Supporting Information

Sequence-dependent optoelectronic and mechanical properties of π-conjugated peptide hydrogelators

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PEPTIDE SYNTHESIS

General Considerations. The chemicals used for 9-fluorenylmethoxycarbonyl (Fmoc)-based solid phase peptide synthesis (*N*-methylpyrrolidone (NMP), *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), *N,N*-diisopropylethylamine (DIPEA), Wang resin, and Fmoc-protected amino acids) were obtained from Oakwood Products, Inc. or Advanced ChemTech. Tetrahydrofuran (THF) was obtained from an Innovative Technologies PureSolv solvent purification system and stored over 4Å molecular sieves (Sigma-Aldrich). *N,N*-dimethylformamide (DMF) was obtained from either Sigma-Aldrich or EMD Millipore Chemicals. DIPEA, THF and DMF were degassed by sparging with nitrogen (N_2) gas for one hour prior to use. Tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$) was obtained from Strem Chemicals. The Biotech-grade cellulose ester dialysis tubings (MWCO 500-1000), with flat widths of either 16-mm or 31-mm were obtained from Spectrum Labs. All other reagents and starting materials were obtained from Sigma-Aldrich and were used as received. 5-bromothiophene-2-carboxylic acid and 5,5'-bis-tributylstannyl-[2,2']-bithiophene were prepared using literature procedures.[1,2] ^{1}H-NMR spectra were obtained using a Bruker Avance 400 MHz (unless otherwise stated) and the data was processed using Bruker Topsin 1.3. Chemical shifts are reported in parts per million relative to residual protio solvent [d_6-DMSO δ: 2.50, D_2O δ: 4.79 (^{1}H NMR)]. The tabulated values for NMR peaks may not reflect the theoretical number of protons expected due to some aggregation previously observed for these materials under basic to neutral conditions.[3]

General Solid Phase Peptide Synthesis (SPPS). All peptides were synthesized using the standard Fmoc solid-phase technique with Wang resin pre-loaded with the terminal amino acid (Wang-Asp= 0.6 mmol/g). To the resin in a peptide chamber, Fmoc-deprotection was accomplished by adding a (1:4) piperidine/DMF solution twice (successive 5- and 10-minute treatment) and then washing with NMP, methanol and dichloromethane (DCM). For the amino acid couplings, 3.0 eq. of the Fmoc-protected amino acid (1.0 eq of the Fmoc-deprotected peptide bound to the resin) underwent external

activation with 2.9 eq. of HBTU and 10 eq. DIPEA. The activated amino acid mixture was mixed for one minute prior to addition in the peptide chamber. The reaction mixture was allowed to mix for 60-120 minutes, after which was rinsed with NMP, methanol and DCM. The completion of all couplings was monitored using a Kaiser test on a few dry resin beads, repeating same amino acid coupling as needed. The general procedure for amino acid coupling was repeated until the desired peptide sequence was obtained.

General *N*-acylation procedure for peptides. Following a procedure reported in the literature,[1] a solution containing 2.1 eq. of 5-bromothiophene-2-carboxylic acid that was activated by HBTU (2.0 eq.) with DIPEA (10 eq.) was mixed for 180 minutes with the resin containing the completed peptide sequence. The resin was rinsed with NMP, methanol and DCM. The resin was treated again with 1.1 eq. of 5-bromothiophene-2-carboxylic acid that was activated by HBTU (1.0 eq.) with DIPEA (10 eq.) for 60 minutes. After rinsing the resin with the standard wash cycle (NMP-methanol-DCM), completion was assessed using a Kaiser test on a few dry resin beads. Treatment with 1.1 eq. of the activated 5-bromothiophene-2-carboxylic acid was repeated as needed.

General on-resin Stille coupling procedure. Following a procedure reported in the literature,[1] the *N*-acylated peptide made by following the general procedures described above were transferred to a Schlenk flask topped with a reflux condenser. The dried resin with $Pd(PPh_3)_4$ (4.0 mol % relative to the amino acid loading in the resin) was kept in the Schlenk flask under a nitrogen (N_2) atmosphere (~10-20 mTorr). In a separate vessel, a ~15 mM solution of 5,5'-bis-tributylstannyl-[2,2']-bithiophene was prepared in DMF. This was then added to the reaction flask via syringe. The reaction mixture was heated up to 80^{o}C while agitating by constantly bubbling nitrogen (N_2) gas in the solution. The said conditions were maintained for 16 hours, and then the reaction mixture was allowed to cool to room temperature. The resin was washed with DMF (3×) in a peptide chamber, followed by the standard wash cycle. The synthesized π-conjugated peptides were then subjected to cleavage procedure.

General cleavage procedure for peptides. The cleavage cocktail was prepared with 9.5 mL of trifluoroacetic acid, 250 μL Milli-Q water, and 250 μL of triisopropylsilane. The resin was treated with

10 mL of cleavage cocktail in a peptide chamber for 3 hours. The filtrate was drained and the resin was washed with DCM (3×). The filtrate was concentrated under reduced pressure. The crude peptide was precipitated out of the filtrate by adding 90 mL of cold Et_2O, allowing the suspension to sit for 5 minutes at 4°C. The pellet formed was isolated by centrifugation, followed by decanting the solvent and drying the solid formed. The pellet was redissolved in Milli-Q water with a few drops of ammonium hydroxide (to completely dissolve the solid) and was subjected to lyophilization. All peptides (both crude and purified) were stored as lyophilized solids at 4°C.

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC). Peptides that underwent Stille coupling were dialyzed prior to HPLC purification in order to completely remove any excess Pd. The HPLC samples were prepared from lyophilized peptide solids after the dialysis procedure and were dissolved in Milli-Q water as basic samples by adding μL amounts of 1 M KOH until the solution reaches pH 8 to 9. Purification and analysis were performed using an Agilent SD1 PrepStar System with a Phenomenex C8 column (Luna 5 μm, 250×21.20 mm and 250×4.60 mm). The mobile phase used consists of an ammonium formate aqueous buffer (~pH 8) and acetonitrile.

Electrospray Ionization Mass Spectrometry (ESI-MS). Samples for ESI-MS analyses were prepared in a 1:1 methanol and water solution with 1.0% (v/v) ammonium hydroxide. Mass spectra were collected using a Thermo Finnigan LCQ Deca Ion Trap Mass Spectrometer in negative mode.

Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) Spectroscopy. All data were obtained on dried peptides using a Thermo Scientific Nicolet iD5 ATR-IR. The spectra of coassemblies were taken from the lyophilized acidic peptide solutions.

DGG Peptide. HO-DGG-4T-GGD-OH. Solid-supported Wang-DGG-NH_2 peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.5 mmol). The peptide was coupled with 5,5'-bis-tributylstannyl-[2,2']-bithiophene (0.25 mmol, 0.186 g) in the presence of $Pd(PPh_3)_4$ (0.02 mmol, 0.023

g) using the general on-resin Stille coupling procedure for 15 hours. Resin was then subjected to the general cleavage procedure. Crude peptide obtained was observed as an orange powder (λ_{max}=418 nm, pH=8, 0.066 g, 30%). MS (ESI-) *m/z* 897.1 ($M\text{-}2H^+ + Na^+$) (calc. 897.1), *m/z* 875.2 ($M\text{-}H^+$) (calc. 875.1), *m/z* 437.2 ($M\text{-}2H^+$) (calc. 437.6). ^{1}H NMR (400 MHz, d_6-DMSO) δ, ppm: 8.91 (m, 1H), 8.26 (m, 1H), 7.78 (d, 2H, *J*= 4 Hz), 7.44 (d, 1H, *J*= 4 Hz), 7.40 (m, 2H), 7.01 (br s, H), 4.15 (m, 1H), 3.90 (d, 2H, *J*= 6 Hz), 3.74 (d, 2H, *J*= Hz), 2.47 (s, 1H), 2.45 (s, 1H), 2.35 (d, 1H, *J*= 2.4 Hz), 2.31 (d, 1H, *J*= 2.4 Hz).

DAA Peptide. HO-DAA-4T-AAD-OH. Solid-supported Wang-DAA-NH_2 peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.5 mmol). The peptide was coupled with 5,5'-bis-tributylstannyl-[2,2']-bithiophene (0.25 mmol, 0.186 g) in the presence of $Pd(PPh_3)_4$ (0.02 mmol, 0.023 g) using the general on-resin Stille coupling procedure for 15 hours. Resin was then subjected to the general cleavage procedure. Crude peptide obtained was observed as an orange powder (λ_{max}=418 nm, pH=8, 0.045 g, 19%). MS (ESI-) *m/z* 953.2 ($M\text{-}2H^+ + Na^+$) (calc. 953.2), *m/z* 931.3 ($M\text{-}H^+$) (calc. 931.2), *m/z* 465.3 ($M\text{-}2H^+$) (calc. 465.1). ^{1}H NMR (400 MHz, d_6-DMSO) δ, ppm: 8.64 (m, H), 7.87 (d, H, *J*= 4.1 Hz), 7.43 (d, H, *J*= 3.8 Hz), 7.39 (d, 4H, *J*= 3.8 Hz) 4.50-4.30 (m, 6H), 4.25 (br s, 2H), 2.65 (m, 2H), 2.32 (m, 2H), 1.37 (d, 6H, *J*= 6.8 Hz), 1.23 (d, 6H, *J*= 6.8 Hz).

DVV Peptide. HO-DVV-4T-VVD-OH. Solid-supported Wang-DVV-NH_2 peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.5 mmol). The peptide was coupled with 5,5'-bis-tributylstannyl-[2,2']-bithiophene (0.25 mmol, 0.186 g) in the presence of $Pd(PPh_3)_4$ (0.02 mmol, 0.023 g) using the general on-resin Stille coupling procedure for 15 hours. Resin was then subjected to the general cleavage procedure. Crude peptide obtained was observed as an orange powder (λ_{max}=418 nm, pH=8, 0.048 g, 18%). MS (ESI-) *m/z* 1065.3 ($M\text{-}2H^+ + Na^+$) (calc. 1065.3), *m/z* 1043.4 ($M\text{-}H^+$) (calc. 1043.4), *m/z* 521.4 ($M\text{-}2H^+$) (calc. 521.1). ^{1}H NMR (400 MHz, d_6-DMSO) δ, ppm: 8.43 (d, 2H, *J*= 8.8 Hz), 8.38 (s, 1H), 8.07 (d, 2H, *J*= 8.8 Hz), 7.95 (d, 2H, *J*= 4.0 Hz), 7.63 (d, 2H, *J*= 6.4 Hz), 7.43 (d, 2H,

J= 4 Hz), 7.38 (dd, 4H, *J*= 3.8 Hz, 1.2 Hz), 4.35 (t, 4H, *J*= 8.4 Hz), 4.16-4.08 (m, 4H), 2.45-2.30 (m, 6H), 2.14 (q, 2H, *J*= 6.4 Hz), 2.04 (q, 4H, *J*= 6.4 Hz), 0.91 (m, 12H), 0.85 (d, 12H, *J*= 6.8 Hz).

DII Peptide. HO-DII-4T-IID-OH. Solid-supported Wang-DII-NH_2 peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.5 mmol). The peptide was coupled with 5,5'-bis-tributylstannyl-[2,2']-bithiophene (0.25 mmol, 0.186 g) in the presence of $Pd(PPh_3)_4$ (0.02 mmol, 0.023 g) using the general on-resin Stille coupling procedure for 16 hours. Resin was then subjected to the general cleavage procedure. Crude peptide obtained was observed as a yellow powder (λ_{max}=416 nm, pH=8, 0.055 g, 20%). MS (ESI-) *m/z* 1099.6 $(M\text{-}H^+)^-$ (calc. 1099.3), *m/z* 549.5 $(M\text{-}2H^+)^{-2}$ (calc. 549.2). ^{1}H NMR (400 MHz, d_6-DMSO) δ, ppm: 8.46 (d, 1H, *J*= 8.3 Hz), 8.09 (d, 1H, *J*= 9.2 Hz), 7.93 (d, 1H, J=4 Hz), 7.65 (br s, 1H), 7.43 (d, 1H, *J*= 4 Hz), 7.38 (m, 2H), 4.37 (t, 2H, *J*= 9 Hz), 4.16 (t, 2H, *J*= 7.8 Hz), 2.43-2.30 (m, 2H), 1.92, (m, 1H), 1.76 (m, 1H), 1.45 (m, 2H), 1.13 (m, 3H), 0.88 (d, 3H, *J*= 6.8 Hz), 0.83 (m, 9H).

DFF Peptide. HO-DFF-4T-FFD-OH. Solid-supported Wang-DFF-NH_2 peptide N-acylated with 5-bromothiophene-2-carboxylic acid was prepared (0.5 mmol). The peptide was coupled with 5,5'-bis-tributylstannyl-[2,2']-bithiophene (0.25 mmol, 0.186 g) in the presence of $Pd(PPh_3)_4$ (0.02 mmol, 0.023 g) using the general on-resin Stille coupling procedure for 18 hours. Resin was then subjected to the general cleavage procedure. Crude peptide obtained was observed as an orange powder (λ_{max}=418 nm, pH=8, 0.017 g, 6%). MS (ESI-) *m/z* 1257.3 $(M\text{-}2H^+\text{+}Na^+)$ (calc. 1257.3), *m/z* 1235.4 $(M\text{-}H^+)$ (calc. 1235.3), *m/z* 628.3 $(M\text{-}3H^+\text{+}Na^+)$ (calc. 628.7), *m/z* 617.4 $(M\text{-}2H^+)$ (calc. 617.2) *m/z* 411.4 $(M\text{-}3H^+)$ (calc. 411.1). ^{1}H NMR (400 MHz, d_6-DMSO) δ, ppm: 8.42 (d, 1H, *J*= 7.6 Hz), 7.82 (d, 1H, *J*= 6.8 Hz), 7.80 (d, 1H, *J*=4 Hz), 7.40 (d, 1H, *J*= 3.6 Hz), 7.36-7.33 (m, 3H), 7.28-7.15 (m, 6H), 4.68 (m, 1H), 4.51 (m, 1H), 4.09 (m, 1H), 3.18-3.06 (m, 4H), 2.96-2.85 (m, 4H), 2.46-2.33 (m, 2H).

PEPTIDE CHARACTERIZATION DATA

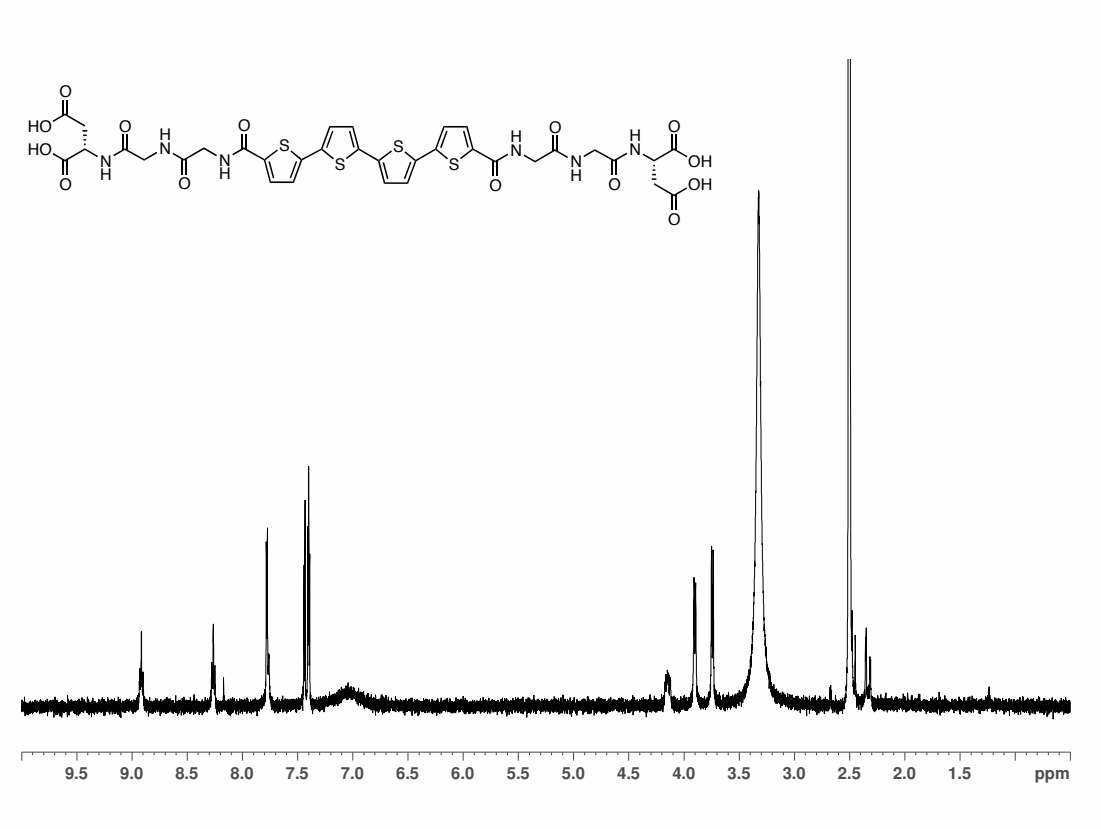

Figure S1. ^{1}H (400 MHz, d_6-DMSO) NMR DGG peptide.

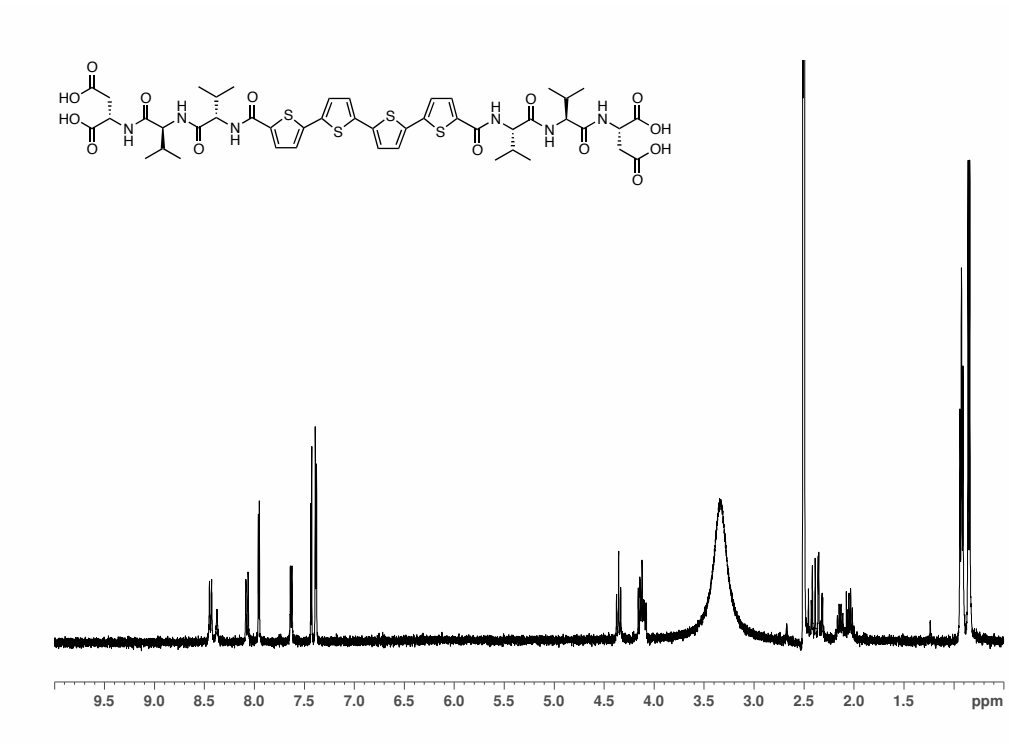

Figure S2. ^{1}H (400 MHz, d_6-DMSO) NMR of DVV peptide.

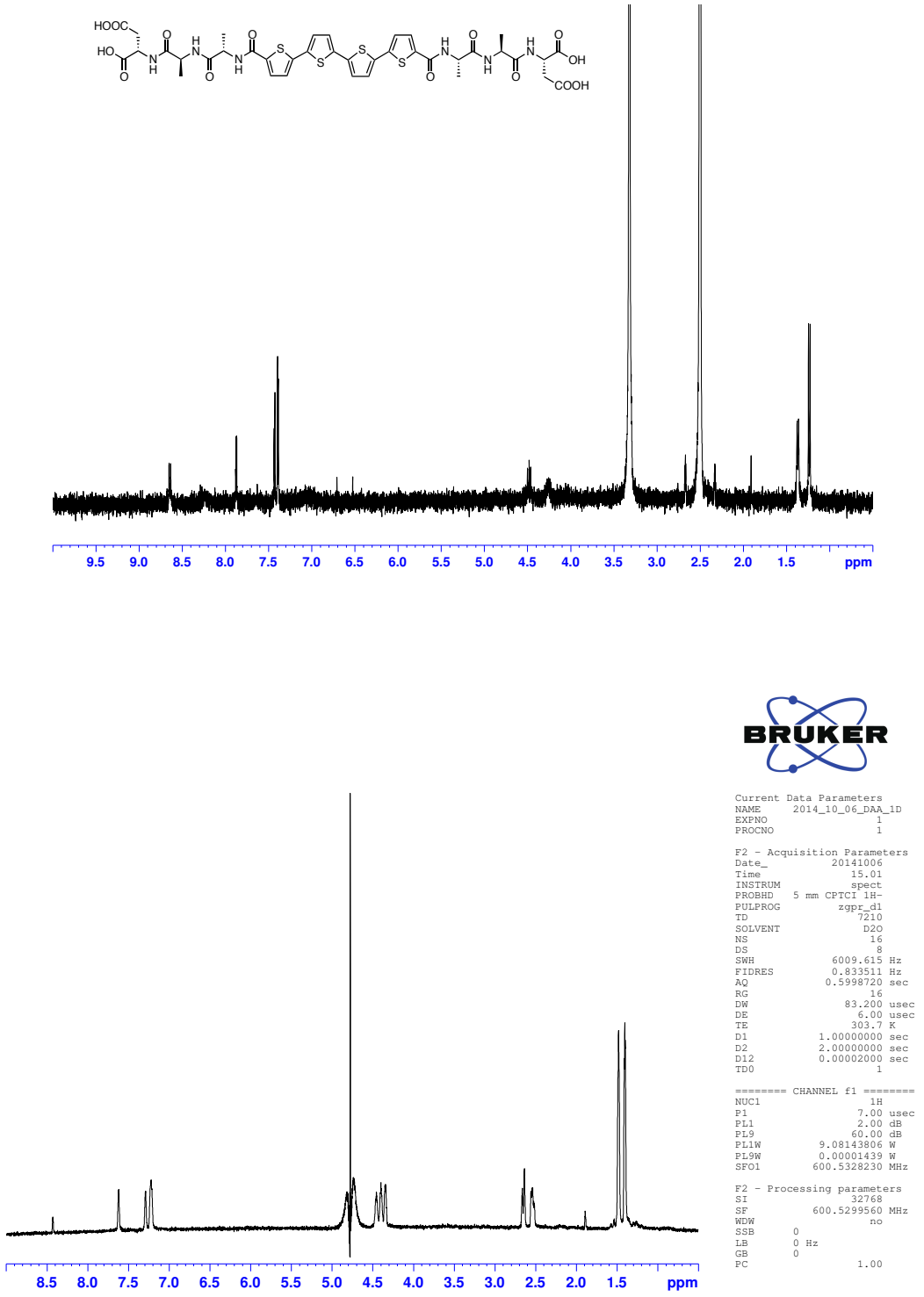

Figure S3. ^{1}H (400 MHz, d_6-DMSO) NMR spectrum (*top*) and ^{1}H (600 MHz, D_2O) NMR spectrum (*bottom*) of DAA peptide.

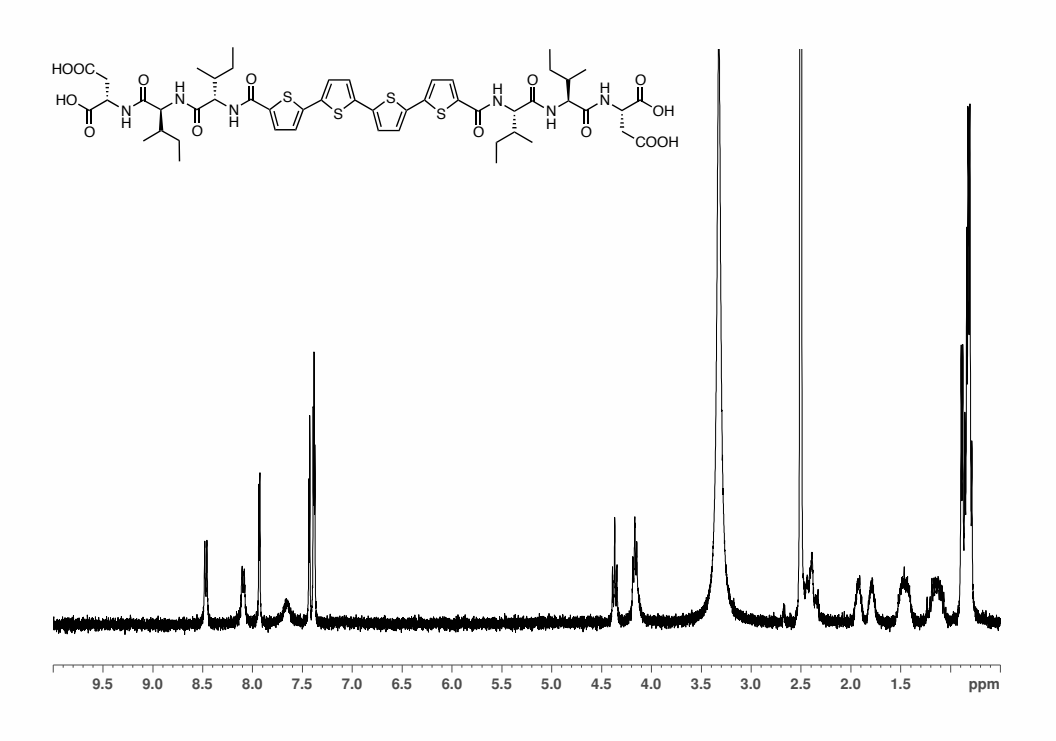

Figure S4. ^{1}H (400 MHz, d_6-DMSO) NMR of DII peptide.

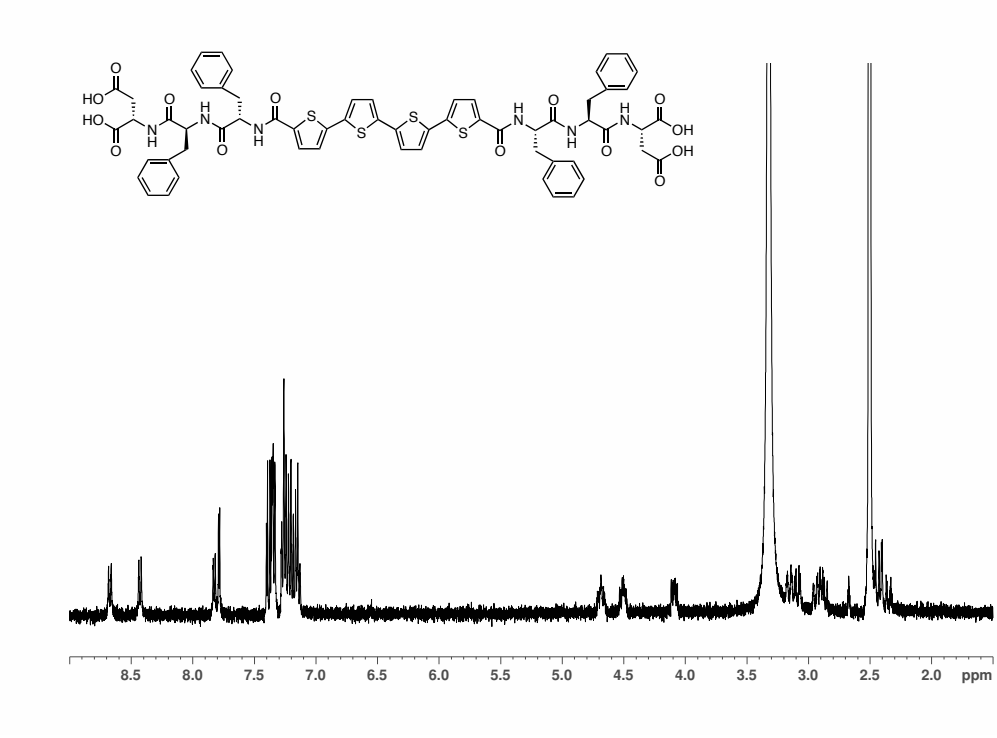

Figure S5. ^{1}H (400 MHz, d_6-DMSO) NMR of DFF peptide.

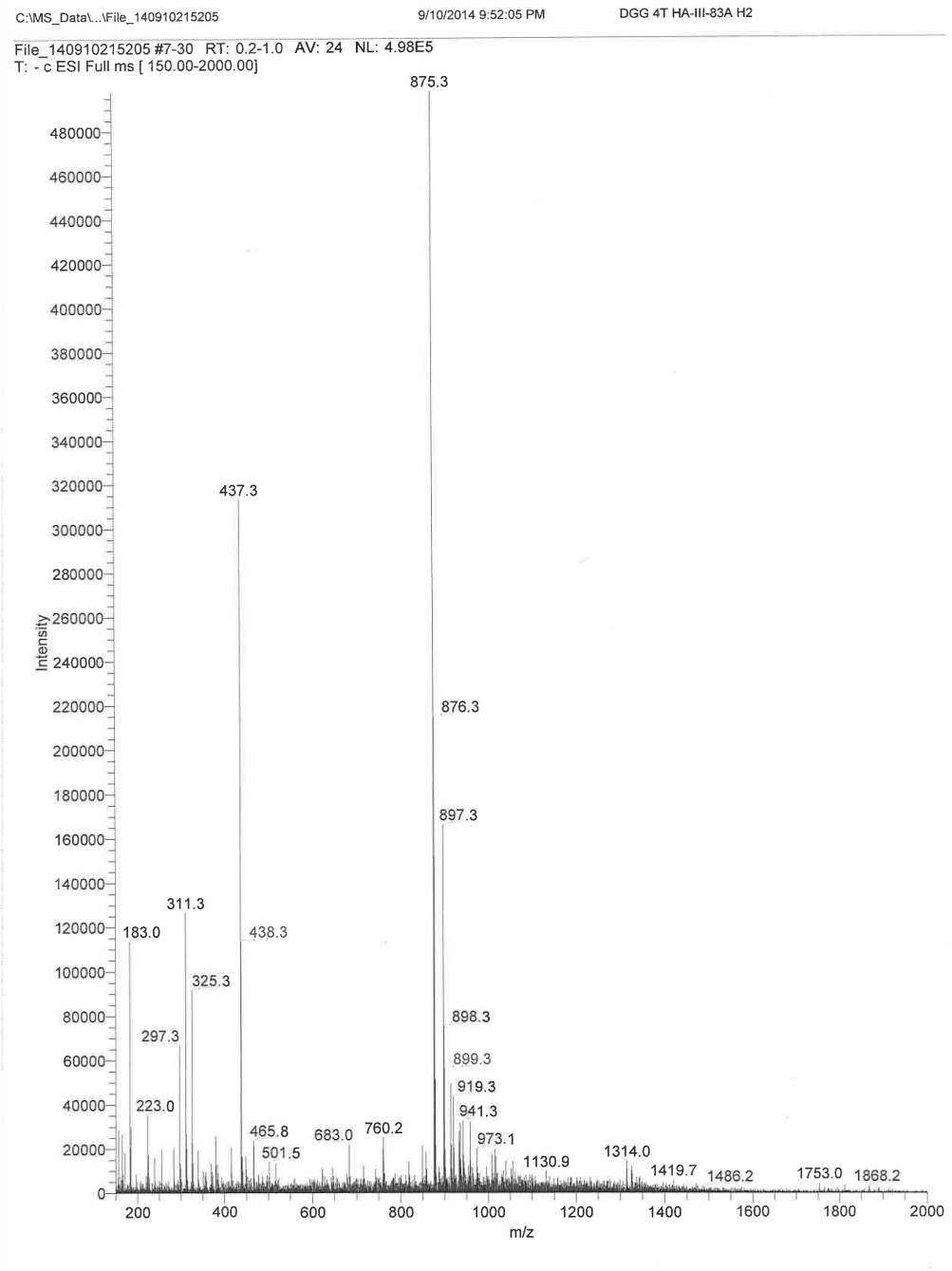

Figure S6. ESI of DGG peptide.

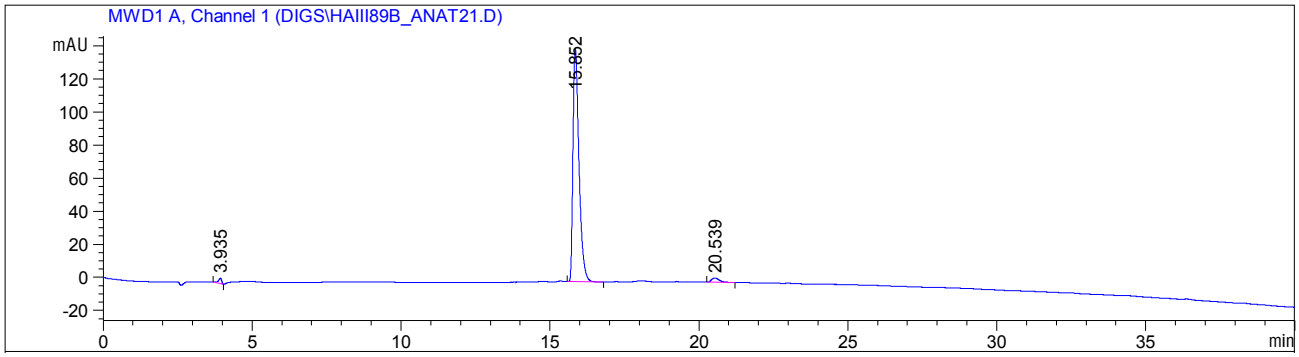

Figure S7. Analytical HPLC trace of DGG, monitoring at 400 nm.

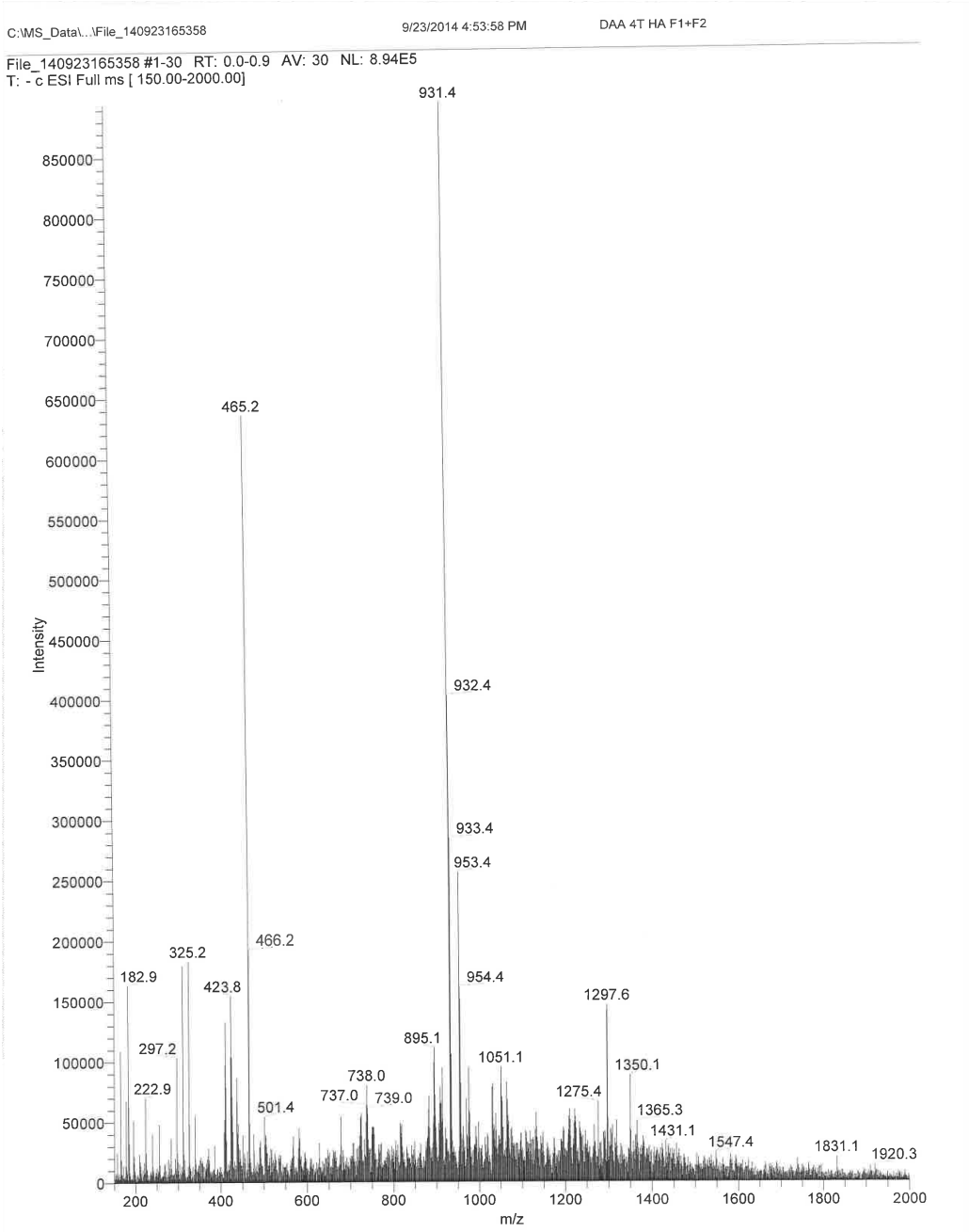

Figure S8. ESI of DAA peptide.

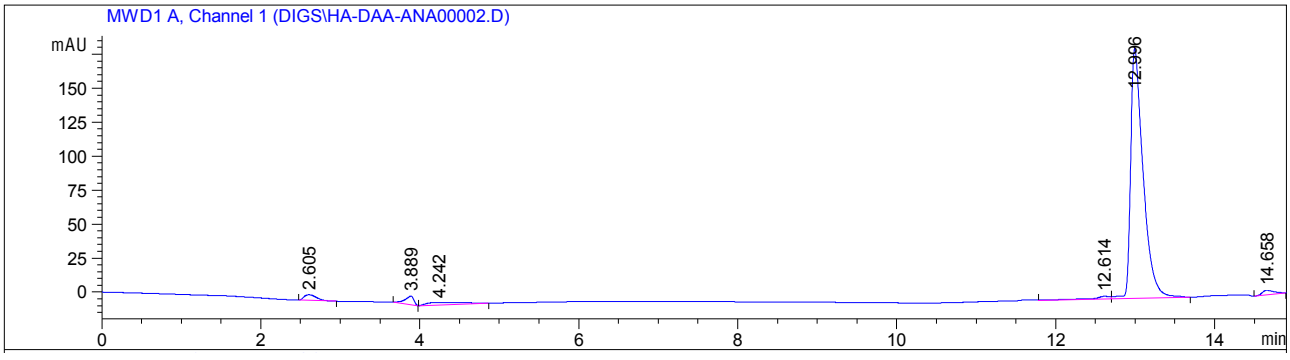

Figure S9. Analytical HPLC trace of DAA, monitoring at 400 nm.

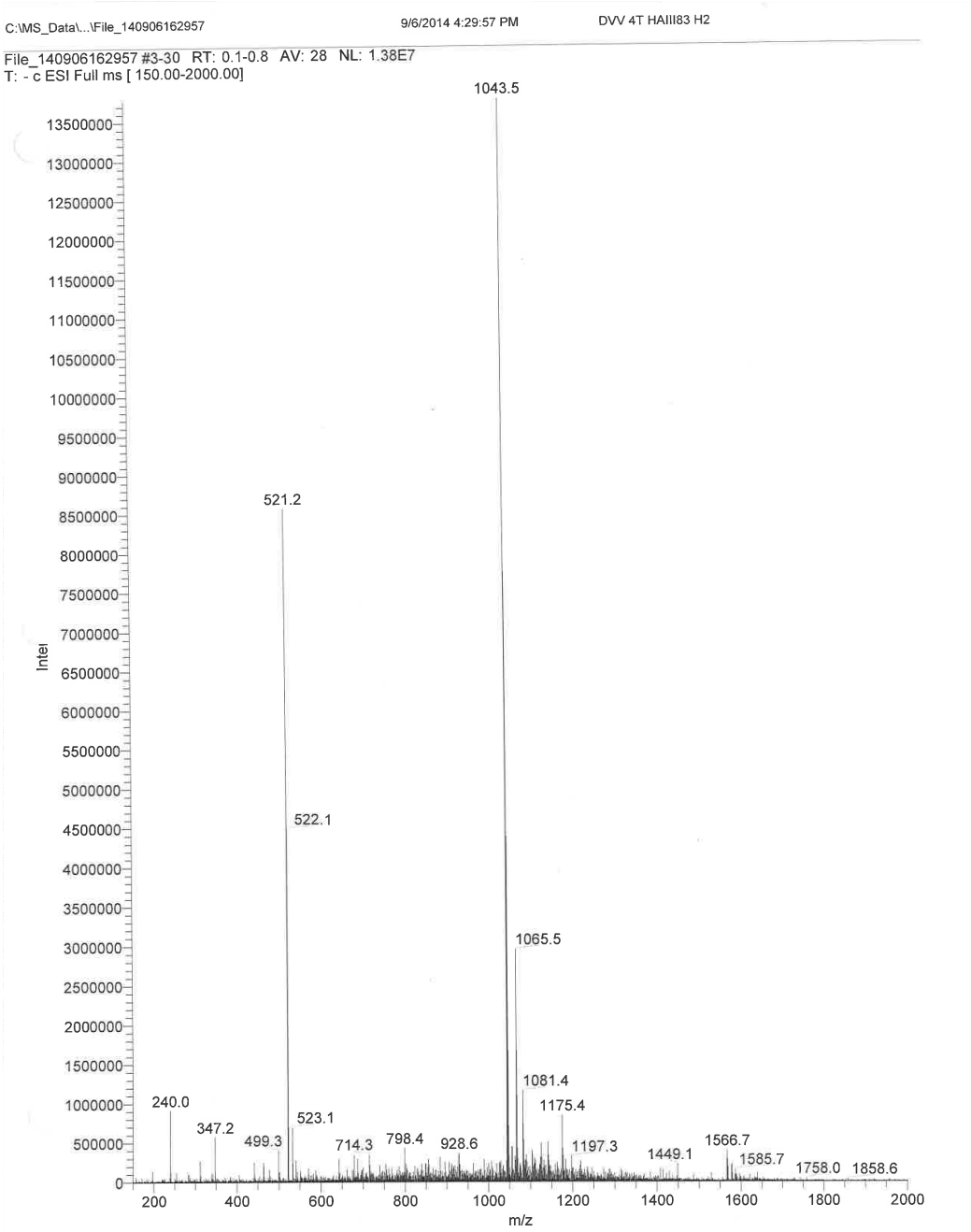

Figure S10. ESI of DVV peptide.

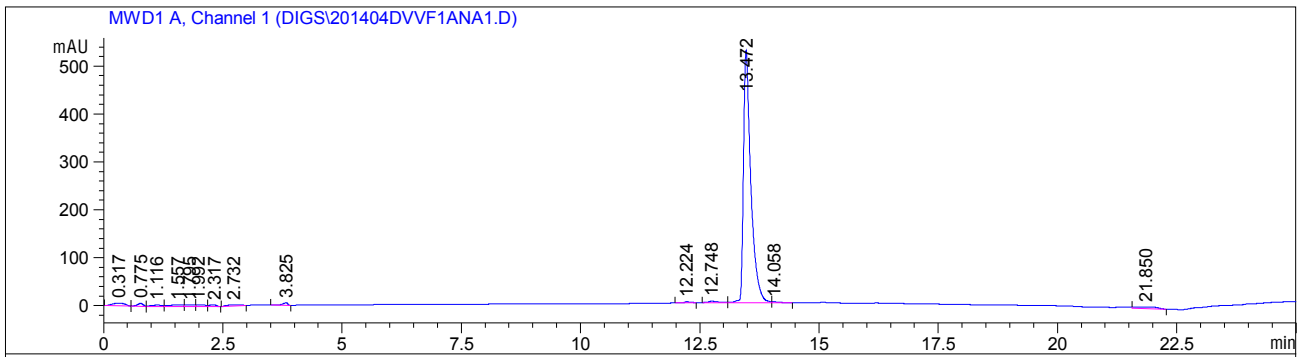

Figure S11. Analytical HPLC trace of DVV, monitoring at 400 nm.

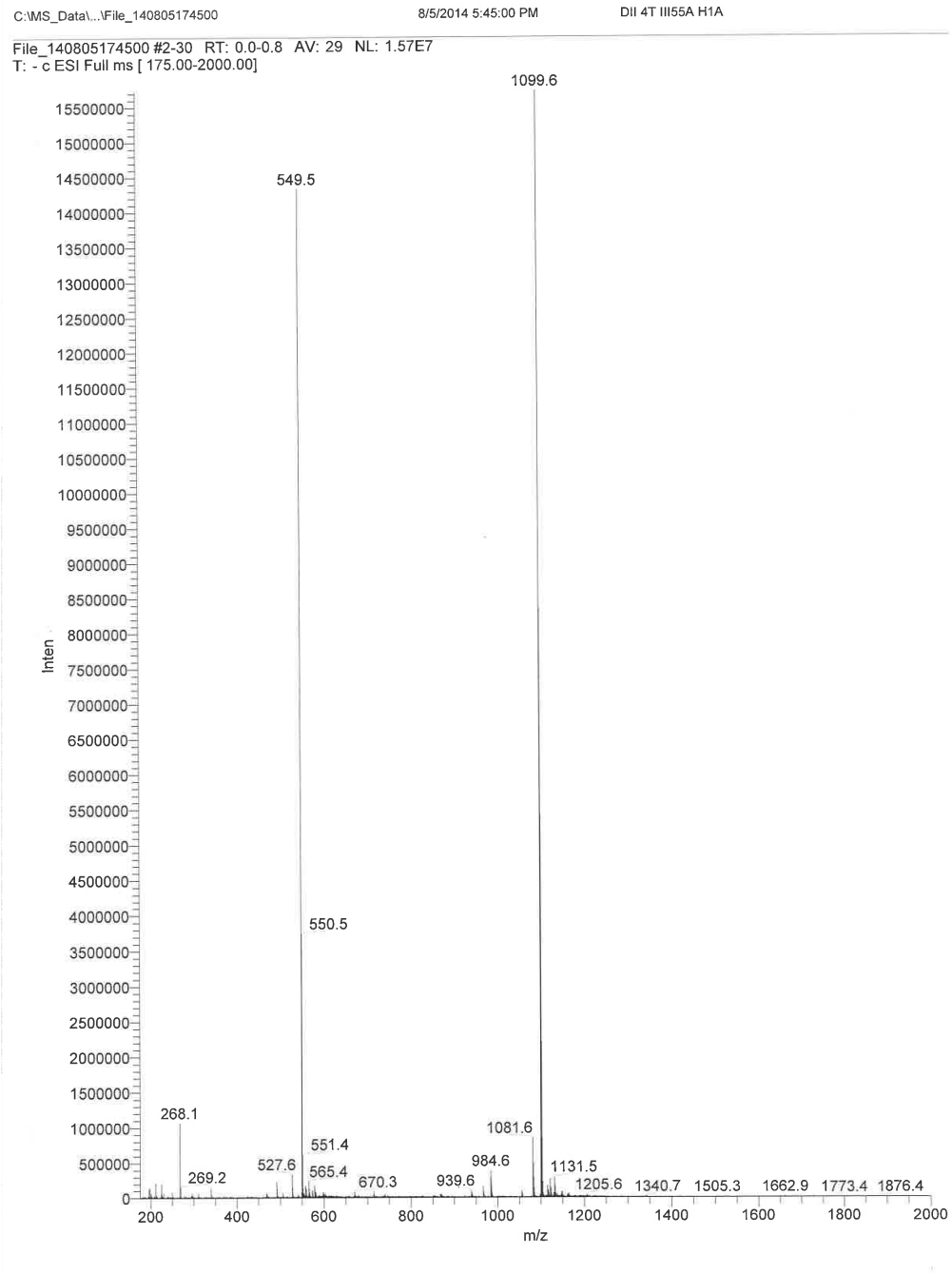

Figure S12. ESI of DII peptide.

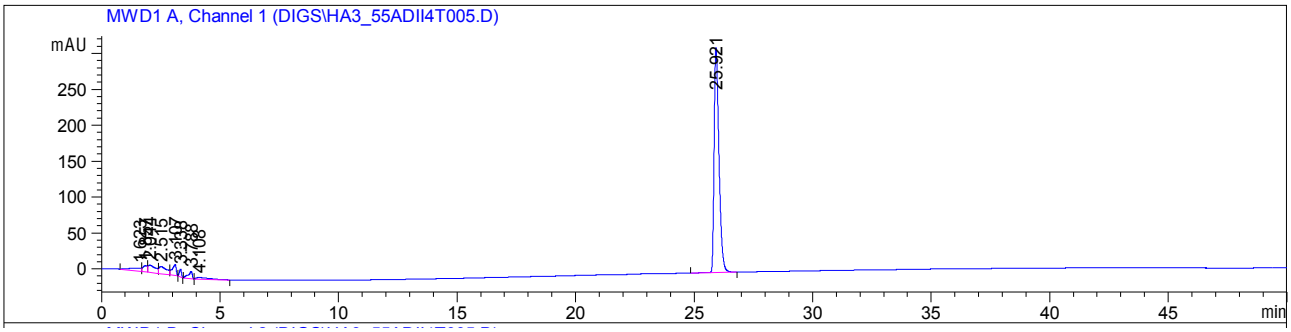

Figure S13. Analytical HPLC trace of DII, monitoring at 400 nm.

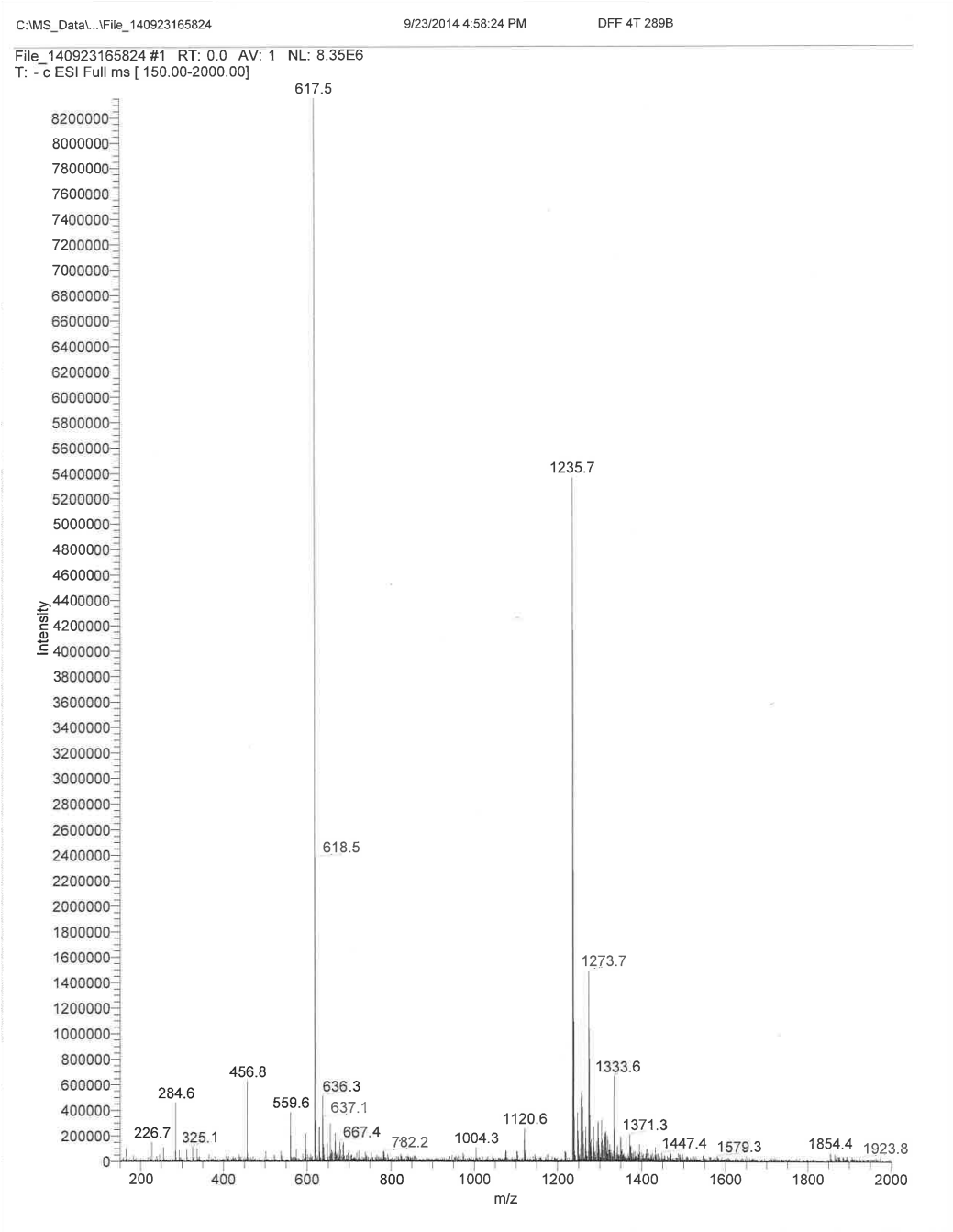

Figure S14. ESI of DFF peptide.

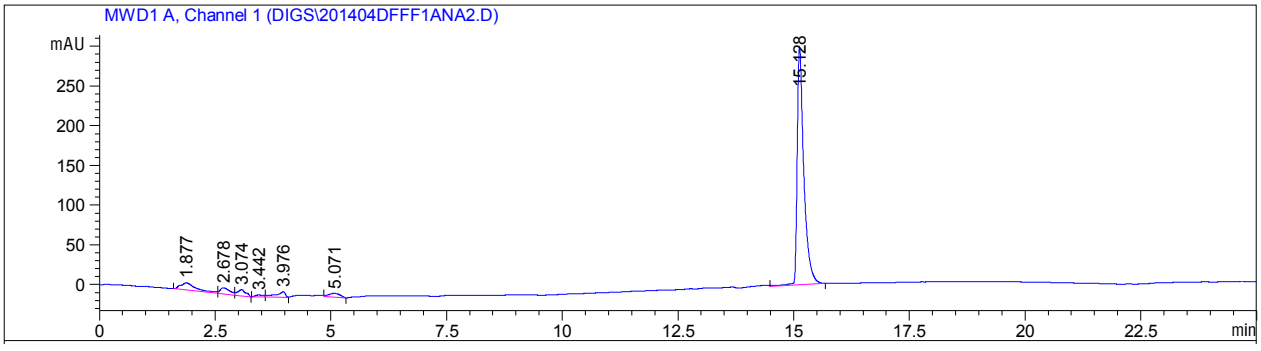

Figure S15. Analytical HPLC trace of DFF, monitoring at 400 nm.

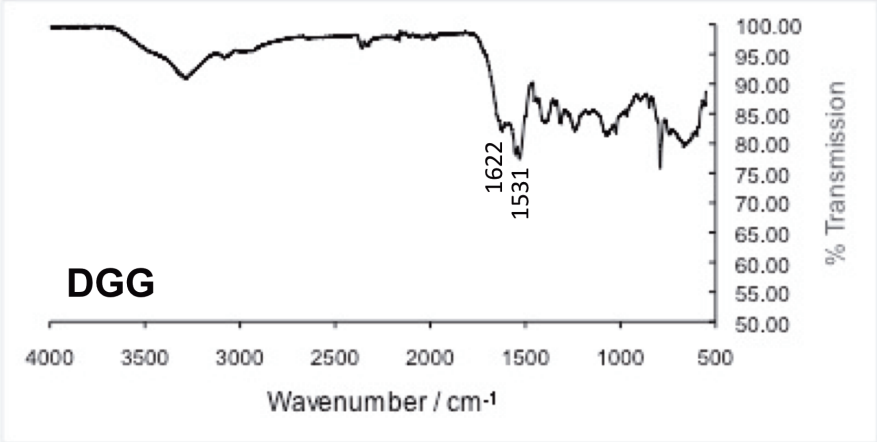

Figure S16. Attenuated total reflectance IR spectra of DGG peptide in solid state.

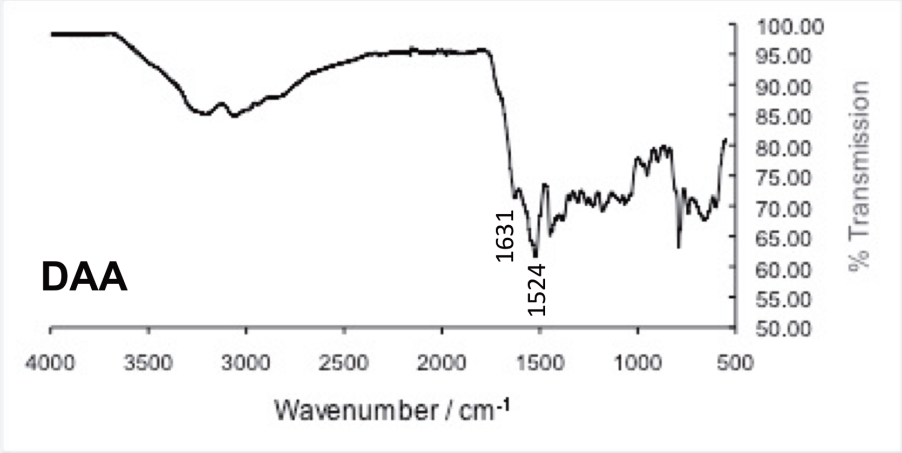

Figure S17. Attenuated total reflectance IR spectra of DAA peptide in solid state.

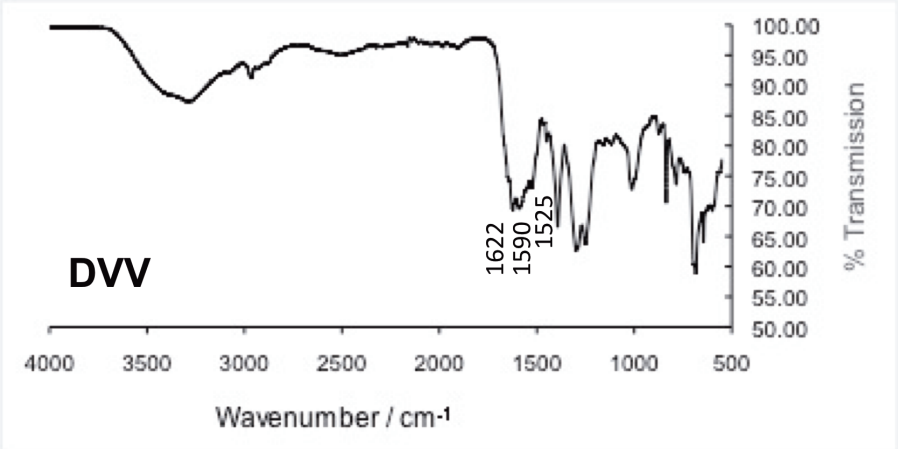

Figure S18. Attenuated total reflectance IR spectra of DVV peptide in solid state.

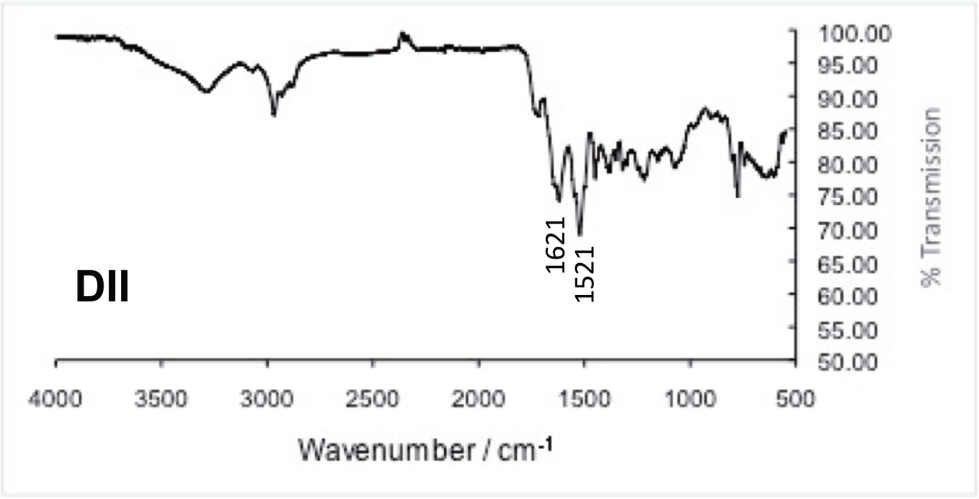

Figure S19. Attenuated total reflectance IR spectra of DII peptide in solid state.

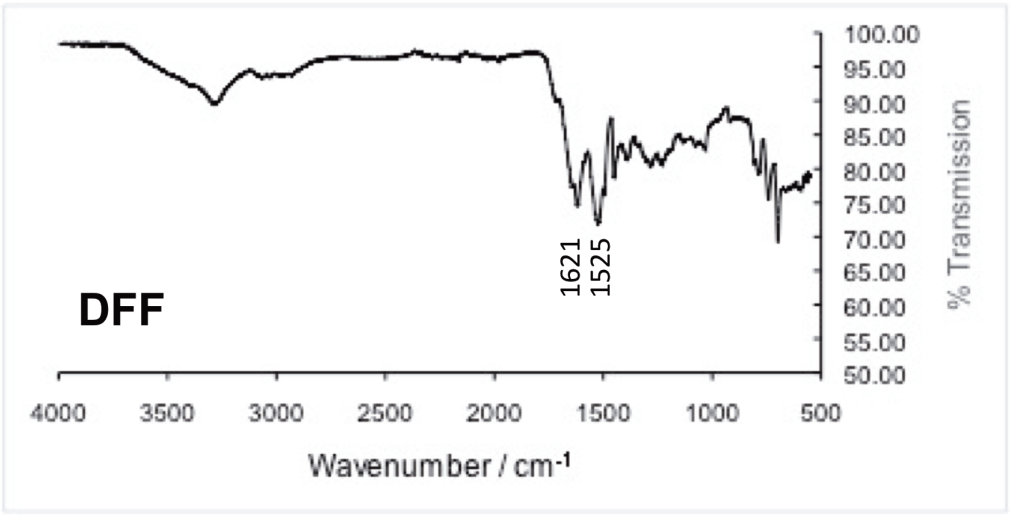

Figure S20. Attenuated total reflectance IR spectra of DFF peptide in solid state.

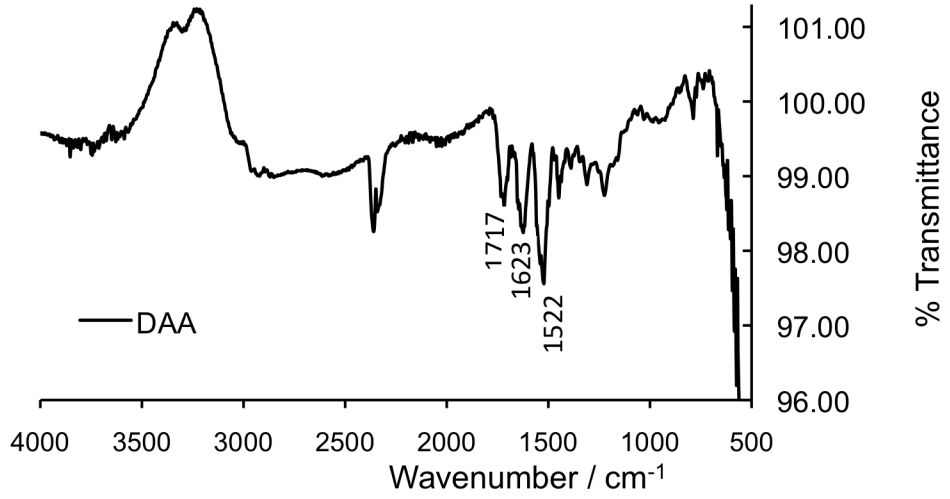

Figure S21. Attenuated total reflectance IR spectra of DAA peptide in gel state.

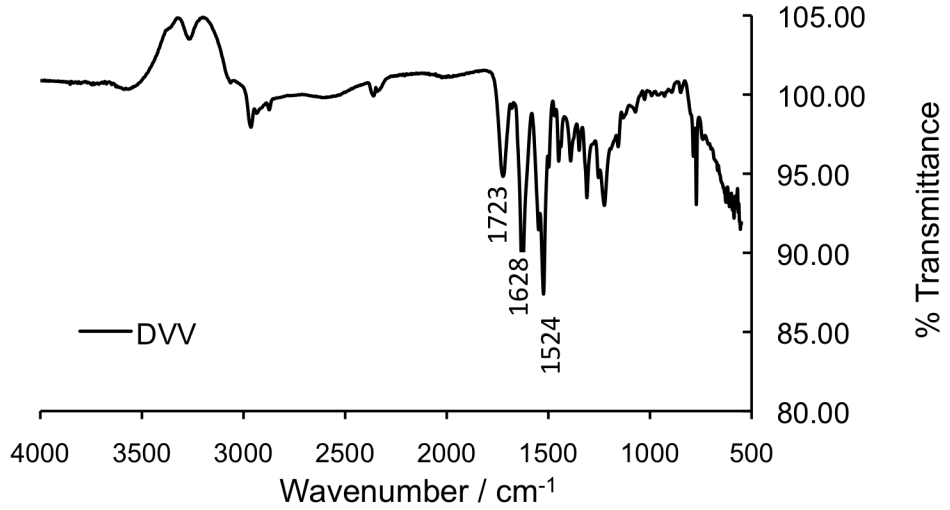

Figure S22. Attenuated total reflectance IR spectra of DVV peptide in gel state.

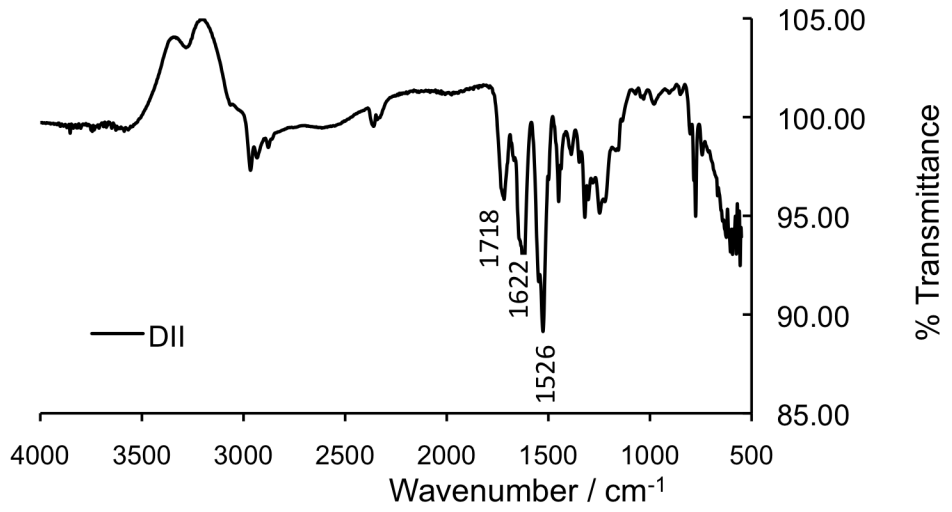

Figure S23. Attenuated total reflectance IR spectra of DII peptide in gel state.

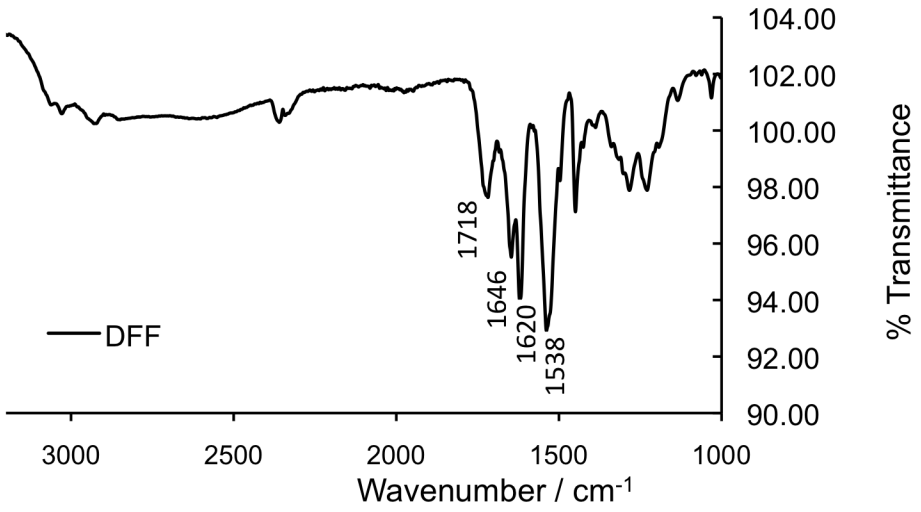

Figure S24. Attenuated total reflectance IR spectra of DFF peptide in gel state.

SUPPLEMENTARY DATA

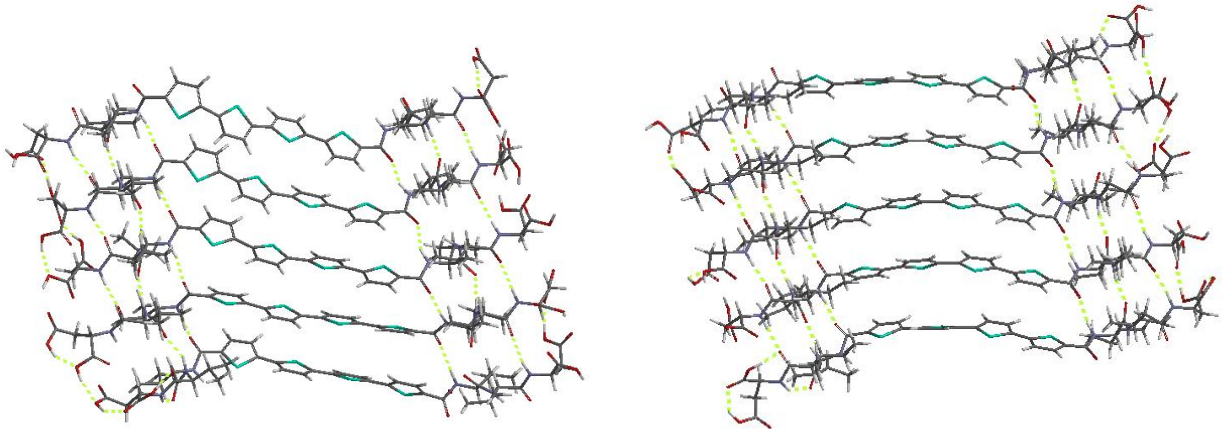

Figure S25. Energy-minimized assembly model for a hypothetical portion of a DVV (*left*) and DII (*right*) peptides. All energy-minimized models shown in this manuscript were done in a Spartan v.1.0.3 module using a Merck Molecular force field.

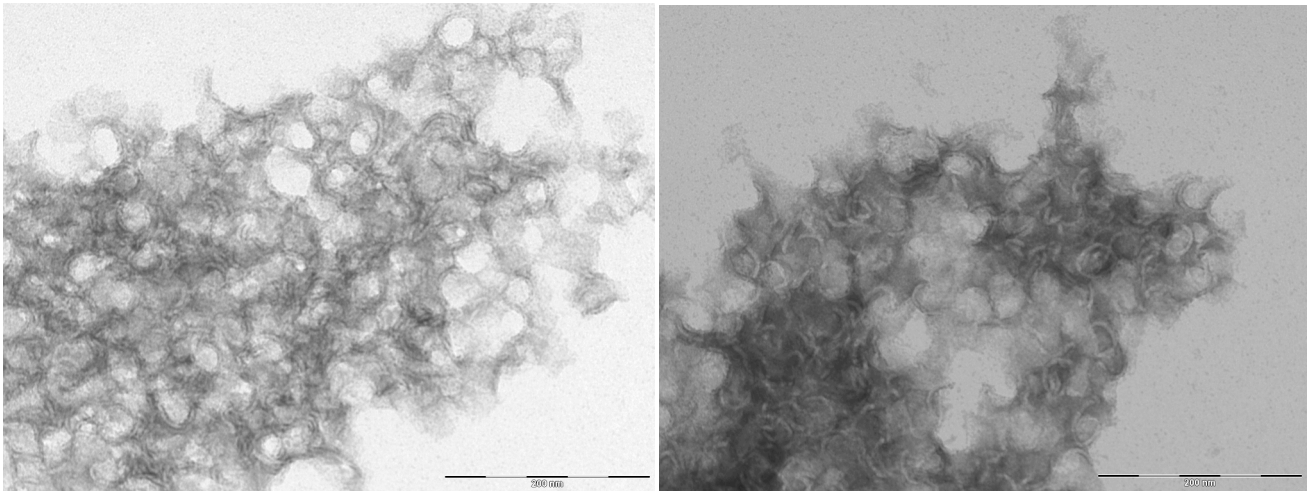

Figure S26. Additional TEM images of 0.1 wt% acidic solution of DGG peptide, to further show the shorter persistence lengths of nanostructures; scale bar= 200 nm.

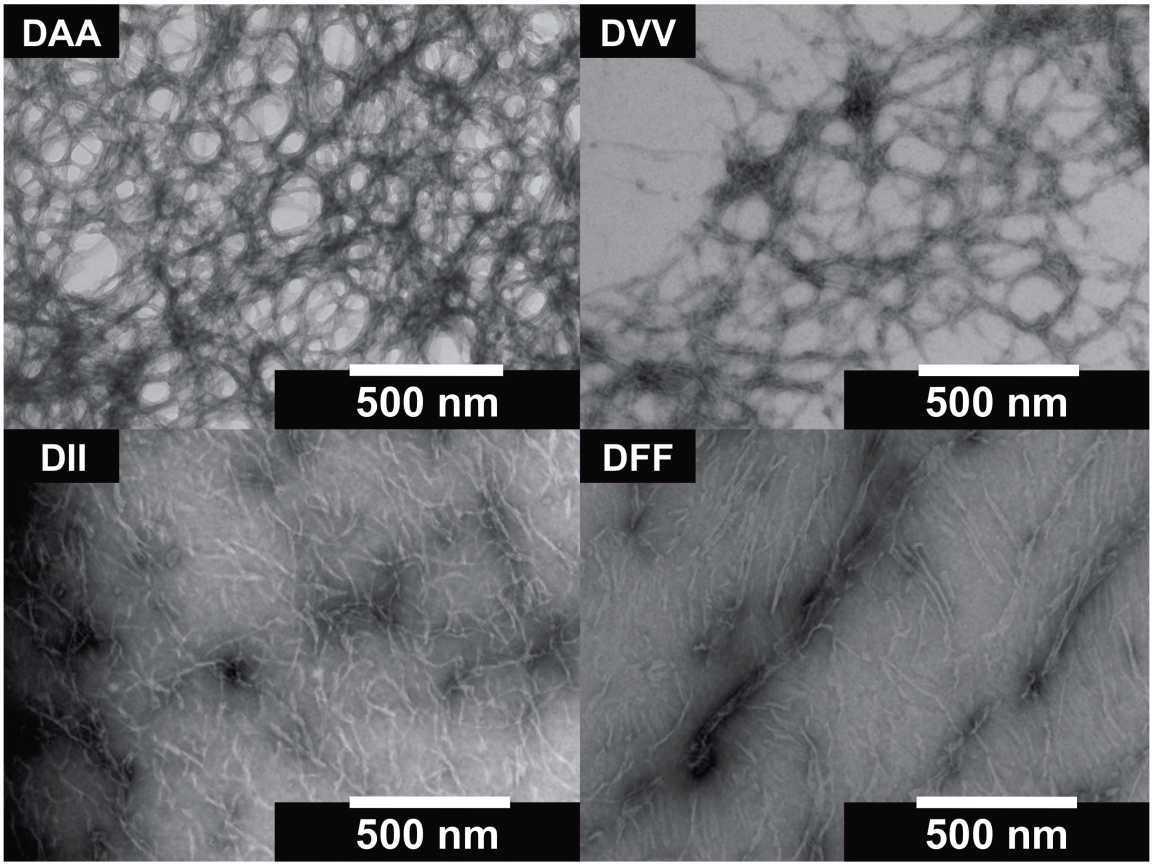

Figure S27. TEM images of the acid-assembled 1wt% D*XX* peptide hydrogels.

Table S1. Quantum yields (Φ_f) and absorption maximum (λ_{max}) of D*XX* peptides relative* to DVV peptide in acidic solutions.

	Relative* Φ_f	**λ_{max} / nm**
DGG	0.008	362
DAA	0.002	366
DVV, *pH 2*	1.00	403
DII	0.39	408
DFF	0.16	412
DVV, *pH 10*	8.40	416

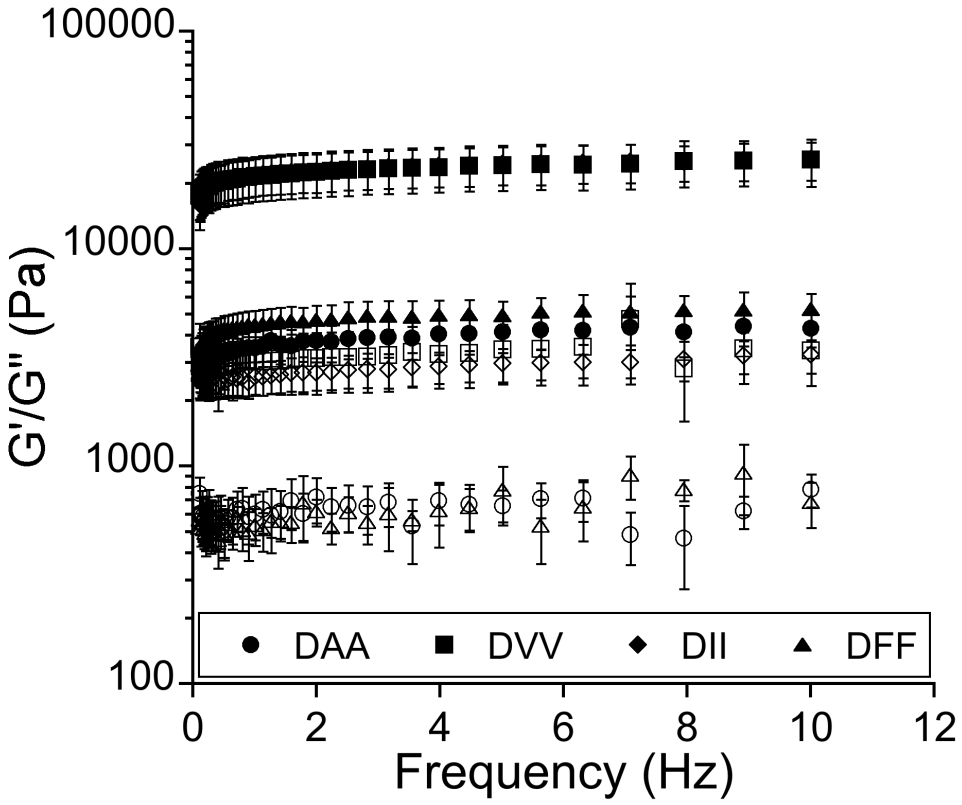

Figure S28. Frequency sweeps (0.1-10 Hz; 0.3% strain; 25°C) for 1 wt% D*XX* peptide hydrogels.

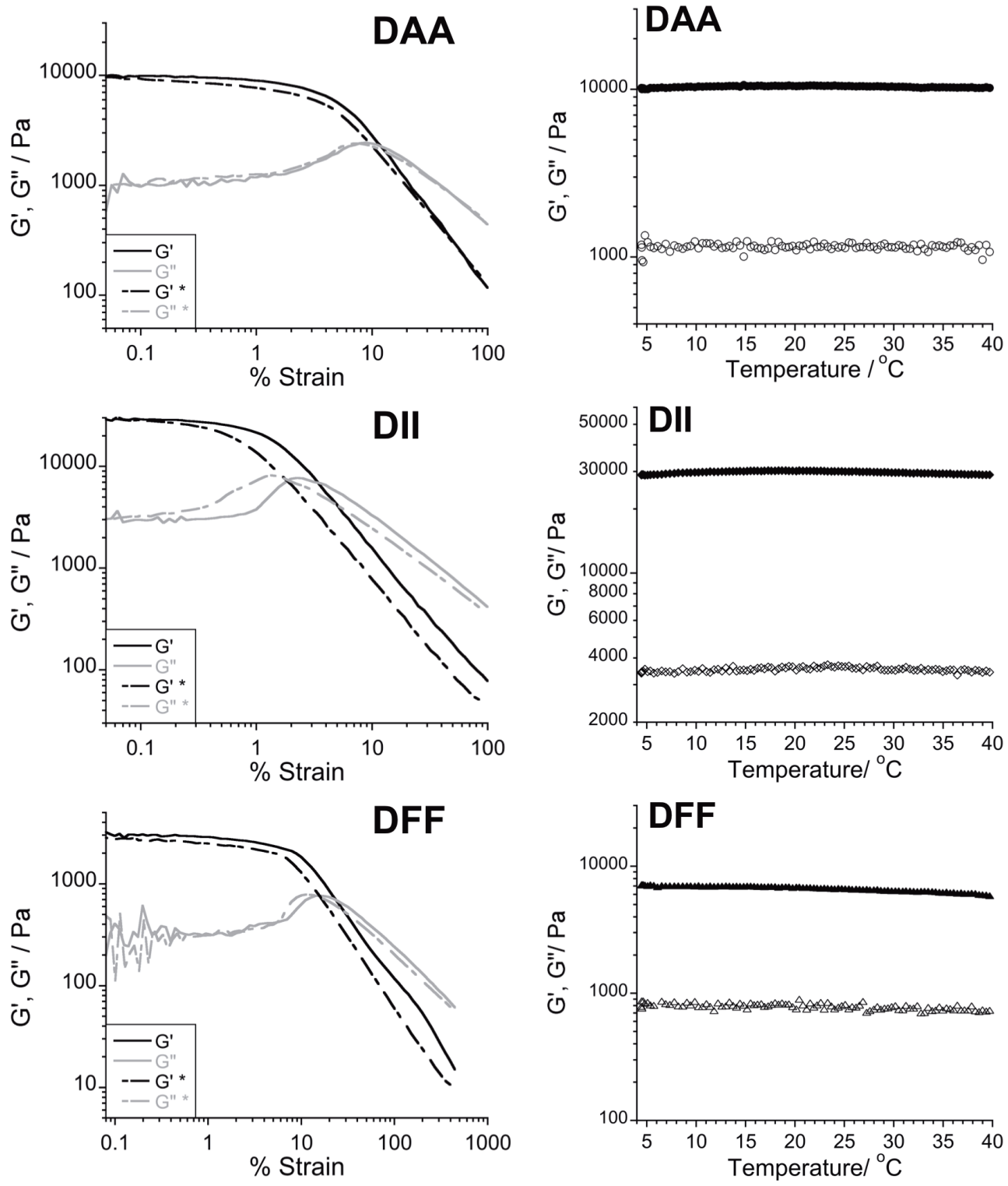

Figure S29. Strain sweeps (*left*, including cyclic sweeps) and temperature sweeps (*right*) for DAA-, DII-, and DFF-4T 1 wt% peptide hydrogels.

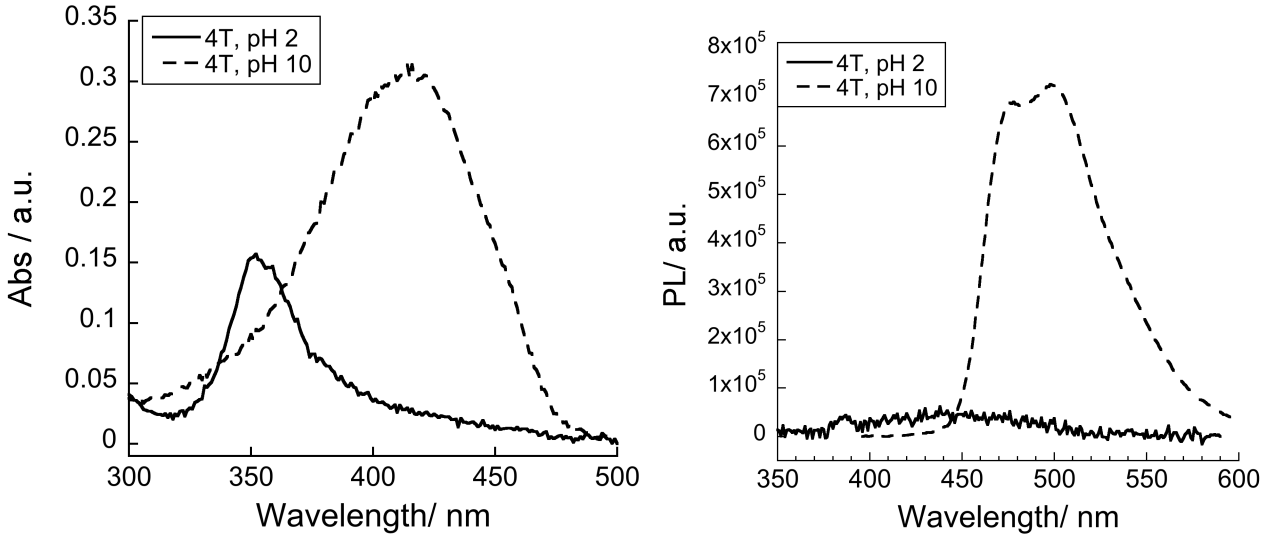

Figure S30. Absorption (*left*) and photoluminescence (*right*) spectra for HOOC-(quaterthiophene)-COOH (**4T**) under basic (---) and acidic (—) conditions, for reference; λ_{exc}= 380 and 340 for PL, respectively.

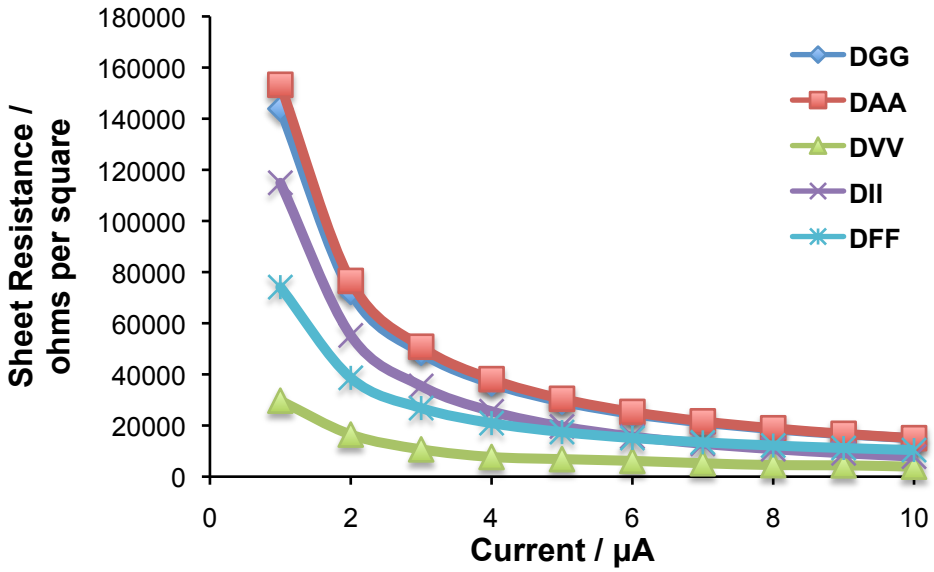

Figure S31. Representative sheet resistance vs. current plots for the dried, acid-assembled D*XX* peptide films.

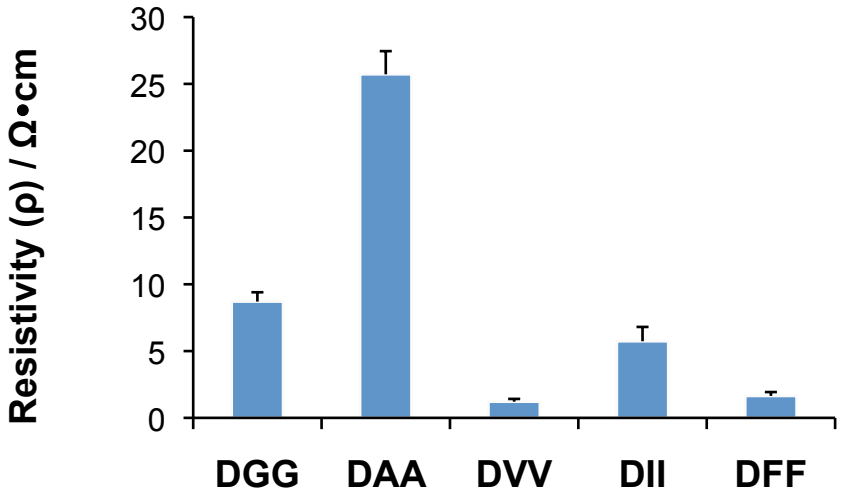

Figure S32. Resistivity data (R_S × measured mean thickness) for dried, acid-assembled D*XX* peptide films.

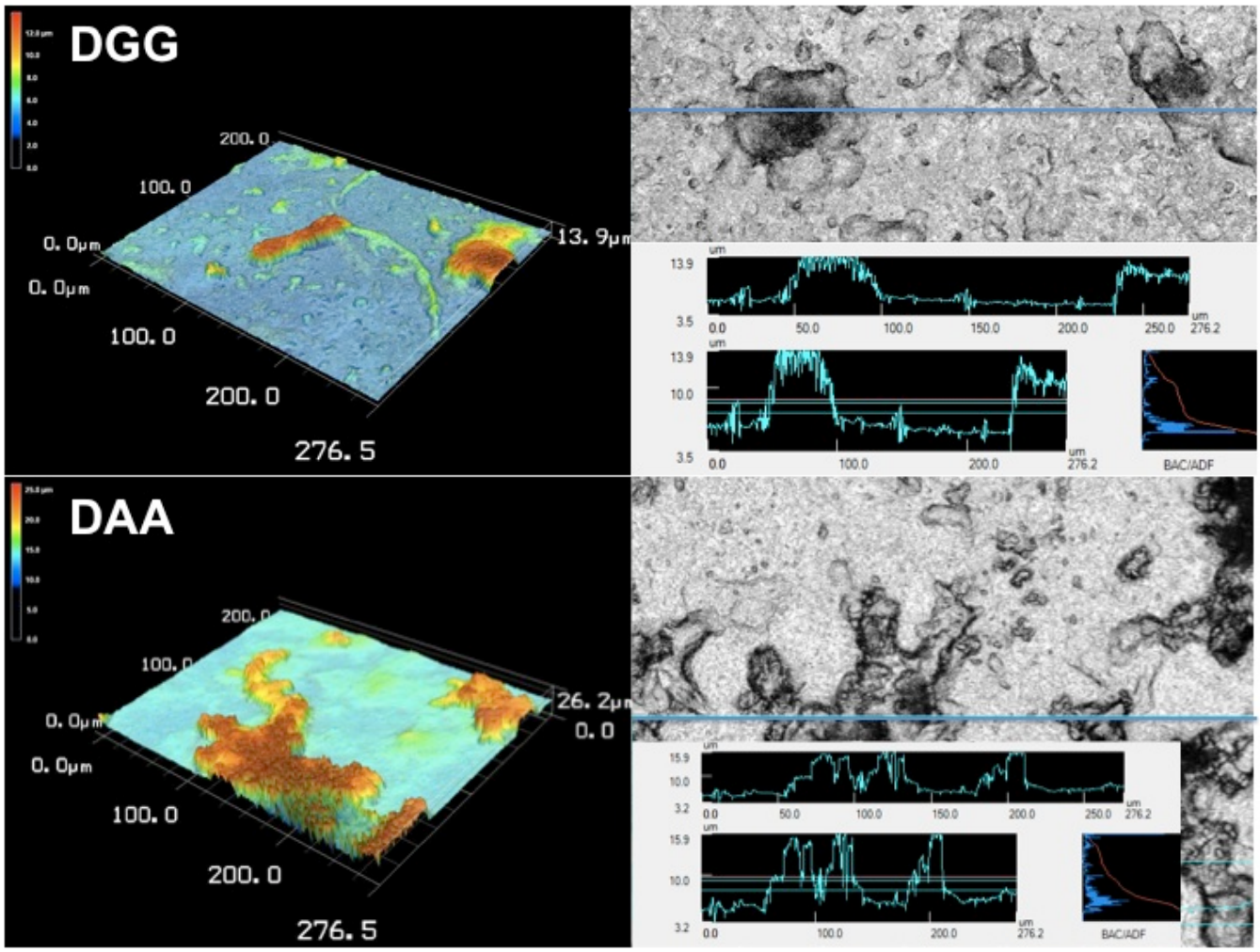

Figure S33. Surface profile of DGG and DAA peptides generated from laser microscopy observations (*left*: generated 3D surface profile; *right*: plotted height profile of a selected section, overlaid with the brightfield image).

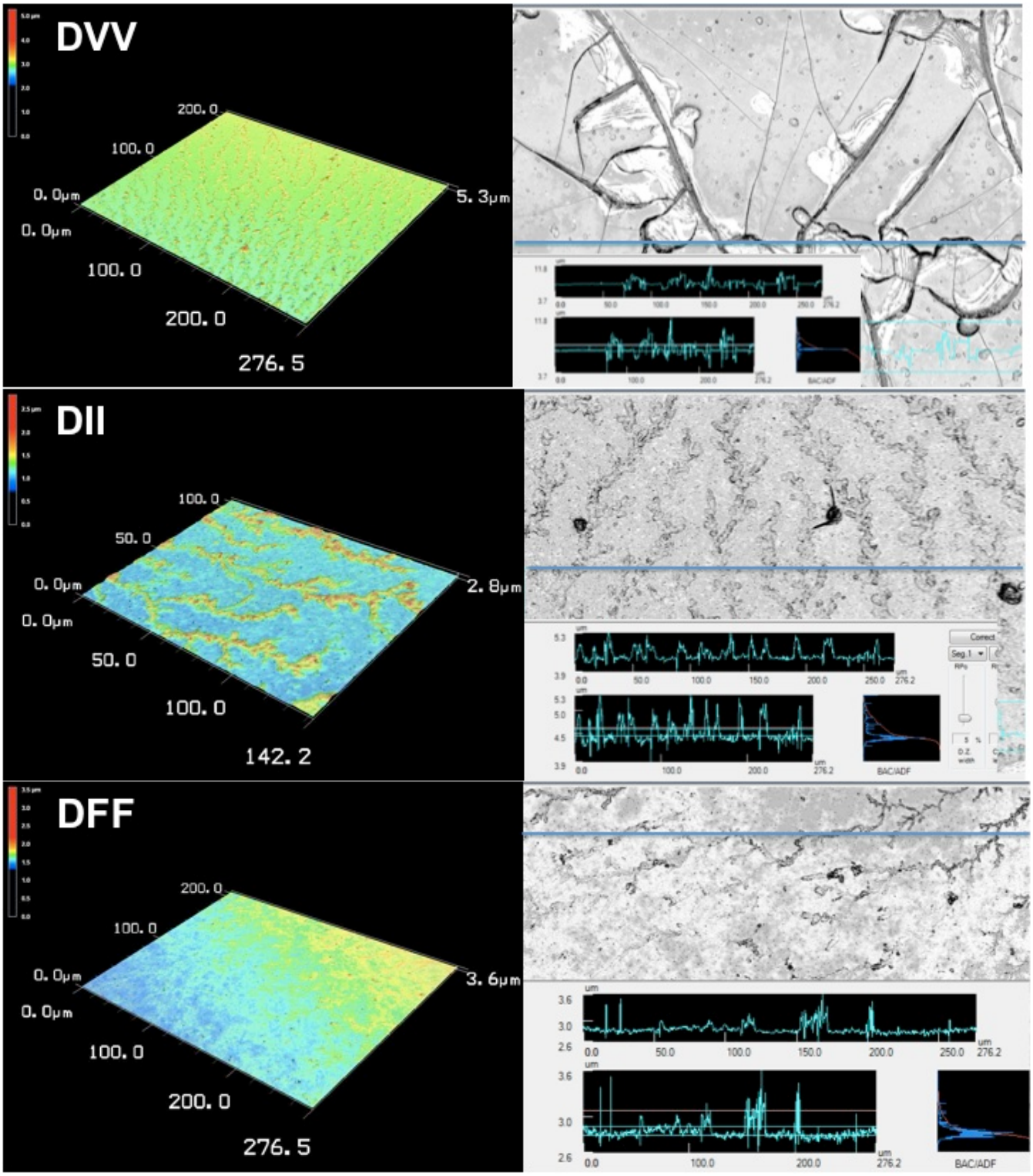

Figure S34. Surface profile of DVV, DII and DFF peptides generated from laser microscopy observations (*left*: generated 3D surface profile; *right*: plotted height profile of a selected section, overlaid with the brightfield image).

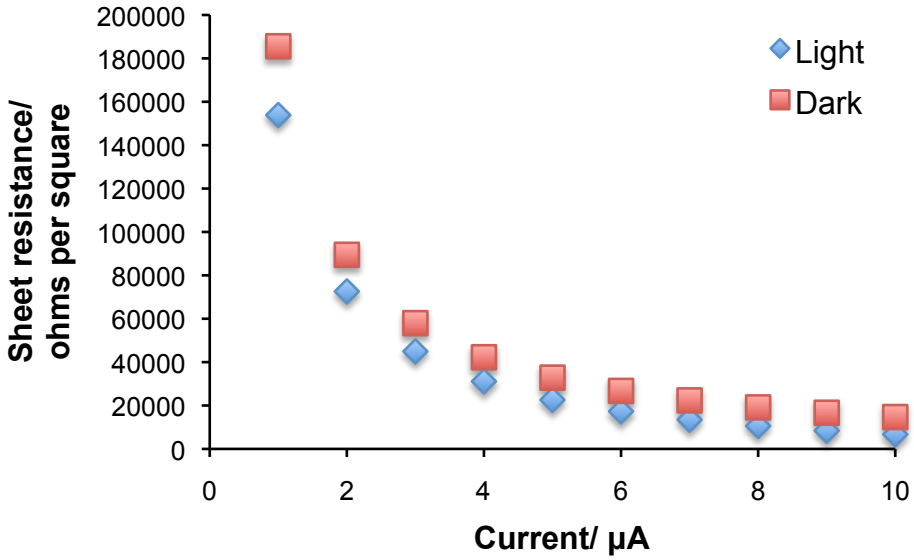

Figure S35. Representative sheet resistance vs. current plots for the dried, acid-assembled DGG peptide films under light and dark conditions.

REFERENCES

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