Electronic Supplementary Information

On resin dimerization incorporates a diverse array of pi-conjugated functionality within aqueous self-assembling peptide backbones

Geeta S. Vadehra1; Brian D. Wall1; Stephen R. Diegelmann1,2; John D. Tovar*1,2,3

1Department of Chemistry, 2Institute for NanoBioTechnology, 3Department of Materials Science and Engineering, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218 (USA)

Table of Contents

Experimental details and characterization data S1
NMR spectra S11
HPLC traces and mass spectra S15
ATR-IR spectra S24
UV-Vis and photoluminescence spectra S27
CD spectra S30
AFM images S33
Assembly Model S34
Macroscopic qualitative hydrogel properties S35
References S35
**Experimental details and characterization data**

**General Considerations.** Reactions were performed in flame-dried glassware under an atmosphere of nitrogen. Non-aqueous solvents were degassed by sparging with nitrogen for 15 minutes prior to use and THF was distilled over sodium/benzophenone. Tetrakis(triphenylphosphine)palladium (Pd(PPh₃)₄) was obtained from Strem Chemicals. Chemicals for solid phase peptide synthesis (N-methylpyrrolidone (NMP), O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), Wang resin, Fmoc-amino acids) were obtained from Advanced ChemTech. All other chemicals were supplied by Sigma-Aldrich or Fisher and used as received. ¹H-NMR and ¹³C-NMR were obtained at 400 MHz and 100 MHz, respectively, using a Bruker Avance 400 MHz FT-NMR spectrometer, unless otherwise noted under the NMR spectrum. Chemical shifts are reported in parts per million relative to residual protio solvent [CDCl₃ δ: 7.26 (¹H) and 77.16 ppm (¹³C), d₆-DMSO δ: 2.50 (¹H) and 39.52 ppm (¹³C), D₂O δ: 4.79 (¹H)].

**General synthesis of peptides.** All peptides were synthesized using standard solid phase 9-fluorenylmethoxy carbonyl (Fmoc) chemistry on a Wang resin preloaded with the Fmoc-protected leading amino acid. Fmoc deprotection was performed by mixing the resin in a piperidine/DMF (2:8) solution for 10 minutes (2x), then rinsing with DMF, MeOH, and CH₂Cl₂. For all standard amino acid couplings, 3.0 eq. (relative to the resin substitution) of Fmoc-protected amino acid was activated externally with 2.9 eq. of HBTU and 10 eq. of diisopropylethylamine (DIPEA) are dissolved in 10 mL of NMP. The activated Fmoc-protected amino acid was then added to a peptide chamber containing the Wang resin and mixed for 3 hours. The resin was then drained and rinsed with NMP, MeOH, and CH₂Cl₂ then allowed to dry. All coupling and deprotection steps were monitored by performing a Kaiser test on a few resin beads which were removed from the peptide chamber after drying. Cleavage from the resin and removal of side-chain protecting groups was accomplished by stirring the resin with trifluoroacetic acid (TFA), water, and triisopropylsilane (TIPS) (95:2.5:2.5) for 3 hours (unless otherwise noted in individual peptide experimental). The resin was removed by filtration and washed with the cleavage mixture. The filtrate volume was then reduced on a rotary evaporator fitted with a KOH (aq) trap and the peptide was precipitated by the addition of cold diethyl ether. Crude peptide was collected by one of two methods; filtered, rinsed with cold diethyl ether and eluted off the filter with ammonium hydroxide and lyophilized, or collected by centrifugation. The peptide was dried under high vacuum followed by purification by triturating with acetonitrile and RP-HPLC.

**UV-Vis and Photoluminescence.** UV-Vis spectra were recorded using a Varian Cary 50 Bio UV-Vis spectrophotometer. Solution photoluminescence were recorded using a PTi Photon Technology International Fluorometer with a Ushio Xenon short arc lamp. Spectroscopic samples were made by diluting HPLC purified fractions with water to achieve an optical density near 0.08 and the pH was adjusted by the addition aliquots of 1M KOH (for ‘basic’) or 1M HCl (for ‘acidic’).

**Circular Dichroism (CD).** CD Measurements were recorded at 20 °C using a Jasco J-810 spectropolarimeter. Spectroscopic samples were made by diluting a basic stock solution of known concentration to 0.20 mg/mL and the pH was adjusted by the addition of 10 µL aliquots of 1M KOH or 1M HCl.

**Atomic Force Microscopy (AFM).** Samples were analyzed by magnetic tapping mode AFM on an Agilent Technologies PicoSPM LE using probes purchased from Micromasch (NSC18...
Co/Cr). **Peptide 2** was prepared for AFM as follows: 100 µL of a 0.7 µM aqueous solution was brought to pH 8 with 1M KOH and heated to 75 °C in a sandbath. 1 µL of 1M HCl was added 2x (30 seconds apart) while agitating. Sample was then removed from heat and allowed to cool for 5 minutes. 10 µL of this sample was added to freshly cleaved mica and allowed to dry before imaging. **Peptides 3 and 6** were prepared in the same manner: 200 µL of a 0.08 mM aqueous solution was placed in an acid chamber (closed vial with concentrated HCl) for 20 minutes. 10 µL of this sample was then deposited onto freshly cleaved mica and allowed to dry before imaging.

**Attenuated Total Reflectance-Infrared (ATR-IR).** ATR-IR spectra were acquired with an attenuated total reflection device (Pike Technologies MIRacle) equipped with a diamond crystal in single reflection mode using a Mattson Infinity Series FTIR spectrometer with a Mercury cadmium telluride detector (2 cm\(^{-1}\) resolution). Each sample was in the solid state used a 4 cm\(^{-1}\) resolution and was accumulated for 500 scans, unless otherwise noted under IR spectrum.

**Electrospray Ionization Mass Spectrometry (ESI-MS)**  
ESI samples were run in negative mode on a Finnigan LCQ ion trap mass spectrometer with electrospray ionization. Samples were run in a 1:1 MeOH:Water solution with 1% ammonium hydroxide.

**Reverse Phase High Performance Liquid Chromatography (RP-HPLC).** Performed on an Agilent Technologies 1100 Series Quaternary LC System fitted with a Phenomenex C8 column (250 x 4.6 mm) with a gradient of buffer B/buffer A (see HPLC trace for each peptide for % B/A, buffer A: Ammonium formate buffer pH 8 in water; buffer B: Methanol) over 30-40 minutes with a flow rate of 0.8 mL/min.

**Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF).** MALDI-TOF spectra were acquired on a Bruker Autoflex III Smartbeam instrument using α-Cyano-4-hydroxycinnamic acid matrix.
**Peptide 1. 1,4,5,8-Naphthalenetetracarboxylic dianhydride (NDA).** (0.008 g, 0.03 mmol) was suspended in pyridine (3 mL) and added to Wang-VDFAG-NH$_2$ resin (0.10 mmol). This mixture was heated to 65 °C followed by the addition of diisopropyl ethyl amine (0.25 mL, 1.5 mmol) and reaction was further heated to 135 °C for 10 hours. A second portion of NDA (0.005 g, 0.02 mmol) was added along with a second portion of pyridine (2 mL). Reaction was maintained at 135 °C and continued for an additional 10 hours. Resin was cooled to room temperature, filtered and washed with MeOH. The resin was then suspended in pyridine (5 mL), heated to 65 °C followed by the addition of diisopropyl ethyl amine (0.25 mL, 1.5 mmol) and further heated to 135 °C for 6 hours. Resin was cooled to room temperature, filtered and washed with one cycle each of H$_2$O, CH$_2$Cl$_2$, 2-propanol, MeOH, NMP, Et$_2$O and CH$_3$CN. The resin was cleaved by mixing with 15 mL of a mixture of trifluoroacetic acid (TFA), water and triisopropylsilane (TIPS) (95:2.5:2.5) for 2 hours. Resin was filtered and washed with 5 mL of the cleavage mixture. The filtrate volume was then reduced by 50% under reduced pressure and product was precipitated by the addition of 150 mL Et$_2$O, and placed in freezer. Pellet was formed by centrifuge. The solid recovered was triturated with Et$_2$O (2x) and CH$_3$CN (2x). Filtrate was lyophilized to yield product as brown powder (0.026 g, 0.020 mmol, 41%).

**ν$_{\text{max}}$(solid)/cm$^{-1}$** 1643, 1548, 1400, 1246, 1219. 1H-NMR (400 MHz, d$_6$-DMSO) δ: 8.71 (4H, s), 7.24 (10H, s), 4.71 (2H, m), 4.56 (2H, m), 4.30 (2H, m), 4.00 (2H, s), 3.06 (2H, m), 2.81 (2H, m), 2.03 (2H, m), 1.15 (6H, d, $J$ = 6.80 Hz), 0.82 (12H, s); MS (MALDI-TOF): m/z 1246.136 (M-H$^+$); calculated 1246.221. Procedure was modified from literature for a mono-imidation.$^1$

**Peptide 2. 3,4,9,10-Perylenetetracarboxylic dianhydride (PDA).** (0.012 g, 0.031 mmol) was suspended in pyridine (3 mL) and added to Wang-VDFAG-NH$_2$ resin (0.10 mmol). This mixture was heated to 65 °C followed by the addition of diisopropyl ethyl amine (0.25 mL, 1.5 mmol) and reaction was further heated to 135 °C for 10 hours. A second portion of PDA (0.008 g, 0.02 mmol) was added along with a second portion of pyridine (2 mL). Reaction was maintained at 135 °C and continued for an additional 10 hours. Resin was cooled to room temperature, filtered and washed with MeOH. The resin was then suspended in pyridine (5 mL), heated to 65 °C followed by the addition of diisopropyl ethyl amine (0.25 mL, 1.5 mmol) and further heated to 135 °C for 6 hours. Resin was cooled to room temperature, filtered and washed with one cycle each of H$_2$O, CH$_2$Cl$_2$, 2-propanol, MeOH, NMP, Et$_2$O and CH$_3$CN. The resin was cleaved by mixing with 15 mL of a mixture of trifluoroacetic acid (TFA), water and triisopropylsilane (TIPS) (95:2.5:2.5) for 2 hours. Resin was filtered and washed with 5 mL of the cleavage mixture. The filtrate volume was then reduced by 50% under reduced pressure and product was precipitated by the addition of 150 mL Et$_2$O, and placed in freezer. Pellet was formed by centrifuge. The solid recovered was triturated with Et$_2$O (2x) and CH$_3$CN (2x). Filtrate was lyophilized to yield product as black powder (0.020 g, 0.015 mmol, 29%).
1248, 1204, 1174, 1141.  $^1$H-NMR (300 MHz, $d_6$-DMSO, 90°C) δ: 8.96 (4H, d, J = 7.9 Hz), 8.63 (4H, d, J = 7.8 Hz), 7.27 (10H, s), 4.80 (4H, s), 4.58 (8H, m), 4.34 (4H, m), 4.15 (4H, broad), 2.07 (6H, m), 1.24 (6H, d, J = 6.8 Hz), 1.01 (2H, m), 0.89 (12H, d, J = 6.7 Hz); MS (ESI-) m/z 684.01 (M-2H$^+$) (calc. 684.24), m/z 455.81 (M-3H$^+$) (calc. 455.83). Procedure was modified from literature for a mono-imidation.$^1$

[2, 2':5', 2''-terthiophene]-5, 5''-dicarboxylic acid (OT-3 diacid). (0.739 g, 2.98 mmol) of 2,2':5',2''-terthiophene was added to a flame dried two-neck round bottom flask and the flask was evacuated and refilled with N$_2$ (3x). 36 mL of dry THF was then added and reaction degassed with N$_2$ for 10 minutes. The reaction mixture was cooled to -78 °C and 11.4 mL of nBuLi in hexane (1.63 M, 18.6 mmol) was added dropwise. The reaction mixture was allowed to warm to 0 °C and stirred for 2 hours. The reaction was then cooled down to -78 °C and solid carbon dioxide was added and stirring was continued for 2 hours at -78 °C and then 18 hours at room temperature. The solid produced was filtered, washed with excess 3% HCl and acetone then dried in vacuum. The product was obtained as a yellow solid (0.934 g, 2.77 mmol, 93%). Procedure adapted from and characterization data matches literature.$^2$

Peptide 3. 0.010 g (0.028 mmol) of OT-3 diacid and (0.031 g, 0.060 mmol) of PyBOP was dissolved in 10 mL 2:1 NMP:CH$_2$Cl$_2$, then 0.174 mL of DIPEA (0.998 mmol) was added and agitated by vortex for 30 seconds. The solution was then added to Wang-DFAG-NH$_2$ resin (0.1 mmol reactive –NH$_2$) in a peptide chamber and the reaction was mixed for 18 hours. The resin was rinsed thoroughly with NMP and CH$_2$Cl$_2$, then mixed for 10 minutes in NMP, and rinsed again with NMP and CH$_2$Cl$_2$. The resin was subjected to a second round of coupling: 0.007 g (0.02 mmol) of OT-3 diacid and 0.021 g (0.040 mmol) of PyBOP was dissolved in 10 mL 2:1 NMP:CH$_2$Cl$_2$, then 0.174 mL of DIPEA (0.998 mmol) was added and stirred for 30 seconds. The solution was then added to the resin in a peptide chamber and the reaction was mixed for 18 hours. The resin was rinsed thoroughly with NMP and CH$_2$Cl$_2$, then mixed for 10 minutes in NMP, and rinsed again with NMP and CH$_2$Cl$_2$. The resin was then washed with CH$_2$Cl$_2$, NMP, acetic acid, and MeOH and placed under high vacuum to dry. The resin was cleaved by mixing with 10 mL of a mixture of trifluoroacetic acid (TFA), water and triisopropylsilane (TIPS) (95:2.5:2.5) for 2 hours. Resin was filtered and washed with 5 mL of the cleavage mixture. The filtrate volume was then reduced by 50% under reduced pressure and product was precipitated by the addition of 150 mL Et$_2$O, and placed in freezer. Pellet was formed by centrifuge. The solid recovered was triturated with Et$_2$O (2x) and CH$_3$CN (2x). Crude peptide was obtained as a yellow powder (0.021 g, 0.019 mmol, 38%). ν$_{max}$(solid)/cm$^{-1}$ 1633, 1540, 1442, 1305, 1203, 1137, 788.  $^1$H NMR (400 MHz, D$_2$O) δ: 7.34-6.99 (m, 14H), 6.51 (broad, 4H), 4.62 (2H, m), 4.44 (s, 2H) 4.30 (2H, d, J = 7.0 Hz), 3.92 (4H, m), 3.23 (2H, d, J = 10.0 Hz), 2.93 (2H, t, J = 9.0 Hz), 2.70 (broad, 4H), 1.27 (6H, d, J = 6.6 Hz). MS (ESI-) m/z 1115.3 (M-H$^+$) (calc. 1115.3), m/z 1137.2 (M-2H$^+$+Na$^+$) (calc. 1137.2), m/z 1159.2 (M-3H$^+$+2Na$^+$) (calc. 1159.2), m/z 556.9 (M-2H$^+$) (calc. 557.1), m/z 567.9 (M-2H$^+$) (calc. 568.1).
2,2':5',2'':5'',2'''-quaterthiophene (OT-4). 5,5'-bis(tributylstannyl)-2,2'-bithiophene (2.16 g, 2.90 mmol) was dissolved in dry DMF (45 mL) and transferred via cannula to Pd(PPh3)4 (0.101 g, 0.087 mmol) in a Schlenk flask. 2-bromothiophene (1.14 g, 6.96 mmol) was added via syringe and the solution was degassed with nitrogen. Reaction was then heated to 90 °C for 18 hours. Reaction was quenched with water, extracted with chloroform (3x), and the combined organics washed with brine. Organics were dried with magnesium sulfate and concentrated by rotary evaporator yielding crude material as a waxy orange solid. The solid was recrystalized from boiling toluene and collected by filtration. Filtered solid was then redissolved in boiling chloroform and stirred with activated charcoal for 15 minutes. The charcoal was removed by filtering through celite and the chloroform removed by rotary evaporator to yield product as a yellow solid (0.676 g, 2.05 mmol, 70%). Characterization data matches literature.3

[2,2':5',2'':5'',2'''-quaterthiophene]-5,5'''-dicarboxylic acid (OT-4 diacid). 0.200 g (0.605 mmol) of 2,2':5',2''':5'',2'''-quaterthiophene (OT-4) was added to a flame dried two-neck round bottom flask and the flask was evacuated and refilled with N2 (3x). 20 mL of dry THF was then added and reaction degassed with N2 for 10 minutes. The reaction mixture was cooled to -78 °C and 2.36 mL of nBuLi in hexane (1.60 M, 3.78 mmol) was added dropwise. The reaction mixture was allowed to warm to 0 °C and stirred for 2 hours. The reaction was then cooled down to -78 °C and solid carbon dioxide was added and stirring was continued for 2 hours at -78 °C and then 18 hours at room temperature. The solid produced was filtered, washed with excess 3% HCl and acetone then dried in vacuum. Procedure adapted from literature.2 The product was obtained as a yellow/orange solid (0.182 g, 0.435 mmol, 72%) and used without further purification.

Peptide 4. 0.013 g (0.031 mmol) of OT-4 diacid and 0.031 g (0.060 mmol) of PyBOP was dissolved in 10 mL NMP, then 0.174 mL of DIPEA (0.998 mmol) was added and mixed by vortex for 30 seconds. The solution was then added to Wang-DFAG-NH2 resin (0.1 mmol reactive – NH2) in a peptide chamber and the reaction was mixed for 18 hours. The resin was rinsed thoroughly with NMP and CH2Cl2, then stirred for 10 minutes in NMP, and rinsed again with NMP and CH2Cl2. The resin was subjected to a second round of coupling: 0.008 g (0.02 mmol) of OT-4 diacid and 0.021 g (0.040 mmol) of PyBOP was dissolved in 10 mL NMP, then 0.174 mL of DIPEA (0.998 mmol) was added and stirred for 30 seconds. The solution was then added to the resin in a peptide chamber and the reaction was mixed for 18 hours. Final cleavage from the resin was performed as described for peptide 3. Crude peptide was obtained as a yellow powder (0.027 g, 0.023 mmol, 45%). \( \nu_{\text{max}}(\text{solid})/\text{cm}^{-1} \) 1730, 1643, 1527, 1454, 1234, 790, 742. 1H NMR (400 MHz, D2O) \( \delta \): 7.34-7.26 (18H, m), 4.39-4.36 (5H, m), 4.22-4.20 (1H, m), 2.92 (5H, q, \( J = 13.7 \) Hz), 2.68-2.53 (13H, m), 2.05 (2H, s), 1.33-1.18 (6H, broad), 1.07 (6H, d, \( J = 7.7 \) Hz). MS (ESI-) m/z 1197.4 (M-H+) (calc. 1197.2), m/z 1219.1 (M-2H+ + Na+) (calc. 1219.2), m/z 597.9 (M-2H+) (calc. 598.1). MS (ESI+) m/z 1197.4 (M+H+) (calc. 1197.2), m/z 1219.1 (M-2H+ + Na+) (calc. 1219.2), m/z 597.9 (M+H+) (calc. 598.1).
Peptide 5. 0.007 g (0.03 mmol) of [1,1'-biphenyl]-4,4'-dicarboxylic acid and 0.031 g (0.060 mmol) of PyBOP was dissolved in 10 mL 2:1 NMP:CH₂Cl₂, then 0.174 mL of DIPEA (0.998 mmol) was added and stirred for 30 seconds. The solution was then added to Wang-DFAG-NH₂ resin (0.1 mmol reactive –NH₂) in a peptide chamber and the reaction was mixed for 18 hours. The resin was rinsed thoroughly with NMP and CH₂Cl₂, then stirred for 10 minutes in NMP, and rinsed again with NMP and CH₂Cl₂. The resin was subjected to a second round of coupling: 0.005 g (0.02 mmol) of [1,1'-biphenyl]-4,4'-dicarboxylic acid and 0.021 g (0.040 mmol) of PyBOP was dissolved in 10 mL 2:1 NMP:CH₂Cl₂, then 0.174 mL of DIPEA (0.998 mmol) was added and stirred for 30 seconds. The solution was then added to the resin in a peptide chamber and the reaction was mixed for 18 hours. Final cleavage from the resin was performed as described for peptide 3. Crude peptide was obtained as an off-white powder (0.023 g, 0.023 mmol, 45%). ν_max(solid)/cm⁻¹ 1635, 1576, 1444, 1396, 842, 752. ¹H NMR (400 MHz, D₂O) δ: 7.81 (4H, d, J = 8.44 Hz), 7.67 (4H, d, J = 8.56 Hz), 7.23 (10H, m), 4.67 (2H, m), 4.38 (2H, m), 4.27 (2H, m), 4.03 (4H, m), 3.25 (2H, dd, J = 14.17, 4.76 Hz), 2.92 (2H, m), 2.61 (4H, m), 1.21 (6H, d, J = 7.16 Hz); MS (ESI⁻) m/z 1043.3 (M-2H⁺+Na⁺) (calc. 1043.3), m/z 1065.4 (M-3H⁺+2Na⁺) (calc. 1065.3), m/z 509.9 (M-2H⁺) (calc. 510.2), m/z 520.8 (M-3H⁺+Na⁺) (calc. 521.2), m/z 531.9 (M-4H⁺+2Na⁺) (calc. 532.2).

Methyl 4-((diethoxyphosphoryl)methyl)benzoate. To a solution of methyl 4-methylbenzoate (2.01 g, 13.3 mmol) and 1,2-dichloroethane (100 mL) was added benzoyl peroxide (catalytic amount) and NBS (2.84 g, 16.0 mmol). The resulting mixture was heated to reflux while being stirred under nitrogen. After three hours, another catalytic amount of benzoyl peroxide was added and the mixture was refluxed for an additional three hours. The reaction mixture was allowed to cool to RT, followed by the addition of Et₂O (20 mL) and filtration of a solid which precipitated out of solution. The filtrate was washed with water (2x) and brine (2x), dried over MgSO₄, filtered and the solvent was removed under reduced pressure to give a light yellow clear oil. Triethyl phosphate (2.28 mL, 13.3 mmol) was added to the oil and the reaction was stirred under nitrogen at 160 °C for three hours. Excess triethyl phosphate was distilled off and the product was purified by column chromatography (EtOAc) to give a transparent light yellow oil (2.28 g, 7.97 mmol, 60%). Characterization data matches literature.⁴

Dimethyl 4,4'-(1E,1'E)-1,4-phenylenebis(ethene-2,1-diyl))dibenzoate (OPV-3 diester). A solution of Methyl 4-((diethoxyphosphoryl)methyl)benzoate (2.02 g, 7.05 mmol) and terephthaldehyde (0.462 g, 3.44 mmol) in THF (12 mL) was added to a suspension of NaOMe (1.12 g, 20.6 mmol) in THF (70 mL). The reaction was stirred under nitrogen for four hours and was then neutralized with 1M HCl. The resulting suspension was filtered, the collected solid was washed with THF, EtOH, and H₂O, then allowed to dry under vacuum to give a yellow solid (1.15 g, 2.88 mmol, 84%). Characterization data matches literature.⁵
**Peptide 6.** A solution of OPV-3 diacid (0.025 g, 0.069 mmol) and PyBOP (0.071 g, 0.14 mmol) was dissolved in a 2:1 solution of NMP/CH₂Cl₂ (10 mL), once dissolved DIPEA (278 µL, 1.60 mmol) was added and the solution was stirred for one minute. This solution was added to Wang-VEVAG-NH₂ resin (0.228 mmol) that had been previously allowed to swell in CH₂Cl₂ for 10 minutes. The resulting mixture was gently agitated for 12 hours. The reaction mixture was then filtered and the resin was washed with NMP, CH₂Cl₂, and MeOH a total of three times. A second coupling solution of OPV-3 diacid (0.017 g, 0.046 mmol) and PyBOP (0.048 g, 0.091 mmol) in 2:1 NMP/CH₂Cl₂ (10 mL) followed by DIPEA (278 µL, 1.60 mmol) was added to the resin and gently agitated for 12 hours. The resin was washed as previously described and a blank coupling cycle with PyBOP (0.071 g, 0.137 mmol) with DIPEA (278 µL, 1.60 mmol) in 2:1 NMP/CH₂Cl₂ (10 mL) was allowed to react for one hour and the resin was washed as previously described. The resin was cleaved in a 1:1 solution of CH₂Cl₂/cleavage cocktail (95:2.5:2.5 TFA/TIPS/H₂O) (10 mL) for two hours. This solution was filtered out and the resin was washed with CH₂Cl₂ (10 mL), the filtrate was evaporated until 50% of the total volume remained, then 90 mL of cold Et₂O was added. The precipitated peptide was centrifuged and the remaining solution was decanted off. This was repeated with 45 mL of cold Et₂O, and then twice with 45 mL of cold MeCN. The final centrifuged peptide was then lyophilized to give a yellow solid (0.070 g, 0.05 mmol, 48%). ν<sub>max</sub>(solid)/cm<sup>-1</sup> 1629, 1529, 1390, 1301, 1222, 958, 844, 756. <sup>1</sup>H NMR (400 MHz, d<sub>6</sub>-DMSO): δ: 7.90 (4H, d, J = 8.0 Hz), 7.70 (4H, d, J = 8.8), 7.67 (4H, s), 7.51 (2H, d, J = 16.4 Hz), 7.35 (4H, d, J = 16.8 Hz), 4.39 (2H, m), 4.26 (2H, m), 4.15 (2H, m), 3.87 (4H, m), 2.20 (4H, m), 2.01 (4H, m), 1.86 (2H, m), 1.76 (2H, m), 1.72 (6H, d, J = 6.8 Hz), 0.82 (24H, m). MS (ESI-) m/z 1280.6 (M-H<sup>+</sup>) (calc. 1279.6), m/z 675.8 (M-4H<sup>+</sup>+2K<sup>+</sup>) (calc. 677.24), m/z 656.8 (M-3H<sup>+</sup>+K<sup>+</sup>) (calc. 658.3), m/z 639.4 (M-2H<sup>+</sup>) (calc. 639.3).

**Peptide 7.** A solution of OPV-3 diacid (0.010 g, 0.026 mmol) and PyBOP (0.027 g, 0.052 mmol) was dissolved in a 2:1 solution of NMP/CH₂Cl₂ (10 mL), once dissolved DIPEA (104 µL,
0.597 mmol) was added and the solution was stirred for one minute. This solution was added to Wang-DFAA-NH₂ resin (0.0896 mmol) that had been previously allowed to swell in CH₂Cl₂ for 10 minutes. The resulting mixture was gently agitated for 12 hours. The reaction mixture was then filtered and the resin was washed with NMP, CH₂Cl₂, and MeOH three times each. A second coupling solution of OPV-3 diacid (0.006 g, 0.02 mmol) and PyBOP (0.018 g, 0.034 mmol) in 2:1 NMP/CH₂Cl₂ (10 mL) followed by DIPEA (104 µL, 0.597 mmol) was added to the resin and gently agitated for 12 hours. The resin was washed as previously described and was cleaved in a 1:1 solution of CH₂Cl₂/cleavage cocktail (95:2.5:2.5 TFA/TIPS/H₂O) (10 mL) for two hours. This solution was filtered out and the resin was washed with CH₂Cl₂ (10 mL), the filtrate was evaporated until 50% of the total volume remained, then 90 mL of cold Et₂O was added. The precipitated peptide was centrifuged and the remaining solution was decanted off. This was repeated with 45 mL of cold Et₂O, and then twice with 45 mL of cold MeCN. The final centrifuged peptide was then lyophilized to give a yellow solid (0.030 g, 0.020 mmol, 30%). ν max(solid)/cm⁻¹ 1641, 1527, 1452, 1396, 1288, 1234, 1186, 962, 846. ¹H NMR (400 MHz, D₂O) δ: 7.80 (4H, d, J = 8.0 Hz), 7.70 (4H, d, J = 8.0 Hz), 7.67 (4H, s), 7.51 (2H, d, J = 16.4 Hz), 7.35 (4H, d, J = 16.8 Hz), 4.39 (2H, m), 4.26 (2H, m), 4.15 (2H, m), 3.87 (4H, m), 2.20 (4H, m), 2.01 (4H, m), 1.86 (2H, m), 1.76 (2H, m), 1.22 (6H, d, J = 6.8 Hz), 0.82 (24H, m). MS (ESI-) m/z 1177.7 (M-H⁺) (calc. 1177.5), m/z 588.3 (M-2H) (calc. 588.2).

Peptide 8. A solution of OPV-3 diacid (0.010 g, 0.028 mmol) and PyBOP (0.029 g, 0.055 mmol) was dissolved in a 2:1 solution of NMP/CH₂Cl₂ (10 mL), once dissolved DIPEA (112 µL, 0.643 mmol) was added and the solution was stirred for one minute. This solution was added to Wang-DFAF-NH₂ resin (0.0992 mmol) that had been previously allowed to swell in CH₂Cl₂ for 10 minutes. The resulting mixture was gently agitated for 12 hours. The reaction mixture was then filtered and the resin was washed with NMP, CH₂Cl₂, and MeOH a total of three times. A second coupling solution of OPV-3 diacid (0.007 g, 0.018 mmol) and PyBOP (0.019 g, 0.037 mmol) in 2:1 NMP/CH₂Cl₂ (10 mL) followed by DIPEA (112 µL, 0.643 mmol) was added to the resin and gently agitated for 12 hours. The resin was washed as previously described and was cleaved in a 1:1 solution of CH₂Cl₂/cleavage cocktail (95:2.5:2.5 TFA/TIPS/H₂O) (10 mL) for two hours. This solution was filtered out and the resin was washed with CH₂Cl₂ (10 mL), the filtrate was evaporated until 50% of the total volume remained, then 90 mL of cold Et₂O was added. The precipitated peptide was centrifuged and the remaining solution was decanted off. This was repeated with 45 mL of cold Et₂O, and then twice with 45 mL of cold MeCN. The final centrifuged peptide was then lyophilized to give a yellow solid (0.043 g, 0.03 mmol, 35%). ν max(solid)/cm⁻¹ 1633, 1531, 1456, 1400, 1232, 1191, 964, 846, 746. ¹H NMR (400 MHz, d₆-DMSO) δ: 7.78 (4H, d, J = 8.4 Hz), 7.66 (8H, m), 7.36 (4H, m), 7.23 (12H, m), 7.15 (8H, m), 4.70 (2H, m), 4.48 (2H, m), 4.29 (4H, m), 3.06 (5H, m), 2.95 (2H, m), 2.8 (4H, m), 1.25 (6H, d, J = 7.2 Hz). MS (ESI-) m/z 1329.5 (M-H⁺) (calc. 1329.5), m/z 664.2 (M-2H⁺) (calc. 664.3).

Dimethyl 2,2'-([2,2':5',2''-terthiophene]-5,5''-dyl)dicacetate (OT-3-CH₂-diester). A flame dried Schlenk flask was cooled under vacuum, refilled with nitrogen and charged with Pd(PPh₃)₄ (0.279 g, 0.241 mmol). The flask was then evacuated and refilled with N₂. Degassed dioxane
(20.0 mL) was cannulated into a flame-dried round-bottom flask followed by the addition of bisstannylated thiophene6 (4.00 g, 6.04 mmol) and brominated thiophene methyl ester7 (4.26 g, 18.1 mmol). The reaction was degassed for 20 minutes then heated to 80°C and stirred for 48 hours. The reaction was cooled to room temperature, diluted with EtOAc, stirred with 1M KF for 10 minutes and filtered through celite. The organic phase was then washed with brine (3x) and water (2x), dried with magnesium sulfate, stirred with charcoal for 20 minutes, filtered through celite again, and concentrated under reduced pressure. The crude material was triturated with hexanes, concentrated, and purified by a silica column chromatography (gradient 20% to 25% EtOAc:hexanes). Final product was obtained as a brown solid (0.837 g, 2.13 mmol, 35%). \(^1^H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\): 7.00 (4H, t, \(J = 3.4\) Hz, Ar-H), 6.84 (2H, d, \(J = 3.4\) Hz, Ar-H), 3.81 (4H, s, -CH\(_2\)-), 3.75 (6H, s, -CH\(_3\)). \(^1^C\) NMR (100 MHz, CDCl\(_3\)) \(\delta\): 170.5 (-COO-), 136.8 (Ar), 135.9 (Ar), 134.2 (Ar), 127.6 (Ar), 124.0 (Ar), 123.2 (Ar), 52.3 (CH\(_3\)O-), 35.4 (-CH\(_2\)-).

2,2'-([2,2':5',2''-terthiophene]-5,5''-diyl)diacetic acid (OT-3-CH\(_2\)-diacid). OT-3-CH\(_2\)-diester (0.837 g, 2.13 mmol) was dissolved in 50 mL diethyl ether and to that was added 15 mL MeOH and 25 mL of 1M KOH (aq). The solution was then stirred for 18 hours at room temperature. The reaction was then acidified with 1M HCl to pH 3 and the organics removed under reduced pressure. The crude material was filtered, washed with excess water, eluted off the filter with 1:1 MeOH:acetone, dried with magnesium sulfate, concentrated under reduced pressure, and triturated with hot hexanes. The product was isolated as a brown solid (0.685 g, 1.88 mmol, 88%). \(^1^H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\): 12.70 (2H, broad), 7.19-6.91 (6H, m), 3.84 (4H, s). \(^1^C\) NMR (100 MHz, CDCl\(_3\)) \(\delta\): 171.4 (-COO-), 136.0 (Ar), 135.2 (Ar), 135.2 (Ar), 127.9 (Ar), 124.4 (Ar), 123.5 (Ar), 35.1 (-CH\(_2\)-). HRMS (FAB) \(m/z\) calculated for (C\(_{16}\)H\(_{12}\)O\(_4\)S\(_3\))^+ 363.9898, found 393.9899.
NMR spectra

Figure S1: $^1$H NMR (400 MHz, $d_6$-DMSO) of Peptide 1 purified by trituration. A list of data is located on page S4.

Figure S2: $^1$H NMR (300 MHz, D$_2$O, 90 °C) of Peptide 2 purified by trituration. A list of data is located on page S4.
Figure S3: $^1$H NMR (400 MHz, D$_2$O) of Peptide 3 purified by trituration. A list of data is located on page S5.

Figure S4: $^1$H NMR (400 MHz, D$_2$O) of Peptide 4 purified by trituration. A list of data is located on page S6.
Figure S5: $^1$H NMR (400 MHz, D$_2$O) of Peptide 5 purified by trituration. A list of data is located on page S7.

Figure S6: $^1$H NMR (400 MHz, d$_6$-DMSO) of Peptide 6 purified by trituration. A list of data is located on page S8.
Figure S7: $^1$H NMR (400 MHz, D$_2$O) of Peptide 7 purified by trituration. A list of data is located on page S9.

Figure S8: $^1$H NMR (400 MHz, d$_6$-DMSO) of Peptide 8 purified by trituration. A list of data is located on page S9.
HPLC traces and mass spectra

Peptide 1

Figure S9: HPLC traces of crude Peptide 1 as cleaved from the resin (top) and of the HPLC purified fraction (bottom). Purification was performed using a linear gradient of 27%-70% buffer B/buffer A over 35 minutes.

Figure S10: MALDI-TOF of Peptide 1. (MALDI-TOF): m/z 1246.136 [M-1]/1; calculated 1246.221.
Peptide 2

Figure S11: ESI- of Peptide 2. (ESI-MS, Negative Ion Mode): m/z calculated for [M-2]/2: 684.24, found 684.01; m/z calculated for [M-3]/3: 455.83, found 455.81.
**Peptide 3**

**Figure S12:** HPLC traces of crude Peptide 3 as cleaved from the resin (top) and of the HPLC purified fraction (bottom). Purification was performed using a linear gradient of 27%-70% buffer B(buffer A over 35 minutes.

**Figure S13:** ESI- of Peptide 3. MS (ESI-) m/z 1115.3 [M-1]⁻/1 (calc. 1115.3), m/z 1137.2 [M-2+Na⁺]⁻/1 (calc. 1137.2), m/z 1159.2 [M-3+2Na⁺]⁻/2 (calc. 1159.2), m/z 556.9 [M-2]²⁻/2 (calc. 557.1), m/z 567.9 [M-3+Na⁺]⁻/3 (calc. 568.1).
**Peptide 4**

**Figure S14:** HPLC traces of crude **Peptide 4** as cleaved from the resin (top) and of the HPLC purified fraction (bottom). Purification was performed using a linear gradient of 35%-60% buffer B/buffer A over 20 minutes then held at 60% for 10 minutes.

**Figure S15:** ESI- of **Peptide 4**. MS (ESI-)  m/z 1197.4 [M-1]⁻/1 (calc. 1197.2), m/z 1219.1 [M-2+Na⁺]⁻/1 (calc. 1219.2), m/z 597.9 [M-2]²⁻/2 (calc. 598.1).
Peptide 5

Figure S16: HPLC traces of crude Peptide 5 as cleaved from the resin (top) and of the HPLC purified fraction (bottom). Purification was performed using a linear gradient of 20%-50% buffer B/buffer A over 20 minutes then held at 50% for 10 minutes.

Figure S17: ESI- of Peptide 5. MS (ESI-) m/z 1043.3 [M-2+Na]^1/-1 (calc. 1043.3), m/z 1065.4 [M-3+2Na]^1/-1 (calc. 1065.3), m/z 509.9 [M-2]^2/-2 (calc. 510.2), m/z 520.8 [M-3+Na]^2/-2 (calc. 521.2), m/z 531.9 [M-4+2Na]^2/-2 (calc. 532.2).
Peptide 6

**Figure S18:** HPLC traces of crude Peptide 6 as cleaved from the resin (top) and of the HPLC purified fraction (bottom). Purification was performed using a linear gradient of 27%-70% buffer B/buffer A over 40.

**Figure S19:** ESI- of Peptide 6. MS (ESI-) m/z 1280.6 [M-1]⁻/₁ (calc. 1279.6), m/z 675.8 [M-4+2K⁺]⁴⁻/₂ (calc. 677.24), m/z 656.8 [M-3+K⁺]⁴⁻/₂ (calc. 658.3), m/z 639.4 [M-2]⁻/₁ (calc. 639.3).
Peptide 7

Figure S20: HPLC traces of crude Peptide 7 as cleaved from the resin (top) and of the HPLC purified fraction (bottom). Purification was performed using a linear gradient of 27%-70% buffer B/buffer A over 40.

Figure S21: ESI- of Peptide 7. MS (ESI-) m/z 1177.7 [M-1]⁻/1 (calc. 1177.5), m/z 588.3 [M-2]²⁻/2 (calc. 588.2).
Peptide 8

Figure S22: HPLC traces of crude Peptide 8 as cleaved from the resin (top) and of the HPLC purified fraction (bottom). Purification was performed using a linear gradient of 27%-70% buffer B/buffer A over 40.

Figure S23: ESI- of Peptide 8. MS (ESI-) m/z 1329.5 [M-1]^1/1 (calc. 1329.5), m/z 664.2 [M-2]^2/2 (calc. 664.3).
**Figure S24:** Crude ESI of a peptide made from OT-3-CH₂-diacid (top) showing products from incomplete dimerization (below).
ATR-IR spectra

Figure S25: Attenuated total reflectance IR spectrum of Peptide 1 in the solid state (purified by trituration). A list of data is located on page S4.

Figure S26: Attenuated total reflectance IR spectrum of Peptide 2 in the solid state (purified by trituration). A list of data is located on page S4.

Figure S27: Attenuated total reflectance IR spectrum of Peptide 3 in the solid state (purified by trituration). A list of data is located on page S5.
Figure S28: Attenuated total reflectance IR spectrum of Peptide 4 in the solid state (purified by trituration). A list of data is located on page S6.

Figure S29: Attenuated total reflectance IR spectrum of Peptide 5 in the solid state (purified by trituration). A list of data is located on page S7.

Figure S30: Attenuated total reflectance IR spectrum of Peptide 6 in the solid state (purified by trituration). A list of data is located on page S8.
**Figure S31:** Attenuated total reflectance IR spectrum (1000 scans) of **Peptide 7** in the solid state (purified by trituration). A list of data is located on page S9.

**Figure S32:** Attenuated total reflectance IR spectrum of **Peptide 8** in the solid state (purified by trituration). A list of data is located on page S9.
UV-Vis and photoluminescence spectra

Figure S33: UV-Vis (left) and photoluminescence (right) of Peptide 2 purified by trituration; taken in neutral (dashed line) and acidic (30 µL 1M HCl into 3 mL, solid line) water.

Figure S34: UV-Vis and photoluminescence of Peptide 4 after triturating with acetonitrile (left) and after HPLC purification (right) taken in neutral (dashed line) and acidic (30 µL 1M HCl into 3 mL, solid line) water.
**Figure S35**: UV-Vis and photoluminescence of Peptide 5 purified by trituration; taken in neutral (dashed line) and acidic (30 µL 1M HCl into 3 mL, solid line) water. The slight fluorescence increase upon assembly is unusual when compared to the other pi-electron linkers reported here. We suspect that the extent of torsional strain and conformational rotational freedom among the phenyl linkages in Peptide 5 is influencing the rates of radiative and nonradiative decay differently as the microscopic environment is becoming more structured and rigid. However, even for molecular oligophenyl chromophores, the interplay of attenuated radiative and non-radiative decay pathways is not general for systems that have free phenyl-phenyl rotation or that have been covalently held into planarity through ring fusion, etc. (see Nijegorodov and Downey, *J. Phys. Chem.*, 1994, **98**, 5639-5643)\(^8\) Our future work will investigate the time-resolved exciton dynamics in order to correlate quantum yields with fluorescence lifetimes as a way to observe how/if radiative and non-radiative pathways for fluorescence decay are being perturbed differently in the assembled structure.
**Figure S36**: UV-Vis and photoluminescence of Peptide 7 purified by trituration; taken in basic (10 µL 1 M KOH into 3 mL, dashed line) and acidic (10 µL 1M HCl into 3 mL, solid line) water.

**Figure S37**: UV-Vis and photoluminescence of Peptide 8 purified by trituration; taken in basic (10 µL 1 M KOH into 3 mL, dashed line) and acidic (10 µL 1M HCl into 3 mL, solid line) water.
CD spectra

**Figure S38:** CD spectrum of 20 µM solution of Peptide 1 purified by trituration; taken in basic (10 µL 1 M KOH into 3 mL, dashed line) and acidic (50 µL 1M HCl into 3 mL, solid line) water.

**Figure S39:** CD spectrum of 20 µM solution of Peptide 2 purified by trituration; taken in basic (10 µL 1 M KOH into 3 mL, dashed line) water. Acidic spectrum could not be collected due to macroscopic aggregates.
Figure S40: CD spectrum of 20 µM solution of Peptide 4 purified by trituration; taken in basic (10 µL 1 M KOH into 3 mL, dashed line) and acidic (50 µL 1M HCl into 3 mL, solid line) water.

Figure S41: CD spectrum of 20 µM solution of Peptide 5 purified by trituration; taken in basic (10 µL 1 M KOH into 3 mL, dashed line) and acidic (50 µL 1M HCl into 3 mL, solid line) water.
**Figure S42:** CD spectrum of Peptide 7 with an optical density of 1.5 purified by trituration; taken in basic (10 µL 1 M KOH into 3 mL, dashed line) and acidic (50 µL 1M HCl into 3 mL, solid line) water.

**Figure S43:** CD spectrum of 27 µM solution of Peptide 8 purified by trituration; taken in basic (10 µL 1 M KOH into 3 mL, dashed line) and acidic (50 µL 1M HCl into 3 mL, solid line) water.
Figure S44: AFM of Peptide 6 showing amplitude (left) and phase (right). Sample preparation: 200 µL of a 0.08 mM aqueous solution was placed in an acid chamber (closed vial with concentrated HCl) for 20 minutes. 10 µL of this sample was then deposited onto freshly cleaved mica and allowed to dry before imaging.
Assembly Model

Figure S45. Energy-minimized illustration of $\beta$-sheets and $\pi$-stacks as space-filling models showing helical twist along a model aggregate (A, Side-on and B, Edge-on). Truncated Peptide 3, omitting aspartic acid residues (HO-FAG-OT3-GAF-OH), is shown for clarity.
Macroscopic qualitative hydrogel properties:

**Peptide 1** (NDI): Formed a translucent brown gel.

**Peptide 2** (PDI): Formed an opaque dark purple/black gel;
Poor HPLC separation and other spectroscopic characterization suggests that the molecule has a high propensity to aggregate even in conditions meant to offer molecularly dissolved solutions.

**Peptide 3** (OT3): Formed a weakly self-supporting and opaque yellow gel.

**Peptide 4** (OT4): Formed a self-supporting and opaque gel.

**Peptide 5** (OP2): Formed a transparent self-supporting gel.

**Peptide 6** (OPV3): Formed a transparent self-supporting yellow gel.

**Peptide 7** (Ala-OPV3): CD signal showed expected features of aggregation in acidic pH.

**Peptide 8** (Phe-OPV3): CD signal showed some as yet undetermined chromophore interaction at basic pH, suggesting some pre-association at basic pH.

---