DESIGN, SYNTHESIS, AND SELF-ASSEMBLY OF SEMICONDUCTING PEPTIDES FOR BIOELECTRONIC MATERIALS

By

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Abstract

Utilization of organic electronic materials has recently become an evolving area of biomedical research, particularly for applications in tissue regeneration. However, aside from the ability to activate electrosensitive tissues, a material’s control over cell fate is dependent on many other factors such as biocompatibility, ability to form hydrogels, mechanical properties, and alignment. We have previously developed self-assembling organic electronic systems based on peptides containing π-conjugated subunits directly in the backbone, which are capable of possessing these crucial properties. To further develop these materials, several investigations were conducted and will be discussed.

First, to simplify the synthesis of these systems and therefore enhance their practicality, new synthetic approaches utilizing solid-phase palladium-catalyzed cross-couplings were developed. These procedures were used to create a library of peptide-π hybrids and the self-assembly and electronic properties of the materials were elucidated by spectroscopy, microscopy, and analysis of field-effect transistor device performance. Next, to alter the assembly properties, novel multivalent architectures were also created by modifying the previously developed synthetic procedures and employing tri- and tetravalent discotic π-conjugated cores. Finally, to enhance the electronic properties of the materials, donor-acceptor peptide-π hybrids were synthesized. Characterization of these systems via steady-state and time-resolved spectroscopy showed that they were capable of photoinduced electron transfer, thus allowing for the creation of charge-separated states with light and in the absence of chemical doping or external electric fields.

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Chapter 1 – Introduction

Bioelectronic Materials

Utilization of organic electronic materials has recently become the forefront of tissue regeneration. This is due to their ability to activate electrosensitive tissues, e.g. nerves and cardiac tissues, which occurs as a result of the conductive microenvironment provided by the electronic materials.\textsuperscript{1-5} For instance, reports have shown that electrical stimulation of neurons within these conducting scaffolds can enhance cell adhesion, promote stem cell differentiation, and accelerate axonal outgrowth.\textsuperscript{6-10} Langer and coworkers illustrated this by deducing the effect of electrical stimulation of PC-12 cells on oxidized polypyrrole films.\textsuperscript{10} The films were prepared and oxidized using poly(styrenesulfonate) as a dopant, then further modified by adsorption of a protein monolayer. The PC-12 cells were incubated on top of the films, where they preferentially adhered (in comparison to a poly(lactic acid) polymer control), most likely due to the superior adsorption of the positively charged matrix proteins on the negatively charged polymer scaffold surface. Electrical stimulation was then performed by subjecting the cells to a steady potential of 100 mV for two hours, using the polypyrrole film as the anode and a gold wire as the cathode. It was determined that median neurite length post-electrical stimulation was nearly double (18.14 µm) in comparison to that of the unstimulated cells (9.5 µm).

Aside from electrical stimulation, there are several other factors that determine the efficiency in which a material can control cell fate. First, the mechanical properties of the material in question are of utmost importance.\textsuperscript{11-15} Hydrogel materials are typically used to promote tissue regeneration due to their high water content and porosity. Furthermore, they can also achieve mechanical properties that are comparable to native tissues which can be used to direct stem cell differentiation. Engler \textit{et. al.} tested this by creating polyacrylamide
gels coated with collagen and varying their elasticity by changing the degree of chemical crosslinking.\textsuperscript{15} They found that the various elasticities promoted differences in the differentiation of human mesenchymal stem cells, as shown in Figure 1.1. Soft gels, in the vicinity of 1 kPa elasticity which featured mechanical properties similar to brain tissue, promoted the formation of neuronal cells. Gels with an elasticity nearer to 10 kPa caused differentiation into the myoblast lineage due to their similarity to striated muscle tissue. Finally, gels at 100 kPa elasticity resembling collagenous bone tissue ultimately differentiated stem cells into osteoblasts.

![Image](image.png)

Figure 1.1. Microenvironment elasticity of various bodily tissues and graph of differentiation marker fluorescence vs substrate elasticity, illustrating the type of cells differentiated from substrates with various mechanical properties. Adapted with permission from ref. 15.

The 3-D shape of the materials also plays a significant role. For instance, the macromolecular alignment of the scaffolds can promote the aligned growth of cells and, in the case of neurons, encourage axonal outgrowth in one dimension through a process called contact guidance which is helpful in the repair of damaged neural axon tracts.\textsuperscript{16-18} Stupp and coworkers have shown this by creating macroscopically aligned scaffolds composed of
supramolecular peptide amphiphile nanofibers.\textsuperscript{18} This was done by heat treating solutions of the peptide amphiphiles which produced aligned lyotropic liquid crystalline regions followed by pipetting the mixture into a CaCl\textsubscript{2} solution, promoting alignment of the self-assembled nanostructures as seen in Figure 1.2a. When the neural cells were seeded within these aligned domains, the neurite segments were found to extend within 20° of the scaffold long axis in contrast to those cells seeded on unaligned scaffolds where no directional bias was evident (Figure 1.2b).

Figure 1.2. a) SEM of macroscopically aligned peptide amphiphile nanostructures and b) diagram of direction of neurite outgrowth relative to the axial direction of aligned and unaligned nanostructures. Adapted with permission from ref. 18.

Finally, the ability of the material to bind cells is imperative. It has been found that this can be achieved through the expression of particular small peptide fragments that mimic certain cell-binding proteins in the extracellular matrix (ECM).\textsuperscript{19-23} For instance, the arginine-glycine-aspartic acid tripeptide (RGD, found in many ECM proteins such as fibronectin), and the isoleucine-lysine-valine-alanine-valine pentapeptide (IKVAV, laminin derived) are known to not only bind cells, but can also be responsible for guiding their growth and promoting specific differentiation. Stupp and coworkers have shown this by integrating an IKVAV
sequence into their peptide amphiphile systems, as shown in Figure 1.3a. These peptide amphiphiles self-assemble into tube-like nanostructures (Figure 1.3a and b), effectively decorating the tube surface with the cell-binding motifs. IKVAV has specifically been shown to encourage neurite sprouting, so this was tested by assembling the peptide in the presence of murine neural progenitor cells. As can be seen in Figure 1.3c, after less than 24 hours, neurite outgrowth from the cells assembled with the IKVAV-containing peptide amphiphile nanostructures is evident, while cells seeded within nanostructures containing a non-cell-binding motif (EQS) had no noticeable outgrowth (Figure 1.3d).

Figure 1.3. a) Structure of IKVAV-containing peptide amphiphile and supramolecular nanostructures formed from their aggregation, b) SEM of the supramolecular nanostructures and light field images of neural progenitor cells assembled with c) IKVAV-containing amphiphile, showing neurite outgrowth and d) EQS-containing amphiphile, showing no significant neurite outgrowth. Adapted with permission from ref. 23.
All of these properties that are vital to tissue regeneration can be obtained through materials composed of peptides containing organic electronic moieties which are capable of supramolecular assembly. The π-conjugated subunits, organized supramolecularly by the peptidic scaffolds, can be used to induce conductive microenvironments for electrical stimulation. The assembly capabilities of the peptide materials can allow for the formation of hydrogels, whose elasticities can be modulated by altering primary amino acid sequence and/or hydrogen bonding interactions.\textsuperscript{24} The assembly can also be regulated to allow for control of the 3-dimensional shape and overall macromolecular alignment of the scaffold materials.\textsuperscript{25,26} And finally, cell-binding peptide fragments can be easily expressed on the surface via synthetic manipulation.
Peptide Self-Assembly

Figure 1.4. General structure and self-assembly of peptide amphiphiles studied by Stupp and coworkers. Adapted with permission from ref. 27.

Nature uses hydrogen bonding as a means to promote self-assembly, specifically as an approach to fold peptides and proteins into secondary structures, including α-helices and β-sheets. One could imagine that hydrogen bonding between peptides could be exploited to initiate the self-assembly of distinct monomers into a variety of different supramolecular structures. Tirrell and Stupp have pioneered this field with the development of peptide amphiphiles.27,28 Systems developed by Stupp, as shown in Figure 1.4, simultaneously rely on hydrophobic interactions and intermolecular hydrogen bonding in order to self-assemble into well-defined 1-D nanostructures.27 The structure of the molecules contains four key features. First, they have a hydrophobic tail in the form of a long, unbranched alkyl chain. This tail gives the peptide its amphiphilic properties and, when assembled in aqueous media, allows for the expression of the peptidic portion of the molecules on the outside of the supramolecular structures. Next to the alkyl tail, the molecules contain a short peptide
sequence made up of hydrophobic amino acids organized into sequences capable of forming β-sheet-like intermolecular hydrogen bonds. These amino acids are responsible for the 1-D nature of the supramolecular structures, in comparison to bilayers and micelles formed by amphiphilic molecules that are not capable of intermolecular hydrogen bonding. Following these amino acids, the next region is comprised of charged amino acid residues to promote aqueous solubility and also allow for the molecules to undergo assembly using pH changes or salt addition as triggers. Finally, the C-terminal end of the peptide can consist of bioactive epitope sequences for interacting with cells or proteins, which are expressed on the exterior of the supramolecular structures. The combination of the amphiphilic nature of these peptides and the β-sheet-like structure forming amino acids ultimately result in 1-D nanotubes, as shown in Figure 1.4.

Figure 1.5. Assembly mechanism of β-amyloid fiber formation. Adapted with permission from ref. 29.

Another type of peptide self-assembly, β-amyloid fiber formation, has also been extensively studied.²⁹,³⁰ Specifically, Boden and coworkers have proposed the assembly mechanism.²⁹ Figure 1.5 illustrates this mechanism, showing the energetic minima in the process. First, a peptide, typically one comprised of sequences capable of intermolecular β-sheet-like hydrogen bonding, adopts a conformation so that these hydrogen bonding interactions can occur. Many peptide fragments assemble in a β-sheet type array, creating a
“tape”, reminiscent of a coiled phone cord. This tape then can adopt a helical ribbon conformation, allowing for facial interactions with other ribbon-like structures to produce fibrils. From there, fibrils can interact facially with one another to create amyloid fibers. This type of peptide self-assembly is distinct from the assembly of peptide amphiphiles, as it does not require synthetically appending hydrophobic alkyl chains to the peptide termini to create amphiphilic molecules. Actually, this general mechanism is known to occur in nature, particularly in the aggregation of Aβ peptides, which is the cause of amyloid plaques that lead to Alzheimer’s disease.31
**π-Conjugated Oligomer Assembly**

Organic semiconductors generally consist of π-electron-rich molecules and have been studied in a variety of forms, including polymers, oligomers, and small molecules. In the case of polymers, defects within the chains, chemical impurities, and variations in chain lengths can have a negative effect on performance.\textsuperscript{32} Using small molecules or oligomeric units in place of polymers is attractive because of their well-defined molecular structures and minimal structural defects, which allow for better control over conjugation length and molecular orientation.\textsuperscript{32-35}

The semiconducting ability of arrays of small molecules and oligomers is dependent on the conjugation length as well as the efficiency of orbital overlap between the intermolecular π-stacks, which is designated by the overall organization of the component molecules.\textsuperscript{32,33,36,37} Garnier and coworkers have shown that changing these two variables can have profound effects on device performance when oligothiophenes are incorporated in field-effect transistors.\textsuperscript{32,33,36} Anthony and coworkers have specifically shown how substantial a role overall organization can play in device performance, using crystals comprised of substituted pentacenes.\textsuperscript{37} In their work, two triisopropylsilyl acetylene-substituted pentacene derivatives were synthesized. The first, \textbf{1}, shown in Figure 1.6a, is substituted on the center ring, creating a symmetric molecule. A model of the solid state ordering of the compound is also shown in Figure 1.6a. With this symmetric substitution, the individual molecules stack in a columnar array in the crystal structure, with the pentacene rings overlapping one another and interacting through π-stacking. The second pentacene derivative, \textbf{2}, shown in Figure 1.6b, is substituted at the second ring, creating a less symmetric molecule. Crystallographic analysis showed that this derivative organizes into a herringbone-like array in the solid state. Resistivity measurements were obtained for both crystals by attaching electrodes in the Montgomery method configuration.\textsuperscript{38} In the case of compound \textbf{1}, the resistivity along the axis perpendicular to the stacked pentacenes (indicated by the arrow in Figure 1.6a) was found to
be $2.5 \times 10^6 \, \Omega \, \text{cm}$. Alternatively, the crystal of compound 2 showed over a 3 orders of magnitude higher resistivity along all axes, the lowest being $8 \times 10^9 \, \Omega \, \text{cm}$. This example stresses the need for organization between small molecules for optimal electron delocalization and, hence, for greater conductivity.

Figure 1.6. Structures, space filling models of solid phase crystal packing, and calculated experimental resistivity of a) 1 and b) 2. Adapted with permission from ref. 37.

Kasha et al. have reported the exciton model of simple dimeric aggregates, which can help gain insight into the orientation of assembled π-conjugated chromophores that are interacting within an ordered supramolecular structure. The models are described by approximating the excited state resonance interaction of the molecules through the electrostatic interaction of their transition dipoles based on a vector model. The two most extreme cases are shown in Figure 1.7a and b. The ovals represent the light-absorbing molecules, while the arrows depict the transition dipoles. In the first case (Figure 1.7a), the
two molecules aggregate such that the transition dipoles are in-line with respect to one another, in a J-aggregation conformation. Upon excitation of the dimer, the dipoles can arrange in an “in-phase” conformation, leading to an electrostatic attraction, and lowering the energy of the E’ excited state in comparison to the monomer. Alternatively, the dipoles could align in an “out of phase” manner, causing electrostatic repulsion, and increasing the energy of the E” excited state. Excitation from the ground state to the lower energy excited state (E’) of the dimer results in a red-shifted absorption spectrum, in comparison to the monomer. Furthermore, J-aggregates commonly exhibit increased photoluminescence intensity.40

Figure 1.7. Energy diagram model for aggregated a) “in-line” (J) dimers and b) “parallel” (H) dimers. Adapted with permission from ref. 39.

The diagram in Figure 1.7b illustrates the model that is obtained from the organization of parallel transition dipoles in the aggregated dimers.39 This type of aggregation, known as H-aggregation, is somewhat more common. Here, the “out of phase” transition results in
electrostatic attraction and lowering of the $E'$ energy level. The “in-phase” transition gives rise to repulsion and an increase in the $E''$ level. Because the excitation from the ground state to the excited state $E'$ is forbidden, excitation occurs to the higher $E''$ state, so the absorption spectrum of an H-aggregated dimer is blue-shifted with respect to its unaggregated monomer. Typically, H-aggregates also exhibit a quenching of photoluminescence intensity.

![Image](image_url)

**Figure 1.8.** a) Structures of ureidotriazine-appended OPV monomers, b) schematic of hydrogen bonding between ureidotriazine units to form homodimers, c) absorption, photoluminescence, and circular dichroism spectra of OPV samples at various temperatures, and d) proposed model of self-assembled structures of OPV homodimers. Adapted with permission from ref. 41 and 42.

To facilitate organization, small molecules can be synthetically manipulated, commonly by the addition of appropriate substituents. These substituents can then interact through intermolecular forces, such as Van-der-Waals forces, π-stacking interactions, or hydrogen bonding, to form supramolecular arrays of small molecules. Forming these arrays can act as a means to organize the components, placing the π-conjugated molecules
within appropriate distances for electron delocalization. For instance, Meijer and coworkers have used a combination of π-stacking and hydrogen bonding interactions to create supramolecular systems of substituted oligophenylenevinylene (OPV) oligomers 3 (n=1), 4 (n=2), and 5 (n=3), as depicted in Figure 1.8a. They installed an ureidotriazine unit onto the OPV π-conjugated subunit. The ureidotriazine moieties are “self-complementary”, meaning that they can produce hydrogen bonded homodimers, as shown in Figure 1.8b. To determine the self-assembly behavior of the system, 5 was subjected to different temperatures, from 283 K to 373 K, while dissolved in dodecane, and the absorption and photoluminescence spectra were acquired (Figure 1.8c). From these data, they determined that 5 is either molecularly dissolved or existing as hydrogen bonded homodimers at high temperatures, but at lower temperatures the molecules aggregate through π-π interactions between OPV units. Circular dichroism spectra were also obtained for 5 in dodecane, which produced a bisignate Cotton effect at the π-π* transition of the chromophore at low temperatures, suggesting interaction of the OPV oligomers within a chiral stack. When heated the signal decreased and at 373 K no meaningful signal was seen, suggesting breakdown of the supramolecular assemblies into monomers or hydrogen bonded dimers. An illustration of the proposed structure of the supramolecular stacks formed from the hydrogen bonded homodimers of these OPV systems, elucidated from the spectroscopic data as well as microscopy, is shown in Figure 1.8d. The helical twist was proposed to arise from the chiral alkoxy substituents on the OPV units.
Due to the well-known assembly capabilities of biological macromolecules, such as DNA, carbohydrates, and peptides, some groups have employed these scaffolds to promote π-conjugated oligomer assembly.\textsuperscript{52-54} In particular, Meijer and coworkers exploited the double helix assembly of DNA by synthesizing thymidylic acid-appended OPV derivatives and investigating their hydrogen bond induced self-assembly with complementary adenylic acid oligomers.\textsuperscript{52} Through spectroscopic and AFM characterization, they determined the two-component system assembles into helical stacks with widths similar to those expected from one OPV molecule and two adenylic acid moieties, leading them to propose the assembly model shown in Figure 1.9. Here, OPV molecules form a hydrogen bonded complex with two adenylic acid oligomers, which ultimately forms the helical stacks with the OPV units embedded in the center.

Figure 1.9. Thymidylic acid-appended OPV derivatives studied by Meijer and coworkers and their hydrogen bond induced self-assembly with complementary adenylic acid oligomers. Adapted with permission from ref. 52.
Table 1.1. Representative structure motifs of π-conjugated peptide systems from the literature, based on substitution position of the electronic subunits.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Connectivity</th>
<th>Example Peptide</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N-terminus <em>π</em> - peptide</td>
<td><img src="image1.png" alt="Peptide Structure" /></td>
<td>62</td>
</tr>
<tr>
<td>2</td>
<td>Single side chain <em>π</em> - peptide</td>
<td><img src="image2.png" alt="Peptide Structure" /></td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>Double side chain <em>π</em> - peptide</td>
<td><img src="image3.png" alt="Peptide Structure" /></td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>Backbone <em>π</em> - peptide</td>
<td><img src="image4.png" alt="Peptide Structure" /></td>
<td>65</td>
</tr>
</tbody>
</table>

We and others have sought to exploit peptide self-assembly as a scaffold to assemble π-conjugated subunits within close distances to facilitate electronic delocalization. This has been done through modifying standard peptides by appending the conjugated oligomers to various positions on the peptide chain. Once these subunits are incorporated, self-assembly can be triggered (generally by addition of a salt or nonsolvent, or a pH change), allowing for the formation of supramolecular structures and the organization of the π-conjugated oligomers. A variety of synthetic strategies have been used to introduce π-conjugated subunits onto a peptide chain, and some key examples from the literature are illustrated in Table 1.1. Meijer, Schenning, and coworkers created a system which appended a substituted OPV unit to the N-terminus of a peptide (entry 1). This was done through an
amide coupling between a carboxylic acid group on the OPV component and the peptide N-terminus. Alternatively, Parquette and coworkers investigated the utilization of reactive groups on amino acid side-chains (entry 2). Specifically, they took advantage of the primary amine of a lysine residue to append a naphthalene dianhydride unit via imidation. Furthermore, Bäuerle and coworkers synthesized a system which embedded a quaterthiophene moiety between two peptide chains (entry 3). This was done through incorporating an unnatural amino acid, 4-azidophenylalanine, into a peptide fragment. Huisgen-type “click” chemistry was then utilized to append one peptide fragment to either side of bisacetylene quaterthiophene.

![Diagram](image)

Figure 1.10. Schematic and space filling illustrations of self-assembled peptides with π-conjugated oligomeric units embedded directly in the backbone, showing the expression of the peptides on the exterior of the supramolecular structures.

Another somewhat less investigated strategy is to incorporate the π-conjugated oligomer directly in the peptide backbone. This technique is desirable because, after self-assembly and β-amyloid-like fiber formation, the organized array of stacked π-conjugated oligomers will be insulated within a peptidic exterior and excluded to a greater extent from
interactions with other external factors such as solvents, as depicted in Figure 1.10. Our group, as well as the Stupp lab, has investigated means to synthesize these types of modified peptides.\textsuperscript{65-67} Stupp and coworkers utilized an entirely solution-phase technique to access their quinquethiophene-containing peptide-\textpi hybrid, which is shown in Table 1.1, entry 4.\textsuperscript{65}

First, a peptide fragment was prepared through successive amino acid couplings, each intermediate being purified via column chromatography. A quinquethiophene dicarboxylic acid was synthesized separately via repeated Stille couplings. After isolation of both reactants, they were combined through double amidation between the carboxylic acid groups of the oligothiophene unit and the N-termini of two peptide fragments, to create a symmetric (C-N N-C) peptide, embedded with the chromophore.
Previous Work on the Synthesis of Peptide-π Hybrids

Scheme 1.1. Synthesis of bithiophene “amino acid” 6 via Stille cross-coupling and peptide-π hybrid 7 via solid-phase peptide synthesis. Reprinted with permission from ref. 66.

Peptide-π hybrids where the π-conjugated chromophore is situated in the peptide backbone could be immensely beneficial to the field of regenerative medicine, due to their electroactive, hydrogel-forming, supramolecular properties. However, to make these system practical for in depth studies, their synthesis must be facile, unlike the extensive solution-phase synthesis used by Stupp and coworkers that was previously described.65 Our group has worked to minimize the synthetic burden by creating procedures that employ solid-phase techniques, specifically solid-phase peptide synthesis.66,67 The first effort was completed by Dr. Stephen Diegelmann. He synthesized bithiophene-containing “amino acid” 6 through a Stille coupling between thiophene species terminated in an Fmoc-protected amine and ester, respectively, according to Scheme 1.1.66 Following saponification, he then used this unnatural π-conjugated amino acid directly in standard Fmoc solid-phase peptide synthesis to obtain the bithiophene-containing peptide-π hybrid 7.
At high pH, the carboxylic acid groups of the peptides are deprotonated, and the peptides remain unassembled due to electrostatic repulsions. However, upon the addition of acid, the carboxylic acid moieties begin to become protonated and self-assembly can occur, ultimately allowing the formation of self-supporting hydrogels at low weight percentages as shown in Figure 1.11a. Furthermore, the supramolecular 1-D nanostructures resulting from the acid-triggered self-assembly were visible by atomic force microscopy (AFM) (Figure 1.11b). The drawback of this synthetic procedure was the relatively difficult preparation of the bithiophene “amino acid”. Due to the non-symmetric nature of the molecule, preparing “amino acids” containing longer oligothiophene moieties and/or other semiconducting π-conjugated chromophores would be a considerable synthetic burden.
Scheme 1.2. “On-resin dimerization” procedure to synthesize peptide-π hybrids. Reprinted with permission from ref. 67.

To simplify the synthetic effort, our group discovered a different solid-phase synthetic strategy to incorporate the π-conjugated subunit within the peptide backbone, according to Scheme 1.2. Rather than preparing a non-symmetric π-conjugated “amino acid”, various π-conjugated dicarboxylic acid derivatives were synthesized and commercially available dianhydrides were used. Then, the peptide was prepared through standard Fmoc solid-phase peptide synthesis, followed by the introduction of the dicarboxylic acid or dianhydride derivative of the π-conjugated subunit to the resin under activating conditions. Site-to-site double amidation or imidation between the dicarboxylic acid or dianhydride and two peptide N-termini, positioned in close proximity to one another on a resin bead, took place. Then, after cleaving from the resin, symmetric peptide-π hybrids were obtained.
It was found that the peptides could also be triggered to self-assemble by lowering the pH of the solution. The self-assembly of these peptides was probed spectroscopically using absorption, photoluminescence, and circular dichroism spectroscopies of basic and acidic samples and representative spectra are shown in Figure 1.12a and b. Upon self-assembly, the UV-Vis absorption of the chromophore (solid line) exhibits a blue shift from that of the unassembled peptide (dashed line). Furthermore, the photoluminescence is quenched significantly. The circular dichroism spectra showed no signal when unassembled at basic pH (dashed line), but upon self-assembly showed a bisignate Cotton effect (solid line), suggesting that the chromophore subunits were experiencing excitonic interactions in a chiral...
environment, brought about by the assembled peptide scaffold. Due to this behavior, the assembly was assigned as an imperfect H-like aggregation, as designated by Kasha et. al., as illustrated in Figure 1.12c. Furthermore, solutions of the peptides at ca. 1 wt% were capable of forming self-supporting hydrogels, as depicted in Figure 1.12d. The nanostructure formation was also characterized through AFM. As shown in Figure 1.12e, the assembled structures had a high aspect ratio, spanning over a micron in length.

This "on-resin dimerization" technique proved useful for synthesizing a variety of peptide-π hybrid materials, however the chromophore dicarboxylic acid moieties still had to be synthesized separately, making the incorporation of more complex subunits difficult due to the synthetic burden and lack of chromophore solubility. These extended π-conjugated oligomer subunits would be beneficial to the overall functionality of self-assembling peptide-π hybrid materials, due to their tendency to have better electronic properties, such as being more efficient semiconductors. Due to this, novel approaches were investigated to access these potentially superior systems.
References


Chapter 2 – Development of Novel Synthetic Procedures for the Formation of Peptide-π Hybrids

Introduction

A potential means to enhance the electronic characteristics of the previously studied peptide materials would be to incorporate chromophores that exhibit better electronic properties. One method to do this is to increase the conjugation length of the π-electronic moiety. For instance, field effect transistors fabricated with oligothiophene thin films as the active layer have shown that conductivities increase with the number of thiophenes in the oligomeric chain from $10^{-11}$ Scm$^{-1}$ for terthiophene to $10^{-6}$ Scm$^{-1}$ for sexithiophene. So, a possible means to impart better electronic properties to the modified peptides, specifically the previously studied quaterthiophene-containing systems, could be to install longer oligothiophene moieties within the peptide backbone, allowing for more efficient intermolecular charge transfer.

Although the “on-resin dimerization” technique that has previously been used and described in Chapter 1 helped to minimize synthetic and purification steps en route to the desired peptide-π hybrids, the chromophore dicarboxylic acids still had to be synthesized separately via solution-phase techniques. This made it difficult to incorporate longer oligothiophenes, such as sexithiophene, because intermolecular interactions of these more complex π-conjugated oligomers cause the dicarboxylic acid analogues to become increasingly insoluble, and hence difficult to synthesize and manipulate. Furthermore, the lengthy solution-phase synthesis and purification of the π-conjugated units slowed the process of creating diverse libraries of peptide-π hybrids. So, in order to access peptide materials embedded with these potentially more conductive subunits, the development of new synthetic strategies was required.
Scheme 2.1. Solid-phase synthesis of oligothiophenes as described by Fréchet.  

Solid-phase syntheses have been employed extensively for the rapid construction of a variety of molecules. Instead of requiring time-consuming solution-phase purification after each step of a synthetic route, catalysts, side products, and reagents can simply be washed from the polymer support prior to cleaving the product from the resin and isolating it in relatively pure form. This method has been widely used for the synthesis of biological materials such as peptides and oligonucleotides and has also been adapted to perform a variety of other chemical transformations, such as palladium-catalyzed cross-couplings to form new carbon-carbon and carbon-heteroatom bonds.\(^{3-5}\) For example, solid-phase Stille and Suzuki cross couplings have been broadly employed for the creation of potential pharmaceuticals and π-conjugated oligomers.\(^{6-15}\) Fréchet and coworkers utilized this technique for the solid-phase synthesis of oligothiophenes, as shown in Scheme 2.1.\(^{11}\) Starting with a 2,2'-bithiophene-5-carboxylic acid moiety attached to a Merrifield-type resin via nucleophilic substitution, repeated cycles of NBS brominations followed by Stille cross-couplings with 2-(trimethylstannyl)-4-octylthiophene ultimately gave a quinquethiophene species which could be cleaved from the resin under basic conditions. The cleavage step could be completed at any stage of the synthesis, allowing for the formation of a variety of oligothiophenes in excellent yield and purity.
Solid-phase chemistry has also been used for intramolecular macrocycle formation due to the “pseudo-dilution effect” inherent to resins with low loading concentrations\textsuperscript{16}, but intermolecular site-to-site reactions between molecules adjacent to one another on a resin bead ("cross-linking") are also known on highly loaded resins. Taking advantage of this site-to-site reactivity allows for the formation of homodimers on the solid-phase, and a wide variety of homodimers have been prepared using transition metal coupling procedures such as ring-closing metathesis to produce alkene linkages and various acetylenic homocouplings to produce butadiyne linkers.\textsuperscript{17-20} For instance, Breinholt and coworkers were able to produce alkene-linked peptide fragments using a solid-phase metathesis procedure, as depicted in Scheme 2.2.\textsuperscript{17} Lysine and phenylalanine containing peptide fragments of various lengths terminated with an alkene through an alkyl linker (n = 1, 2, 3, 4, or 8) were synthesized via Fmoc solid-phase peptide synthesis on Rink resin. Then, solid-phase metathesis was performed in the presence of 1\textsuperscript{st} Generation Grubbs catalyst. Site-to-site reactivity allowed for the formation of alkene-linked homodimers, which could then be cleaved from the resin with trifluoroacetic acid to give mixtures of E and Z peptide alkene “dimers”.

Because solution-phase purification procedures are not needed for solid-phase syntheses, insolubility of reaction intermediates, such as quinque- and sexithiophene moieties, would not pose a synthetic challenge. So, to circumvent the solubility issue that plagued the synthesis of more complex oligothiophene-containing peptides, we sought to
utilize solid-phase Pd-mediated cross-couplings in conjunction with solid-phase peptide synthesis (SPPS).
Solid-Phase Palladium-Catalyzed Cross-Coupling Procedure

A peptide was synthesized as a test case via the solid-phase palladium-catalyzed cross-coupling procedure (Scheme 2.3). Following SPPS, 2-bromothiophene carboxylic acid (prepared by lithiation of 2,5-dibromothiophene, followed by quenching with CO$_2$) was coupled to the amine termini of the peptide chains via amidation under HBTU activation conditions. Stille cross-coupling conditions were used to couple the resin-bound 2-bromothiophene carboxamide moiety with stannylated Fmoc-protected thiophene amine (previously prepared by Dr. Stephen Diegelmann$^{21}$) to give a resin-bound peptide bearing a terminal bithiophene. This unit was then treated under standard SPPS to continue peptide growth, and subsequent cleavage from the resin afforded compound 1. In principle, we could use more complex Ar$^2$ units, but in practice, their synthesis with the requisite Fmoc groups is quite difficult, requiring multiple steps due to the asymmetry of the molecule, as well as multiple protecting group interconversions due to the sensitivity of the Fmoc protecting group to $n$-butyllithium, which is
necessary for stannylation. This method then requires additional SPPS in order to extend the peptide backbone. While this allows for the variation of primary amino acid sequence on either side of the chromophore, difficulties in the preparation of the Ar²-Fmoc segment led us to pursue an alternative strategy.
We sought a new strategy that capitalized on the ease of the on-resin dimerization crosslinking and on the versatility of Pd-mediated cross-coupling. This led to the development of the solid-phase palladium-catalyzed cross-coupling dimerization procedure, summarized in Scheme 2.4. After completion of the peptide synthesis via SPPS, diverse N-acyl end caps consisting of thiophene, bithiophene, and phenyl arenes (Ar\(^1\)) were coupled to the resin via standard HBTU-mediated amidation. The resin was then treated with a doubly-reactive second arene (Ar\(^2\)) under various palladium-catalyzed cross-coupling conditions. Double cross-coupling between Ar\(^2\) moieties and two Ar\(^1\) terminal units adjacent to one another on a resin bead occurred, resulting in a locally crosslinked structure and forming the full Ar\(^1\)-Ar\(^2\)-Ar\(^1\) π-conjugated subunit on the resin. This structure could then be cleaved from the resin to give the desired symmetric peptide-π hybrid.
Table 2.1. Peptide-π hybrids 2-9 synthesized via solid-phase palladium-catalyzed cross-coupling dimerization procedure.

<table>
<thead>
<tr>
<th>Peptide-π hybrids</th>
<th>Method</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Stille</td>
<td>DFAG-NH₃-S₃-S₃-N-GAFD</td>
</tr>
<tr>
<td>3</td>
<td>Stille</td>
<td>DFAG-NH₃-S₃-S₃-N-GAFD</td>
</tr>
<tr>
<td>4</td>
<td>Stille</td>
<td>DADGG-NH₃-S₃-S₃-N-GGDAD</td>
</tr>
<tr>
<td>5</td>
<td>Stille</td>
<td>DADDG-NH₃-S₃-S₃-N-GDDAD</td>
</tr>
<tr>
<td>6</td>
<td>Suzuki</td>
<td>VEVAG-NH₃-S₃-S₃-N-GAVEV</td>
</tr>
<tr>
<td>7</td>
<td>Sonogashira</td>
<td>DFAG-NH₃-S₃-S₃-N-GAFD</td>
</tr>
<tr>
<td>8</td>
<td>Stille</td>
<td>VEVAG-NH₃-S₃-S₃-N-GAVEV</td>
</tr>
<tr>
<td>9</td>
<td>Suzuki</td>
<td>VEVAG-NH₃-S₃-S₃-N-GAVEV</td>
</tr>
</tbody>
</table>

The library of peptide-π hybrids created using this procedure are shown in Table 2.1. Utilizing Stille cross-coupling conditions with thiophene and bithiophene-based end capped peptides and disubstituted transmetallating agents, peptides embedded with oligothiophene chromophores of varying length were synthesized, from terthiophene (2) to sexithiophene (5). 4-Iodophenyl carboxamide-capped peptides were subjected to Suzuki conditions in the
presence of 1,4-benzene diboronic acid and Sonogashira conditions with 1,4-diethynyl benzene to give oligophenylene-containing 6 and oligophenyleneethynylene-containing 7, respectively. Typically, following cross-coupling, the peptide is cleaved from the resin using a 2.5:2.5:9.5 water:TIPS:TFA cocktail, where TIPS is included as a radical scavenger to avoid undesired reactions from taking place during cleavage. Interestingly, in the case of 7, the addition of TIPS resulted in the partial hydrogenation of the alkynes, potentially due to a silane-mediated palladium-catalyzed hydrogenation reaction with a small amount of remaining palladium catalyst. To remedy this, TIPS was removed from the cleavage cocktail for this peptide. Finally, Stille or Suzuki conditions were used to give peptides embedded with chromophores consisting of alternating patterns of thiophene and benzene subunits, to produce 8 and 9, respectively. Due to the more readily available cross-coupling partners, this method has been much more widely explored in our lab.
Self-Assembly and Microscopy of Peptide-π Hybrids

Figure 2.1. Illustration of peptide-π hybrids and their self-assembly into 1-D nanostructures.

By design, these peptides remain molecularly dissolved in aqueous solution at basic pH due to repulsion between negatively charged carboxylic acid endgroups and sidechains. Intermolecular self-assembly of the peptide is triggered upon protonating these groups under acidic conditions by reducing the Coulombic repulsion among the molecules and therefore encouraging interpeptide hydrogen bonding and simultaneously inducing intimate π-electron interactions between the embedded chromophores. The end result is the production of 1-D nanostructures as illustrated ideally in Figure 2.1.
To visualize the nanostructures that are formed from the self-assembly of the chromophore-embedded peptides, transmission electron microscopy (TEM) was employed. Micrographs of assembled samples of 3, 4, 5, and 6 are shown in Figure 2.2. In general, each peptide forms 1-D nanostructures on the order of microns in length. Nanostructures of 3 and 4 displayed fairly uniform widths of approximately 6-9 nm (Figure 2.2a and b). Molecular modeling of these peptides show that their lengths in their most extended conformation are
4.1 and 4.9 nm, respectively. The nanostructures observed by TEM are comprised of individual nanostructures as well as bundles of 2 intertwined but well-resolved structures. 6 also appears to form consistent, well-defined nanostructures with diameters of 5-7 nm, which interact with other individual structures to form bundles of various widths, some reaching nearly 40 nm (Figure 2.2d). Furthermore, these extended bundled structures display a distinct helical twist. Alternatively, 5 appears to assemble into much smaller and less well-defined structures (Figure 2.2c). This could potentially be due to quadrupole interactions between the embedded sexithiophene cores prohibiting extended assembly.

Figure 2.3. 1 wt% solutions (upright vials, illuminated by a UV source) and hydrogel formation following solution acidification (inverted vials) of a) 2, b) 3, c) 4, and d) 7.

Upon acidification of 1 wt% aqueous peptide solutions, the formation of self-supporting hydrogels occurs. Figure 2.3 depicts the hydrogel formation. The upright vials contain 1 wt% solutions of peptides 2, 3, 4, and 7 at neutral pH. The solutions are emissive under a handheld UV lamp due to the unaggregated photoluminescent chromophore cores. Upon acidification via acid vapor diffusion, the self-supporting hydrogels form, as shown in the inverted vials. The emission of these hydrogels is quenched, due to the H-like aggregation of the internal π-conjugated moieties.
A key aspect of these materials is the exciton coupling between the transition dipoles of the chromophore moieties that occurs upon assembly, leading to well-characterized perturbations in the absorption and photoluminescence spectra. Therefore, absorption and
photoluminescence data were acquired for all peptides to observe the extent of intermolecular exciton coupling. Figure 2.4 shows these data for 2-9 in both their unassembled (pH 8, dashed lines) and assembled (pH 6, solid lines) states. In general, each peptide displays a blue shift in the absorption $\lambda_{\text{max}}$, and a quenching and red shift of photoluminescence upon assembly, which is indicative of cofacial H-like aggregation and/or excimeric interactions of the chromophore moieties. Although each peptide studied displayed these similar spectroscopic characteristics, slight differences are observed for individual peptides, presumably due to the differences in the identities of the chromophore subunits and their flanking peptides that collectively influence the electronic interactions within the aggregates. For instance, 4 and 5 display larger blue shifts in their absorption upon assembly (38 and 32 nm respectively) in comparison to 6 and 7, which show only a 15 and 7 nm shift, respectively. Differences in the magnitudes of the observed exciton couplings among these different chromophore units can be attributed to differences in their respective isolated excitation energies and/or the respective oscillator strengths of these transitions. 7 shows a large 82 nm red-shift in the photoluminescence $\lambda_{\text{max}}$ upon assembly, whereas this shift is only 32-39 nm in the cases of 2, 3, 6, 8 and 9, highlighting the different contributions of excimer-like emission within the different aggregates. Relatedly, the quenching of photoluminescence upon assembly occurs with varying degrees for each peptide. The photophysics associated with these materials are quite complex and require systematic molecular variations on a per-chromophore basis to explicate in more depth. Regardless of these specific photophysical differences, it is remarkable that all peptide-$\pi$ systems reported here undergo H-like self-association. Even longer oligomers embedded within 4, 5, and 7 undergo $\pi$-stacking and 1-D nanostructure formation.
Circular dichroism (CD) can also be utilized to deduce exciton coupling between chromophore moieties, particularly those experiencing coupling in a chiral environment. CD spectra for 2-9 are shown in Figure 2.5. Each peptide displays no meaningful absorption above 250 nm (corresponding to the chromophore π-π* transition) when unassembled in basic solution, but upon assembly all show bisignate Cotton effects with the crossovers.
occurring at the $\lambda_{\text{max}}$ of the respective chromophore UV-Vis absorptions. This suggests that the respective chromophores are interacting through exciton coupling within a chiral environment, which is created by the inherently chiral assembly of the peptide scaffolds, organized into hydrogen bonded sheet-like structures that display an overall helical twist. Slight differences are evident between individual peptides in the amide absorption region of the CD spectra between 200 and 250 nm. 2, 6, and 8 displayed relatively more intense signal in this region in comparison to the other studied peptides. This is possibly due to differences in peptide assembly, brought about by variations in primary amino acid sequences. We have found that the spectral signatures associated with classical peptide secondary structures ($\alpha$-helix, $\beta$-sheet, etc.) are not particularly diagnostic for these types of self-assembled materials due to their relatively disordered nature compared to standard $\beta$-sheet peptide models.

This developed procedure had given us a method that could be used to incorporate more complex $\pi$-conjugated subunits within peptide backbones than was previously possible such as the sexithiophene unit of 5. Sexithiophene is a well-established high-performance $p$-channel organic semiconductor that is usually included into transistor architectures via thermal evaporation. To elucidate the electrical properties that are possible with this compound in a biomimetic framework, assembled nanostructures of 5 were incorporated as the active layer of a field-effect transistor (Figure 2.6) in collaboration with Prof. Howard E. Katz’s group at Johns Hopkins University. A solution of 5 was dropcast on an SiO$_2$ substrate and then treated with concentrated HCl vapor to induce assembly. Once the sample was dry, gold source and drain electrodes were evaporated to complete the device. The hole mobility was calculated to be $3.8 \times 10^{-5}$ cm$^2$V$^{-1}$s$^{-1}$ by fitting the current-voltage data to the linear equation for transistor current. Although the mobility was possibly reduced by crystal grain boundaries due to the film’s polycrystalline morphology, the measurement demonstrates that these materials can transport charges through self-assembled networks. It is also important to recognize that these materials are ca. 70% peptidic “insulation” that would be inherently expected to impede
carrier mobility. Nevertheless, these mobilities are larger than those extracted from many other peptide-based field-effect transistor devices reported recently.\textsuperscript{26-28}

Figure 2.6. a) Schematic of assembly of 5, b) illustration of field-effect transistor, and Current-Voltage response plots (c) at gate voltages of 20V to -80V, with varied applied drain voltage and (d) at an applied drain voltage of -80V, with varied applied gate voltage.
Conclusion

Two novel synthetic procedures to create peptides embedded with \( \pi \)-conjugated subunits were developed. Each procedure utilized solid-phase palladium-catalyzed cross-couplings in conjunction with standard Fmoc solid-phase peptide synthesis. The new methods minimized solution-phase synthetic and purification steps, allowing for the creation of highly conjugated peptide-\( \pi \) hybrid materials that were previously inaccessible due to solubility issues. The self-assembly properties of peptides synthesized via the solid-phase palladium-catalyzed cross-coupling dimerization procedure were elucidated by absorption, photoluminescence, and circular dichroism spectroscopies, as well as visualized by TEM. It was found that each peptide in the library self-assembled into helical stacks, causing intimate electronic interaction between the embedded \( \pi \)-conjugated subunits in an H-like sense, and ultimately creating 1-D nanostructures. To determine the electronic properties of the materials, an assembled sample of sexithiophene-containing peptide 5 was incorporated as the active layer of a field-effect transistor. The dried hydrogel was found to have a hole mobility of 3.8x10\(^{-5}\) cm\(^2\)V\(^{-1}\)s\(^{-1}\), approximately an order of magnitude higher than other similar peptide based materials previously reported, proving that the assembled structures of these novel, highly conjugated peptide-\( \pi \) hybrids are capable of transporting charge.
Experimental

General considerations: THF was freshly distilled from sodium/benzophenone, or acquired from an Innovative Technologies Pure Solv solvent purification system and dried over 4 Å molecular sieves. DMF and diisopropylamine were purchased from Sigma-Aldrich and dried over 4 Å molecular sieves. Solvents were degassed by sparging with nitrogen for 30 to 90 minutes before use. All non-SPPS reactions were performed under an inert N2 atmosphere using flame-dried glassware. Tetrakis(triphenylphosphine)palladium was obtained from Strem Chemicals. N-Methylpyrrolidone (NMP), Wang resin (preloaded with an amino acid), and Fmoc-protected amino acids were obtained from Advanced ChemTech. O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU) was purchased from Oakwood Products Inc. 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. 2,5-bis(tributylstannyl)thiophene, 5,5′-bis-tributylstannyl-[2,2′]-bithiophene, and 5′-bromo-[2,2′-bithiophene]-5-carboxylic acid were prepare using literature procedures.29-31

NMR Spectroscopy: 1H-NMR spectra were obtained using a Bruker Avance 400 MHz FT-NMR spectrometer, and processed with Bruker Topspin 1.3. Peptide 1H NMR spectra were acquired using a 1 second presaturation pulse to suppress water.

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 0.1% ammonium hydroxide.

MALDI: MALDI-TOF samples were collected using a Bruker AutoFlexIII MALDI-TOF/TOF in positive mode. Samples were prepared in a 1:1 acetonitrile:water solution with 0.1% TFA with α-cyano-4-hydroxycinnamic acid as a matrix.

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 with an Ushio Xenon short arc lamp. Spectroscopic samples were prepared by diluting the peptide solution to the appropriate concentration in Millipore water to achieve an optical density near 0.1. The pH was then adjusted by adding 10 μL of either 1M KOH (basic) or 1M 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 in Millipore water. The pH was then adjusted by adding 10 μL of either 1M KOH (basic) or 1M HCl (acidic).

**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 endcappping columns with silica solid support. An ammonium formate aqueous buffer (pH 8) and acetonitrile were used as the mobile phase.

**Transmission Electron Microscopy (TEM):** Imaging was performed on a Philips EM 420 transmission electron microscope equipped with an SIS Megaview III CCD digital camera and a FEI Tecnai 12 TWIN transmission electron microscope equipped with a SIS Megaview III wide-angle camera. The samples were prepared by pipetting a drop of 1 mg/mL solution of assembled peptide in water 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 sample was then stained with a 2% uranyl acetate solution and excess moisture was wicked off. The grid was allowed to dry in air before imaging.

**Device Fabrication:** A neutral 1 wt% solution of 4 was dropcast atop a piranha cleaned SiO2 substrate and subjected to HCl vapor in a closed chamber for 3 minutes. After drying, 50 nm thick gold electrodes were thermally evaporated, at a rate of 0.4 angstroms/second, using a 200 mesh TEM grid as a shadow mask. The electrical readings were obtained using an Agilent 4155c semiconductor parameter analyzer. The mobility was calculated by fitting the transfer curve to the linear region of the field effect transistor equation:
\[ I_D = \mu C_i \left( \frac{W}{L} \right) V_D (V_G - V_{th}) \]

Where \( I_D \) is the drain current, \( \mu \) is the hole mobility, \( C_i \) is the gate insulator capacitance per unit area, \( W \) and \( L \) are the width and length of the channel, respectively, \( V_D \) is the drain voltage, \( V_G \) is the gate voltage, and \( V_{th} \) is the threshold voltage. The capacitance value for the 300 nm \( \text{SiO}_2 \) layer was 11.5 nF/cm\(^2\) and the electrodes had a width of 60 \( \mu \)m and a length of 8 \( \mu \)m.

**5-bromothiophene-2-carboxylic acid:** THF (50 mL) was added via cannula to a flame-dried 100 mL Schlenk flask. 2,5-dibromothiophene (10 mmol, 2.42 g) was added via syringe. The reaction vessel was cooled to -78°C and n-butyllithium (1.61M solution in hexanes, 11 mmol, 6.8 mL) was added dropwise over 5 minutes. The solution was allowed to stir at -78°C for 1 hr. Dry ice (approx. 50 g) was added to the reaction mixture, and was allowed to stir and return to room temperature for 18 hrs. under nitrogen. The light gray suspension was filtered and the white solid was stirred in 2M HCl for 1 hr. The suspension was filtered to yield a white solid (5.7 mmol, 1.2 g, 57%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta: 11.45 \) (br s, 1H), 7.64 (d, 1H, \( J = 4.0 \) Hz), 7.11 (d, 1H, \( J = 4.0 \) Hz).

**1,4-bis((trimethylsilyl)ethynyl)benzene:** 40 mL THF was transferred to a flame-dried Schlenk flask. 1,4-dibromobenzene (2.5 mmol, 0.060 g), Pd(PPh\(_3\))\(_4\) (0.10 mmol 0.12 g), and Cul (0.2 mmol, 0.004 g) were added. Trimethylsilylacetylene (6.0 mmol, 0.87 mL) and 10 mL DIPA were added via syringe. The mixture was heated to 45°C for 24 hrs. under nitrogen. The resulting suspension was filtered and the filtrate was concentrated under reduced pressure. The residual solid was dissolved in dichloromethane and washed with an aqueous ammonium chloride solution. The organic layer was concentrated and crude product was subjected to column chromatography (silica, hexanes) to afford a white solid (2.3 mmol, 0.61 g, 90% yield). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta: 7.37 \) (s, 4H), 0.22 (s, 18H).

**1,4-diethynylbenzene:** Literature procedure was modified as follows\(^{32}\): Methanol was added to a 100 mL round-bottomed flask. 1,4-bis((trimethylsilyl)ethynyl)benzene (0.63 mmol, 0.17
g) and K$_2$CO$_3$ (3.2 mmol, 0.44 g) were added. The mixture was allowed to stir at room temperature for 1 hr. under nitrogen. Methanol was removed under reduced pressure. Residual white solid was dissolved in water and washed twice with ether. The organic layer was collected and solvent removed under reduced pressure to yield a white solid (0.47 mmol, 0.059 g, 15%), which was used immediately without further purification. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 7.42 (s, 2H), 3.15 (s, 1H).

**General Solid Phase Peptide Synthesis (SPPS) procedure:** Peptides were synthesized via standard SPPS using Fmoc-protected amino acids, starting from Wang resin preloaded with the first amino acid, within a peptide chamber (Wang-Asp = 0.8 mmol/g, Wang-Val = 0.67 mmol/g). Fmoc deprotection was completed through treating the resin with a 20% piperidine solution in DMF twice for 10 minutes. The resin was washed 3x each with NMP, methanol, and DCM. Amino acid couplings were performed by external activation of 3 eq. of the Fmoc-protected amino acid, relative to resin loading, with 2.9 eq. of HBTU and 10 eq. of diisopropylethylamine, which was then added to the peptide chamber and agitated for 45-90 minutes. After coupling, the resin was again washed 3x each with NMP, methanol, and DCM. All couplings were monitored using a Kaiser test on a few dry resin beads. The procedure was repeated until the desired oligopeptide sequence was obtained.

**General N-acylation Procedure of Peptides:** Following completion and deprotection of the oligopeptide, the resin was treated with an aryl halide carboxylic acid (3 eq.) that was activated by HBTU (2.9 eq.) and diisopropylethylamine (10 eq.) for 2-3 hours, leading to an N-acylated peptide capped with the desired aryl halide. The coupling was monitored using a Kaiser test on a few dry resin beads. After completion, the resin was subjected to a standard wash cycle: 3x NMP, 3x DMF, 2x isopropanol, 2x water, 2x (2x THF, 2x isopropanol), 2x acetonitrile, 2x diethyl ether, 2x hexanes.

**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. Excess solvent was removed from the resin by placing it under vacuum. \( \text{Pd(PPh}_3\text{)}_4 \) (4 mol%, relative to resin loading) was added to the reaction vessel. An approximately 15 mM solution of the bis-stannylated aryl reagent (0.5 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 hrs. 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. Excess solvent was removed from the resin by placing it under vacuum. \( \text{Pd(PPh}_3\text{)}_4 \) (4 mol% relative to resin loading) and benzene-1,4-diboronic acid (0.55 eq.) was added to the reaction vessel. \( \text{K}_2\text{CO}_3 \) (8 eq.) was dissolved in 0.5 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 hrs. 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, 2x isopropanol, 2x water, 2x (2x THF, 2x isopropanol), 2x acetonitrile, 2x diethyl ether, 2x hexanes. The resin was treated with 9.5 mL of trifluoroacetic acid, 250 μL water, and 250 μL of triisopropylsilane for 3 hrs. 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 90 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 2 mL of water and 30 μL ammonium hydroxide and lyophilized. The crude peptide was then dissolved in 5-10 mL of water. The solution was placed inside dialysis tubing of the appropriate length. The tubing was stirred in 1L of water for 2 hours. After 2 hours, the water was exchanged and the tubing was allowed to stir for another 2 hours. The water was exchanged once again, and the tubing stirred overnight (approx. 15 hours). The tubing was then removed from water, and the peptide solution transferred to a separate container and lyophilized.

**Nonsymmetric IG-OT2-GI peptide (1):** Solid supported Wang-IG-NH₂ peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.1 mmol). Pd(PPh₃)₄ (0.002 g, 0.002 mmol) was added. (9H-fluoren-9-yl)methyl ((5-tributylstannyl)thiophen-2-yl)methyl)carbamate, (0.075 g, 0.12 mmol) was weighed out in a syringe and diluted in 3 mL DMF. The solution was added to the reaction flask. The mixture was heated to 80°C for 17 hrs with constant N₂ bubbling. Following general cleavage and work-up, the crude peptide was obtained as a yellow powder and was not further purified. MS (MALDI-TOF) m/z 580.2 (M+H⁺) (calc. 580.2), m/z 602.2 (M+Na⁺) (calc. 602.2).

**DFAG-OT3-GAFD peptide (2):** Solid supported Wang-DFAG-NH₂ peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.1 mmol). The resin was subjected to the standard Stille coupling procedure in the presence of 2,5-bis(tributylstannyl)thiophene (0.050 mmol, 0.033 g) and Pd(PPh₃)₄ (0.004 mmol, 0.005 g) for 21 hours. Following general cleavage and work-up, the peptide was obtained as a yellow powder (0.016 mmol, 0.018 g, 32% yield). Following HPLC purification, 0.006 mmol, 0.007 g, 12% yield. ¹H NMR (400 MHz, D₂O) δ: 7.51 (d, 2H, J = 3.9 Hz), 7.31-7.26 (m, 8H), 7.21-7.18 (m, 2H), 7.16 (br s, 2H), 7.09 (br s, 2H), 4.70 (dd, 2H, J = 7.3, 4.9 Hz), 4.41 (dd, 2H, J = 8.3, 4.5 Hz), 4.3 (q, 2H, J = 7.2 Hz), 4.05 (d, 2H, J = 16.6 Hz), 3.93 (d, 2H, J = 16.9 Hz), 3.29 (dd, 2H, J = 16.0, 4.5 Hz), 3.0 (dd, 2H, J = 12.0, 10.1 Hz), 2.70 (dd, 2H, J = 16.0, 4.5 Hz), 2.59 (dd, 2H, J = 16.0, 8.4 Hz), 1.27 (d, 6H, J = 7.2 Hz). UV-Vis (H₂O) λ/nm (log ε): 392 (4.57). MS (ESI) m/z 1115.4 (M-H)-
DFAG-OT4-GAFD peptide (3): Solid supported Wang-DFAG-NH$_2$ peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.3 mmol). The resin was subjected to the standard Stille coupling procedure in the presence of 5,5'-bis-tributylstannyl-[2,2']-bithiophene (0.25 mmol, 0.19 g) and Pd(PPh$_3$)$_4$ (0.012 mmol, 0.014 g) for 18 hours. Following general cleavage and work-up, the peptide was obtained as a light orange powder (0.063 mmol, 0.075 g, 42% yield). Following HPLC purification, 0.016 mmol, 0.019 g, 11% yield. $^1$H NMR (400 MHz, D$_2$O) δ: 7.45-6.20 (m, 18H), 2H, 4.65-4.61 (m, 2H), 4.40 (dd, 2H, $J = 7.0$, 5.7 Hz), 4.31 (q, 2H, $J = 7.2$ Hz), 3.98 (d, 2H, $J = 16.8$ Hz), 3.88 (d, 2H, $J = 17.3$ Hz), 3.4-3.15 (m, 2H), 3.00-2.87 (m, 2H), 2.65 (dd, 2H, $J = 16.7$, 5.2 Hz), 2.58 (dd, 2H, $J = 15.5$, 7.5 Hz), 1.28 (d, 6H, $J = 7.0$ Hz). UV-Vis (H$_2$O) λ/nm (log ε): 419 (4.64). MS (ESI) m/z 1197.4 (M-H)- (calc. 1197.2), m/z 598.6 (M-2H)-2 (calc. 598.1), m/z 399.0 (M-3H)-3 (calc. 398.4), m/z 1219.4 (M-2H+Na)-1 (calc. 1219.2).

DADGG-OT5-GGDAD peptide (4): Solid supported Wang-DADGG-NH$_2$ peptide N-acylated with 5'-bromo-[2,2'-bithiophene]-5-carboxylic acid was prepared (0.3 mmol). The resin was subjected to the standard Stille coupling procedure in the presence of 2,5-bis(tributylstanny1)thiophene (0.15 mmol, 0.10 g) and Pd(PPh$_3$)$_4$ (0.012 mmol, 0.014 g) for 18 hours. Following general cleavage and work-up, the peptide was obtained as an orange powder (0.037 mmol, 0.049 g, 25% yield). Following HPLC purification, 0.015 mmol, 0.020 g, 10% yield. $^1$H NMR (400 MHz, D$_2$O) δ: 7.52 (d, 2H, $J = 3.4$), 7.23-7.15 (m, 2H), 7.14-7.09 (m, 2H), 7.09-7.02 (m, 4H), 4.64 (dd, 2H, $J = 8.9$, 4.6 Hz), 4.39-4.33 (m, 4H), 4.14-3.96 (m, 8H), 2.74 (dd, 2H, $J = 16.1$, 4.6), 2.66 (dd, 2H, $J = 15.8$, 4.3), 2.62 (dd, 2H, $J = 14.6$, 9), 2.54 (dd, 2H, $J = 15.7$, 9.2), 1.36 (d, 6H, $J = 7.2$). UV-Vis (H$_2$O) λ/nm (log ε): 433 (4.65). MS (ESI) m/z 1329.4 (M-H)- (calc. 1329.2), m/z 664.7 (M-2H)-2 (calc. 664.1), m/z 442.9 (M-3H)-3 (calc. 442.4), m/z 332.1 (M-4H)-4 (calc. 331.5), m/z 1373.4 (M-3H+2Na)-1 (calc. 1374.3).
DADDG-OT6-GDDAD peptide (5): Solid supported Wang-DADDG-NH₂ peptide N-acylated with 5'-bromo-[2,2'-bithiophene]-5-carboxylic acid was prepared (0.3 mmol). The resin was subjected to the standard Stille coupling procedure in the presence of 5,5'-bis-tributylstannyl-[2,2']-bithiophene (0.150 mmol, 0.112 g) and Pd(PPh₃)₄ (0.012 mmol, 0.014 g) for 16 hours. Following general cleavage and work-up, the peptide was obtained as an orange powder (0.048 mmol, 0.073 g, 32% yield). Following HPLC purification, 0.004 mmol, 0.007 g, 3% yield. ¹H NMR (400 MHz, D₂O) δ: 7.47 (s, 2H), 7.20-6.80 (m, 4H), 6.80-6.46 (m, 2H), 4.65 (dd, 2H, J = 5.5, 2.0 Hz), 4.35 (dd, 2H, J = 8.7, 4.6 Hz), 4.32-4.22 (m, 2H), 4.20-4.00 (m, 2H), 2.85-2.69 (m, 4H), 2.69-2.58 (m, 6H), 2.53 (dd, 2H, J = 15.0, 8.7 Hz), 1.34 (br s, 6H). UV-Vis (H₂O) λ/nm (log ε): 447 (4.76). MS (ESI) m/z 763.3 (M-2H)² (calc. 763.1), m/z 381.2 (M-4H)² (calc. 381.0), m/z 386.7 (M-5H+Na)² (calc. 386.5), m/z 515.9 (M-4H+Na)³ (calc. 515.7), m/z 774.3 (M-3H+Na)² (calc. 774.1).

VEVAG-OP3-GAVEV peptide (6): Solid supported Wang-VEVAG-NH₂ peptide N-acylated with 4-iodobenzoic acid was prepared (0.5 mmol). The resin was subjected to the standard Suzuki coupling procedure for 20 hours. Following general cleavage and work-up, the peptide was obtained as a white powder (0.53 mmol, 0.65 g, 211% yield). Following HPLC purification, 0.014 mmol, 0.017 g, 5% yield. ¹H NMR (400 MHz, D₂O) δ: 7.94 (d, 4H, J = 8.5 Hz), 7.90-7.85 (m, 8H), 4.45 (q, 2H, J = 7.1 Hz), 4.36 (dd, 2H, J = 9.3, 5.3 Hz), 4.22-4.14 (m, 4H), 4.11 (d, 2H, J = 8.4 Hz), 4.05 (d, 2H, J = 5.88 Hz), 2.34-2.16 (m, 4H), 2.16-2.00 (m, 6H), 1.98-1.86 (m, 2H), 1.41 (d, 6H, J = 7.2 Hz), 0.95 (d, 6H, J = 6.8 Hz), 0.95 (d, 6H, J = 6.7 Hz), 0.90 (d, 6H, J = 7.1 Hz), 0.88 (d, 6H, J = 7.0 Hz). UV-Vis (H₂O) λ/nm (log ε): 301 (4.74). MS (ESI) m/z 1227.9 (M-H)⁻ (calc. 1227.6), m/z 613.8 (M-2H)-² (calc. 613.3), m/z 624.8 (M-3H+Na)-² (calc. 624.3), m/z 408.8 (M-3H)-³ (calc. 408.5), m/z 306.6 (M-4H)-⁴ (calc. 306.1), m/z 1249.8 (M-2H+Na)⁻ (calc. 1249.5), m/z 1293.8 (M-4H+3Na)-¹ (calc. 1293.5).

DFAG-OPE3-GAFD peptide (7): Solid supported Wang-DFAG-NH₂ peptide N-acylated with 4-iodobenzoic acid was prepared following the general SPPS and N-acylation procedures.
The resin (0.3 mmol) was transferred to a Schlenk flask and dried under vacuum. Pd(PPh₃)₄ (0.015 mmol, 0.017 g) Cul (0.03 mmol, 0.006 g) and 1,4-diethynylbenzene (0.17 mmol, 0.021 g) were added to the reaction vessel. 3 mL of diisopropyl amine and 7 mL of DMF were added to the flask via syringe. The mixture was agitated constantly by bubbling nitrogen through the solution at room temperature for 18 hrs. The resin was washed with water and then subjected to the standard cleavage (triisopropylsilane was omitted from the cleavage cocktail) and work-up procedure. The peptide was obtained as a white powder (0.054 mmol, 0.062 g, 36% yield). Following HPLC purification, 0.002 mmol, 0.018 g, 11% yield. ¹H NMR (400 MHz, D₂O) δ:

7.87-7.50 (m, 12H), 7.50-7.10 (m, 10H), 4.39 (dd, 2H, J = 8.8, 4.2 Hz), 4.30-4.20 (m, 2H), 4.11 (d, 2H, J = 17.1 Hz), 4.00 (d, 2H, J = 15.0 Hz), 3.37-3.23 (m, 2H), 2.94 (dd, 2H, J = 13.2, 10.8 Hz), 2.65 (dd, 2H, J = 15.8, 3.9 Hz), 2.54 (dd, 2H, J = 16.0, 9.2 Hz), 1.23 (d, 6H, J = 5.8 Hz). UV-Vis (H₂O) λ/nm (log ε): 332 (4.80). (ESI) m/z 572.5 (M-2H)⁻² (calc. 572.1), m/z 1167.4 (M-2H+Na)⁻² (calc. 1167.4), m/z 381.4 (M-3H)⁻³ (calc. 381.1).

VEVAG-PTP-GAVEV peptide (8): Solid supported Wang-VEVAG-NH₂ peptide N-acylated with 4-iodobenzoic acid was prepared (0.1 mmol). The resin was subjected to the standard Stille coupling procedure in the presence of 2,5-bis(tributylstannyl)thiophene (0.050 mmol, 0.037 g) and Pd(PPh₃)₄ (0.004 mmol, 0.005 g) for 21 hours. Following general cleavage and work-up, the peptide was obtained as a pale yellow powder (0.028 mmol, 0.034 g, 54% yield). Following HPLC purification, 0.005 mmol, 0.006 g, 10% yield. ¹H NMR (400 MHz, D₂O) δ:

7.74 (d, 4H, J = 8.4 Hz), 7.65 (d, 4H, J = 8.4 Hz), 7.44 (s, 2H), 4.43 (q, 2H, J = 7.3 Hz), 4.35 (dd, 2H, J = 9.2, 5.4 Hz), 4.15 (d, 2H, J = 7.6 Hz), 4.13-4.00 (m, 6H), 2.34-2.16 (m, 4H), 2.14-1.99 (m, 6H), 1.98-1.84 (m, 2H), 1.40 (d, 6H, J = 7.2 Hz), 0.94 (d, 12H, J = 6.7 Hz), 0.90 (d, 6H, J = 7.3 Hz), 0.88 (d, 6H, J = 7.1 Hz). UV-Vis (H₂O) λ/nm (log ε): 352 (4.54). MS (ESI) m/z 1233.6 (M-H)⁻ (calc. 1233.5), m/z 616.5 (M-2H)-² (calc. 616.3), m/z 627.5 (M-3H+Na)-² (calc. 627.2), m/z 638.6 (M-4H+2Na)-² (calc. 638.2), m/z 1277.6 (M-3H+2Na)⁻² (calc. 1277.5), m/z 1299.6 (M-4H+3Na)-¹ (calc. 1299.5).
VEVAG-TPT-GAVEV peptide (9): Solid supported Wang-VEVAG-NH₂ peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.1 mmol). The resin was subjected to the standard Suzuki coupling procedure for 27 hours. Following general cleavage and work-up, the peptide was obtained as a white powder (0.016 mmol, 0.020 g, 32% yield). Following HPLC purification, 0.001 mmol, 0.002 g, 2.5% yield. ¹H NMR (400 MHz, D₂O) δ: 7.77 (s, 4H), 7.71 (d, 2H, J = 4.0 Hz), 7.50 (d, 2H, J = 4.0 Hz), 4.43 (q, 2H, J = 7.2 Hz), 4.34 (dd, 2H, J = 9.3, 5.3 Hz), 4.16 (d, 2H, J = 7.6 Hz), 4.14 (d, 2H, J = 16.6 Hz), 4.07 (d, 2H, J = 16.8 Hz), 4.05 (d, 2H, J = 5.8 Hz), 2.33-2.16 (m, 4H), 2.14-1.99 (m, 6H), 1.97-1.86 (m, 2H), 1.41 (d, 6H, J = 7.2 Hz), 0.95 (d, 6H, J = 6.8 Hz), 0.94 (d, 6H, J = 6.8 Hz), 0.90 (d, 6H, J = 6.8 Hz), 0.88 (d, 6H, J = 6.8 Hz). UV-Vis (H₂O) λ/νm (log ε): 356 (4.51). MS (ESI) m/z 1239.6 (M-H)-(calc. 1239.5), m/z 1283.7 (M-3H+2Na)-(calc. 1283.4), m/z 1305.7 (M-4H+3Na)-(calc. 1305.4), m/z 619.8 (M-2H)-2 (calc. 619.2).

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A. M. Sanders; T. J. Dawidczyk; H. E. Katz; J. D. Tovar

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and

Solid-Phase Pd-Catalysed Cross-Coupling Methods for the Construction of π-Conjugated Peptide Nanomaterials
A. M. Sanders; J. D. Tovar

Supramolecular Chemistry 2014, 26 (3-4), 259-266
References


Chapter 3 – Multivalent Trimeric and Tetrameric Peptide-π Hybrids

Introduction

C3-symmetric compounds capable of intermolecular hydrogen bonding have been widely explored for their self-assembling abilities. Due to the liquid-crystalline properties of these molecules and the availability of three axes to participate in hydrogen bonding, they are able to form highly stable supramolecular assemblies. Typically, these types of systems are known to form cylindrical shaped fibers arising from columnar stacking of the molecules, giving the structures 1-D directionality and the potential for anisotropic properties, which is attractive for organic electronic applications. The presence of three hydrogen bond-forming axes can enhance the thermodynamic strength, create more extended cylindrical stacks, and possibly lower the concentration necessary to form organo- or hydrogels.

![Figure 3.1. General structure of 1,3,5-benzenetricarboxamide (BTA).](image)

One of the most simple and highly explored C3-symmetric cores is benzene-1,3,5-tricarboxamide (BTA) unit, shown in Figure 3.1. This molecule features a simple aromatic core and three amide substituents capable of intermolecular hydrogen bonding. Monomers consisting of this core have been explored heavily by Meijer, as well as other groups, and the R groups have been varied from simple alkyl or glycol chains to π-conjugated chromophores and to amino acids and oligopeptides.
Utilizing amino acid or oligopeptide side chains as self-assembly scaffolds is beneficial due to their ability to create intermolecular hydrogen bonds, which work to strengthen the supramolecular assemblies. Furthermore, altering the identity of the amino acids or changing oligopeptide sequences allows for control over the self-assembly of the systems. For instance, Meijer and coworkers have explored BTA cores substituted with a variety of dipeptide sequences and have determined that the thermodynamic stability of the supramolecular stacks is influenced greatly by amino acid composition. Furthermore, sequence engineering gives the opportunity for external control over the assembled materials via stimuli such as changes in pH. This possibility was explored by Besenius and coworkers. They synthesized four C3-symmetric BTA-containing peptide trimers via solution-phase couplings between the N-termini of peptide fragments and benzene-1,3,5-tricarbonyl chloride, utilizing alternating hydrophobic/hydrophilic amino acids to encourage β-sheet-like assembly. These molecules are shown in Figure 3.2. 1 and 2 contain lysine amino acids while 3 and 4 include glutamic acid moieties, causing the individual molecules to be positively and negatively charged, respectively, near neutral pH. Mixing the co-monomers at a 1:1 ratio allowed for copolymerization through Coulombic attraction and intermolecular hydrogen bonding in a pH range from 6.1 to 7.0, as determined through circular dichroism spectra.

Figure 3.2. BTA-containing peptide trimers studied by Besenius and coworkers.
Lowering the pH to 3.6 or increasing it to 8.9 caused the complete dissociation of the supramolecular polymers into their molecularly dissolved components. Interestingly, homopolymerization was reportedly not seen at these extreme acidic and basic pH regimes. The resulting nanorod assemblies could also be visualized with HRTEM.

Figure 3.3. a) Structure and b) self-assembly of peptide 5 into spherical micelles, investigated by Kimizuka and coworkers. Adapted with permission from ref. 4.

Although most supramolecular assemblies based on BTA-containing monomers have been reported to form 1-D nanostructures, Kimizuka and coworkers investigated a peptidic system that instead preferred to organize into micelle-like nanospheres. This BTA trimer 5, shown in Figure 3.3a, is terminated by CFKFEFKFE oligopeptides. Atomic force microscopy (AFM) of the assembled peptide trimer showed spherical structures on the order of 2-3 nanometers in height. Alternatively, AFM images of assemblies of solely the CFKFEFKFE peptide showed 1-D fibrils with heights of approximately 1 nm. They hypothesized that the assembly of 5 is occurring via anti-parallel β-sheet stacking between peptide chains in the array shown in Figure 3.3b to form the spherical structures.
Figure 3.4. Structures and circular dichroism spectra of monomer 6, dimer 7, and trimer 8 assemblies studied by Meijer and coworkers. Adapted with permission from ref. 9.

One particular study from the literature that emphasizes the assembly advantages of discotic molecules possessing three axes capable of hydrogen bonding (instead of one or two) was performed by Meijer and coworkers. For this experiment, molecules 6, 7, and 8 were synthesized (Figure 3.4). Each molecule consists of the same basic structure, differing in the number of hydrogen bond forming axes based on urea moieties from one in 6 to three in 8. Compound 8 is known to assemble in nonpolar solvents to form 1-D chiral stacks where the helicity is caused by the chiral alkyl substituents. When assembled in a nonpolar hydrocarbon solvent, 8 displayed a Cotton effect in its circular dichroism spectrum, indicative of the formation of the chiral assemblies (Figure 3.4). However, upon similar preparation of compound 7 at the same concentration, a much less intense Cotton effect was evident, which also differed in shape and sign. In the case of compound 6, which provides only one axis for hydrogen bonding, no Cotton effect was seen.
The previous experiment illustrated the advantage of three axes for the formation of chiral supramolecular assemblies in these types of systems. Meijer and coworkers also studied the effect of three axes on the thermodynamic strength of the assemblies. Assembled solutions of 7 and 8 were subjected to heating and the intensities of their circular dichroism maxima were monitored. The results are shown in Figure 3.5. Complete loss of signal was found for compound 8 at 135°C, while that for compound 7 occurred at a much lower temperature, 80°C. The addition of a third hydrogen bonding axis gave rise to assemblies that were stable at 40% higher temperatures.

Our group has previously taken advantage of the self-assembly properties of peptide fragments as a means to assemble linear π-conjugated, semiconducting oligomers. To create these systems, we have developed three unique synthetic procedures, each based on “dimerizations” between π-conjugated chromophores and the N-termini of peptide fragments immobilized on a solid support. Each procedure begins with the solid-phase synthesis of the peptide fragment, followed by the incorporation of the π-conjugated unit on the solid-phase through either a double amidation (with π-conjugated dicarboxylic acids), double imidation (with dianhydrides), or a piecewise synthesis via N-terminus “capping” with an aryl halide followed by palladium-catalyzed cross-coupling. All of these methods have proved versatile.
in the production of peptidic “dimers” containing a variety of π-conjugated cores. However, the methods thus far have solely been utilized to create “dimers”, as opposed to higher-order oligomers, e.g. symmetric trimeric or tetrameric peptides. Due to the potential benefits of creating these multivalent peptide-π hybrids, we sought to expand the previously developed synthetic procedures to create trimeric and tetrameric peptides, which can undergo self-assembly into 1-D nanostructures.
Benzotrithiophene-Containing Peptide Trimers

Introduction

Figure 3.6. Benzotrithiophene derivative investigated by Roncali and coworkers.\textsuperscript{15}

Benzotrithiophene (or trithienobenzene or BTT) analogues have previously been investigated for their use as donor molecules in molecular heterojunction solar cells.\textsuperscript{15} Roncali and coworkers substituted the core with hexyl-terminated bithiophene moieties to create the C3-symmetric star-shaped oligothiophene molecule 9, shown in Figure 3.6. An alkylated perylene derivative was combined with 9 and incorporated as the active material in solar cell devices. The devices showed an efficiency of 1\%, among the best for similar entirely small-molecule based cells at the time of the publication of these results, demonstrating the electronic potential for this particular C3-symmetric π-conjugated core.
To incorporate these C3-symmetric π-conjugated units within peptide backbones, the core was first synthesized and substituted with appropriate substituents for peptide coupling. The preparation of the desired C3-symmetric benzotrithiophene core was initially carried out using a synthesis described in the literature which is outlined in Scheme 3.1. First, 1,3,5-trichlorobenzene was triply iodinated using iodine and periodic acid in sulfuric acid to give 10. Next, a triple Sonogashira coupling with TMS-acetylene was carried out to give 11. This reaction gave the desired compound in nearly 50% yield on a small scale, but scaling up the reaction proved difficult. This was probably due to the volatile TMS-acetylene reagent evaporating during the course of the reaction, which was carried out under reflux conditions. 11 was then subjected to conditions as described in the literature with sodium sulfide nonahydrate to yield benzotrithiophene 12. Again, this step provided the desired compound in reasonable yield on a small scale, but upon scale-up, the yields diminished. Due to these difficulties, a different synthetic route was investigated.
The new synthetic route was designed to take advantage of a photochemical ring-closure previously described in the literature to ultimately obtain the desired core. It has been shown that various terthiophene units can undergo this ring-closure to obtain different variations of the aromatic core, as shown in Figure 3.7. For instance, compound 13, which is comprised of a central thiophene ring that is attached to the 2 position of two other thiophene rings at its 2 and 3 positions, yields the C3-symmetric 12 after photochemical treatment, but also yields the non-symmetric 14. To obtain exclusively the desired symmetry of the benzotrithiophene unit, compound 15 must be synthesized. This molecule differs from 13 in that the thiophene ring attached to the 2 position of the central ring is bound through its 3 position.

To obtain this isomer, 2,3-dibromothiophene was subjected to Stille conditions in the presence of 3-(tributylstannyl)thiophene (Scheme 3.2). This reaction proved to be regioselective and gave 16 in reasonable yield. 16 was then carried on to a second Stille coupling with 2-(tributylstannyl)thiophene to give the desired terthiophene compound 15. 15 was then treated with the photochemical conditions described in the literature using a 450 Watt mercury lamp in the presence of oxygen and a catalytic amount of iodine to give the desired benzo[1,2-b:3,4-b’:5,6-b’’]trithiophene core 12. Initially, the reaction produced a
mixture of starting material 15 and desired product 12 that was inseparable by chromatography, recrystallization, and vacuum sublimation. However, recycling the mixture and treating it to the same conditions eventually yielded entirely desired product. Next, the core was triply stannylated via lithiation with n-BuLi and quenching with tributyltin chloride to give tristannylated BTT 17.

Scheme 3.2. Synthesis of stannylated benzotrithiophene 17 via photocyclization.
Scheme 3.3. Synthesis of benzotrithiophene-containing peptide trimers 18 and 19 via solid-phase palladium-catalyzed trimerizations.

To synthesize the peptidic trimers containing the benzotrithiophene core, solid-supported peptides consisting of the amino acid sequence VEVAG-NH$_2$ were prepared and acylated with aryl bromides (either thiophene or bithiophene), as previously described.$^{13,14}$ Then, as shown in Scheme 3.3, 17 was introduced to the resin under Stille cross-coupling conditions modified from the solid-phase Stille cross-coupling procedure previously described (details in Chapter 2)$^{13}$, facilitating a triple coupling between 17 and three peptide fragments adjacent to one another on a resin bead. Following cleavage from the resin, the desired peptide trimers 18 and 19 were obtained.
Characterization of Assembly

Figure 3.8. Absorption, photoluminescence (a, c), and circular dichroism (b, d) spectra of unassembled (dashed lines, pH 8) and assembled (solid lines, pH 6) 18 (a, b) and 19 (c, d).

The absorption and photoluminescence changes observed for 18 and 19 upon assembly via acidification are somewhat dissimilar to those previously seen for peptides embedded with linear oligoarene chromophores. Typically, these “1-D” peptides exhibit a blue shift in their absorption $\lambda_{\text{max}}$ (usually accompanied by a weak, red-shifted shoulder) and a significant quenching in the photoluminescence upon assembly, which is consistent with an imperfect H-like aggregation arrangement of the linear chromophores as described by Kasha. However, due to the C3-symmetric nature of the BTT core, and hence different intermolecular interactions of the transition dipoles, the same perturbations in the optical spectra of the trimeric peptides were not expected. 18 and 19 displayed similar absorption and emission spectra while unassembled at basic pH (Figure 3.8a and c, dashed lines), with
the $\lambda_{\text{max}}$ occurring at a lower energy for 19 due to the increased conjugation of the bithiophene linkers (410 vs 363 nm and 565 vs 520 nm). Upon assembly, the absorption spectrum of 19 shows minimal perturbation, while the emission is significantly quenched (Figure 3.8c, solid lines). 18 shows a slight (9 nm) blue shift in the absorption, accompanied by an enhancement of emission intensity, with respect to the molar absorptivity of the assembled peptide (Figure 3.8a, solid lines). At acidic pH, the circular dichroism spectra of 18 and 19 display bisignate Cotton effects in the vicinity of the absorbance ranges of the chromophores, suggesting the transition dipoles of the self-assembled $\pi$-conjugated moieties are interacting within the chiral environment caused by the assembly of the peptide scaffolds. Interestingly, even at a basic pH where significant charge repulsion due to the deprotonated carboxylic acid endgroups and side chains would be expected to inhibit intermolecular assembly, Cotton effects are still evident, suggesting some assembly occurs even at high pH. Furthermore, despite the similar structure of the two peptide-$\pi$ hybrid molecules, the CD spectra depicts Cotton effects of opposite handedness and different intensities. Assembled 18 induces a fairly strong positive Cotton effect, suggesting an overall right-handed or clockwise helicity in the supramolecular assemblies, while 19 induces a weaker negative Cotton effect, proposing some bias toward left-handed or counterclockwise helicity.\textsuperscript{19-21}

The assemblies of 18 and 19 were also visualized using TEM. Interestingly, the two related compounds seem to organize into quite different nanostructures. 18, as shown in Figure 3.9a, assembles into long 1-D nanostructures which appear to be on the order of a few microns in length. The thinnest structures display a width of 5.0 ± 0.7 nm, while 3.7 nm would be the expected width for a single columnar stack of the molecules in their most extended conformation. Most structures appear thicker, which is likely due to bundling interactions between two or more individual structures. Alternatively, 19 (Figure 3.9b) appears to organize into relatively amorphous aggregates, as opposed to well-defined 1-D nanostructures, under
the same conditions. These differences could be due to variances in solubility due to the more extended and more hydrophobic core of 19.

Figure 3.9. TEM images of assembled samples of a) 18 and b) 19.
Decacyclene Triimide-Containing Peptide Trimers

Introduction

Electron deficient and hence potentially n-type semiconducting C3-symmetric cores have also been investigated. In particular, decacyclene triimide (DTI) derivatives have been prepared by Wudl and coworkers. These derivatives, shown in Figure 3.10a, are substituted with different branched alkyl chains. The molecules showed interesting assembly properties, driven by intermolecular π-π stacking, which were characterized by SEM. Intriguingly, the structures formed were highly dependent on the identity of the alkyl substituents. 20 preferred to form hexagonal pillars that were approximately 1 μm wide and 3 μm long when drop-cast from a THF solution as shown in Figure 3.10b. Alternatively, 21 formed long fibers about 0.5 μm wide and up to a millimeter in length when dropcast from o-DCB solution (Figure 3.10c).

Optoelectronic properties of the molecules were elucidated from cyclic voltammetry and DFT calculations and compared to other rylene imides. Although DTI contains three electron withdrawing substituents, the LUMO of the DTI core was found to be approximately the same or slightly higher than that of naphthalene diimide and perylene diimide, due to the fact that
the π-electrons in DTI cannot be delocalized over the entire core. This means the acenaphthalimide units are essentially independent of one another. However, some communication exists between the imide groups, as its LUMO is indeed lower than that of naphthalene monoimide.
Decacyclene trianhydride 22 was supplied by Prof. Wudl. By modifying the method previously developed in our lab for solid-phase coupling of peptides to anhydrides (detailed in Chapter 1), peptide trimers 23 and 24 were synthesized according to Scheme 3.4 via a triple imidation between 22 and the N-termini of three peptides situated adjacent to one another on a resin bead. Presumably due to strong intermolecular interactions, HPLC purification proved difficult for both compounds, giving rise to broad HPLC traces and making purification of the systems difficult (Figure 3.11a). To impart better solubility and minimize aggregation during purification, the triple imidation was carried out utilizing a longer (11 amino acids) peptide containing more ionizable aspartic acid residues, resulting in trimer 25. Satisfyingly, as shown...
in Figure 3.11b, the crude HPLC trace appeared much more manageable, and the peptide was able to be purified for further study.

Figure 3.11. Crude HPLC traces of a) 23 and b) 25.
Characterization of Assembly

![Figure 3.12](image)

Figure 3.12. Absorption, photoluminescence (a), and circular dichroism (b) spectra of unassembled (dotted lines, pH 8) and assembled (solid lines, pH 4) 25.

The absorption and photoluminescence changes observed for 25 upon assembly are again dissimilar to those seen for the previously studied linear peptide “dimers”. 25 displayed a decrease in the molar absorptivity of the $\lambda_{\text{max}}$ upon acidification and assembly, however this decrease did not coincide with a spectral shift (either bathochromic or hypsochromic), as shown in Figure 3.12a. Furthermore, acidification of the sample caused a fairly dramatic enhancement of the emission intensity, as opposed to the significant quenching that was previously seen for H-aggregated linear peptide-π hybrids. Interestingly, the CD spectra of 25 does not show evidence of any signal within the absorbance range of the chromophore upon assembly (Figure 3.12b), which had previously been seen with assembled samples of the peptide-π hybrids containing linear chromophores and benzotriothiophene, although significant changes in the amide region are apparent. This could be due to the supramolecular assemblies either not exhibiting any helical twist, or not showing a bias for right-handed or left-handed helicity.
TEM was employed to visualize the supramolecular assemblies of 25 and are shown in Figure 3.13. The 1-D nanostructures are very well-defined and appear to be several microns in length, while maintaining a very uniform thickness of 5.1 ± 0.2 nm. The most extended conformation of 25 is expected to be approximately 4.2 nm in length, thus it is postulated that the nanostructures are composed of single columnar stacks of the molecules in their extended conformations. In comparison to the nanostructures of peptide-π hybrids previously discussed, assembled samples of 25 show a relative lack of interstructure interactions or “bundling”. This could be a consequence of the trivalent architecture, coupled with the rigidity of the decacyclene core, which allows for the formation of very uniform and distinct supramolecular structures.
Hexa-\textit{peri}-hexabenzocoronene-Containing Peptide Trimers

Introduction

![Diagram a) Structure of HBC and b) self-assembly mechanism of alkyl-substituted HBC derivatives. Adapted with permission from ref. 23.](Image)

Another family of electronically desirable π-conjugated materials is the polycyclic aromatic hydrocarbon (PAH) family. These molecules are described as 2-dimensional sections of graphite, consisting entirely of sp$^{2}$ hybridized carbons. One of the most studied discotic PAH is hexa-\textit{peri}-hexabenzocoronene (HBC, Figure 3.14a). This molecule, made up of 13 fused benzene rings, has been established as a discotic liquid crystal and has also been shown to exhibit local charge carrier mobilities as high as 1.1 cm$^{2}$V$^{-1}$s$^{-1}$.\textsuperscript{23} Furthermore, the hydrocarbon-based molecule is ambipolar, which could allow for electronic modification through the incorporation of electron donating or withdrawing substituents. The processability of this inherently insoluble compound can be enhanced in organic solvents by substituting the core with long alkyl chains, however processability in aqueous solution has been less studied.\textsuperscript{24}

The assembly of HBC analogues has been studied extensively by groups such as Aida and Müllen.\textsuperscript{24-28} A schematic of the aggregation of alkyl-substituted HBC molecules is shown in Figure 3.14b. As concentration increases (or temperature is decreased), monomers interact through π-stacking to form columnar assemblies. Continued increase in
concentration (or decrease in temperature) results in the aggregation of the columns into columnar arrays, where the disordered alkyl chains fill the gaps between the stacked HBC cores.\textsuperscript{23} Introducing hydrophilic and hydrophobic side chains on opposite sides of the HBC core has been shown to produce complex nanotube-like bilayer structures.\textsuperscript{25-28}

![Figure 3.15](image.png)

Figure 3.15. a) Structure and b) self-assembly of HBC-containing molecule 26 into helical stacks, as investigated by Müllen and coworkers. Adapted with permission from ref. 29.

Utilizing this core to create C3-symmetric molecules has been somewhat unexplored, presumably due to synthetic difficulty. However, there are some examples of these types of molecules in the literature, synthesized by Müllen and coworkers.\textsuperscript{29} One of these examples, methoxy and alkyl substituted 26, is shown in Figure 3.15a. Although the semiconducting properties of the resulting supramolecular materials were not investigated, the assembly of the molecule was explored. It was found to form helical stacks as shown in Figure 3.15b, ultimately assembling into nanostructures that could be visualized by TEM. The helicity of the assembly occurred without the presence of chiral substituents, and was hypothesized to be due to repulsion between methoxy substituents in conjunction with the π-π stacking of the HBC cores.
Figure 3.16. a) Schematic of the “zone-casting” method developed by Müllen and coworkers and b) HR-TEM image of the resulting aligned films. Adapted with permission from ref. 31.

As previously mentioned, HBC exhibits extremely high local charge carrier mobilities, however, because creating long-range organization of the HBC units is difficult, the performances of HBC-containing devices are plagued by imperfections and grain boundaries in thin films.\textsuperscript{23} To remedy this, several methods have been established to impart macroscopic order to functionalized HBC thin films. Müllen developed a “zone-casting” method to impart macroscopic order of HBC-containing solutions on surfaces.\textsuperscript{23,30,31} This was done by releasing the solution from a nozzle onto a surface that is moving away from the supply, as shown in the schematic in Figure 3.16. Filtered inverse FFT and HR-TEM images of films created through this type of deposition proved that aligned columnar structures are created. Field effect transistors of these aligned films showed a field-effect mobility of 5x10\textsuperscript{-3} cm\textsuperscript{2}V\textsuperscript{-1}s\textsuperscript{-1}.
Another method of supramolecularly organizing the PAH moieties is installing substituents on the HBC cores that are capable of intermolecular hydrogen bonding.\textsuperscript{24,32} Because hydrogen bonding is generally a stronger interaction than \(\pi\)-stacking, incorporating hydrogen bonding in the system could allow for more organized self-assembly and more thermodynamically stable structures. HBC cores have been substituted with alkyl-tethered carboxylic acids, ureido, and amido groups, which can self-assemble into helical twisting nanostructures via hydrogen bonding, as depicted in Figure 3.17.\textsuperscript{32} A variety of experiments led to the conclusion that the presence of hydrogen bonding influences the supramolecular order a great deal, and can allow for more thermally stable organization. However, the effects of hydrogen bonding on the electronic properties of HBC systems in devices have not yet been elucidated.
Scheme 3.5. Different synthetic routes used to form substituted HBC derivatives.

The formation of substituted HBCs is generally performed through Scholl-type aromatization of a hexaphenylbenzene precursor. The preparation of these precursors can be completed through various techniques, as summarized in Scheme 3.5, each allowing for different substitution patterns on the HBC core. Hexasubstituted HBC derivatives can be synthesized through the formation of hexasubstituted hexaphenylbenzene molecules via $\text{Co}_2(\text{CO})_8$-mediated 2+2+2 cyclotrimerizations of disubstituted diphenylacetylenes, as shown in Route A. However, this method is generally not adequate for forming HBC derivatives that display a more complicated arrangement of substituents, because the cyclotrimerization is normally not regioselective. More complicated substitution patterns of the hexaphenylbenzene precursors can be created through Diels-Alder reactions between tetraphenylcyclopentadienone and diphenylacetylene derivatives, as depicted in Route B.
Furthermore, C3-symmetric tri-substituted HBCs can be obtained through Scholl aromatization of tris(biphenyl)benzene derivatives (Route C).
Hexa-\textit{peri}-hexabenzocoronene Core Synthesis

Scheme 3.6. Attempted synthesis of HBC derivatives 31 and 32 via Route A.

To create a C3-symmetric HBC-containing peptide trimer, an appropriately triply substituted HBC core needed to be synthesized. This was initially attempted by Dr. Stephen Diegelmann utilizing the Route A strategy.\textsuperscript{34} As shown in Scheme 3.6, 1-iodo-4-benzoic acid was converted to the methyl ester 27, and then subjected to Sonogashira cross-coupling conditions with phenylacetylene to yield methyl 4-(phenylethynyl)benzoate (28). Then, Co\textsubscript{2}(CO)\textsubscript{8}-mediated tricyclization was performed to yield a statistical mixture of the two hexaphenylbenzene isomers 29 and 30. It was hoped that subjecting this mixture to Scholl reaction conditions would allow for the oxidative dehydrogenation of 29 and 30 to the corresponding HBC triesters 31 and 32. However, no reaction occurred.
The literature has shown that the Scholl reaction of these types of species most likely proceeds through an arenium cation intermediate, much like electrophilic aromatic substitution, as depicted in Figure 3.18.\textsuperscript{35} The presence of electron withdrawing substituents, such as the ester substituents of 29 and 30, is destabilizing of these positively charged intermediates, so it is understandable that the reaction did not occur with this deactivated system.

Reports have shown that the formation of hexafluoro- and hexaiodo-HBC derivatives from their hexaphenylbenzene counterparts is possible, suggesting that halide substituents will not deactivate the system and prevent the Scholl aromatization from occurring.\textsuperscript{36-38} Due to this, a new route to the symmetric, trisubstituted HBC derivative was designed, utilizing halides (specifically bromides) as the substituents which could later be converted to appropriate functional groups for peptide attachment. This synthesis is shown in Scheme 3.7.
Scheme 3.7. Attempted synthesis of HBC derivatives 36 and 37 via Route A.

1-Bromo-4-iodobenzene was coupled with phenylacetylene under Sonogashira conditions to yield 33. Co$_2$(CO)$_8$-mediated tricyclization was conducted to yield a mixture of tribromo-hexaphenylbenzene isomers 34 and 35. This mixture was subjected to Scholl reaction conditions to form the tribromo-HBC isomers 36 and 37, however, treatment with FeCl$_3$ did not yield the desired product. Some literature suggests that, once again, this system is too deactivated for the arenium cation intermediates to be stable enough for the reaction to proceed. However, the aforementioned fact that the hexafluoro-HBC molecule, a presumably more deactivated system, has been synthesized under these conditions suggests that this conclusion is incorrect. Perhaps the true explanation revolves around the directing nature of the bromide substituents, as opposed to their level of deactivation. Gortari and coworkers have shown that the reaction does proceed in similar bromide substituted systems, under similar reaction conditions, but only in systems where the bromide substituent is situated \textit{para} to the nascent bond.$^{39}$ Moving the substituents \textit{meta} to the site of desired bond-making yielded oligomeric species, due to bonds being made intermolecularly at the \textit{para} position. As halides are considered \textit{ortho}, \textit{para} directors in electrophilic aromatic substitution, this
conclusion is reasonable; the arenium cation stability and steric effects would likely encourage bond formation at the *para* position, as opposed to the desired *meta* position in this system.

Scheme 3.8. Various synthetic strategies to form tris(biphenyl)benzene derivatives en route to C3-symmetric HBC analogues.

Utilizing the Route A strategy will eventually create a presumably inseparable mixture of desired symmetric and nonsymmetric regioisomers in an approximately 1:3 ratio, due to the statistics of the arrangement of the molecules during the Co$_2$(CO)$_8$ mediated 2+2+2 trimerization. Although it is possible that the two regioisomers could be separated via HPLC purification of the final peptide trimers, this would require wasting a significant amount of peptide, as well as limiting the maximum theoretical yield. Due to this and the failures of the previously attempted Route A methods, synthetic routes were designed around the Route C strategy to ultimately allow for the preparation of solely the C3-symmetric core through utilization of a tris(biphenyl)benzene precursor. These derivatives can be synthesized through multiple means, as shown in Scheme 3.8. Strategies 1 and 2 involve Suzuki-type couplings between benzene derivatives triply substituted at the 1, 3, and 5 positions and biphenyl compounds. Strategy 3 is performed through Suzuki-type couplings between halide substituted triphenylbenzene derivatives and benzene boronic acids or boronate esters. Alternatively, Strategy 4 is completed through tricyclization/dehydration of biphenyl
acetophenone compounds. Each of these strategies were investigated en route to the desired C3-symmetric HBC core.

Scheme 3.9. Attempted synthesis of boronate ester 41 and boronic acid 42.

First, Strategy 1 was investigated. To synthesize the biphenyl boronic acid or boronate ester coupling partner (Scheme 3.9), 1,3-dibromobenzene was treated with n-butyllithium, then quenched with TMSCl to yield 38. 38 was subjected to Miyaura conditions with (dppf)PdCl₂ and bis(pinacolato)diboron to yield boronate ester 39. Under Suzuki conditions, 39 and 1-bromo-2-iodobenzene were couple to afford 40. From here, 40 was to be converted to the analogous boronic acid or boronate ester. The Miyaura route to give boronate ester 41 actually afforded debrominated starting material and other unidentifiable side products. Lithiation and quenching with trimethylborane gave a complex mixture instead of desired boronic acid 42 despite the use of a literature procedure.³⁷
Scheme 3.10. Attempted synthesis of TMS-substituted tris(biphenyl)benzene derivative 44 via Strategy 2.

Next, Strategy 2 was investigated. According to Scheme 3.10, 1,3,5-tribromobenzene was subjected to Miyaura conditions, which gave 43, although in fairly poor yield. However, the following Suzuki coupling between 43 and 40 resulted in only unreacted starting material, not desired tris(biphenyl)benzene compound 44. This could presumably be due to steric hindrance of the bromide substituent, situated *ortho* to the TMS substituted ring within biphenyl 40.

Scheme 3.11. Attempted synthesis of TMS-substituted tris(biphenyl)benzene derivative 44 via Strategy 3.
To remedy this, Strategy 3 was investigated (Scheme 3.11). 43 was subjected to Suzuki conditions with 1-bromo-2-iodobenzene, which yielded the triphenylbenzene derivative 45 in good yield. It was also found that 45 could be prepared directly via trimerization and subsequent dehydration of 2'-bromoacetophenone in the presence of triflic acid, which was found to be a more viable option for scaling up the synthesis. From here, previously synthesized boronate ester 39 was used under Suzuki conditions in an attempt to form desired HBC precursor 44. However, once again, the reaction gave only unreacted starting material.

The lack of reaction progression could possibly have been due to the less reactive, bulky boronate ester inhibiting transmetallation to the palladium complex of the hindered substrate. To rectify this, the same reaction was attempted with the analogous boronic acid, as summarized in Scheme 3.12.

![Scheme 3.12](image)

Scheme 3.12. Various Suzuki conditions investigated to form TMS-substituted tris(biphenyl)benzene derivative 44 via Strategy 3.

The analogous boronic acid 46 was prepared by lithiation of 38 and quenching with trimethylborane. However, general Suzuki conditions (Pd(PPh₃)₄, K₂CO₃, in
toluene/ethanol/water) between 46 and 45 and gave mainly unreacted starting material, despite the use of a literature procedure\textsuperscript{41}, although evidence of a small amount of desired product 44 was seen. Other Suzuki conditions were employed to enhance product yield. First, the catalyst was replaced with Pd\textsubscript{2}(dba)\textsubscript{3} in the presence of a triphenylphosphine ligand. This yielded a slightly more complex mixture, signifying more cross-coupling was taking place. Next, the SPhos ligand was employed with K\textsubscript{3}PO\textsubscript{4} as a base in toluene. It has been previously shown that this more electron donating ligand can allow for cross-coupling to occur with compounds containing hindered bromides.\textsuperscript{42} On a small scale, this yielded the desired product 44 almost exclusively. An attempt to scale up the reaction, however, led to minimal conversion.


In an attempt to enhance product yield on a larger scale by avoiding the Suzuki coupling step, Strategy 4 was investigated to provide the crucial tris(biphenyl)benzene intermediate as summarized in Scheme 3.13. Here, 2'-bromoacetophenone was treated with boronic acid 46 under Suzuki conditions to yield biphenyl acetophenone 47. Treatment with ICl gave iodinated compound 48. Adaptation a literature procedure\textsuperscript{43} for trimerization and dehydration to form 49, however, resulted mainly in a dehydrated dimeric product and unreacted starting material.
Following the failure of Strategy 4, Strategy 3 was further investigated. In an attempt to enhance the turnover of the reaction, iodide substituted starting materials were employed (Scheme 3.14). First, cyclization/dehydration of 2'-iodobenzophenone was performed to give iodinated triphenylbenzene compound 50. 50 was subjected to Suzuki cross-coupling conditions in the presence of 46 to, satisfyingly, give the crucial tris(biphenyl)benzene compound 44 in good yield. Iodination via treatment with ICl gave iodinated compound 49, which was then subjected to Scholl reaction conditions with FeCl₃ to give the desired triiodinated HBC compound 51.

Hexa-\textit{peri}-hexabenzocoronene-Containing Peptide Trimer Synthesis

Scheme 3.15. Attempted synthesis of HBC-containing peptide trimers via solid-phase Sonogashira cross-coupling trimerization.

Utilizing the iodide substituents as synthetic handles for eventual peptide attachment, several routes were investigated. First, triiodo-HBC 51 was treated with TIPS-acetylene under Sonogashira conditions to yield the TIPS protected trialkynyl HBC derivative 52 (Scheme 3.15). The silyl protecting groups were removed to give deprotected analogue 53. 53 was then introduced to a resin-bound peptide, which had been acylated with 4-iodobenzoic acid, under Sonogashira conditions using a modified version of the solid-phase Sonogashira cross-coupling procedure previously developed in the lab (details in Chapter 2). Unfortunately, no reaction took place, presumably because the complete insolubility of 53 prevented its intercalation into the voids of the resin beads, therefore not allowing cross-coupling between the terminal alkynes of the HBC core and the aryl iodide-capped peptides to occur.
Scheme 3.16. Attempted synthesis of HBC-containing peptide trimers via solid-phase a) sila-Sonogashira and b) in situ deprotection/Sonogashira cross-coupling trimerizations.

Because it was suspected that the insolubility of the HBC derivative was preventing the solid-phase cross-coupling from occurring, other methods were attempted which were designed to allow for some solubility (or impediment of macroscopic aggregation) of the HBC cores during the reaction. First, as shown in Scheme 3.16a, a direct solid-phase coupling between the silated alkynes of TMS-protected trialkynyl HBC 54 (prepared through Sonogashira coupling of 51 and TMS-acetylene) and a resin-bound aryl iodide capped peptide was investigated, by modifying a solution-phase literature protocol. Sonication of 54 in DMF prior to addition to the reaction mixture created a heterogeneous suspension (in contrast to the plates of 53 which could not be suspended in solution). It was hoped that this would allow for the intercalation of 54 into the resin voids, however no reaction was observed. Assuming that employing bulkier TIPS groups as the alkyne protecting group could allow for greater HBC solubility or preclusion from aggregation, an in-situ deprotection/cross-coupling method with
52 was investigated, using standard Sonogashira conditions in addition to TBAF, as adapted from a solution-phase procedure in the literature (Scheme 3.16b). But again, no reaction between the aryl iodide capped peptides and the HBC core was observed. This reaction does proceed in the solution phase with aryl iodides (as will be shown later), so the failure of the solid-phase reaction is once again presumably due to lack of HBC intercalation into the resin beads.

Scheme 3.17. a) Attempted synthesis of tricarboxylic acid HBC derivative 55, and synthesis of tricarboxylic acid HBC derivatives b) 56 and c) 58.

Because it had become apparent that facilitating peptide attachment via the solid-phase Sonogashira cross-coupling procedure was not effective, attachment through a
modified solid-phase PyBOP-mediated amidation, which has previously been shown by our lab to proceed in the presence of insoluble π-conjugated cores (details in Chapter 1), was investigated. To accomplish this, carboxylic acid moieties needed to be installed on the triiodo HBC core 51. This was first attempted by lithiation and quenching of 51 with CO\textsubscript{2} as shown in Scheme 3.17. However, this reaction failed to provide the desired tricarboxylic acid HBC 55. Next, 51 was subjected to Suzuki conditions in the presence of 4-carboxybenzene boronic acid to give tricarboxylic acid 56. Alternatively, 52 was subjected to \textit{in-situ} desilation-Sonogashira conditions with methyl 4-iodobenzoate to give triester 57, and saponified to give alkynyl-spaced tricarboxylic acid 58.

56 and 58 were each utilized in PyBOP-mediated solid-phase amidations with various resin-bound peptides as shown in Scheme 3.18 to give the HBC-containing peptide trimer library 59 – 63. Each trimer contains either the 56 or 58 core and three peptide fragments ranging in length from 5 to 11 amino acids with differing ratios of ionizable aspartic acid residues. These peptide lengths and primary amino acid sequences were tweaked in an attempt to impart solubility and lessen the aggregation of the systems under basic conditions, despite the insoluble aromatic core. While increasing the length and number of aspartic acid residues of the peptide fragments seemed to allow for better solubility in basic aqueous media, none of the systems could be adequately purified by HPLC. A representative HPLC trace corresponding to compound 63 is shown in Figure 3.19. As was similar with all of the peptides in the library, the trace displays a broad peak spanning nearly 15 minutes. This may be due to aggregation of the compounds on the reverse-phase column, even under basic buffer conditions. The entirety of the peak was collected and characterization was attempted, anyway. While absorption spectra suggested that the samples collected did indeed contain the HBC core, neither ESI nor MALDI-TOF mass spectrometry showed evidence of the desired compounds. However, TEM of 62 showed evidence of the formation of 1-D nanostructures upon assembly, as shown in Figure 3.20.

Figure 3.19. Crude HPLC trace of 63.
The evidence of nanostructures formed from the assembly of impure peptide 62 prompted the investigation of a new procedure which could allow for adequate purification of the desired product. Because it is presumed that the purification difficulty stems from aggregation of the peptides on the HPLC column, it could be remedied by subjecting the peptides to HPLC purification prior to the Scholl-type cyclization to form the HBC core, because the uncyclized oligophenyl precursor is more soluble and less prone to aggregation. To achieve this, the peptide fragments would be attached via solid-phase PyBOP-mediated amidation to an uncyclized oligophenyl tricarboxylic acid core, HPLC purified, then subjected to Scholl conditions. To form the uncyclized oligophenyl core, a coupling between the triiodo-oligophenyl precursor 49 and 4-carboxybenzene boronic acid under standard Suzuki conditions was initially performed, as illustrated in Scheme 3.19a. However, after allowing the coupling to proceed for 18 hours, the major product was found to be [1,1'-biphenyl]-4-
To rule out possible undesirable couplings through the carboxylic acid moiety, the reaction was repeated using 4-((methoxycarbonyl)phenyl)boronic acid (Scheme 3.19b), resulting in the analogous biphenyl ester 65. These conditions were then repeated and monitored closely by TLC. It was found that the desired triester oligophenyl product 66 is indeed formed within the first few hours of heating and after 3 hours the desired compound was isolated in 34% yield (Scheme 3.19c). These results suggest that the biphenyl side product is ultimately formed by some sort of elimination-like side reaction that occurs upon extended heating. With this information, the analogous reaction using 4-carboxybenzene boronic acid as the coupling partner was attempted with the shorter heating time (Scheme 3.19d), however this also resulted in [1,1'-biphenyl]-4-carboxylic acid 64.

Scheme 3.19. Attempted and successful synthetic routes to form uncyclized triester 66. Routes a), b), and d) ultimately formed undesired biphenyl compounds 64 and 65.
Scheme 3.20. Synthesis of uncyclized peptide trimer 68 via solid-phase PyBOP-mediated triple amidation.

Following its preparation, uncyclized ester 66 was saponified to tricarboxylic acid 67 and subjected to solid-phase PyBOP-mediated triple amidation with resin-bound peptides to give peptidic trimer 68 (Scheme 3.20). HPLC purification of this compound proved to be much more facile, as shown by the HPLC trace of the crude mixture in Figure 3.21. In the trace, the first large peak corresponds to the desired trimer 68, while the second and third peaks correspond to the analogous dimer (69) and monomer peptides, as concluded through ESI mass spectral analyses.

Figure 3.21. Crude HPLC spectrum of 68 and 69 product mixture.
Scheme 3.21. Attempted Scholl reaction to form HBC-containing peptide trimer 70.

Test reactions for the Scholl-type dehydrogenation were performed on the purified peptidic dimer 69, as to not waste material. However, both FeCl₃ and DDQ/triflic acid Scholl conditions (Scheme 3.21) ultimately resulted in an insoluble brown/orange solid that could not be characterized and unreacted starting material instead of HBC peptide dimer 70. The failure of the reaction most likely due to 69 not being soluble in the reaction solvent (dichloromethane), perhaps resulting in intermolecular bond formation between the cores of neighboring aggregated molecules. Alternative approaches will need to be developed in order to synthesize an HBC-containing peptide trimer that can be adequately purified to elucidate its self-assembly and electronic properties. For instance, preparation and HPLC purification of the peptide fragment followed by a solution-phase coupling of the peptide to the HBC core could potentially alleviate purification issues.
Porphyrin-Containing Peptide Tetramers

Introduction

Porphyrrins are known to be particularly efficient ligands, catalysts, light harvesters, photoinduced electron transfer donors, and photosensitizers. Nature takes advantage of these properties in processes such as photosynthesis (chlorophyll) and various enzymatic reactions of hemoproteins. Porphyrrins have been used for a multitude of applications from biomedical therapy to electronic materials. Furthermore, peptides have also been employed as a means to enhance the effectiveness of porphyrrins for certain applications.

![Diagram](image-url)

Figure 3.22. Porphyrin-peptide surfactants synthesized by Manoharan and coworkers.

One application where these porphyrrin-peptide conjugates have found use is in photodynamic therapy. Because porphyrrins are efficient photosensitizers, they can be used to generate reactive oxygen species. These reactive oxygen species are cytotoxic and can be effective as a cancer treatment when generated in the vicinity of a tumor. However, their cytotoxicity is restricted to the area in which they are concentrated, so the porphyrrin-based photosensitizer must be delivered to the site. To accomplish this, Manoharan and
coworkers employed a peptide surfactant to aid in cellular uptake, which can form a complex with the porphyrin through electrostatic interactions.\textsuperscript{51} As shown in Figure 3.22, they utilized mixtures of meso-tetrakis(4-sulfonatophenyl)porphyrin 71 (negatively charged at physiological pH) and short, lysine-containing peptide 72 (positively charged at physiological pH). Although increasing concentrations of peptide did result in the desired porphyrin aggregation (according to absorption and photoluminescence studies), any enhancement of cellular uptake caused by the peptide surfactant was not explored.

![Figure 3.23. Porphyrin-peptide hybrids synthesized by Vicente and coworkers.\textsuperscript{52}](image)

Vicente and coworkers took this concept a step further and designed a series of porphyrin-peptide hybrids that contained amino acid sequences chosen to enhance the cellular uptake of the molecules into specific organelles.\textsuperscript{52} The structures are shown in Figure 3.23. The sequences, ranging from 25-32 amino acid residues in length, consist of combinations of known cell penetrating peptide (CPP) sequences and nuclear localization signal (NLS) sequences. The peptides were then attached covalently to the porphyrin moiety through a linker to give 73-76. No peptide was attached to control porphyrin 77. The cellular uptake of the hybrids into human HEp2 cells was found to be 3-8 times higher than for the
control 77, with 75 predominating. Upon 20 minute illumination with a 100 W halogen lamp, 75 also displayed the lowest IC$_{50}$ concentration of 6.9 µM.

Figure 3.24. Porphyrin-peptide-quinone donor-acceptor dyads synthesized by Bolton and coworkers.$^{53}$

Porphyrrin-peptide conjugates have also found uses in electron transfer and photovoltaic applications.$^{53,54}$ Porphyrins are capable of photoinduced electron transfer as well as light harvesting while the peptides can be used as scaffolds to create donor-acceptor dyads or promote supramolecular organization of multiporphyrin arrays. Bolton and coworkers studied a series of porphyrin-peptide-quinone donor-acceptor dyads to explore the photoinduced electron transfer of the systems and how it relates to distance.$^{53}$ The structures of the studied dyads are shown in Figure 3.24. The “peptide” bridges that were employed included a simple amide linker (78) glycine (79), and phenylalanine (80). The electron transfer rates were deduced for each system in a variety of solvents. In most solvents, the rates were found to follow the trend $80>78>79$. These results suggest that while a shorter distance between the electron donor and acceptor yields a faster rate of electron transfer (78 vs. 79),
the presence of an aromatic moiety in the linker works to enhance electron transfer rates even more, presumably due to the phenyl group of phenylalanine aiding the transfer.

Figure 3.25. a) Structures, and b) schematic of assembly with fullerenes of porphyrin-peptide oligomers synthesized by Fukuzumi and coworkers. Adapted with permission from ref. 54.

More recently, Fukuzumi and coworkers synthesized a series of porphyrin-peptide oligomers capable of supramolecular assembly with fullerenes to create novel photovoltaic cells that combine the simple solution-processability of conjugated polymer-based photovoltaics with the light harvesting abilities of porphyrins. The oligomers, structures shown in Figure 3.25a, are comprised of 1 (81), 2 (82), 4 (83), or 8 (84) lysine residues functionalized with a porphyrin macrocycle. The peptide backbone provides a scaffold to align the porphyrin rings, and was found to be flexible enough to allow for accommodation of a C\textsubscript{60} fullerene between the porphyrins, creating supramolecular arrays of electron donors and acceptors (Figure 3.25b). They assembled the porphyrin-peptide-C\textsubscript{60} arrays onto nanostructured SnO\textsubscript{2} substrates and compared the efficiencies of the resulting photovoltaic devices. It was ultimately discovered that the system which possessed the highest number
of porphyrin rings (84) also displayed the highest power conversion efficiency (1.3%) and maximum incident photon-to-photocurrent efficiency (42%).

These examples illustrate the utility of porphyrin-peptide hybrids for therapeutic and electronic applications. They also show that peptide incorporation enhances the effectiveness of the porphyrins by facilitating cellular recognition and/or supramolecular organization. Due to these reasons, the creation of novel porphyrin-peptide tetrameric hybrids was explored.
To create a novel porphyrin-peptide conjugate system as well as test the limitations of the previously developed solid-phase chromophore-peptide couplings, we envisioned constructing a tetrameric peptide system containing a free-base porphyrin core. This was completed through a modified version of the solid phase PyBOP-mediated amidation procedure previously described (details in Chapter 1)\(^\text{(12)}\), utilizing commercially available meso-(tetra(4-carboxyphenyl))porphine and Wang resin-bound peptides with free terminal amine positions, as shown in Scheme 3.22, which provided peptide tetramer $85$. 

Characterization of Assembly

Figure 3.26. Absorption (a, b) and photoluminescence (c, d) spectra of 85 in water (a, c) and 6mM CaCl₂(aq) (b, d) at pH 8 (blue), pH 6 (green) and pH 4 (red). The photoluminescence spectra were acquired by exciting the samples at the $\lambda_{\text{max}}$ of the Soret band (415 nm – solid lines, 437 nm – dashed lines).

Visually, solutions of 85 appear as a deep purple at basic pH and a vivid green at acidic pH. As shown in Figure 3.26a, the absorption spectrum of 85 at pH 8 shows the Soret band at 415 nm as well as four Q-bands at 514, 553, 580, and 631 nm (blue line). Upon acidification, the Soret band red-shifts 22 nm and only two Q-bands are apparent, one at 595 nm and one at 649 nm (red line). This loss of two Q-bands is indicative of a doubly protonated “diacid” version of the porphyrin core, due to increased symmetry upon protonation. At pH 6, the absorption spectrum shows evidence of both of these species in solution (green line).
Similar behavior is seen in the emission spectra of 85 at each pH (Figure 3.26c). The basic and acidic samples were excited at the $\lambda_{\text{max}}$ of the Soret bands, resulting in emission peaks at 640 nm and 665 nm, respectively. Spectra for the sample at pH 6 was collect by exciting at both Soret bands, resulting in two different emission profiles.

Interestingly, aggregation of the diacid form of the porphyrin typically occurs in water at low pH and the assembly is dependent on the counterion of the acid used. For instance, the aggregated diacid form of tetra(p-carboxyphenyl)porphyrin in a pH 0.9 water/HCl mixture shows a blue-shifted Soret band (in comparison to the unaggregated diacid), suggesting mostly H-like aggregation. Alternatively, the same sample prepared in a pH 0.9 water/HNO$_3$ mixture shows both red-shifted and blue-shifted Soret bands, suggesting the existence of both H- and J-like aggregate in the same assembled sample. However, neither of these situations seem to be occurring upon acidification of 85, which appears to be remaining unaggregated by comparison to literature spectra. This may be due to coulombic repulsion of the doubly charged porphyrin cores, preventing the assembly of the peptides.

While the previously studied peptide-$\pi$ hybrids have all been designed with acidic amino acid residues to allow for pH triggered aggregation, another method to elicit assembly is by increasing the ionic strength of the solution with salt, such as CaCl$_2$. This technique is especially useful for future biological and cell culture applications of the peptide assemblies, where pH changes are not well tolerated. Due to the pH sensitivity of the porphyrin core of 85 possibly inhibiting acid-induced aggregation, absorption and photoluminescence studies were performed using 6mM CaCl$_2$ aqueous solutions at various pH levels (Figure 3.26b,d). The spectrum at pH 4 + CaCl$_2$ appeared identical to the acidic spectrum without CaCl$_2$ (pH 4 - CaCl$_2$), however the spectra at pH 8 and 6 + CaCl$_2$ showed significant differences, ultimately resulting in two nearly identical spectra. Both of these absorption profiles display four Q bands and a slightly broader Soret band at 415 nm with a relatively low molar absorptivity. Similarly, the photoluminescence spectrum at pH 4 + CaCl$_2$ was identical to that pH 4 - CaCl$_2$, while
those at pH 6 and 8 + CaCl$_2$ were altered in intensity. It is assumed that the spectral changes are not caused by the coordination of Ca$^{2+}$ inside the porphyrin macrocycle, as it has previously been shown that this interaction is not energetically desirable due to size discrepancy between the Ca$^{2+}$ cation and the interior diameter of the porphyrin$^{57}$, although porphyrin-Ca$^{2+}$-porphyrin sandwich complexes could potentially be formed.

Figure 3.27. Circular dichroism spectra of 85 in water (a, c) and 6mM CaCl$_2$(aq) (b, d) at pH 8 (blue), pH 6 (green) and pH 4 (red).

To further investigate the effects of pH and ionic strength on the assembly of 85, circular dichroism spectroscopy was employed. As shown in Figure 3.27a and c, no meaningful signal was seen in the spectra of samples prepared at pH 6 and 8 without the addition of CaCl$_2$, aside from significant scattering between 400 and 450 nm that was seen in
all samples. However, spectra at pH 4 - CaCl₂ showed a weak signal near 450 nm. Alternatively, the spectra of 85 at pH 6 and 8 in a 6mM CaCl₂ solution (Figure 3.27b and d) exhibited significant bisignate Cotton effects within the Soret band absorbance range, while that at pH 4 + CaCl₂ was identical to its spectrum without CaCl₂. These data suggest that the addition of CaCl₂ to near neutral pH solutions of 85 is either triggering the assembly of the porphyrin-peptide hybrid, or altering the assembly in a manner that induces a bias in helical handedness.¹⁹⁻²¹

![Figure 3.28. TEM images of 85 from 1 mg/mL solutions assembled via acidification.](image)

To visualize the supramolecular assemblies created from acidification and CaCl₂ addition to solutions of 85, TEM was employed. The resulting images are shown in Figure 3.28 and Figure 3.29. Using acidification as the assembly trigger resulted in 1-D nanostructure several microns in length, despite any Coulombic repulsion caused by the protonation of the porphyrin core (Figure 3.28). These structures also undergo significant inter-structure interactions to form larger bundled assemblies. Single structures range in
thickness from 6.5 nm – 9 nm, while the expected width of the most extended conformation of 85 is approximately 5.0 nm.

Figure 3.29. TEM images of 85 from 1 mg/mL solutions assembled via CaCl₂ addition.

Interestingly, when the addition of CaCl₂ was employed as the assembly trigger, TEM images showed larger areas of less well-defined structures as well as small areas of defined 1-D structures (Figure 3.29). These more well-defined regions displayed structures significantly shorter in length and generally more uniform in width (6.6 ± 0.4 nm). Although comparison between the solution-phase assembly studies and the visualization of the assemblies through TEM may be complicated by drying and staining as well as variability in assembly kinetics due to using different methods of introducing the trigger (diffusion via acid chamber vs. direct injection of CaCl₂(aq)), it is reasonable to deduce that both assembly triggers produce supramolecular aggregation of the porphyrin-peptide hybrid and that the resulting structures are unique in comparison to one another. 58
Conclusion

Multivalent self-assembling peptide-π hybrid materials containing a variety of tri- and tetravalent discotic cores (benzothiophene, decacyclene triimide, hexa-peri-hexabenzocoronene, and meso-substituted porphyrin) were investigated. Following the preparation of the π-conjugated subunits, these peptide systems were synthesized by modifying solid-phase amidation, imidation, and Stille cross-coupling procedures previously developed in our lab, thus expanding these methodologies for the production of these complex multivalent architectures. Although purification via HPLC was an issue with systems containing highly conjugated cores due to insolubility and untriggered aggregation, particularly in the case of the HBC-containing peptide trimers, ultimately four novel multivalent peptide-π hybrids were synthesized, purified, and further investigated.

Each multivalent peptide was capable of self-assembling into 1-D nanostructures, however absorption, photoluminescence, and circular dichroism data suggested that the assemblies differed on a molecular level. For instance, while the assembly of BTT-containing peptide 19 corresponded with a quenching of the BTT subunit’s photoluminescence, that of related peptide 18 and DTI-containing peptide 25 triggered enhancements in photoluminescence. Furthermore, the circular dichroism spectra of 18 and 19 showed Cotton effects upon aggregation of opposite sign, suggesting helical chirality of the assemblies with opposite handedness, while that of 25 showed no signal in the vicinity of the chromophore, signifying either a lack of helicity or directional bias of the helicity. The assembly characterization of porphyrin-containing peptide tetramer 85 was complicated by the protonation of the porphyrin core upon acidification, however it was seen that differing assemblies arose from aggregation via acidification vs. salt addition.

Visualization with TEM also showed differences in the 1-D nanostructures formed by each multivalent peptide-π hybrid. 18, 25, and 85 each formed high aspect ratio 1-D nanostructures, each with widths corresponding to the length of the peptides in their most
extended conformation, suggesting a one-dimensional stacking of the molecules in the supramolecular structures, while 19 appeared to form more undefined aggregates under the same conditions. Furthermore, the nanostructures differed in the amount of interstructure interactions with 85 experiencing extreme bundling, 18 displaying minor structure intertwining, and 25 showing a relative lack of interstructure bundling. These differences may be due to the different multivalent architectures (trimeric vs. tetrameric), as well as the variances in the conjugated cores’ structural rigidities.
Experimental

General considerations: Toluene, THF, and DCM were acquired from an Innovative Technology Pure Solv solvent purification system. Solvents were degassed by sparging with nitrogen for 30 to 90 minutes before use and nonaqueous solvents were dried over 4 Å molecular sieves. All glassware was flame-dried prior to use and all reactions were performed under an inert N₂ atmosphere unless otherwise noted. Tetrakis(triphenylphosphine)palladium was obtained from Strem Chemicals. FeCl₃ was purchased from Acros. N-Methylpyrrolidine (NMP), Wang resin substituted with the first amino acid, and Fmoc-protected amino acids were obtained from Advanced ChemTech. HBTU, 2,3-dibromothiophene, PyBOP, 4-carboxybenzene boronic acid, 4-(methoxycarbonyl)phenyl boronic acid, TFA, 1,3-dibromobenzene, and 2'-iodoacetophenone were purchased from Oakwood Products Inc. Meso-tetra(4-carboxyphenyl)porphine was obtained from Frontier Scientific. Decacycylene trianhydride was obtained from Professor Fred Wudl. All other reagents, starting materials, and solvents were obtained from Sigma-Aldrich. 1,3,5-trichloro-2,4,6-triodobenzene (10), 3-(tributylstannyl)thiophene, and 2,2''-diiodo-5'-(2-iodophenyl)-1,1':3',1''-terphenyl (50) were prepare using literature procedures.⁵⁹-⁶¹

NMR Spectroscopy: ¹H-NMR spectra were obtained using a Bruker Avance 400 MHz FT-NMR spectrometer, and processed with Bruker Topspin 1.3. Peptide ¹H NMR spectra were acquired using a 1 second presaturation pulse to suppress water.

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 0.1% ammonium hydroxide.

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 with an Ushio Xenon short arc lamp. Spectroscopic samples were prepared by diluting in Millipore water until achieving an absorbance near 0.1.
The pH was then adjusted by adding 10 μL of either 1M KOH (basic) or 1M HCl (acidic). Ionic strength was adjusted by the addition of 200 mM CaCl$_2$(aq) to bring the final CaCl$_2$ concentration to 6 mM.

**MALDI-TOF:** MALDI-TOF samples were collected using a Bruker AutoFlexIII MALDI-TOF/TOF in positive mode. Samples were prepared in a 1:1 acetonitrile:water solution with 0.1% TFA with α-cyano-4-hydroxycinnamic acid as a matrix.

**Circular Dichroism (CD):** CD spectra were obtained using a AVIV 420 CD spectropolarimeter. Spectroscopic samples were prepared by diluting to the appropriate concentration in Millipore water. The pH was then adjusted by adding 10 μL of either 1M KOH (basic) or 1M HCl (acidic). Ionic strength was adjusted by the addition of 200 mM CaCl$_2$(aq) to bring the final CaCl$_2$ concentration to 6 mM.

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

**Transmission Electron Microscopy (TEM):** Imaging was performed on a Philips EM 420 transmission electron microscope equipped with an SIS Megaview III CCD digital camera. The samples were prepared by pipetting a drop of 1 mg/mL solution of assembled peptide in water onto 200 mesh Formvar coated copper grids and incubated for 5 minutes at 25°C. Excess solution was wicked off by touching the side of the grid to filter paper. The sample was then stained with a 2% uranyl acetate solution and excess moisture was wicked off. The grid was allowed to dry in air before imaging.

$((2,4,6\text{-trichlorobenzene-1,3,5-triyl)}\text{tris(ethyne-2,1-diyl))tris(trimethylsila...})$ (11):

Literature procedure was modified as follows$^{16}$: Compound 10 (1.40 g, 2.50 mmol), Pd(PPh$_3$)$_4$ (0.26 g, 0.23 mmol, 9 mol%), and Cul (0.086 g, 0.45 mmol, 18 mol%) were added to a Schlenk flask. THF (10 mL), diisopropylamine (2 mL), and TMS-acetylene (1.60 mL, 11.3 mmol) were
added. The mixture was heated to 75°C for 18 hours. TMS-acetylene (0.20 mL, 1.4 mmol) was added. The mixture was allowed to remain at 75°C for an additional 24 hours. The resulting suspension was diluted with dichloromethane and washed with a saturated aqueous NH₄Cl solution three times. The solvent was removed under vacuum. The crude material was purified via column chromatography (silica, hexane) to yield 11 (0.58 g, 1.2 mmol, 49%) as an orange/yellow solid. ¹H NMR (400 MHz, CDCl₃) δ: 0.29 (s, 27 H). ¹³C NMR (100 MHz, CDCl₃) δ: 139.1, 122.8, 108.1, 97.4, -0.2.

**benzo[1,2-b:3,4-b′:5,6-b′′]trithiophene (12):** Following a procedure reported in the literature¹⁶: Sodium sulfide nonahydrate (2.88 g, 12.0 mmol) was placed in a round bottomed flask. NMP (50 mL) was and 11 (0.94 g, 2.0 mmol) were added. The mixture was heated to 190°C for 17 hours. The solution was poured into a saturated aqueous NH₄Cl solution and filtered. The brown solid was dissolved in chloroform and washed three times with a saturated aqueous NaCl solution. The organic layer was collected and the solvent was removed under vacuum. The crude material was purified via column chromatography (silica, 30% chloroform in hexanes) to yield 12 (0.32 g, 7.8 mmol, 65%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ: 7.62 (d, 3 H, J = 5.2 Hz), 7.52 (d, 3 H, J = 5.6 Hz).

**3-bromo-2,3'-bithiophene (16):** 2,3-dibromothiophene (9.69 g, 40.0 mmol) and Pd(PPh₃)₄ (0.92 g, 0.80 mmol, 2 mol%) was placed in a Schlenk flask. DMF (200 mL), was added and the mixture was heated to 80°C. 3-(tributylstannyl)thiophene (16.4 g, 44.0 mmol) was added dropwise and the mixture was allowed to stir at 80°C for 115 hours. The resulting solution was allowed to cool to room temperature, diluted with ether, and stirred in a 1M aqueous KF solution for 10 minutes. The mixture was filtered and the filtrate washed with a saturated aqueous NH₄Cl solution three times. The organic layer was collected and the solvent removed under vacuum. The crude product was purified via column chromatography (silica, hexanes) to yield 16 (6.47 g, 26.4 mmol, 66%) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ: 7.77
(dd, 1 H, J = 3, 1.4 Hz), 7.48 dd, 1 H, J = 5.2, 1.2 Hz), 7.39 (dd, 1 H, J = 5.0, 3.0 Hz), 7.20 (d, 1 H, J = 5.6 Hz), 7.05 (d, 1 H, J = 5.2 Hz).

2,3':2',3''-terthiophene (15): 16 (6.33 g, 25.8 mmol), and Pd(PPh3)4 (0.59 g, 0.52 mmol, 2 mol%) were placed in a Schlenk flask. DMF (100 mL) was added and the mixture was heated to 80°C. 2-(tributylstannyl)thiophene (10.8 g, 28.8 mmol), was added dropwise via syringe. The solution was allowed to stir at 80°C for 17 hours. The resulting solution was allowed to cool to room temperature, diluted with ether, and stirred in a 1M aqueous KF solution for 10 minutes. The mixture was filtered and the filtrate washed with a saturated aqueous NH4Cl solution twice. The organic layer was collected and the solvent removed under vacuum. The crude product was purified via column chromatography (silica, hexanes) to yield 15 (5.80 g, 23.3 mmol, 91%) as a white solid. 1H NMR (400 MHz, CDCl3) δ: 7.34-7.30 (m, 2 H), 7.28-7.23 (m, 2 H), 7.21 (d, 1 H, J = 5.2 Hz), 7.09 (dd, 1 H, J = 4.8, 1.6 Hz), 7.02-7.00 (m, 2 H).

benzo[1,2-b:3,4-b':5,6-b'']trithiophene (12): Literature procedure was modified as follows17: 15 (5.48 g, 22.0 mmol) and I2 (0.17 g, 0.66 mmol, 3.0 mol%) were dissolved in toluene (1800 mL). The mixture was stirred at room temperature for 21 hours with air bubbling through the solution while irradiated by a 450 Watt mercury vapor lamp equipped with a cooling water jacket. The solution was washed with a saturated aqueous Na2S2O3 solution. The organic layer was collected and solvent removed under vacuum. The crude product was purified via column chromatography (silica/10% DCM in hexanes) to give an inseparable mixture of 12 and 15. This mixture was put under the same conditions as previously described for 19 hours, then worked up and purified the same as previously described to give pure 12 (3.05 g, 12.3 mmol, 56%) as a pale yellow solid. 1H NMR (400 MHz, CDCl3) δ: 7.62 (d, 3 H, J = 5.2 Hz), 7.52 (d, 3 H, J = 5.6 Hz).

2,5,8-tris(tributylstannyl)benzo[1,2-b:3,4-b':5,6-b'']trithiophene (17): Literature procedure was modified as follows15: A Schlenk flask was charged with 12 (0.25 g, 1.0 mmol) and dissolved in 40 mL of THF. The reaction vessel was cooled to 0°C and n-BuLi (1.6 M in
hexanes, 2.0 mL, 3.1 mmol) was added dropwise over 5 minutes. The mixture was allowed to stir and return to room temperature for 1.5 hours. SnBu₃Cl (0.84 mL, 3.1 mmol) was added dropwise. The solution was allowed to stir for 18 hours and was then diluted with DCM and washed with a saturated aqueous NH₄Cl solution and brine three times. The organic layer was collected and the solvent removed under vacuum. The crude product was purified by column chromatography (alumina/hexanes) to yield 17 (0.84 g, 0.75 mmol, 75%) as a pale yellow oil. ¹H NMR (400 MHz, CDCl₃) δ: 7.72 (s, 3H), 1.67 (quin, 18H, J = 8.0 Hz), 1.44 (sext, 18H, J = 8.0 Hz), 1.29-1.23 (m, 18H), 0.97 (t, 27H, J = 8.0 Hz).

**VEVAG-T-BTT peptide trimer (18):** Wang resin-bound VEVAG peptide capped with a thiophene bromide (0.3 mmol) (synthesis detailed in Chapter 2 and ref. 13) was placed in a Schlenk flask and excess solvent removed under vacuum. Pd(PPh₃)₄ (0.017 g, 0.015 mmol, 5 mol%) was added. 17 (0.11 g, 0.10 mmol), was dissolved in 7 mL DMF and the solution was added to the reaction vessel via syringe. The mixture was heated to 80°C with constant nitrogen bubbling through the solution to agitate the resin beads for 20 hours. The mixture was allowed to cool and the resin was transferred to a peptide chamber. The resin was subjected to a wash cycle (3x NMP, 3x DMF, 2x iPrOH, 2x H₂O, 2x (THF, iPrOH), 2x acetonitrile, 2x ether, 2x hexanes). The resin was then treated with a mixture of 9.5 mL TFA, 250 µL TIPS, 250 µL H₂O, and 5 mL DCM for 3 hours. The resulting solution was separated from the resin and was concentrated to approximately 5 mL under reduced pressure. 90 mL of ether was added and the orange solid was isolated via centrifugation. The solid was dissolved in approximately 5 mL of water and 10 µL NH₄OH and lyophilized. The product was further purified by HPLC to give 18 (0.0071 mmol, 0.014 g, 7.1%) as a yellow powder. MS (ESI) m/z 1989.1 (M-H) (calc. 1988.6), m/z 1016.2 (M-4H+2Na+)-2 (calc. 1015.8), m/z 1005.3 (M-3H+Na+)-2 (calc. 1004.8), m/z 994.3 (M-2H)-2 (calc. 993.8), m/z 662.5 (M-3H)-3 (calc. 662.2).
**VEVAG-BT-BTT peptide trimer (19):** Wang resin-bound VEVAG peptide capped with a bithiophene bromide (0.3 mmol) (synthesis detailed in Chapter 2 and ref. 13) was placed in a Schlenk flask and excess solvent removed under vacuum. Pd(PPh₃)₄ (0.017 g, 0.015 mmol, 5 mol%) was added. 17 (0.11 g, 0.10 mmol), was dissolved in 7 mL DMF and the solution was added to the reaction vessel via syringe. The mixture was heated to 80°C with constant nitrogen bubbling through the solution to agitate the resin beads for 20 hours. The mixture was allowed to cool and the resin was transferred to a peptide chamber. The resin was subjected to a wash cycle (3x NMP, 3x DMF, 2x iPrOH, 2x H₂O, 2x (THF, iPrOH), 2x acetonitrile, 2x ether, 2x hexanes). The resin was then treated with a mixture of 9.5 mL TFA, 250 μL TIPS, 250 μL H₂O, and 5 mL DCM for 3 hours. The resulting solution was separated from the resin and was concentrated to approximately 5 mL under reduced pressure. 90 mL of ether was added and the orange solid was isolated via centrifugation. The solid was dissolved in approximately 5 mL of water and 10 μL NH₄OH and lyophilized. The product was further purified by HPLC to give 19 (0.0032 mmol, 0.0071 g, 3.2%) as an orange powder. MS (ESI) m/z 1117.2 (M-2H)-2 (calc. 1117.3), m/z 744.2 (M-3H)-3 (calc. 744.5).

**General triple imidation procedure for decacyclene triimide peptide trimers (23-25):** Wang resin-bound peptide displaying free terminal amines was placed in a Schlenk flask and excess solvent removed under vacuum. 22 (0.33 eq) and pyridine were added. The mixture was heated to 65°C, and then DIPEA (15 eq) was added. The mixture was heated to 135°C for 18 hours with N₂ bubbling constantly to agitate the resin. The reaction was allowed to cool and the resin was transferred to a peptide chamber. The resin was subjected to a wash cycle (3x NMP, 3x DMF, 2x iPrOH, 2x H₂O, 2x (THF, iPrOH), 2x acetonitrile, 2x ether, 2x hexanes). The resin was then treated with a mixture of 9.5 mL TFA, 250 μL TIPS, 250 μL H₂O, and 5 mL DCM for 3 hours. The resulting solution was separated from the resin and was concentrated to approximately 5 mL under reduced pressure. 90 mL of ether was added and
the orange solid was isolated via centrifugation. The solid was dissolved in approximately 5 mL of water and 10 μL NH₄OH and lyophilized.

**(VEVAG)₃-decacyclene triimide peptide trimer (23):** Solid supported Wang-VEVAG-NH₂ peptide was prepared (0.1 mmol). The resin was subjected to the standard triple imidation procedure in the presence of 22 (0.022 g, 0.033 mmol) and DIPEA (0.25 mL, 1.5 mmol) in pyridine (4 mL) for 17 hours to give 23 as an orange powder (0.061 g, crude). Peptide could not be purified further due to solubility issues. MS (ESI) m/z 1012.5 (M-2H)² (calc. 1011.9).

**(DADGG)₃-decacyclene triimide peptide trimer (24):** Solid supported Wang-DADGG-NH₂ peptide was prepared (0.1 mmol). The resin was subjected to the standard triple imidation procedure in the presence of 22 (0.022 g, 0.033 mmol) and DIPEA (0.25 mL, 1.5 mmol) in pyridine (4 mL) for 17 hours to give 24 as an orange powder (0.056 g, crude). Peptide could not be purified further due to solubility issues. MS (ESI) m/z 641.7 (M-3H+Na⁺)² (calc. 641.5).

**(DADADADADGG)₃-decacyclene triimide peptide trimer (25):** Solid supported Wang-DADADADADGG-NH₂ peptide was prepared (0.5 mmol). The resin was subjected to the standard triple imidation procedure in the presence of 22 (0.11 g, 0.17 mmol) and DIPEA (1.3 mL, 7.5 mmol) in pyridine (8 mL) for 18 hours and was further purified by HPLC to give 25 (0.033 g, 0.009 mmol, 5.5%) as an orange powder. MS (ESI) m/z 1193.5 (M-3H)³ (calc. 1192.7), m/z 894.5 (M-4H)⁴ (calc. 894.3), m/z 715.4 (M-5H)⁵ (calc. 715.2), m/z 595.8 (M-6H)⁶ (calc. 595.8), m/z 446.8 (M-8H)⁸ (calc. 446.6).

**1-bromo-4-(phenylethynyl)benzene (33):** Literature procedure was modified as follows: 250 mL of THF was transferred to a flame-dried Schlenk flask. 1-bromo-4-iodobenzene (4.33 g, 15 mmol), Pd(PPh₃)₄ (0.121 g, 0.105 mmol), and CuI (0.041 g, 0.11 mmol) were added. Phenylacetylene (1.70 mL, 15.1 mmol) was added via syringe, as was 50 mL of deoxygenated DIPA. The mixture was allowed to stir at room temperature for 18 hrs under nitrogen. The resulting suspension was filtered and the filtrate was concentrated under reduced pressure. The residual solid was dissolved in dichloromethane and washed with a saturated ammonium
chloride solution. The organic layer was concentrated and crude product was subjected to column chromatography (silica, hexanes), to yield a white solid (3.75 g, 14.5 mmol, 97%). $^1$H NMR (400 MHz, CDCl$_3$) δ: 7.35-7.37 (m, 3H), 7.40 (d, 2H, $J = 8.4$ Hz), 7.49 (d, 2H, $J = 8.4$ Hz), 7.52-7.54 (m, 2H).

4-bromo-3',5'-bis(4-bromophenyl)-4',6'-diphenyl-1,1':2',1''-terphenyl (34) and 4,4''-dibromo-4'- (4-bromophenyl)-3',5',6'-triphenyl-1,1':2',1''-terphenyl (35): Adapted from the literature as follows$^{63}$: A 3-neck round bottom flask equipped with a reflux condenser was charged with 1-bromo-4- (phenylethynyl)benzene (33) (1.68 g, 5.00 mmol). 1,4-dioxane (100 mL) was added to the reaction flask via cannula. Co$_2$(CO)$_8$ (0.17 g, 0.5 mmol) was added and the solution was allowed to reflux for 19 hrs under nitrogen. The mixture was allowed to cool and purified by column chromatography (silica, 50:50 dichloromethane:hexanes). The resulting solid was triturated with acetone to yield a white powder (1.06 g, 58%). $^1$H NMR (400 MHz, CDCl$_3$) δ: 7.00 (dd, 6H, $J = 8.4$ Hz), 6.91 (t, 3H, $J = 3.2$ Hz), 6.88-6.87 (m, 6H), 6.78-6.75 (m, 6H), 6.66 (d, 6H, $J = 8.4$ Hz). $^{13}$C NMR (100 MHz, CD$_2$Cl$_2$) δ: 133.30, 131.52, 130.29, 130.10, 130.07, 127.32, 127.14, 126.12, 125.92. HR-MS (EI): found m/z = 767.9660 (M$^+$); calc. for C$_{42}$H$_{27}$Br$_{79}$3: 767.9663, found m/z = 769.9660 (M$^+$); calc. for C$_{42}$H$_{27}$Br$_{79}$2Br$_{81}$: 769.9642, found m/z = 771.9634 (M$^+$); calc. for C$_{42}$H$_{27}$Br$_{79}$Br$_{81}$2: 771.9622, found m/z = 773.9615 (M$^+$); calc. for C$_{42}$H$_{27}$Br$_{81}$3: 773.9601.

1-bromo-3-(trimethylsilyl)benzene (38): Following a procedure reported in the literature$^{64}$, 1,3-dibromobenzene (11.9 g, 50 mmol), n-butyllithium (1.58 M, 36.7 mL, 58 mmol), and TMSCI (8.3 mL, 66 mmol), in diethyl ether (100 mL), yielded 38 (10.2 g, 44.5 mmol, 88%) and was used without further purification. $^1$H NMR (400 MHz, CDCl$_3$) δ: 7.69-7.67 (m, 1H), 7.52 (ddd, 1H, $J = 8.0$, 1.2, 0.8 Hz), 7.47 (dt, 1H, $J = 7.6$, 0.8 Hz), 7.26 (t, 1H, $J = 7.6$ Hz), 0.33 (s, 9H).

Trimethyl(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yI)phenyl)silane (39): A three neck round bottom flask equipped with a condenser was charged with 38 (9.4 g, 41.3 mmol),
bis(pinacolato) diboron (11.6 g, 45.5 mmol), PdCl$_2$(dppf) (0.29 g, 0.4 mmol, 1 mol%), and KOAc (12.6 g, 128 mmol). 1,4-dioxane (300 mL) was added and the mixture was heated to reflux for 15 hours. The solution was allowed to cool to room temperature. It was diluted with ether and washed with brine three times. The first aqueous layer was washed with ether. The organic fractions were combined and concentrated under reduced pressure. The crude material was purified via column chromatography (silica, 35% DCM in hexane) to yield 39 (8.70 g, 31.5 mmol, 76%) as a white powder. $^1$H NMR (400 MHz, CDCl$_3$) δ: 8.10 (s, 1H), 7.94 (dt, 1H, $J = 7.2, 1.2$ Hz), 7.73 (dt, 1H, $J = 7.2, 1.6$ Hz), 7.46 (t, 1H, $J = 7.6$ Hz), 1.44 (s, 12H), 0.40 (s, 9H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ: 139.7, 139.5, 136.3, 135.5, 127.2, 25.0, -0.9.

HR-MS (EI): found m/z = 276.1720 (M$^+$); calc. for C$_{15}$H$_{25}$BO$_2$Si: 276.1717, found m/z = 261.149 (M-CH$_3$$^+$); calc. for C$_{14}$H$_{26}$BO$_2$Si: 261.148.

2-bromo-3’-(trimethylsilyl)biphenyl (40): Adapted from the literature as follows$^{37}$: 39 (6.0 g, 21.7 mmol), 1-bromo-2-iodobenzene (5.60 g, 19.8 mmol), and Pd(PPh$_3$)$_4$ (0.23 g, 0.20 mmol, 1 mol %) were added to a three neck round bottom flask equipped with a condenser. Toluene (240 mL), ethanol (80 mL), and 1M K$_2$CO$_3$(aq) (80 mL) were added to the reaction vessel. The mixture was heated to reflux for 18 hours. After cooling, the organic layer was separated, diluted with toluene, and washed with water twice. The solvent was removed under vacuum and the crude mixture was purified by column chromatography (silica, hexane), to yield 40 (5.30 g, 17.4 mmol, 88%) as a colorless liquid. $^1$H NMR (400 MHz, CDCl$_3$) δ: 7.71 (d, 1H, $J = 8.0$ Hz), 7.62-7.61 (m, 1H), 7.60-7.57 (m, 1H), 7.47-7.44 (m, 2H), 7.40-7.39 (m, 2H), 7.38 (s, 1H), 7.25-7.20 (m, 1H), 0.35 (s, 9H).

1,3,5-Tris(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzene (43): Literature procedure was modified as follows$^{66}$: 1,3,5-tribromobenzene, bis(pinacolato) diboron, PdCl$_2$(dppf), and KOAc were placed in a three neck round bottom flask equipped with a condenser. DMSO (200 mL) was added. The mixture was heated to 80°C for 19 hours. The mixture was allowed to cool, quench slowly with water, and diluted with DCM. The organic
layer was washed with water 3 times and the solvent was removed under vacuum. The crude product was purified by column chromatography (silica, DCM) and recrystallized from ethanol to yield 43 (2.64 g, 5.80 mmol, 39%) as a white solid. \( ^1\text{H NMR} \ (400 \text{ MHz, CDCl}_3) \delta: 8.36 \ (s, 3\text{H}), 1.33 \ (s, 36 \text{ H}). \)

\( ^{13}\text{C NMR} \ (100 \text{ MHz, CDCl}_3) \delta: 144.3, 83.8, 25.0. \)

1,3,5-tris-2'-bromophenylbenzene (45): 43 (0.46 g, 1 mmol), 1-bromo-2-iodobenzene (0.99 g, 3.5 mmol), and Pd(PPh\(_3\))\(_4\) (0.08 g, 0.07 mmol, 2 mol %) were added to a three neck round bottom flask equipped with a condenser. Toluene (55 mL), ethanol (15 mL), and 1M K\(_2\)CO\(_3\) (aq) (15 mL) were added to the reaction vessel. The mixture was heated to reflux for 19 hours. After cooling, the organic layer was separated and washed with brine three times. The solvent was removed under vacuum and the crude mixture was purified by column chromatography (silica, 20% DCM in hexane), to yield 45 (0.38 g, 0.70 mmol, 70%) as a white powder. \( ^1\text{H NMR} \ (400 \text{ MHz, CDCl}_3) \delta: 7.70 \ (dd, 3\text{H}, J = 8, 1.2 \text{ Hz}), 7.52 \ (s, 3\text{H}), 7.47 \ (dd, 3\text{H}, J = 7.6, 1.6 \text{ Hz}), 7.38 \ (td, 3\text{H}, J = 7.6, 1.2 \text{ Hz}), 7.21 \ (td, 3\text{H}, J = 13.5, 2.0 \text{ Hz}). \)

\( ^{13}\text{C NMR} \ (100 \text{ MHz, CDCl}_3) \delta: 142.1, 140.6, 133.4, 131.7, 129.8, 129.1, 127.6, 122.8. \)

1,3,5-tris-2'-bromophenylbenzene (45): Following a procedure reported in the literature\(^40\), 2'-bromoacetophenone (3.9 g, 20.0 mmol) and trifluoromethanesulfonic acid (0.7 mL, 8 mmol) yielded 45 (1.06 g, 5.90 mmol, 30%) as a white solid after column chromatography (silica, 15% DCM in hexane). \( ^1\text{H NMR} \ (400 \text{ MHz, CDCl}_3) \delta: 7.70 \ (dd, 3\text{H}, J = 8, 1.2 \text{ Hz}), 7.52 \ (s, 3\text{H}), 7.47 \ (dd, 3\text{H}, J = 7.6, 1.6 \text{ Hz}), 7.38 \ (td, 3\text{H}, J = 7.6, 1.2 \text{ Hz}), 7.21 \ (td, 3\text{H}, J = 13.5, 2 \text{ Hz}). \)

\( ^{13}\text{C NMR} \ (100 \text{ MHz, CDCl}_3) \delta: 142.1, 140.6, 133.4, 131.7, 129.8, 129.1, 127.6, 122.8. \)

(3-(trimethylsilyl)phenyl)boronic acid (46): Literature procedure was modified as follows\(^66\): 38 (2.35 g, 10.3 mmol) was added to a Schlenk flask. Toluene (80 mL), and THF (20 mL) were added. The solution was cooled to -78°C in a dry ice/acetone bath. N-butyllithium (1.59 M, 7.75 mL, 12.3 mmol) was added dropwise over 5 minutes. The mixture was allowed to stir at -78°C for 1 hour. Trimethyl borate (4.3 g, 41 mmol), was added dropwise. The mixture was allowed to stir and return to room temperature over 24 hours. The vessel was cooled to
0°C and 50 mL of degassed 1M HCl was added slowly. The mixture was diluted with ether and washed with brine. The solvent was removed under vacuum. The crude product was recrystallized from hexane to yield 46 (1.20 g, 6.20 mmol, 60%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ: 8.45 (s, 1H), 8.24 (dt, 1H, J = 7.2, 1.6 Hz), 7.79, (dt, 1H, J = 7.2, 1.2 Hz), 7.53 (td, 1H, J = 7.4, 0.8 Hz), 0.38 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ: 140.9, 140.1, 137.8, 136.2, 127.6, -1.0.

1,3,5-tris[3''-(trimethylsilyl)-2'-biphenyl]ylbenzene (44): 45 (0.14 g, 0.25 mmol), 46 (0.22 g, 1.1 mmol), Pd₂(dba)₃ (0.014 g, 0.015 mmol, 6 mol%), SPhos (0.025 g, 0.060 mmol, 24 mol%), and K₃PO₄ (0.48 g, 2.3 mmol) were added to a Schlenk tube equipped with a condenser. Toluene (2 mL) was added. The mixture was heated to 110°C for 42 hours. After cooling, the mixture was diluted with ether and washed with brine twice. The solvent was removed under vacuum. The crude material was purified via column chromatography (silica, 50% DCM in hexane) to yield 44 (0.93 g, 0.12 mmol, 50%) as a white powder. ¹H NMR (400 MHz, CDCl₃) δ: 7.55 (dt, 3H, J = 7.2, 1.2 Hz), 7.52-7.47 (m, 6H), 7.42 (td, 3H, J = 7.4, 1.6 Hz), 7.38-7.30 (m, 6H), 7.01 (dt, 3H, J = 7.6, 1.8 Hz), 6.83 (s, 3H), 6.77 (dd, 3H, J = 7.6, 1.2 Hz), 0.30 (s, 27H). ¹³C NMR (100 MHz, CDCl₃) δ: 141.2, 141.0, 140.6, 140.4, 140.3, 135.1, 131.5, 130.9, 130.4, 130.2, 130.1, 127.3, 127.3, -1.0.

1-(3''-(trimethylsilyl)-[1,1''-biphenyl]-2-yl)ethanone (47): 2'-bromoacetophenone (0.36 g, 1.8 mmol), 46 (0.52 g, 2.7 mmol), and Pd(PPh₃)₄ (0.04 g, 0.04 mmol, 2 mol %) were added to a three neck round bottom flask equipped with a condenser. Toluene (30 mL), ethanol (8 mL), and 1M K₂CO₃(aq) (8 mL) were added to the reaction vessel. The mixture was heated to reflux for 19 hours. After cooling, the organic layer was separated and washed with brine three times. The solvent was removed under vacuum and the crude mixture was purified by column chromatography (silica, 50% DCM in hexane), to yield 47 (0.42 g, 1.6 mmol, 88%) as a colorless viscous oil. ¹H NMR (400 MHz, CDCl₃) δ: 7.62-7.57 (m, 2H), 7.56-7.51 (m, 2H), 7.48-7.42 (m, 2H), 7.42-7.34 (m, 2H), 2.01 (s, 3H), 0.33 (s, 9H). ¹³C NMR (100 MHz, CDCl₃)
δ: 204.8, 141.2, 141.1, 140.9, 140.0, 133.9, 132.8, 130.7, 130.2, 129.1, 128.1, 127.9, 127.4, 30.4, -1.1.

**1-(3'-iodo-[1,1'-biphenyl]-2-yl)ethanone (48):** 47 (0.39 g, 1.5 mmol) was added to a Schlenk flask. Chloroform (50 mL) was added via cannula. ICl (1 M in DCM, 2.9 mL, 2.9 mmol) was added dropwise to the solution. The mixture was allowed to stir at room temperature for 1 hour. 1 M NaHSO₃(aq) was added. The mixture was washed with water 3 times. The organic phase was collected and the solvent was removed under reduced pressure to yield 48 (0.42 g, 1.3 mmol, 88%) as a viscous pale yellow oil. ¹H NMR (400 MHz, CDCl₃) δ: 7.72-7.67 (m, 2H), 7.56 (dd, 1H, J = 6.0, 1.6 Hz), 7.48 (td, 1H, J = 7.6, 1.6 Hz), 7.40 (td, 1H, J = 7.4, 1.6 Hz), 7.31 (ddd, 1H, J = 7.6, 1.2, 0.4 Hz), 7.25 (ddd, 1H, J = 9.0, 1.6, 1.2 Hz), 7.11 (td, 1H, J = 7.6, 0.8 Hz), 2.09 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ: 203.6, 142.9, 140.3, 138.8, 137.4, 136.7, 130.9, 130.3, 130.1, 128.2, 128.1, 127.9, 94.5, 30.4.

**1,3,5-tris[3''-(trimethylsilyl)-2'-biphenyl]ylbenzene (44):** A 2 neck round bottomed flask equipped with a reflux condenser was charged with 50 (1.5 g, 2.2 mmol), 46 (2.60 g, 13.2 mmol), and Pd(PPh₃)₄ (0.31 g, 0.26 mmol). Toluene (35 mL), ethanol (10 mL), and a 2M aqueous solution of K₂CO₃ (10 mL), were added via syringe. The mixture was heated to reflux for 90 hours. The solution was allowed to cool to room temperature and was diluted with dichloromethane. It was washed with a saturated aqueous NaCl solution and the solvent was removed from the organic layer under vacuum. The crude compound was purified via column chromatography (silica, 30% DCM in hexanes) to yield 44 (1.60 g, 2.07 mmol, 94%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ: 7.55 (dt, 3H, J = 7.2, 1.2 Hz), 7.52-7.47 (m, 6H), 7.42 (td, 3H, J = 7.4, 1.6 Hz), 7.38-7.30 (m, 6H), 7.01 (dt, 3H, J = 7.6, 1.8 Hz), 6.83 (s, 3H), 6.77 (dd, 3H, J = 7.6, 1.2 Hz), 0.30 (s, 27H). ¹³C NMR (100 MHz, CDCl₃) δ: 141.2, 141.0, 140.6, 140.4, 140.3, 135.1, 131.5, 130.9, 130.4, 130.2, 130.1, 127.3, 127.3, -1.0.
3,3'''-diiodo-5''-(3'-iodo-[1,1'-biphenyl]-2-yl)-1,1':2',1''':3'',1'''':2''',1'''''-quinquephenyl (49): Following a procedure reported in the literature\textsuperscript{37}: A Schlenk flask was charged with 44 (0.19 g, 0.25 mmol) and dissolved in chloroform (55 mL). ICl (1M solution in dichloromethane, 1.5 mL, 1.5 mmol), was added dropwise. The mixture was allowed to stir at room temperature for 1 hour. The resulting solution was quenched with 1M aqueous NaHSO\textsubscript{3} solution. The organic layer was washed with water three times and concentrated under reduced pressure until approximately 5 mL remained. The product was crashed out of solution upon the addition of cold ethanol to yield 49 (0.16 g, 0.18 mmol, 72%) as a white powder. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) δ: 7.64-7.59 (m, 6H), 7.38-7.31 (m, 9H), 7.03 (t, 3H, J = 3.0 Hz), 6.93 (dt, 3H, J = 7.6, 1.2 Hz), 6.86-6.81 (m, 3H), 6.78 (s, 3H).

2,8,14-triiodohexabenzocoronene (51): Following a procedure reported in the literature\textsuperscript{37}: 49 (0.10 g, 0.11 mmol) was added to a Schlenk flask and dissolved in dichloromethane (30 mL). FeCl\textsubscript{3} (0.64 g, 4.0 mmol), was dissolved in nitromethane (2 mL) and added dropwise to the reaction flask. The mixture was allowed to stir at room temperature for 1 hour. The mixture was quenched with 30 mL of methanol. The resulting solid was filtered off to give 50 as an insoluble yellow powder (0.64 g, 0.07 mmol, 64%). Characterization could not be obtained due to insolubility.

2,8,14-tris((triisopropylsilyl)ethynyl)hexabenzocoronene (52): 51 (0.36 g, 0.40 mmol) and Pd(PPh\textsubscript{3})\textsubscript{4} (0.069 g, 0.060 mmol, 15 mol%), and Cul (0.023 g, 0.12 mmol, 30 mol%) were placed in a Schlenk flask. THF (9 mL) and DIPA (3 mL) were added via syringe. TIPS-acetylene (0.27 mL, 1.2 mmol) was added and the mixture was heated to 45°C for 22 hours. The resulting solution was allowed to cool to room temperature, diluted with DCM, and washed with a saturated aqueous NH\textsubscript{4}Cl solution. The organic layer was collected and the solvent removed under vacuum. The crude product was suspended in 10 mL DCM and crashed out with 50 mL MeOH. The resulting solid was filtered to give 52 (0.40 g, 0.37 mmol, 93%) as a
yellow solid. NMR data could not be obtained due to insolubility. MS (MALDI-TOF) m/z 884.7 (C$_{64}$H$_{59}$Si$_2^+$) (calc. 883.4), m/z 904.6 (C$_{64}$H$_{58}$NaSi$_2^+$) (calc. 905.4).

**2,8,14-triethynylhexabenzocoronene (53):** 52 (0.13 g, 0.13 mmol) was placed in a Schlenk flask. THF (30 mL) was added via syringe. TBAF (1.0 M in THF, 0.76 mL, 0.76 mmol) was added and the mixture was allowed to stir at room temperature for 3 hours. The suspension was poured onto 100 mL of methanol. The resulting solid was filtered to give 53 (0.058 g, 0.098 mmol, 77%) as brown/orange plates which was carried on immediately. NMR data could not be obtained due to insolubility.

**2,8,14-tris((trimethylsilyl)ethynyl)hexabenzocoronene (54):** 51 (0.45 g, 0.50 mmol) and Pd(PPh$_3)_4$ (0.087 g, 0.075 mmol, 15 mol%), and Cul (0.029 g, 0.15 mmol, 30 mol%) were placed in a Schlenk flask. THF (12 mL) and DIPA (5 mL) were added via syringe. TMS-acetylene (0.27 mL, 1.2 mmol) was added and the mixture was heated to 45°C for 20 hours. The resulting solution was allowed to cool to room temperature, diluted with DCM, and washed with a saturated aqueous NH$_4$Cl solution. The organic layer was collected and the solvent removed under vacuum. The crude product was suspended in 10 mL DCM and crashed out with 50 mL MeOH. The resulting solid was filtered to give 54 (0.35 g, 0.43 mmol, 87%) as yellow plates. NMR data could not be obtained due to insolubility. MS (MALDI-TOF) m/z 809.7 (M$^+$) (calc. 810.3), m/z 794.6 (C$_{56}$H$_{39}$Si$_3^+$) (calc. 795.2).

**4,4',4''-(hexabenzo[bc,ef,hi,kl,no,qr]coronene-2,8,14-triyl)tribenzoic acid (56):** 51 (0.36 g, 0.40 mmol) and Pd(PPh$_3)_4$ (0.056 g, 0.048 mmol, 12 mol%), and 4-carboxybenzene boronic acid (0.40 g, 2.4 mmol) were placed in a Schlenk flask. Toluene (6 mL) and ethanol (2 mL) and a 4.8 M aqueous solution of K$_2$CO$_3$ (2 mL) were added via syringe. The mixture was heated to reflux for 45 hours. The resulting solution was allowed to cool to room temperature and was acidified to pH 1 with concentrated HCl. The resulting orange solid was isolated via filtration and washed with water, methanol, and dichloromethane to give 56 (0.35 g, 0.40 mmol, 100%). NMR data could not be obtained due to insolubility. MS (MALDI-TOF) m/z
trimethyl 4,4',4''-(hexabenzocoronene-2,8,14-triyltris(ethyne-2,1-diyl))tribenzoate (57): 52 (0.21 g, 0.20 mmol), Pd(PPh3)4 (0.016 g, 0.014 mmol, 7 mol%), and Cul (0.005 g, 0.03 mmol, 14 mol%) were placed in a Schlenk flask. THF (4 mL) and DIPA (1 mL) were added via syringe. TBAF (1.0 M in THF, 0.80 mL, 0.80 mmol) was added dropwise via syringe and the mixture was heated to 45°C for 45 hours. The resulting solution was allowed to cool to room temperature, diluted with DCM, and washed with a saturated aqueous NH4Cl solution. The organic layer was collected and the solvent removed under vacuum. The crude product was suspended in 10 mL DCM and crashed out with 50 mL MeOH. The resulting solid was filtered to give 57 (0.20 g, 0.20 mmol, 99%) as yellow plates. NMR data could not be obtained due to insolubility. MS (MALDI-TOF) m/z 996.2 (M+)(calc. 996.3), m/z 838.2 (C62H30O4+)(calc. 838.2), m/z 680.1 (C52H24O2+) (calc. 680.2).

trimethyl 4,4',4''-(hexabenzocoronene-2,8,14-triyltris(ethyne-2,1-diyl))tribenzoic acid (58): 57 (0.048 g, 0.048 mmol) was suspended in 2 mL THF in a Schlenk flask. In a separate flask, KOH (0.041 g, 0.72 mmol) was dissolved in 2 mL of water. The solution was added to the reaction vessel, and the mixture allowed to stir at 75°C for 37 hours. The resulting solution was acidified to pH 1 with 2 M HCl. The resulting suspension was filtered and the precipitate collected and washed with water to give 58 as a brown solid (0.042 g, 0.044 mmol, 92%). NMR data could not be obtained due to insolubility. MS (MALDI-TOF) m/z 953.8 (M+) (calc. 954.2), m/z 809.8 (C60H25O4+) (calc. 809.2), m/z 665.8 (C51H21O2+) (calc. 665.2).

General procedure for the preparation of HBC peptide trimers (59-63): 56 (0.088 g, 0.10 mmol) or 58 (0.096 g, 0.10 mmol) and PyBOP (0.17 g, 0.33 mmol) was placed in a scintillation vial and suspended in 5 mL NMP. DIPEA (0.78 mL, 4.5 mmol) was added and the vial was agitated for 1 minute. The suspension was poured into a peptide chamber containing a Wang resin-bound peptide. The vial was rinsed with 5 mL NMP, which was also added to the peptide
chamber. The chamber was allowed to spin for approximately 40 hours at room temperature. The suspension was drained from the peptide chamber and the resin was subjected to a wash cycle (3x NMP, 3x DMF, 2x iPrOH, 2x H$_2$O, 2x (THF, iPrOH), 2x acetonitrile, 2x ether, 2x hexanes). The resin was then treated with a mixture of 9.5 mL TFA, 250 μL TIPS, 250 μL H$_2$O, and 5 mL DCM for 3 hours. The resulting solution was separated from the resin and was concentrated to approximately 5 mL under reduced pressure. 90 mL of ether was added and the yellow solid was isolated via centrifugation. The solid was dissolved in approximately 5 mL of water and 10 μL NH$_4$OH and lyophilized. Following attempts to purify by HPLC, the product was obtained as a yellow powder, which could not be characterized due to insolubility.

[1,1'-biphenyl]-4-carboxylic acid (64): 49 (0.55 g, 0.60 mmol), Pd(PPh$_3$)$_4$ (0.083 g, 0.072 mmol, 12 mol%), and 4-carboxybenzene boronic acid (0.60 g, 3.6 mmol) were placed in a Schlenk flask. Toluene (9 mL) and ethanol (3 mL) and a 4.8 M aqueous solution of K$_2$CO$_3$ (3 mL) were added via syringe. The mixture was heated to reflux for 3 or 18 hours. The resulting solution was allowed to cool to room temperature and was acidified to pH 1 with concentrated HCl. The resulting white solid was isolated via filtration and washed with water to give 64 (0.30 g, 1.5 mmol). $^1$H NMR (400 MHz, CDCl$_3$) δ: 8.19 (d, 2H, $J = 8.4$ Hz), 7.71 (d, 2H, $J = 8.4$ Hz), 7.65 (d, 2H, $J = 7.2$ Hz), 7.49 (t, 2H, $J = 7.7$ Hz), 7.41 (t, 1H, $J = 7.2$ Hz).

methyl [1,1'-biphenyl]-4-carboxylate (65): 49 (0.55 g, 0.60 mmol) and Pd(PPh$_3$)$_4$ (0.083 g, 0.072 mmol, 12 mol%), and (4-(methoxycarbonyl)phenyl)boronic acid (0.65 g, 3.6 mmol) were placed in a Schlenk flask. Toluene (9 mL) and ethanol (3 mL) and a 4.8 M aqueous solution of K$_2$CO$_3$ (3 mL) were added via syringe. The mixture was heated to reflux for 18 hours. The resulting solution was allowed to cool to room temperature, was diluted with dichloromethane, and the organic layer was extracted and washed with water. The solvent was removed under vacuum and the resulting solid was purified via column chromatography (silica, 80% DCM in hexanes) to give 65 (0.32 g, 1.5 mmol) as a white solid. $^1$H NMR (400 MHz, DMSO-d$_6$) δ:
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8.04 (dd, 2H, J = 8.4, 1.4 Hz), 7.83 (dd, 2H, J = 8.5, 1.8 Hz), 7.74 (d, 2H, J = 8.2 Hz), 7.51 (t, 2H, J = 7.9 Hz), 7.41 (t, 1H, J = 7.4 Hz), 3.88 (s, 3H).

1,3,5-tri(methyl[1,1′:3′,1″-terphenyl]-4-carboxylate)benzene (66): 49 (0.55 g, 0.60 mmol), Pd(PPh₃)₄ (0.083 g, 0.072 mmol, 12 mol%), and (4-(methoxycarbonyl)phenyl)boronic acid (0.65 g, 3.6 mmol) were placed in a Schlenk flask. Toluene (9 mL) and ethanol (3 mL) and a 4.8 M aqueous solution of K₂CO₃ (3 mL) were added via syringe. The mixture was heated to reflux for 3 hours. The resulting solution was allowed to cool to room temperature, was diluted with dichloromethane, and the organic layer was extracted and washed with water. The solvent was removed under vacuum and the resulting solid was purified via column chromatography (silica, 80% DCM in hexanes) to give 66 (0.19 g, 0.21 mmol, 34%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ: 7.96 (d, 6H, J = 7.9 Hz), 7.64 (d, 6H, J = 8.0 Hz), 7.56 (d, 3H, J = 7.7 Hz), 7.48 (s, 3H), 7.43 (d, 3H, J = 7.6 Hz), 7.36 (t, 3H, J = 7.4 Hz), 7.23 (t, 3H, J = 7.4 Hz), 7.16 (t, 3H, J = 7.6 Hz), 6.93 (d, 3H, J = 7.6 Hz), 6.81 (s, 3H), 6.76 (d, 3H, J = 7.5 Hz), 3.85 (s, 9H). ¹³C NMR (100 MHz, DMSO-d₆) δ: 166.0, 144.5, 141.7, 140.2, 139.5, 139.4, 138.9, 134.6, 134.2, 130.3, 129.8, 129.7, 129.5, 128.6, 128.5, 128.4, 127.9, 127.7, 126.9, 52.1.

1,3,5-tri([1,1′:3′,1″-terphenyl]-4-carboxylic acid)benzene (67): 66 (0.29 g, 0.31 mmol) was dissolved in 10 mL of THF in a 3 neck round bottomed flask equipped with a reflux condenser. In a separate flask, KOH (0.26 g, 4.7 mmol) was dissolved in 10 mL of water. The mixture was added to the round bottomed flask. The mixture was heated to reflux for 16 hours. The solution was allowed to cool to room temperature, acidified to pH 1 with concentrated HCl, and diluted with DCM. The organic layer was extracted and washed with 1 M KOH(aq). The aqueous layer was extracted and acidified to pH 1 with concentrated HCl. The white solid was filtered off to give 67 (0.25 g, 0.28 mmol, 91%). ¹H NMR (400 MHz, DMSO-d₆) δ: 12.9 (br s, 3H), 7.96 (d, 6H, J = 8.0 Hz), 7.65 (d, 6H, J = 7.7 Hz), 7.58 (d, 3H, J = 7.7 Hz), 7.50 (s,
3H), 7.45 (d, 3H, J = 7.4 Hz), 7.38 (t, 3H, J = 7.6 Hz), 7.25 (t, 3H, J = 7.2 Hz), 7.17 (t, 3H, J = 7.4 Hz), 6.92 (d, 3H, J = 7.5 Hz), 6.81 (s, 3H), 6.77 (d, 3H, J = 7.4 Hz).

(DADADADADGG)₃-decaphenyl peptide trimer (68) and dimer (69): Adapted from the literature as follows¹²: To resin bound DADADADADGG-NH₂ peptide (1 mmol) within a peptide chamber was added 5 mL of dichloromethane. In a separate vial, 67 (0.15 g, 0.017 mmol) and PyBOP (0.29 g, 0.56 mmol) was dissolved in 15 mL NMP. DIPEA (1.48 mL, 8.5 mmol) was added to the vial and the solution was agitated for 1 minute. The solution was then added to the peptide chamber and mixed for 18 hours. The resin was subjected to a washing cycle (3x NMP, 3x DMF, 2x iPrOH, 2x water, 2x (2x THF, 2x iPrOH), 2x acetonitrile, 2x ether, 2x hexanes). The resin was again swelled in 5 mL of dichloromethane and subjected to a second round of coupling with 67 (0.15 g, 0.017 mmol), PyBOP (0.29 g, 0.56 mmol) dissolved in 15 mL NMP, which was mixed with DIPEA (1.48 mL, 8.5 mmol), agitated for 1 minute and added to the peptide chamber. The chamber was agitated for 23 hours. The resin was again subjected to the same wash cycle. The resin was treated with 9.5 mL of trifluoroacetic acid, 250 μL water, and 250 μL of triisopropylsilane for 3 hrs. The peptide solution was filtered from the resin beads, which were then washed 3x with DCM, and the resulting solution concentrated by evaporation under reduced pressure. The crude peptide was then precipitated from solution with 90 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 2 mL of water and 30 μL ammonium hydroxide and lyophilized. The crude product was purified via HPLC to give 68 and 69 as white solids. 68: MS (ESI) m/z 1907.5 (M-2H)² (calc. 1906.6), m/z 1271.4 (M-3H)³ (calc. 1270.8), m/z 953.3 (M-4H)⁴ (calc. 952.8), m/z 762.5 (M-5H)⁵ (calc. 762.0), m/z 635.3 (M-6H)⁶ (calc. 634.9). 69: MS (ESI) m/z 1420.3 (M-2H)² (calc. 1420.0), m/z 946.7 (M-3H)³ (calc. 946.3), m/z 709.7 (M-4H)⁴ (calc. 709.5), m/z 567.7 (M-5H)⁵ (calc. 567.4), m/z 472.9 (M-6H)⁶ (calc. 472.8).
**Tetraphenylporphyrin(DADGG)$_4$ peptide tetramer (85):** Adapted from the literature as follows$^2$: To resin bound DADGG-NH$_2$ peptide (0.5 mmol) within a peptide chamber was added 5 mL of dichloromethane. In a separate vial, meso-(tetra(4-carboxyphenyl))porphine (0.060 g, 0.075 mmol) and PyBOP (0.017 g, 0.33 mmol) was dissolved in 10 mL NMP. DIPEA (0.78 mL, 4.5 mmol) was added to the vial and the solution was agitated for 1 minute. The solution was then added to the peptide chamber and mixed for 15 hours. The resin was subjected to a washing cycle (3x NMP, 3x DMF, 2x iPrOH, 2x water, 2x (2x THF, 2x iPrOH), 2x acetonitrile, 2x ether, 2x hexanes). The resin was again swelled in 5 mL of dichloromethane and subjected to a second round of coupling with meso-(tetra(4-carboxyphenyl))porphine, (0.040 g, 0.05 mmol), PyBOP (0.12 g, 0.22 mmol) dissolved in 10 mL NMP, which was mixed with DIPEA (0.52 mL, 3.0 mmol), agitated for 1 minute and added to the peptide chamber. The chamber was agitated for 24 hours. The resin was again subjected to the same wash cycle. The resin was treated with 9.5 mL of trifluoroacetic acid, 250 μL water, and 250 μL of triisopropylsilane for 3 hrs. The peptide solution was filtered from the resin beads, which were then washed 3x with DCM, and the resulting solution concentrated by evaporation under reduced pressure. The crude peptide was then precipitated from solution with 90 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 2 mL of water and 30 μL ammonium hydroxide and lyophilized. The crude product was purified via HPLC to give 85 (0.028 g, 0.011 mmol, 9.2%) as a purplish-brown solid. MS (ESI) $m/z$ 1224.9 (M-2H)-2 (calc. 1224.9), $m/z$ 1236.6 (M-3H+Na+)-2 (calc. 1235.9), $m/z$ 816.4 (M-3H)-3 (calc. 816.2), $m/z$ 612.1 (M-4H)-4 (calc. 611.9).
References


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Chapter 4 – Donor-Acceptor Peptide-π Hybrids

Introduction

Semiconducting, biocompatible materials based on organic electronic polymers and oligomers have been used to create a variety of bioelectronics devices that can be used for sensing/signaling\(^1\)–\(^3\) and controlling cell growth\(^4,5\), migration\(^6\), and differentiation\(^7\)–\(^10\). However, the conductivity of the materials, i.e. the ability to create charge-carriers and generate electric fields, typically relies on chemical doping and/or the use of external electronics. An interesting next step in the development of these materials, specifically to aid in their potential future uses in regenerative medicinal applications, is to investigate a means to create charge-separation in the absence of chemical doping and external electronics. One possible method to achieve this is to design donor-acceptor systems capable of photoinduced charge transfer.

Figure 4.1. Donor-acceptor-donor triads 1 and 2 and controls 3 and 4 studied by Janssen and coworkers.\(^1^9\)

Photoinduced electron transfer is a method used by nature to perform redox reactions, particularly in the process of photosynthesis. Much work has been done to mimic this process,
typically through the design of covalently bound donor-acceptor dyads, triads, and tetrads.\textsuperscript{11-18} The covalent connection of the two redox-active chromophores allows for charge transfer to not be limited by diffusion. Furthermore, work has been done to study the self-assembly of these donor-acceptor systems into supramolecular arrays and the effect of this on the charge transfer processes.\textsuperscript{19-23} One particular study by Janssen investigates both of these concepts.\textsuperscript{19} Two donor-acceptor-donor triads were studied, each containing two oligophenylenevinylene units covalently attached to a perylene diimide moiety (Figure 4.1). The triads differed by the substitution of the perylene subunit at the bay positions, ranging from chloride (1) to tert-butyl phenoxide (2). Donor and acceptor controls (3 and 4, respectively) were also prepared for comparison.

![Schematic](image1)

**Figure 4.2.** a) Schematic of J-like assembly and b) absorption spectra of 1 (black squares), 3 (black triangles) and 4 (white triangles). Adapted with permission from ref. 19.
The absorption spectra of the systems were studied in methylcyclohexane at concentrations that allowed for aggregation of the compounds. It was deduced that the systems tended to form J-like aggregates upon assembly (Figure 4.2a) due to the hindrance of the bay substituents preventing face-to-face stacking of the perylene units. A low energy absorbance in the range of 800 nm was seen for compound 1 (Figure 4.2b, black squares), which was determined to correspond to a charge transfer band, because this low energy peak was absent in the spectra for the isolated donor and acceptor controls 3 and 4 (black and white triangles, respectively). A solution of both controls (1:1) gave rise to only a weak charge transfer band (white squares), illustrating the importance of the covalent attachment of the donor and acceptor moieties. Furthermore, the charge transfer kinetics of the assemblies were compared to the molecularly dissolved triads by changing the solvent. It was found, in the case of compound 2, that electron transfer occurs nearly an order of magnitude faster for the assemblies in methylcyclohexane ($1.2 \times 10^{12}$ s$^{-1}$) than for the molecularly dissolved samples in toluene ($2.7 \times 10^{11}$ s$^{-1}$), presumably due to the closer proximity of the donor and acceptor moieties in the J-like aggregated state.

Figure 4.3. Structures of peptide-based donor-acceptor system studied by Fox and coworkers.$^{26,27}$

Utilizing peptide assembly to organize donor and acceptor moieties in space has also been investigated$^{24,25}$, including work performed by Fox and coworkers.$^{26,27}$ They synthesized peptide 5 shown in Figure 4.3, which contains unnatural amino acids containing pyrene and
dimethylaniline groups. The peptide was designed to assemble into an α-helical structure, thus placing the chromophore substituents within appropriate distances to facilitate charge transfer. It was found that electron transfer occurred nearly 25 times faster when the peptide was dissolved in a solvent that encouraged its self-assembly (acetonitrile), in comparison to solvents that disrupted assembly (2,2,2-trifluoroethanol).²⁶ These examples illustrate that supramolecular assemblies of covalently-bound donor-acceptor chromophores, specifically those that rely on peptide assembly, can be used to facilitate charge transfer and induce polar charge-separated states with light.

Figure 4.4. Illustration of self-assembly and electron transfer of proposed donor-acceptor peptide-π hybrids.

These concepts were extended to the peptide-π hybrid systems that have been previously studied in our lab²⁸-³⁰ by incorporating acceptor units onto amino acid side chains in the peptide backbone, in a general structure as shown in Figure 4.4. Lowering the pH
allows supramolecular self-assembly to occur through favorable intermolecular hydrogen bonding, ultimately creating noncovalent 1-D nanostructures as illustrated in Figure 4.4. Upon excitation of the internal donor moiety, electron transfer to the peripheral acceptors can occur. An oligothiophene chromophore, specifically quaterthiophene (OT4), was chosen for the donor as the synthesis of peptides embedded with this π-conjugated moiety has already been optimized (details in Chapter 2). Electron deficient naphthalene diimide (NDI) was selected for the acceptor, due to the option of facile incorporation via lysine side-chains. Venkataraman and coworkers have previously used this donor-acceptor pair and found it to be an ideal system for electron transfer due to the lack of spectral overlap between the emission wavelengths of OT4 and absorption wavelengths of NDI (to discourage non-polar energy transfer) and beneficial LUMO level positioning (to encourage transfer of excited electrons).
Synthesis of Donor-Acceptor and Control Peptide-π Hybrids


The synthesis of the OT4-NDI donor-acceptor peptide 6 (Scheme 4.1) began with the synthesis of N-propyl-1,4,5,8-naphthalenetetracarboxylic acid monoanhydride through imidation with propylamine to prevent diimidation with two lysine residues within peptide chains. Solid-supported peptides consisting of sequence K(Mtt)VEVGG-NH₂ were prepared and N-acylated with 5-bromothiophene-2-carboxylic acid, as previously described (details in Chapter 2).²⁹ The Mtt protecting group was chosen for the lysine residue because it can be removed in a weakly acidic environment, making it an orthogonal protecting group in Fmoc peptide synthesis. The peptide was subjected to solid-phase Stille cross-coupling conditions
in the presence of 5,5'-bis(tributylstannyl)-2,2'-bithiophene, as previously described (details in Chapter 2). Next, the Mtt protecting group was removed from the lysine residue by treatment with a 5% TFA cocktail, followed by imidation with the naphthalene monoanhydride. The resulting peptide was then cleaved from the resin to give the final product 6, where the NDI acceptor moiety is situated 6 amino acid residues away from the OT4 donor.

![Chemical Structures](image)

Figure 4.5. Structures of donor-acceptor (6, 7, and 8) and acylated control (9, 10 and 11) peptides.

To study the effect of donor-acceptor distance on the electron transfer capabilities of the peptides, 7 and 8 were synthesized using the same method, with donor-acceptor distances of 3 and 2 amino acids, respectively (Figure 4.5). The valine-glutamic acid-valine
sequence was maintained in each peptide to preserve similar overall hydrophilicity/hydrophobicity of the peptide chains. Analogous “donor-only” control peptides 9, 10, and 11 were also prepared, where the NDI substituted lysine residues are replaced by acylated lysines (Figure 4.5). The controls were used to determine the contribution of the NDI acceptor unit on the electron transfer and assembly behavior of the peptides and also as a means to “dilute” the acceptor moieties within self-assembled samples of donor-acceptor and control mixtures through heterostructure assembly.
Microscopy of Donor-Acceptor and Control Peptide-π Hybrids

Figure 4.6. Transmission electron micrographs of a) 6, b) 7, c) 8, d) 25% 6:75% 9, e) 25% 7:75% 10, f) 25% 8:75% 11, g) 9, h) 10, and i) 11.

The peptide-π hybrids are designed to maintain a relatively dissolved state in aqueous solution at high pH due to repulsion between negatively charged carboxylic acid groups. At low pH, the protonation of these groups reduces the Coulombic repulsion and triggers intermolecular self-assembly into 1-D nanostructures. To visualize these nanostructures and compare the assembly behavior of each donor-acceptor and control peptide, TEM was employed. Micrographs of assembled samples of each peptide and mixtures of each donor-acceptor peptide with its analogous control (25%DA:75%C) are shown in Figure 4.6. Samples
were prepared by acidification via acid vapor diffusion of the unassembled peptide or peptide mixture. In general, each peptide and mixture forms 1-D nanostructures displaying fairly uniform widths of 5-7 nm and significant intertwining of 2 or more structures is evident. The nanostructures formed by 6 are substantially thicker, at approximately 10-12 nm. This could be due to the positioning of the NDI moiety on the external amino acid side chain, causing the peptide to exhibit a higher degree of hydrophobicity at its termini in comparison to the other peptides, thus altering its assembly. Furthermore, the donor-acceptor peptide structures appear to generally have lower aspect ratios in comparison to those of the acylated controls. The structures formed from a mixture of 8 and 11 appear to be unique with less structure intertwining, slightly thicker diameters (8-10 nm), and relatively longer aspect ratios. These differences in nanostructure formation are common and unavoidable to these types of materials upon varying the amino acid sequence and introducing hydrophobic substituents to various positions on the peptide backbone. Nonetheless, useful assembly and electronic properties can be extracted from the self-assembled materials.
Figure 4.7. Steady-state absorption spectra of donor-acceptor (DA, blue lines) and control peptides (C, red lines) and mixtures (10:90, 25:75, and 50:50 DA:C, purple lines). a) 6 and 9, unassembled (pH 8), b) 6 and 9, assembled (pH 4), c) 7 and 10, unassembled (pH 8), d) 7 and 10, assembled (pH 4), e) 8 and 11, unassembled (pH 8), f) 8 and 11, assembled (pH 8)

The acid-triggered self-assembly of the peptides also simultaneously induces intimate \(\pi\)-electron interactions between the embedded OT4 moieties, thus encouraging exciton coupling between the transition dipoles of the chromophores. These interactions produce
perturbations in the steady-state absorption spectra. Therefore, these data were acquired for each donor-acceptor peptide (DA, blue lines), acylated control peptide (C, red lines), and various DA:C mixtures (10:90, 25:75, and 50:50 DA:C, purple lines) in both basic and acidic aqueous environments, and are shown in Figure 4.7. In general, each acylated control peptide displays a blue shift in the absorption $\lambda_{\text{max}}$ of the OT4 chromophore upon acid-triggered assembly, from 425 nm to 390-410 nm, which is indicative of cofacial H-like aggregation.\textsuperscript{32} Interestingly, while control peptides 9 and 10 display larger blue shifts (32-34 nm), 11 shifts only 13 nm and exhibits a low energy shoulder at 450 nm. This difference in behavior is possibly due to the presence of the bulky acylated lysine residues in close proximity to the OT4 chromophore. Upon the addition of increasing amounts of the analogous donor-acceptor peptides, the absorptivity of the OT4 absorption at 420 nm decreases, while that of the NDI chromophore at 390 nm is enhanced. 100% DA peptide spectra (blue lines) shows minimal perturbation of the NDI absorption between basic and acidic environments and are similar for each peptide. The OT4 chromophore contribution to the absorption spectra is more varied for the series. From unassembled to assembled, 6 shows a slight perturbation of the OT4 absorption, while in 7, this absorption is essentially unperturbed, suggesting assembly is occurring even in a basic environment. 8 displays the most perturbation of the OT4 absorption, showing a similar $\lambda_{\text{max}}$ blue shift and low energy shoulder as seen with control 11.
Figure 4.8. Steady-state emission spectra of donor-acceptor (DA, blue lines) and control peptides (C, red lines) and mixtures (10:90, 25:75, and 50:50 DA:C, purple lines). a) 6 and 9, unassembled (pH 8), b) 6 and 9, assembled (pH 4), c) 7 and 10, unassembled (pH 8), d) 7 and 10, assembled (pH 4), e) 8 and 11, unassembled (pH 8), f) 8 and 11, assembled (pH 8). Plots of relative (to 100% C) quantum yield vs mol% DA for each donor-acceptor and control pair (6 and 9 – solid lines, 7 and 10 – dashed lines, 8 and 11 – dotted lines) while g) unassembled and h) assembled.
Steady-state photoluminescence can also be perturbed upon interaction of emissive chromophores through self-assembly. As seen in Figure 4.8a-f, following excitation of the OT4 chromophore, each acylated control peptide displays nearly identical emission at 510 nm in a basic environment and dramatic (approx. 100 fold) quenching and red-shifting to 550-560 nm upon assembly, indicative of excimer formation (red lines). Due to electron transfer occurring in the donor-acceptor peptides, radiative emission does not occur in unassembled or assembled samples (blue lines). Therefore, increasing ratios of DA:C causes enhanced photoluminescence quenching (purple lines), in both unassembled and assembled samples. Interestingly, more dramatic quenching of the photoluminescence occurs at the same mole percent of donor-acceptor peptide within assembled samples, as shown in the relative photoluminescence quantum yield vs mol% DA plots in Figure 4.8g and h. While minimal change occurs for the unassembled mixtures of 10% donor-acceptor to 90% acylated control relative to the 100% control samples, assembled 10:90 mixtures retain only 55%-46% relative quantum yield. Unassembled samples containing 50% donor-acceptor peptide are quenched to 87%-64% relative quantum yield, while assembled samples are much more dramatically reduced to 13%-11%. These results suggests that a larger fraction of the photogenerated excitons are undergoing photoinduced electron transfer to the NDI acceptor units during the lifetime of the OT4 excited state when the peptides are assembled into nanostructures. This could be due to the ability of the excitons to delocalize within the assembled arrays prior to electron transfer to the NDI acceptor units, which cannot occur within the unassembled mixtures. Differences due to donor-acceptor distances within the series could not be definitively determined due to variances in assembly properties between the systems.
Figure 4.9. Circular dichroism spectra of donor-acceptor (a, b, c) and control (d, e, f) peptides under assembled (pH 4, solid lines) and unassembled (pH 8, dashed lines) conditions. a) 6, b) 7, c) 8 d) 9, e) 10, f) 11.

Circular dichroism (CD) can be utilized to characterize electronic coupling between chromophores within a chiral environment. CD spectra for each donor-acceptor (6, 7, and 8) and acylated control (9, 10, and 11) peptide in basic (pH 8) and acidic (pH 4) aqueous environments are shown in Figure 4.9. While each control peptide displays minimal absorption within the wavelength range of the OT4 chromophore π-π* transition (dashed lines), upon acidification and assembly Cotton effects are evident (solid lines), suggesting chromophore interaction within the chiral environment created by the assembled peptide scaffolds. Control peptides 9 and 10 show similar behavior, each inducing a negative bisignate signal, although that for 10 is over twice as intense. As was previously seen in the absorption studies, 11 also exhibits much different behavior in its CD spectrum, suggesting a unique supramolecular assembly in comparison to the other acylated control peptides. Again, this could possibly be due to the relatively bulky lysine residue situated in close proximity to the embedded quaterthiophene chromophore. Each donor-acceptor peptide (6, 7, and 8)
displays vastly different CD behavior. While 6 shows no meaningful signal corresponding to the OT4 chromophore in either acidic or basic environments, weak signals consistent with the NDI subunit are evident. Alternatively, 7 exhibits a strong negative bisignate Cotton effect in the absorption range of the OT4 and NDI moieties while assembled in acidic solution. This signal is also still evident even at basic pH, as was predicted by the lack of perturbation of its absorption spectrum with pH change. 8 shows essential no meaningful signal in the chromophore absorbance range in acidic solution, but upon increasing the pH, a weak positive Cotton effect is revealed, suggesting that some aggregation may be occurring at high pH. This could be a consequence of the large, hydrophobic NDI subunit in the vicinity of the peptide core.

![Graphs](image)

**Figure 4.10.** Circular dichroism spectra of donor-acceptor (DA, blue lines) and control peptides (C, red lines) and mixtures (10:90 DA:C, purple lines) under assembled conditions (pH 4). a) 6 and 9, b) 7 and 10, c) 8 and 11.

CD spectra of donor-acceptor/control mixtures in acidic solution (10:90 DA:C, purple lines) were also obtained and compared to those of the pure components (blue and red lines) (Figure 4.10). In the case of donor-acceptor/control pairs 6/9 and 8/11, addition of donor-acceptor peptide correlates with decreasing molar ellipticity. Alternatively, the mixture of 7 and 10 causes a slight increase in signal intensity, due to the fact that the molar ellipticity of assembled 7 is significantly larger than that of 10. These spectral studies demonstrate that,
while the structure of each peptide is quite similar and each have been shown to form 1-D nanostructures by TEM, the position of the lysine residues elicit differences in aggregation at the molecular level, which is typical of related materials upon alteration of the amino acid sequence/substitution.\(^{33}\)

Photoinduced electron transfer between the OT4 and NDI moieties within the peptide assemblies should create charge-separated states consisting of OT4\(^{+}\) / NDI\(^{-}\) pairs upon excitation. There are many examples in the literature where the formation of these radicals within covalent or supramolecular donor-acceptor systems (upon donor excitation by laser pulse) is confirmed by transient absorption spectroscopy.\(^{34-37}\) To investigate the charge transfer capabilities of the systems, transient absorption spectra was obtained by Timothy Magnanelli of Professor Art Bragg’s lab at Johns Hopkins University for each donor-acceptor peptide (following excitation of the OT4 moiety by high fluence 400 nm light). Spectra for 8 in both unaggregated and aggregated states are shown in Figure 4.11a and b. Both unaggregated and aggregated samples show evidence of a short-lived OT4 singlet excited state between 700 and 850 nm\(^{38,39}\), as well as longer-lived absorbances. The peaks arising between 650 and 700 nm are indicative of the OT4\(^{+}\) species\(^{35}\), while those with maximums at 500 and 1100 are attributed to the NDI\(^{-}\) species\(^{34,36,40}\), confirming that these systems are indeed capable of photoinduced electron transfer in an aqueous environment. Relative to the unaggregated samples, the assembled structures give rise to longer lived OT4\(^{+}\) / NDI\(^{-}\) pairs, whose lifetimes extend past 1000 ps. The NDI\(^{-}\) peak assignments were confirmed by obtaining transient absorption spectra for control 10, shown in Figure 4.11c and d. The unaggregated sample shows evidence of the excited OT4 singlet, as well as peaks arising at longer timescales which were attributed to a triplet excited state following intersystem crossing. Furthermore, a negative absorption is seen below 600 nm, which is due to stimulated emission (SE) of the excited OT4 chromophore.\(^{36}\) Alternatively, in the aggregated control sample, peaks corresponding to the OT4\(^{+}\) species are again evident, which is likely
due to the close proximity of the π-conjugated moieties within the supramolecular structures allowing for charge delocalization to occur. However, it is not clear if the corresponding reduced species of the charge pair appears in the spectrum. Interestingly, the appearance of the OT4⁺ species in assembled samples of control 10 was dependent on the fluence of the light source, with the cationic species only appearing upon excitation with high-fluence light. Alternatively, the appearance of charged species within the samples of 8 was independent on fluence, suggesting the creation of the charge-separated state is more favorable in the donor-acceptor systems.

Figure 4.11. Transient absorption spectra and peak assignments of donor-acceptor peptide 8 (a,b) and acylated control peptide 10 (c, d) under unassembled (a,c) and assembled (b,d) conditions.
Conclusion

Three donor-acceptor peptides containing an OT4 donor core and NDI acceptor moieties peripherally attached to lysine residues were synthesized. The synthesis was completed by using the solid-phase Stille cross-coupling dimerization procedure previously developed in the lab, followed by the deprotection of the orthogonally protected lysine residues and solid-phase imidation with the naphthalene moiety. The three peptides differed in the spacial distance between the electron donor and acceptor subunits (6, 3, and 2 amino acids). Analogous “donor-only” control peptide-π hybrids were also prepared for comparison by replacing the NDI moiety with acyl groups.

The self-assembly of each peptide and mixtures of each donor-acceptor and control pair was investigated by TEM, absorption, photoluminescence, and circular dichroism. It was seen that each peptide and mixture formed 1-D nanostructures upon assembly via acidification, however the assembly and interaction of the embedded OT4 cores varied between peptides due to the different positioning of the lysine residues (either acylated or NDI-substituted). Assembly of the acylated control peptides corresponded with a quenching in photoluminescence due to the H-like aggregation of the embedded OT4 subunits and the addition of increasing amounts of the non-emissive donor-acceptor peptide also caused photoluminescence quenching. More dramatic quenching was seen within assembled donor-acceptor/control mixtures in comparison to unassembled samples containing the same mol% of the donor-acceptor peptides, suggesting a larger fraction of the photogenerated excitons undergo electron transfer to the acceptor NDI subunits following excitation in the assemblies. This could be due to exciton delocalization within the assembled arrays prior to electron transfer occurring. Unfortunately, different behaviors within the series due to varied donor-acceptor distances could not be determined due to variances in the assembly properties of each peptide.
Finally, the photoinduced electron transfer capabilities of the systems were elucidated via transient absorption. Upon donor excitation of assembled and unassembled samples of the donor-acceptor peptides, absorbances corresponding to OT4\textsuperscript{+} / NDI\textsuperscript{−} pairs became evident and appeared longer-lived within the assembled samples, showing that the materials are capable of creating charge-separated states upon photoexcitation.
Experimental

General considerations: DMF was purchased from Sigma-Aldrich and dried over 4 Å molecular sieves. Solvents were degassed by sparging with nitrogen for 30 to 90 minutes before use. Tetrakis(triphenylphosphine)palladium was obtained from Strem Chemicals. Wang-Lys(Mtt)-Fmoc was purchased from Chem-Impex International. N-Methylpyrrolidone (NMP), Wang-Val-Fmoc resin, Wang-Lys-Fmoc resin, and Fmoc-protected amino acids were obtained from Advanced ChemTech. O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) was purchased from Oakwood Products Inc. All other reagents and starting materials were obtained from Sigma-Aldrich and were used as received. Wang-Lys-Fmoc resin was acylated via treatment with acetic anhydride. 5,5'-bis-tributylstannyl-[2,2']-bithiophene, and 5-bromothiophene-2-carboxylic acid were prepare using literature procedures.41,29

NMR Spectroscopy: 1H-NMR spectra were obtained using a Bruker Avance 400 MHz FT-NMR spectrometer, and processed with Bruker Topspin 1.3. Peptide 1H NMR spectra were acquired using a 1 second presaturation pulse to suppress water.

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 0.1% ammonium hydroxide.

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 with an Ushio Xenon short arc lamp. Spectroscopic samples were prepared at 2.2 µM in Millipore water. The pH was then adjusted by adding 10 µL of either 1M KOH (basic) or 1M HCl (acidic).

Circular Dichroism (CD): CD spectra were obtained using a AVIV 420 CD spectropolarimeter. Spectroscopic samples were prepared at 44 µM in Millipore water. The pH was then adjusted by adding 10 µL of either 1M KOH (basic) or 1M HCl (acidic).
**Reverse-Phase HPLC:** HPLC purification was performed on a Varian PrepStar SD-1 instrument using Luna 5 μm particle diameter C8 with TMS endcapping columns with silica solid support. An ammonium formate aqueous buffer (pH 8) and acetonitrile were used as the mobile phase.

**Transmission Electron Microscopy (TEM):** Imaging was performed on a Philips EM 420 transmission electron microscope equipped with an SIS Megaview III CCD digital camera. The samples were prepared by pipetting a drop of 1 mg/mL solution of assembled peptide in water onto 200 mesh Formvar coated copper grids and incubated for 5 minutes at 25°C. Excess solution was wicked off by touching the side of the grid to filter paper. The sample was then stained with a 2% uranyl acetate solution and excess moisture was wicked off. The grid was allowed to dry in air before imaging.

**Transient Absorption:** Samples were prepared in water and the pH was then adjusted by adding 10 μL of either 1M KOH (basic) or 1M HCl (acidic). All samples were excited by high fluence 400 nm laser pulse. All images were produced by chirp correcting and combining signals from regions of stable white light.

**N-propyl-1,4,5,8-naphthalenetetracarboxylic acid monoanhydride:** Adapted from the literature as follows42: 1,4,5,8-Naphthalenetetracarboxylic acid dianhydride (10.7 g, 40.0 mmol) was added to a three neck round bottomed flask equipped with a condenser and dissolved in DMF (300 mL). The mixture was heated to 140°C. Propylamine (3.28 mL, 40.0 mmol), was diluted in DMF (25 mL) and added dropwise over 2.5 hours to the reaction flask via syringe pump. The mixture was further heated to reflux for 15 hours, then allowed to return to room temperature. 500 mL of a saturated NH₄Cl aqueous solution was added. The light brown solid was collected by filtration, then washed with water. The crude solid was suspended in boiling chloroform, and the suspension was filtered. The solvent was removed from the mother liquor to give the desired product as an off-white solid (5.26 g, 17.0 mmol, 43%) that was used without further purification. ^1H NMR (400 MHz, CDCl₃) δ: 8.82 (s, 4H),
4.21-4.15 (m, 2H), 1.78 (sext, 2H, $J = 7.6$ Hz), 1.03 (t, 3H, $J = 7.4$ Hz). $^{13}$C NMR (100 MHz, CDCl$_3$) δ: 162.4, 159.0, 133.3, 131.4, 129.0, 128.1, 122.9, 42.8, 21.5, 11.6.

**General Solid Phase Peptide Synthesis (SPPS), N-terminus-acylation, and On-Resin Stille Coupling procedure:** Peptides were synthesized via standard SPPS using Fmoc-protected amino acids, starting from Wang resin preloaded with the first amino acid (Wang-Val = 0.7 or 0.4 mmol/g, Wang-Lys(Mtt) = 0.47 mmol/g, Wang-Lys(Ac) = 0.37 mmol/g), acylated with 5-bromothiophene-2-carboxylic acid and subjected to Stille cross-coupling conditions in the presence of 5,5′-bis-tributylstannyl-[2,2′]-bithiophene as previously described in Chapter 2 and ref. 29.

**General On-Resin Deprotection and Imidation Procedure:** Following SPPS, N-terminus-acylation, and On-Resin Stille coupling procedures, the resin was dried and placed in a Schlenk tube equipped with a reflux condenser. N-propyl-1,4,5,8-naphthalenetetracarboxylic acid monoanhydride (3 eq.) and DMF (10 mL) was added and the mixture was heated to 50°C for 1 hour, with continuous N$_2$ bubbling through the solution. The suspension was then heated to 110°C for 22 hours. The mixture was allowed to cool, then the resin was transferred to a peptide chamber and subjected to a wash cycle (3x NMP, 3x DMF, 2x iPrOH, 2x H$_2$O, 2x (2x THF, 2x iPrOH), 2x acetonitrile, 2x diethylether, 2x hexanes).

**General cleavage, work-up procedure of peptides:** Following solid-phase cross-coupling and imidation (if applicable), the peptide was cleaved from the resin with a 95% TFA cocktail, isolated, and HPLC purified as previously described in Chapter 2 and ref. 29.

**K(NDI)VEVGG-OT4-GGVEVK(NDI) peptide (6):** Solid supported Wang-K(Mtt)VEVGG-NH$_2$ peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.46 mmol) and subjected to the standard Stille coupling procedure in the presence of 5,5′-bis-tributylstannyl-[2,2′]-bithiophene (0.23 mmol, 0.17 g) and Pd(PPh$_3$)$_4$ (0.018 mmol, 0.021 g) for 19 hours. The lysine residues of the resulting solid-supported peptide were then subjected to the deprotection and imidation procedure with N-propyl-1,4,5,8-naphthalenetetracarboxylic acid
monoanhydride (1.4 mmol, 0.43 g) for 22 hours. Following general cleavage, work-up, and HPLC purification, the peptide was obtained as a light orange powder (0.0032 mmol, 0.0063 g, 1.3% yield). UV-Vis (H$_2$O) λ/nm (log ε): 386 (4.32). MS (ESI) m/z 1068.8 (M-2H)$^-$ (calc. 1068.8), m/z 712.3 (M-3H)$^-$ (calc. 712.2), m/z 534.2 (M-3H)$^-$ (calc. 533.9).

**VEVK(NDI)GG-OT4-GGK(NDI)VEV peptide (7):** Solid supported Wang-VEVK(Mtt)GG-NH$_2$ peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.50 mmol) and subjected to the standard Stille coupling procedure in the presence of 5,5'-bis-tributylstannyl-[2,2']-bithiophene (0.25 mmol, 0.19 g) and Pd(PPh$_3$)$_4$ (0.020 mmol, 0.023 g) for 18 hours. The lysine residues of the resulting solid-supported peptide were then subjected to the deprotection and imidation procedure with N-propyl-1,4,5,8-naphthalenetetracarboxylic acid monoanhydride (1.5 mmol, 0.46 g) for 22 hours. Following general cleavage, work-up, and HPLC purification, the peptide was obtained as an orange powder (0.0068 mmol, 0.014 g, 2.7% yield). UV-Vis (H$_2$O) λ/nm (log ε): 388 (4.54). MS (ESI) m/z 1068.9 (M-2H)$^-$ (calc. 1068.8), m/z 712.3 (M-3H)$^-$ (calc. 712.4), m/z 534.1 (M-3H)$^-$ (calc. 533.9).

**VEVGK(NDI)G-OT4-GK(NDI)GVEV peptide (8):** Solid supported Wang-VEVK(Mtt)GG-NH$_2$ peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.50 mmol) and subjected to the standard Stille coupling procedure in the presence of 5,5'-bis-tributylstannyl-[2,2']-bithiophene (0.25 mmol, 0.19 g) and Pd(PPh$_3$)$_4$ (0.020 mmol, 0.023 g) for 18 hours. The lysine residues of the resulting solid-supported peptide were then subjected to the deprotection and imidation procedure with N-propyl-1,4,5,8-naphthalenetetracarboxylic acid monoanhydride (1.5 mmol, 0.46 g) for 22 hours. Following general cleavage, work-up, and HPLC purification, the peptide was obtained as an orange-brown powder (0.010 mmol, 0.022 g, 4.1% yield). UV-Vis (H$_2$O) λ/nm (log ε): 387 (4.60). MS (ESI) m/z 1068.9 (M-2H)$^-$ (calc. 1068.8), m/z 712.4 (M-3H)$^-$ (calc. 712.2), m/z 534.1 (M-3H)$^-$ (calc. 533.9).

**K(Ac)VEVGG-OT4-GGVEVK(Ac) peptide (9):** Solid supported Wang-K(Ac)VEVGG-NH$_2$ peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.43 mmol) and
subjected to the standard Stille coupling procedure in the presence of 5,5’-bis-tributylstannyl-[2,2’]-bithiophene (0.22 mmol, 0.16 g) and Pd(PPh$_3$)$_4$ (0.017 mmol, 0.020 g) for 20 hours. Following general cleavage, work-up, and HPLC purification, the peptide was obtained as a light orange powder (0.0075 mmol, 0.012 g, 3.5% yield). UV-Vis (H$_2$O) $\lambda$/nm (log $\varepsilon$): 420 (4.48). MS (ESI) $m/z$ 819.7 (M-2H)-2 (calc. 819.3), $m/z$ 546.2 (M-3H)-3 (calc. 545.9), $m/z$ 409.5 (M-3H)-3 (calc. 409.1).

**VEVK(Ac)GG-OT4-GGK(Ac)VEV peptide (10):** Solid supported Wang-VEVK(Ac)GG-NH$_2$ peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.5 mmol) and subjected to the standard Stille coupling procedure in the presence of 5,5’-bis-tributylstannyl-[2,2’]-bithiophene (0.25 mmol, 0.19 g) and Pd(PPh$_3$)$_4$ (0.020 mmol, 0.023 g) for 20 hours. Following general cleavage, work-up, and HPLC purification, the peptide was obtained as a light orange powder (0.018 mmol, 0.029 g, 7.1% yield). UV-Vis (H$_2$O) $\lambda$/nm (log $\varepsilon$): 417 (4.59). MS (ESI) $m/z$ 819.7 (M-2H)-2 (calc. 819.3), $m/z$ 546.2 (M-3H)-3 (calc. 545.9), $m/z$ 409.5 (M-3H)-3 (calc. 409.1).

**VEVGK(Ac)G-OT4-GK(Ac)GVEV peptide (11):** Solid supported Wang-VEVGK(Ac)G-NH$_2$ peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.5 mmol) and subjected to the standard Stille coupling procedure in the presence of 5,5’-bis-tributylstannyl-[2,2’]-bithiophene (0.25 mmol, 0.19 g) and Pd(PPh$_3$)$_4$ (0.020 mmol, 0.023 g) for 20 hours. Following general cleavage, work-up, and HPLC purification, the peptide was obtained as a light orange powder (0.010 mmol, 0.016 g, 3.9% yield). UV-Vis (H$_2$O) $\lambda$/nm (log $\varepsilon$): 420 (4.61). MS (ESI) $m/z$ 819.8 (M-2H)-2 (calc. 819.3), $m/z$ 546.2 (M-3H)-3 (calc. 545.9), $m/z$ 409.5 (M-3H)-3 (calc. 409.1).
References


Chapter 5 – Appendix

NMR

NMR Spectroscopy: $^1$H-NMR spectra were obtained using a Bruker Avance 400 MHz FT-NMR spectrometer, and processed with Bruker Topspin 1.3. Peptide $^1$H NMR spectra were acquired using a 1 second presaturation pulse to suppress water.

Figure 5.1. $^1$H NMR of DFAG-OT3-GAFD peptide (2-2) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. $^1$H NMR (400 MHz, D$_2$O) δ: 7.51 (d, 2H, $J = 3.9$ Hz), 7.31-7.26 (m, 8H), 7.21-7.18 (m, 2H), 7.16 (br s, 2H), 7.09 (br s, 2H), 4.70 (dd, 2H, $J = 7.3$, 4.9 Hz), 4.41 (dd, 2H, $J = 8.3$, 4.5 Hz), 4.3 (q, 2H, $J = 7.2$ Hz), 4.05 (d, 2H, $J = 16.6$ Hz), 3.93 (d, 2H, $J = 16.9$ Hz), 3.29 (dd, 2H, $J = 16.0$, 4.5 Hz), 3.0 (dd, 2H, $J = 12.0$, 10.1 Hz), 2.70 (dd, 2H, $J = 16.0$, 4.5 Hz), 2.59 (dd, 2H, $J = 16.0$, 8.4 Hz), 1.27 (d, 6H, $J = 7.2$ Hz).
Figure 5.2. $^1$H NMR of DFAG-OT4-GAFD peptide (2-3) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. $^1$H NMR (400 MHz, D$_2$O) δ: 7.45-6.20 (m, 18H), 2H, 4.65-4.61 (m, 2H), 4.40 (dd, 2H, $J = 7.0$, 5.7 Hz), 4.31 (q, 2H, $J = 7.2$ Hz), 3.98 (d, 2H, $J = 16.8$ Hz), 3.88 (d, 2H, $J = 17.3$ Hz), 3.4-3.15 (m, 2H), 3.00-2.87 (m, 2H), 2.65 (dd, 2H, $J = 16.7$, 5.2 Hz), 2.58 (dd, 2H, $J = 15.5$, 7.5 Hz), 1.28 (d, 6H, $J = 7.0$ Hz).
Figure 5.3. $^1$H NMR of DADGG-OT5-GGDAD peptide (2-4) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. $^1$H NMR (400 MHz, D2O) $\delta$: 7.52 (d, 2H, $J = 3.4$), 7.23-7.15 (m, 2H), 7.14-7.09 (m, 2H), 7.09-7.02 (m, 4H), 4.64 (dd, 2H, $J = 8.9, 4.6$ Hz), 4.39-4.33 (m, 4H), 4.14-3.96 (m, 8H), 2.74 (dd, 2H, $J = 16.1, 4.6$), 2.66 (dd, 2H, $J = 15.8, 4.3$), 2.62 (dd, 2H, $J = 14.6, 9$), 2.54 (dd, 2H, $J = 15.7, 9.2$), 1.36 (d, 6H, $J = 7.2$).
Figure 5.4. $^1$H NMR of DADDG-OT6-GDDAD peptide (2-5) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. $^1$H NMR (400 MHz, D$_2$O) $\delta$: 7.47 (s, 2H), 7.20-6.80 (m, 4H), 6.80-6.46 (m, 2H), 4.65 (dd, 2H, $J = 5.5$, 2.0 Hz), 4.35 (dd, 2H, $J = 8.7$, 4.6 Hz), 4.32-4.22 (m, 2H), 4.20-4.00 (m, 2H), 2.85-2.69 (m, 4H), 2.69-2.58 (m, 6H), 2.53 (dd, 2H, $J = 15.0$, 8.7 Hz), 1.34 (br s, 6H).
Figure 5.5. $^1$H NMR of VEVAG-OP3-GAVEV peptide (2-6) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. $^1$H NMR (400 MHz, D$_2$O) $\delta$: 7.94 (d, 4H, $J = 8.5$ Hz), 7.90-7.85 (m, 8H), 4.45 (q, 2H, $J = 7.1$ Hz), 4.36 (dd, 2H, $J = 9.3$, 5.3 Hz), 4.22-4.14 (m, 4H), 4.11 (d, 2H, $J = 8.4$ Hz), 4.05 (d, 2H, $J = 5.88$ Hz), 2.34-2.16 (m, 4H), 2.16-2.00 (m, 6H), 1.98-1.86 (m, 2H), 1.41 (d, 6H, $J = 7.2$ Hz), 0.95 (d, 6H, $J = 6.8$ Hz), 0.95 (d, 6H, $J = 6.7$ Hz), 0.90 (d, 6H, $J = 7.1$ Hz), 0.88 (d, 6H, $J = 7.0$ Hz).
Figure 5.6. $^1$H NMR of DFAG-OPE3-GAFD peptide (2-7) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. $^1$H NMR (400 MHz, D$_2$O) δ: 7.87-7.50 (m, 12H), 7.50-7.10 (m, 10H), 4.39 (dd, 2H, $J = 8.8$, 4.2 Hz), 4.30-4.20 (m, 2H), 4.11 (d, 2H, $J = 17.1$ Hz), 4.00 (d, 2H, $J = 15.0$ Hz), 3.37-3.23 (m, 2H), 2.94 (dd, 2H, $J = 13.2$, 10.8 Hz), 2.65 (dd, 2H, $J = 15.8$, 3.9 Hz), 2.54 (dd, 2H, $J = 16.0$, 9.2 Hz), 1.23 (d, 6H, $J = 5.8$ Hz).
Figure 5.7. $^1$H NMR of VEVAG-PTP-GAVEV peptide (2-8) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. $^1$H NMR (400 MHz, D$_2$O) $\delta$: 7.74 (d, 4H, $J = 8.4$ Hz), 7.65 (d, 4H, $J = 8.4$ Hz), 7.44 (s, 2H), 4.43 (q, 2H, $J = 7.3$ Hz), 4.35 (dd, 2H, $J = 9.2$, 5.4 Hz), 4.15 (d, 2H, $J = 7.6$ Hz), 4.13-4.00 (m, 6H), 2.34-2.16 (m, 4H), 2.14-1.99 (m, 6H), 1.98-1.84 (m, 2H), 1.40 (d, 6H, $J = 7.2$ Hz), 0.94 (d, 12H, $J = 6.7$ Hz), 0.90 (d, 6H, $J = 7.3$ Hz), 0.88 (d, 6H, $J = 7.1$ Hz).
Figure 5.8. $^1$H NMR of VEVAG-TPT-GAVEV peptide (2-9) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. $^1$H NMR (400 MHz, D$_2$O) $\delta$: 7.77 (s, 4H), 7.71 (d, 2H, $J = 4.0$ Hz), 7.50 (d, 2H, $J = 4.0$ Hz), 4.43 (q, 2H, $J = 7.2$ Hz), 4.34 (dd, 2H, $J = 9.3$, 5.3 Hz), 4.16 (d, 2H, $J = 7.6$ Hz), 4.14 (d, 2H, $J = 16.6$ Hz), 4.07 (d, 2H, $J = 16.8$ Hz), 4.05 (d, 2H, $J = 5.8$ Hz), 2.33-2.16 (m, 4H), 2.14-1.99 (m, 6H), 1.97-1.86 (m, 2H), 1.41 (d, 6H, $J = 7.2$ Hz), 0.95 (d, 6H, $J = 6.8$ Hz), 0.94 (d, 6H, $J = 6.8$ Hz), 0.90 (d, 6H, $J = 6.8$ Hz), 0.88 (d, 6H, $J = 6.8$ Hz).
Figure 5.9. $^1$H NMR of VEVAG-T-BTT peptide trimer (3-18) in $\text{D}_2\text{O}$.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. Peak broadness presumably due to aggregation of the peptide during the experiment.
Figure 5.10. $^1$H NMR of VEVAG-BT-BTT peptide trimer (3-19) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. Peak broadness presumably due to aggregation of the peptide during the experiment.
Figure 5.11. $^1$H NMR of (DADADADADGG)$_3$-decacyclene triimide peptide trimer (25) in D$_2$O. Spectrum was acquired using a 1 second presaturation pulse to suppress water. Integration discrepancy likely due to aggregation and water suppression. $^1$H NMR (400 MHz, D$_2$O) $\delta$: 8.55-7.0 (m, 12H), 4.75-3.80 (m, 23H), 3.00-2.30 (m, 30H), 1.36 (s, 36H).
$^1$H NMR of 4-bromo-3',5'-bis(4-bromophenyl)-4',6'-diphenyl-1,1':2',1''-terphenyl (34) and 4,4''-dibromo-4'-(4-bromophenyl)-3',5',6'-triphenyl-1,1':2',1''-terphenyl (3-35) in CDCl$_3$.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 7.00 (dd, 6H, $J = 8.4$ Hz), 6.91 (t, 3H, $J = 3.2$ Hz), 6.88-6.87 (m, 6H), 6.78-6.75 (m, 6H), 6.66 (d, 6H, $J = 8.4$ Hz).
Figure 5.13. $^{13}$C NMR of 4-bromo-3',5'-bis(4-bromophenyl)-4',6'-diphenyl-1,1':2',1"-terphenyl (34) and 4,4"-dibromo-4'-(4-bromophenyl)-3',5',6'-triphenyl-1,1':2',1"-terphenyl (3-35) in CD$_2$Cl$_2$.

$^{13}$C NMR (100 MHz, CD$_2$Cl$_2$) δ: 133.30, 131.52, 130.29, 130.10, 130.07, 127.32, 127.14, 126.12, 125.92.
Figure 5.14. $^1$H NMR of Trimethyl(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)silane (3-39) in CDCl$_3$.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 8.10 (s, 1H), 7.94 (dt, 1H, $J = 7.2, 1.2$ Hz), 7.73 (dt, 1H, $J = 7.2, 1.6$ Hz), 7.46 (t, 1H, $J = 7.6$ Hz), 1.44 (s, 12H), 0.40 (s, 9H).
Figure 5.15. $^{13}$C NMR of Trimethyl(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)silane (3-39) in CDCl$_3$.

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$: 139.7, 139.5, 136.3, 135.5, 127.2, 25.0, -0.9.
Figure 5.16. $^1$H NMR of 1-(3′-(trimethylsilyl)-[1,1′-biphenyl]-2-yl)ethanone (3-47) in CDCl$_3$.

$^1$H NMR (400 MHz, CDCl$_3$) δ: 7.62-7.57 (m, 2H), 7.56-7.51 (m, 2H), 7.48-7.42 (m, 2H), 7.42-7.34 (m, 2H), 2.01 (s, 3H), 0.33 (s, 9H).
Figure 5.17. $^{13}$C NMR of 1-(3'-(trimethylsilyl)-[1,1'-biphenyl]-2-yl)ethanone (3-47) in CDCl$_3$.

$^{13}$C NMR (100 MHz, CDCl$_3$) δ: 204.8, 141.2, 141.1, 140.9, 140.0, 133.9, 132.8, 130.7, 130.2, 129.1, 128.1, 127.9, 127.4, 30.4, -1.1.
Figure 5.18. $^1$H NMR of 1-(3'-iodo-[1,1'-biphenyl]-2-yl)ethanone (3-48) in CDCl$_3$.

$^1$H NMR (400 MHz, CDCl$_3$) δ: 7.72-7.67 (m, 2H), 7.56 (dd, 1H, $J = 6.0$, 1.6 Hz), 7.48 (td, 1H, $J = 7.6$, 1.6 Hz), 7.40 (td, 1H, $J = 7.4$, 1.6 Hz), 7.31 (ddd, 1H, $J = 7.6$, 1.2, 0.4 Hz), 7.25 (ddd, 1H, $J = 9.0$, 1.6, 1.2 Hz), 7.11 (td, 1H, $J = 7.6$, 0.8 Hz), 2.09 (s, 3H).
Figure 5.19. $^1$H NMR of 1-(3'-iodo-[1,1'-biphenyl]-2-yl)ethanone (3-48) in CDCl$_3$.

$^{13}$C NMR (100 MHz, CDCl$_3$) δ: 203.6, 142.9, 140.3, 138.8, 137.4, 136.7, 130.9, 130.3, 130.1, 128.2, 128.1, 127.9, 94.5, 30.4.
Figure 5.20. $^1$H NMR of 1,3,5-tri(methyl[1,1':3',1"-terphenyl]-4-carboxylate)benzene (3-66) in CDCl$_3$.

$^1$H NMR (400 MHz, CDCl$_3$) δ:  7.96 (d, 6H, $J = 7.9$ Hz), 7.64 (d, 6H, $J = 8.0$ Hz), 7.56 (d, 3H, $J = 7.7$ Hz), 7.48 (s, 3H), 7.43 (d, 3H, $J = 7.6$ Hz), 7.36 (t, 3H, $J = 7.4$ Hz), 7.23 (t, 3H, $J = 7.4$ Hz), 7.16 (t, 3H, $J = 7.6$ Hz), 6.93 (d, 3H, $J = 7.6$ Hz), 6.81 (s, 3H), 6.76 (d, 3H, $J = 7.5$ Hz), 3.85 (s, 9H).
Figure 5.21. $^{13}$C NMR of 1,3,5-tri(methyl[1,1′:3′,1″-terphenyl]-4-carboxylate)benzene (3-66) in DMSO-d6.

$^{13}$C NMR (100 MHz, DMSO-d6) δ: 166.0, 144.5, 141.7, 140.2, 139.5, 139.4, 138.9, 134.6, 134.2, 130.3, 129.8, 129.7, 129.5, 128.6, 128.5, 128.4, 127.9, 127.7, 126.9, 52.1.
Figure 5.22. $^1$H NMR of 1,3,5-tri([1,1':3',1"-terphenyl]-4-carboxylic acid)benzene (3-67) in DMSO-d6.

$^1$H NMR (400 MHz, DMSO-d6) δ: 12.9 (br s, 3H), 7.96 (d, 6H, $J$ = 8.0 Hz), 7.65 (d, 6H, $J$ = 7.7 Hz), 7.58 (d, 3H, $J$ = 7.7 Hz), 7.50 (s, 3H), 7.45 (d, 3H, $J$ = 7.4 Hz), 7.38 (t, 3H, $J$ = 7.6 Hz), 7.25 (t, 3H, $J$ = 7.2 Hz), 7.17 (t, 3H, $J$ = 7.4 Hz), 6.92 (d, 3H, $J$ = 7.5 Hz), 6.81 (s, 3H), 6.77 (d, 3H, $J$ = 7.4 Hz).
Figure 5.23. $^1$H NMR of tetraphenylporphyrin(DADGG)$_4$ peptide tetramer (85) in D$_2$O. Spectrum was acquired using a 1 second presaturation pulse to suppress water. Integration discrepancy likely due to aggregation and water suppression. $^1$H NMR (400 MHz, D$_2$O) $\delta$: 8.88 (br s, 4H), 8.18 (br s, 8 H), 7.65 (br s, 7H), 7.07 (br s, 2H), 4.70 (br s, 1H), 4.50-4.00 (m, 16H), 2.80-2.50 (m, 16H), 1.40 (s, 12H).
Figure 5.24. $^1$H NMR of N-propyl-1,4,5,8-naphthalenetetracarboxylic acid monoanhydride in CDCl$_3$.

$^1$H NMR (400 MHz, CDCl$_3$) δ: 8.82 (s, 4H), 4.21-4.15 (m, 2H, $J$), 1.78 (sext, 2H, $J = 7.6$ Hz), 1.03 (t, 3H, $J = 7.4$ Hz).
Figure 5.25. $^{13}$C NMR of N-propyl-1,4,5,8-naphthalenetetracarboxylic acid monoanhydride in CDCl$_3$.

$^{13}$C NMR (100 MHz, CDCl$_3$) δ: 162.4, 159.0, 133.3, 131.4, 129.0, 128.1, 122.9, 42.8, 21.5, 11.6.
Figure 5.26. $^1$H NMR of K(NDI)VEVGG-OT4-GGVEVK(NDI) peptide (4-6) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. Peak broadness presumably due to aggregation of the peptide during the experiment.
Figure 5.27. $^1$H NMR of VEVK(NDI)GG-OT4-GGK(NDI)VEV peptide (4-7) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. Peak broadness presumably due to aggregation of the peptide during the experiment.
Figure 5.28. $^1$H NMR of VEGK(NDI)G-OT4-GK(NDI)GVEV peptide (4-8) in D$_2$O. Spectrum was acquired using a 1 second presaturation pulse to suppress water. Peak broadness presumably due to aggregation of the peptide during the experiment.
Figure 5.29. $^1$H NMR of K(Ac)VEVGG-OT4-GGVEVK(Ac) peptide (4-9) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. Peak broadness presumably due to aggregation of the peptide during the experiment.
Figure 5.30. $^1$H NMR of VEVK(Ac)GG-OT4-GGK(Ac)VEV peptide (4-10) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. Peak broadness presumably due to aggregation of the peptide during the experiment.
Figure 5.31. $^1$H NMR of VEGK(Ac)G-OT4-GK(Ac)GVEV peptide (4-11) in D$_2$O.

Spectrum was acquired using a 1 second presaturation pulse to suppress water. Peak broadness presumably due to aggregation of the peptide during the experiment.
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 0.1% ammonium hydroxide.

Figure 5.32. ESI spectrum of DFAG-OT3-GAFD peptide (2-2).

MS (ESI) m/z 1115.4 (M-H)- (calc. 1115.3), m/z 1137.4 (M-2H+Na)- (calc. 1137.2), m/z 1159.5 (M-3H+2Na)- (calc. 1159.2), m/z 1181.5 (M-4H+3Na)- (calc. 1181.2), m/z 557.3 (M-2H)-2 (calc. 557.1).
Figure 5.33. ESI spectrum of DFAG-OT4-GAFD peptide (2-3).

MS (ESI) m/z 1197.4 (M-H) (calc. 1197.2), m/z 598.6 (M-2H)-2 (calc. 598.1), m/z 399.0 (M-3H)-3 (calc. 398.4), m/z 1219.4 (M-2H+Na)-1 (calc. 1219.2).
Figure 5.34. ESI spectrum of DADGG-OT5-GGDAD peptide (2-4).

MS (ESI) m/z 1329.4 (M-H)- (calc. 1329.2), m/z 664.7 (M-2H)-2 (calc. 664.1), m/z 442.9 (M-3H)-3 (calc. 442.4), m/z 332.1 (M-4H)-4 (calc. 331.5), m/z 1373.4 (M-3H+2Na)-1 (calc. 1374.3).
Figure 5.35. ESI spectrum of DADDG-OT6-GDDAD peptide (2-5).

MS (ESI) m/z 763.3 (M-2H)-2 (calc. 763.1), m/z 381.2 (M-4H)-4 (calc. 381.0), m/z 386.7 (M-5H+Na)-4 (calc. 386.5), m/z 515.9 (M-4H+Na)-3 (calc. 515.7), m/z 774.3 (M-3H+Na)-2 (calc. 774.1).
Figure 5.36. ESI spectrum of VEVAG-OP3-GAVEV peptide (2-6).

MS (ESI) m/z 1227.9 (M-H) (calc. 1227.6), m/z 613.8 (M-2H)-2 (calc. 613.3), m/z 624.8 (M-3H+Na)-2 (calc. 624.3), m/z 408.8 (M-3H)-3 (calc. 408.5), m/z 306.6 (M-4H)-4 (calc. 306.1), m/z 1249.8 (M-2H+Na)-1 (calc. 1249.5), m/z 1293.8 (M-4H+3Na)-1 (calc. 1293.5).
Figure 5.37. ESI spectrum of DFAG-OPE3-GAFD peptide (2-7).

(ESI) \( m/z \) 572.5 (M-2H)-2 (calc. 572.1), \( m/z \) 1167.4 (M-2H+Na)- (calc. 1167.4), \( m/z \) 381.4 (M-3H)-3 (calc. 381.1).
Figure 5.38. ESI spectrum of VEVAG-PTP-GAVEV peptide (2-8).

MS (ESI) m/z 1233.6 (M-H) (calc. 1233.5), m/z 616.5 (M-2H) (calc. 616.3), m/z 627.5 (M-3H+Na) (calc. 627.2), m/z 638.6 (M-4H+2Na) (calc. 638.2), m/z 1277.6 (M-3H+2Na) (calc. 1277.5), m/z 1299.6 (M-4H+3Na) (calc. 1299.5).
Figure 5.39. ESI spectrum of VEVAG-TPT-GAVEV peptide (2-9).

MS (ESI) m/z 1239.6 (M-H)- (calc. 1239.5), m/z 1283.7 (M-3H+2Na)- (calc. 1283.4), m/z 1305.7 (M-4H+3Na)- (calc. 1305.4), m/z 619.8 (M-2H)-2 (calc. 619.2).
Figure 5.40. ESI spectrum of VEVAG-T-BTT peptide trimer (3-18).

MS (ESI) m/z 1989.1 (M-H)- (calc. 1988.6), m/z 1016.2 (M-4H+2Na+)-2 (calc. 1015.8), m/z 1005.3 (M-3H+Na+)-2 (calc. 1004.8), m/z 994.3 (M-2H)-2 (calc. 993.8), m/z 662.5 (M-3H)-3 (calc. 662.2).
Figure 5.41. ESI spectrum of VEVAG-BT-BTT peptide trimer (3-19).

MS (ESI) $m/z$ 1117.2 (M-2H)-2 (calc. 1117.3), $m/z$ 744.2 (M-3H)-3 (calc. 744.5).
Figure 5.42. ESI spectrum of (VEVAG)$_3$-decacyclene triimide peptide trimer (3-23).

MS (ESI) $m/z$ 1012.5 (M-2H)$^-$ (calc. 1,011.9).
Figure 5.43. ESI spectrum of (DADGG)$_3$-decacyclene triimide peptide trimer (3-24).

MS (ESI) $m/z$ 641.7 ($M-3H+Na^+$)$^2$ (calc. 641.5).
Figure 5.44. ESI spectrum of (DADADADADGG)$_3$-decacylene triimide peptide trimer (3-25).

MS (ESI) m/z 1192.7 (M-3H)$^3$ (calc. 1192.7), m/z 894.6 (M-4H)$^4$ (calc. 894.3), m/z 715.4 (M-5H)$^5$ (calc. 715.2), m/z 596.1 (M-6H)$^6$ (calc. 595.8), m/z 446.8 (M-8H)$^8$ (calc. 446.6).
Figure 5.45. ESI spectrum of (DADADADADG)_{3}-decaphenyl peptide trimer (3-68).

MS (ESI) \( m/z \ 1907.5 \) (M-2H)\(^2\) (calc. 1906.6), \( m/z \ 1271.4 \) (M-3H)\(^3\) (calc. 1270.8), \( m/z \ 953.3 \) (M-4H)\(^4\) (calc. 952.8), \( m/z \ 762.5 \) (M-5H)\(^5\) (calc. 762.0), \( m/z \ 635.3 \) (M-6H)\(^6\) (calc. 634.9).
Figure 5.46. ESI spectrum of ESI spectrum of (DADADADADGG)$_3$-decaphenyl peptide dimer (3-69).

MS (ESI) $m/z$ 1420.3 (M-2H)$^2$ (calc. 1420.0), $m/z$ 946.7 (M-3H)$^3$ (calc. 946.3), $m/z$ 709.7 (M-4H)$^4$ (calc. 709.5), $m/z$ 567.7 (M-5H)$^5$ (calc. 567.4), $m/z$ 472.9 (M-6H)$^6$ (calc. 472.8).
Figure 5.47. ESI spectrum of tetraphenylporphyrin(DADGG)₄ peptide tetramer (3-85).

MS (ESI) m/z 1224.9 (M-2H)-2 (calc. 1224.9), m/z 1236.6 (M-3H+Na+)-2 (calc. 1235.9), m/z 816.4 (M-3H)-3 (calc. 816.2), m/z 612.1 (M-4H)-4 (calc. 611.9).
Figure 5.48. ESI spectrum of K(NDI)VEVGG-OT4-GGVEVK(NDI) peptide (4-6).

MS (ESI) m/z 1068.8 (M-2H)-2 (calc. 1068.8), m/z 712.3 (M-3H)-3 (calc. 712.2), m/z 534.2 (M-3H)-3 (calc. 533.9).
Figure 5.49. ESI spectrum of VEVK(NDI)GG-OT4-GGK(NDI)VEV peptide (4-7).

MS (ESI) m/z 1069.1 (M-2H)-2 (calc. 1068.9), m/z 712.8 (M-3H)-3 (calc. 712.4), m/z 534.5 (M-3H)-3 (calc. 533.9).
Figure 5.50. ESI spectrum of VEVGK(NDI)G-OT4-GK(NDI)GVEV peptide (4-8).

MS (ESI) $m/z$ 1069.0 (M-2H)$^{-2}$ (calc. 1068.8), $m/z$ 712.6 (M-3H)$^{-3}$ (calc. 712.2), $m/z$ 534.3 (M-3H)$^{-3}$ (calc. 533.9).
Figure 5.51. ESI spectrum of K(Ac)VEVGG-OT4-GGVEVK(Ac) peptide (4-9).

MS (ESI) m/z 819.7 (M-2H)-2 (calc. 819.3), m/z 546.2 (M-3H)-3 (calc. 545.9), m/z 409.5 (M-3H)-3 (calc. 409.1).
Figure 5.52. ESI spectrum of VEVK(Ac)GG-OT4-GGK(Ac)VEV peptide (4-10).

MS (ESI) m/z 819.7 (M-2H)-2 (calc. 819.3), m/z 546.2 (M-3H)-3 (calc. 545.9), m/z 409.5 (M-3H)-3 (calc. 409.1).
Figure 5.53. ESI spectrum of VEVGK(Ac)G-OT4-GK(Ac)GVEV peptide (4-11).

MS (ESI) $m/z$ 819.8 (M-2H)-2 (calc. 819.3), $m/z$ 546.2 (M-3H)-3 (calc. 545.9), $m/z$ 409.5 (M-3H)-3 (calc. 409.1).
MALDI-TOF

Matrix-assisted Laser Desorption/Ionization Time of Flight Mass Spectroscopy (MALDI-TOF): MALDI-TOF samples were collected using a Bruker AutoFlexIII MALDI-TOF/TOF in positive mode. Samples were prepared in a 1:1 acetonitrile:water solution with 0.1% TFA with α-cyano-4-hydroxycinnamic acid as a matrix.

Figure 5.54. MALDI-TOF spectrum of nonsymmetric IG-OT2-GI peptide (2-1).

MS (MALDI-TOF) m/z 580.2 (M+H⁺) (calc. 580.2), m/z 602.2 (M+Na⁺) (calc. 602.2).
Figure 5.55. MALDI-TOF spectrum of 2,8,14-tris((triisopropylsilyl)ethynyl)hexabenzocoronene (3-52).

MS (MALDI-TOF) m/z 884.9 ($C_{64}H_{59}Si_2^+$) (calc. 883.4), m/z 904.9 ($C_{64}H_{58}NaSi_2^+$) (calc. 905.4).
Figure 5.56. MALDI-TOF spectrum of 2,8,14-tris((trimethylsilyl)ethynyl)hexabenzocoronene (3-54).

MS (MALDI-TOF) m/z 809.2 (M⁺) (calc. 810.3), m/z 794.4 (C₅₆H₃₉Si₃⁺) (calc. 795.2).
Figure 5.57. MALDI-TOF spectrum of 4,4',4''-(hexabenzo[bc,ef,hi,kl,no,qr]coronene-2,8,14-triyl)tribenzoic acid (3-56).

MS (MALDI-TOF) m/z 903.0 (C_{63}H_{30}NaO_6^+) (calc. 905.2), m/z 783.0 (C_{56}H_{26}NaO_4^+) (calc. 785.2), m/z 641.9 (C_{49}H_{22}O_2^+) (calc. 642.2), m/z 521.9 (C_{42}H_{18}^+) (calc. 522.1)
Figure 5.58 MALDI-TOF spectrum of trimethyl 4,4',4''-(hexabenzocoronene-2,8,14-triytris(ethyne-2,1-diyl))tribenzoate (3-57).

MS (MALDI-TOF) m/z 996.1 (M⁺) (calc. 996.3), m/z 838.0 (C₆₂H₃₀O₄⁺) (calc. 838.2), m/z 680.0 (C₅₂H₂₄O₂⁺) (calc. 680.2).
Figure 5.59. MALDI-TOF spectrum of trimethyl 4,4',4''-(hexabenzocoronene-2,8,14-
triyltris(ethyne-2,1-diyl))tribenzoic acid (3-58).

MS (MALDI-TOF) m/z 953.8 (M⁺) (calc. 954.2), m/z 809.8 (C₆₀H₂₅O₄⁺) (calc. 809.2), m/z 665.8
(C₅₁H₂₁O₂⁺) (calc. 665.2).
Figure 5.60. EI spectrum for 4-bromo-3',5'-bis(4-bromophenyl)-4',6'-diphenyl-1,1':2',1"-terphenyl (3-34) and 4,4"-dibromo-4'-(4-bromophenyl)-3',5',6'-triphenyl-1,1':2',1"-terphenyl (3-35).

HR-MS (EI): found m/z = 767.9660 (M⁺); calc. for C₄₂H₂₇Br₇₉: 767.9663, found m/z = 769.9660 (M⁺); calc. for C₄₂H₂₇Br₇₉Br₈₁: 769.9642, found m/z = 771.9634 (M⁺); calc. for C₄₂H₂₇Br₇₉Br₈₁: 771.9622, found m/z = 773.9615 (M⁺); calc. for C₄₂H₂₇Br₈₁: 773.9601.
Figure 5.61. EI spectrum for trimethyl(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)silane (3-39):

HR-MS (EI): found \( m/z = 276.1720 \) (\( M^+ \)); calc. for \( C_{15}H_{25}BO_2Si \): 276.1717, found \( m/z = 261.149 \) (\( M-CH_3^+ \)); calc. for \( C_{14}H_{26}BO_2Si \): 261.148.
HPLC

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 were used as the mobile phase.

Figure 5.62. Analytical HPLC trace of purified DFAG-OT3-GAFD peptide (2-2). Traces monitoring 260 nm (top) and 394 nm (bottom). Method: 25% acetonitrile-buffer for 5 minutes, then linear gradient of 25%-67% over 25 minutes.
Figure 5.63. Analytical HPLC trace of purified DFAG-OT4-GAFD peptide (2-3).

Traces monitoring 260 nm (top) and 419 nm (bottom). Method: Linear gradient of 11%-67% acetonitrile-buffer over 45 minutes.
Figure 5.64. Analytical HPLC trace of purified DADGG-OT5-GGDAD peptide (2-4).

Traces monitoring 260 nm (top) and 435 nm (bottom). Method: Linear gradient of 11%-67% acetonitrile-buffer over 45 minutes.
Figure 5.65. Analytical HPLC trace of purified DADDG-OT6-GDDAD peptide (2-5).
Traces monitoring 260 nm (top) and 435 nm (bottom). Method: Linear gradient of 11%-67% acetonitrile/buffer over 40 minutes.
Figure 5.66. Analytical HPLC trace of purified VEVAG-OP3-GAVEV peptide (2-6).

Traces monitoring 260 nm (top) and 310 nm (bottom). Method: Linear gradient of 11%-67% acetonitrile-buffer over 45 minutes.
Figure 5.67. Analytical HPLC trace of purified DFAG-OPE3-GAFD peptide (2-7).

Traces monitoring 260 nm (top) and 332 nm (bottom). Method: Linear gradient of 11%-67% acetonitrile-buffer over 45 minutes.
Figure 5.68. Analytical HPLC trace of purified VEVAG-PTP-GAVEV peptide (2-8).

Traces monitoring 260 nm (top) and 354 nm (bottom). Method: Linear gradient of 11%-67% acetonitrile-buffer over 30 minutes, then 400% for 10 minutes.
Figure 5.69. Analytical HPLC trace of purified VEVAG-TPT-GAVEV peptide (2-9).

Traces monitoring 260 nm (top) and 354 nm (bottom). Method: Linear gradient of 11%-67% over 30 minutes, then 400% for 10 minutes.
Figure 5.70. Analytical HPLC trace of purified VEVAG-T-BTT peptide trimer (3-18). Traces monitoring 260 nm (top) and 375 nm (bottom). Method: Linear gradient of 11%-82% acetonitrile-buffer over 30 minutes, then linear gradient of 82%-11% over 5 minutes.

Figure 5.71. Analytical HPLC trace of purified VEVAG-BT-BTT peptide trimer (3-19). Traces monitoring 260 nm (top) and 400 nm (bottom). Method: Linear gradient of 11%-82% acetonitrile-buffer over 30 minutes, then linear gradient of 82%-11% over 5 minutes.
Figure 5.72. Analytical HPLC trace of purified (DADADADADGG)₃-decacyclene trimide peptide trimer (3-25).
Traces monitoring 220 nm (top) and 415 nm (bottom). Method: 5% acetonitrile/buffer for 5 minutes, then linear gradient of 5%-54% over 25 minutes, then linear gradient of 54%-5% over 5 minutes.

Figure 5.73. Analytical HPLC trace of purified tetraphenylporphyrin(DADGG)₄ peptide tetramer (3-85).
Traces monitoring 422 nm (top) and 230 nm (bottom). Method: Linear gradient of 5%-43% acetonitrile/buffer over 30 minutes, then linear gradient of 43%-5% over 5 minutes.
Figure 5.74. Analytical HPLC trace of purified K(NDI)VEVGG-OT4-GGVEVK(NDI) peptide (4-6).
Traces monitoring 430 nm (top) and 260 nm (bottom). Method: Linear gradient of 11%-67% acetonitrile-buffer over 35 minutes, then linear gradient of 67%-11% over 5 minutes.

Figure 5.75. Analytical HPLC trace of purified VEVK(NDI)GG-OT4-GGK(NDI)VEV peptide (4-7).
Traces monitoring 430 nm (top) and 260 nm (bottom). Method: Linear gradient of 11%-67% acetonitrile-buffer over 35 minutes, then linear gradient of 67%-11% over 5 minutes.
Figure 5.76. Analytical HPLC trace of purified VEVGK(NDI)G-OT4-GK(NDI)GVEV peptide (4-8).

Traces monitoring 430 nm (top) and 260 nm (bottom). Method: Linear gradient of 11%-67% acetonitrile-buffer over 35 minutes, then linear gradient of 67%-11% over 5 minutes.

Figure 5.77. Analytical HPLC trace of purified K(Ac)VEVGG-OT4-GGVEVK(Ac) peptide (4-9).

Traces monitoring 430 nm (top) and 260 nm (bottom). Method: Linear gradient of 11%-67% acetonitrile-buffer over 35 minutes, then linear gradient of 67%-11% over 5 minutes.
Figure 5.78. Analytical HPLC trace of purified VEVK(Ac)GG-OT4-GGK(Ac)VEV peptide (4-10).
Traces monitoring 430 nm (top) and 260 nm (bottom). Method: Linear gradient of 11%-67% acetonitrile/buffer over 35 minutes, then linear gradient of 67%-11% over 5 minutes.

Figure 5.79. Analytical HPLC trace of purified VEVGK(Ac)G-OT4-GK(Ac)GVEV peptide (4-11).
Traces monitoring 430 nm (top) and 260 nm (bottom). Method: Linear gradient of 11%-67% acetonitrile/buffer over 35 minutes, then linear gradient of 67%-11% over 5 minutes.
Transient Absorption

**Transient Absorption:** Samples were prepared in water and the pH was then adjusted by adding 10 μL of either 1M KOH (basic) or 1M HCl (acidic). All samples were excited by high fluence 400 nm laser pulse. All images were produced by chirp correcting and combining signals from regions of stable white light.

![Figure 5.80](image)

Figure 5.80. Transient absorption spectrum of unassembled K(NDI)VEVGG-OT4-GGVEVK(NDI) peptide (4-6).
Figure 5.81. Transient absorption spectrum of assembled K(NDI)VEVGG-OT4-GGVEVK(NDI) peptide (4-6).

Figure 5.82. Transient absorption spectrum of unassembled VEVK(NDI)GG-OT4-GGK(NDI)VEV peptide (4-7).
Figure 5.83. Transient absorption spectrum of assembled VEVK(NDI)GG-OT4-GGK(NDI)VEV peptide (4-7).

Figure 5.84. Transient absorption spectrum of unassembled VEVGK(NDI)G-OT4-GK(NDI)GVEV peptide (4-8).
Figure 5.85. Transient absorption spectrum of assembled VEVGK(NDI)G-OT4-GK(NDI)GVEV peptide (4-8).

Figure 5.86. Transient absorption spectrum of unassembled VEVK(Ac)GG-OT4-GGK(Ac)VEV peptide (4-10).
Figure 5.87. Transient absorption spectrum of assembled VEVK(Ac)GG-OT4-GGK(Ac)VEV peptide (4-10).

Figure 5.88. Transient absorption spectrum of assembled VEVGK(Ac)G-OT4-GK(Ac)GVEV peptide (4-11).
Chapter 6 – Curriculum Vitae

Allix M. Sanders was born in Lebanon, Pennsylvania in the United States of America on March 26th, 1988. She attended Annville-Cleona Junior-Senior High School in Annville, Pennsylvania. Following graduation, she entered Lebanon Valley College in 2006, where she studied chemistry under Dr. Marc Harris. Her undergraduate research was based on the design and synthesis of open-chain host compounds for encapsulating and sensing cationic guests. She graduated in 2010 and began her Ph.D. studied at Johns Hopkins University under the guidance of Professor John D. Tovar. Her Ph.D. research centered on the design, synthesis, and assembly of chromophore-bearing peptides for the preparation of supramolecular biologically-compatible electronic nanomaterials.
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Research Interests


Education & Research Experience

Ph.D. Chemistry, Johns Hopkins University, September 2015
   Research Advisor: John D. Tovar
   Design, synthesis, and assembly of chromophore-bearing peptides for the preparation of supramolecular biologically-compatible electronic nanomaterials.

M.A. Chemistry, Johns Hopkins University, June 2012 (GPA: 3.96)

B.S. Chemistry (ACS), Lebanon Valley College, May 2010 (GPA: 3.86, Summa Cum Laude)
   Research Advisor: Marc A. Harris
   Design and synthesis of open-chain host compounds for encapsulating and sensing cationic guests.

Publications & Patents

Journal Articles


**Patents**


**Conference Presentations**


“Characterization and complexation studies of novel bipyridine oligomeric host systems and their Cu(II), Ag(I), and Pt(II) metallomacrocyclic analogs by liquid-liquid extraction and ESI mass spectral analysis.” Poster Presentation: 238th ACS National Meeting, Washington, D.C. August 16 – 20, 2009.

“Complexation and selectivity studies of multi-receptor bipyridine oligomeric host systems and their metallated Cu(II), Ag(I), Pt(II), and Ru(II) analogues by liquid-liquid extraction, mass spectrometry, and spectroscopic analyses.” Poster Presentation: 12th Annual Undergraduate Research Symposium in the Chemical and Biological Sciences, University of Maryland Baltimore County, Baltimore, MD. October 10, 2009.


“Cu(II), Ag(I), and Pt(II) bipyridine oligomer metallomacrocycles that function as efficient host complexes for the encapsulation of alkali ion guests.” Poster Presentation: 11th Annual Undergraduate Research Symposium in the Chemical and Biological Sciences, University of Maryland Baltimore County, Baltimore, MD. October 14, 2008.
Awards & Fellowships

Harry and Cleio Greer Fellowship, Johns Hopkins University: Full year stipend support and supplement awarded to an outstanding advanced-year graduate student. 2014.

Selected to attend the 63rd Lindau Nobel Laureate Meeting, Council for the Lindau Nobel Laureate Meetings. 2013.


Outstanding Senior Chemistry Major Award, Southeastern Pennsylvania Section of the American Chemical Society: Awarded to an outstanding senior chemistry major. 2010.

Dr. Judith Bond Endowment Award, Southeastern Pennsylvania Section of the American Chemical Society: Awarded to an outstanding junior chemistry major in Southeastern Pennsylvania. 2009.

1st place poster presentation in the Chemical Sciences: 12th Annual Undergraduate Research Symposium in the Chemical and Biological Sciences, University of Maryland Baltimore County, Baltimore, MD. 2009.


Middle Atlantic Conference Scholar Athlete of the Year Award, Middle Atlantic Athletic Conference: Top senior student-athlete in Track and Field out of 16 MAC schools based on athletic and academic achievement. 2010.


Middle Atlantic Conference Academic Honor Roll, Middle Atlantic conference. Maintaining a minimum 3.20 GPA each semester while participating in an NCAA sport. 2006 – 2010.

Teaching Experience

Johns Hopkins University
Head Teaching Assistant – Organic Chemistry I. Fall 2012.

Lebanon Valley College
**Instrument & Lab Proficiency**

Multi-step organic synthesis utilizing air-free Schlenk line technique, solid-phase peptide synthesis, coordination complex synthesis, NMR, ESI, MALDI-TOF, TEM, FTIR, UV-Vis, Photoluminescence, CD, HPLC, Prep LC

**Activities**

Johns Hopkins University Homewood Campus Safety Committee. 2014 – Present.
Johns Hopkins University Chemistry Department Safety Committee. 2014 – Present.