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