Electronic Supplementary Material (ESI) for Biomaterials Science.

Electronic Supporting Information

Self-Assembly of Amphiphilic Tripeptides with Sequence-Dependent Nanostructure

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Contents

1. Materials

All materials are purchased commercially and used as received without any further purification.

All solvents used are purchased commercially and used without further purification.

Materials Used

Fmoc-Phe-OH	02443	ChemImpexInt'L INC
Fmoc-Ile-OH	02425	ChemImpexInt'L INC
Fmoc-Asp (OtBu)-OH	00494	ChemImpexInt'L INC
Fmoc-Ala-OH	02369	ChemImpexInt'L INC
Fmoc-Gly-OH	02416	ChemImpexInt'L INC
Fmoc-Val-OH	02470	ChemImpexInt'L INC
Fmoc-Leu-OH	00145	ChemImpexInt'L INC
Acetic Anhydride	125580	Beantown Chemicals
Diisopropyl Ethyl Amine	D125806	Sigma-Aldrich
DiisopropylCarbodiimide (DIC)	00110	ChemImpexInt'L INC
Piperidine	104094	Sigma-Aldrich
Rink Amide AM Resin (100-200 Mesh)	06761	ChemImpexInt'L INC
Trifluoro Acetic Acid	T6508	Sigma-Aldrich
Tri isopropyl Silane	233781	Sigma-Aldrich

2. Methods

2.1.Peptide Synthesizer

Microwave-assisted automated solid phase peptide synthesis (Liberty Blue CEM corporation) was used to synthesize the peptides. Rink Amide Resin (0.91 meq/g, 100-200 mesh) was used and Fmoc deprotection was completed using 20% piperidine in DMF. Each Fmoc amino acid coupling step was achieved using microwave heating in the presence of diisopropylcarbodiimide (DIC) and Oxyma in DMF. Peptide bound Resin was transferred to a shaker vessel followed by washing with dichloromethane and allowed to air dry. The peptides were acetylated using Acetic anhydride, DMF and diisopropyl ethylamine(1:10:0.25 v/v/v ratio respectively, 2X). Following acetylation, the resins were washed with Dichloromethane (DCM) and the acetylated peptides were cleaved from the resin and protecting groups were removed using trifluoroacetic acid/triisopropylsilane/DI H_2O(95: 2.5: 2.5, v/v/v) for 2h at room temperature. The cleaved peptide-TFA solution was isolated and evaporated in a rotary evaporator under vacuum. The product was further washed with toluene and precipitated in ice cold diethyl ether (added drop-wise). The solid powder was washed in cold diethyl ether and isolated by centrifugation (3x, 4000 RPM, 5 min). It was then dried under vacuum to collect a powder.

2.2.High performance liquid chromatography (HPLC)

Analytical HPLC (Gilson) was used to verify purity of the acetylated tripeptide amides used in these studies. Sample were prepared at concentrations of approximately 1-2 mg/mL in deionised (DI) water and 25 μL was injected onto a Gemini (Phenomenex) C18 column (250 mm x 4.6 mm) with 5 μm fused silica beads at a flow rate of 1 mL/ min. A linear gradient from 0-100%

(v/v) acetonitrile (+0.1% NH_3OH) in water (+0.1% NH_3OH) was run over 10 min. UV absorbances at four wavelengths (220, 260, 280 and 350 nm) were monitored and purity was calculated from integration of each peak in the resulting chromatogram.

2.3.Rheology

The mechanical properties of the peptide hydrogels were tested using ATS Rheosystems Viscoanalyzer device equipped with parallel plate geometry using a gap distance of 0.5 mm. All experiments were done in deionized water 24h after solubilization. First, a strain sweep was performed for each hydrogel to determine the linear viscoelastic range for the material. Subsequently, materials were strained at this determined value (strain of approximately 2% for all hydrogels) and a frequency sweep from 0.1 to 10 Hz was performed to measure the storage modulus (G′) and loss modulus (G″) for each hydrogel. All rheology measurements were collected at ambient temperatures (~22 °C).

2.4.Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) images were captured using a JEOL filtering transmission electron microscope operating at 200 kV fitted with CCD camera. Carbon-coated copper grids (200 mesh) were used as received for microscopy. The support film was contacted to hydrogel materials for 3 seconds and blotted using filter paper. For tripeptides that did not form hydrogels, 10 μL of the sample was drop-cast onto the grid. Each sample was dried and subsequently stained with uranyl acetate (10μL, 2 % aqueous), blotting with filter paper to remove excess. The dried grids were then imaged using the microscope.

2.5.Dynamic Light Scattering (DLS)

For DLS on FGD samples, a Malvern Zetasizer instrument was used. Before measurement, the samples were filtered in a 150 nm filter to remove any dust from the solution. 1 mL of the sample was added to the cuvette for the measurement. To ensure accuracy, three different measurements were collected.

2.6.Circular Dichroism (CD)

Near-ultraviolet circular dichroism spectroscopy was performed on an Aviv 202SF spectrometer, collecting ellipticity (mdeg) and photomultiplier HT (V) over the range of 250-190 nm. Samples of tripeptides in water were prepared at 20 mM in deionized water and diluted to 5 mM immediately prior to collecting specra. Samples of ~30 µl were placed between two quartz plates with a pathlength of 0.1 mm, and three acquisitions were collected and averaged for each sample. Data where HT exceeded 500 V (<200 nm) was excluded from presentation.

2.7.Fourier Transform Infrared Spectroscopy (FT-IR)

For the FTIR measurement, tripeptides were dissolved at 20 mM and 5 mM in DI H_2O. The measurements were performed on samples 24h following solubilization using a liquid sample holder. FTIR spectra were acquired using a JASCO 6300 FTIR with attenuated total reflectance attachment (ATR) and spectra resolution of 4 cm^{-1}.

2.8.Cell Culture

NIH-3T3 fibroblasts (ATCC) were cultured in high glucose DMEM containing penicillin/streptomycin and 10% fetal bovine serum. Upon reaching confluency, cells were isolated with trypsin/EDTA. Hydrogel-forming tripeptides were prepared by dissolving in deionized water, and 100 μL was combined with 20 μL of cells in media and suspended using gentle pipetting. The target cell density was 50,000 cells per gel. After suspending cells in the hydrogels, 200 μL of culture media was added gently on top of the gels in a 96-well plate. Cells were assessed for viability by staining with calcien-AM (green, Live) and ethidiumhomodimer (red, dead) according to manufacturer recommendations. Cells within the hydrogel were then imaged using a EVOS Automated Fluorescent Microscopy system (ThermoFisher).

3. Chemical Characterization of synthesized Tripeptides

Ac-FGD-NH_2:

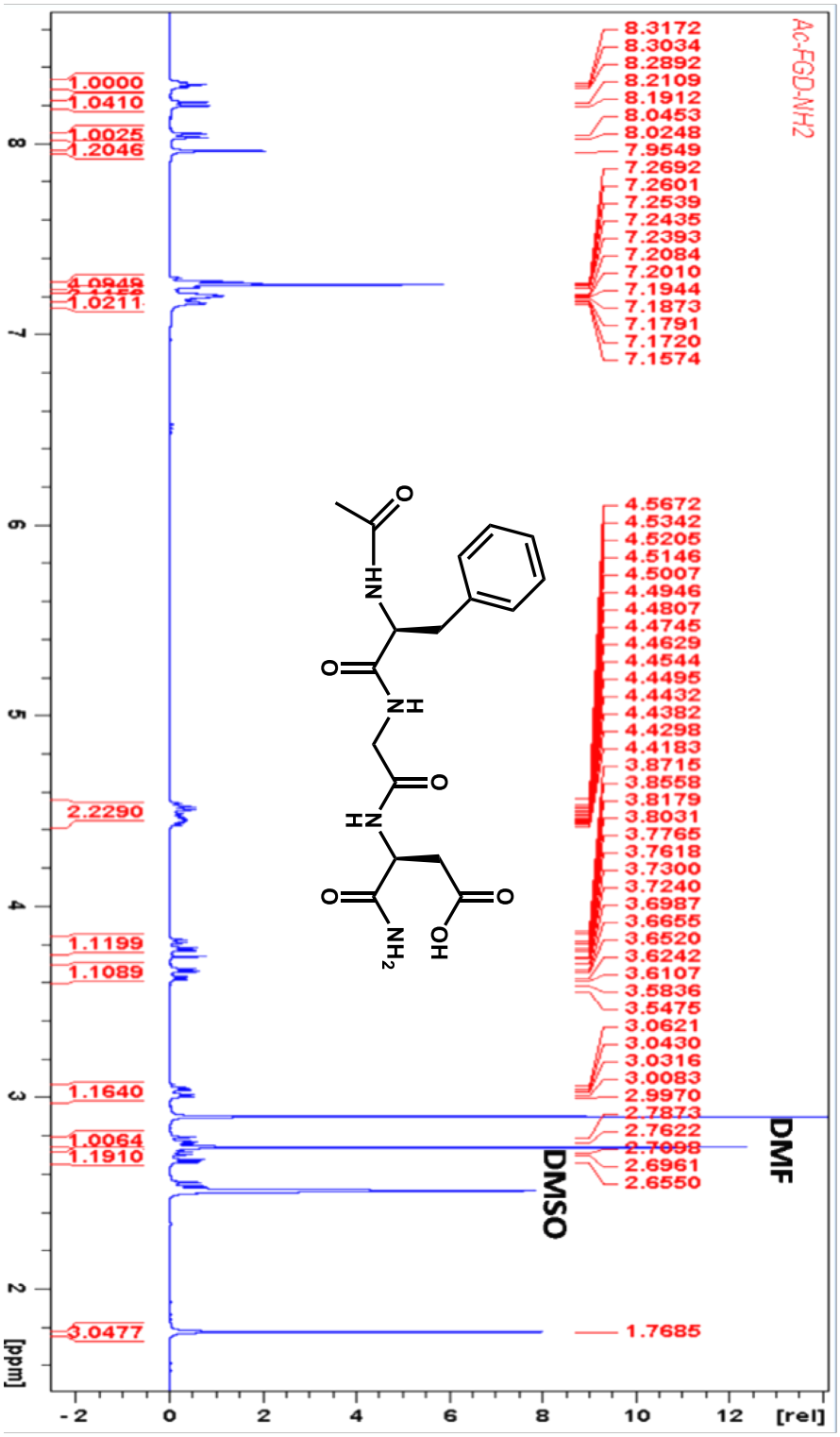

Figure S1: ^{1}H NMR spectroscopy of Ac-FGD-NH_2

ESI-MS: Calculated- 378.39 g/mol; Found- 378.8 g/mol

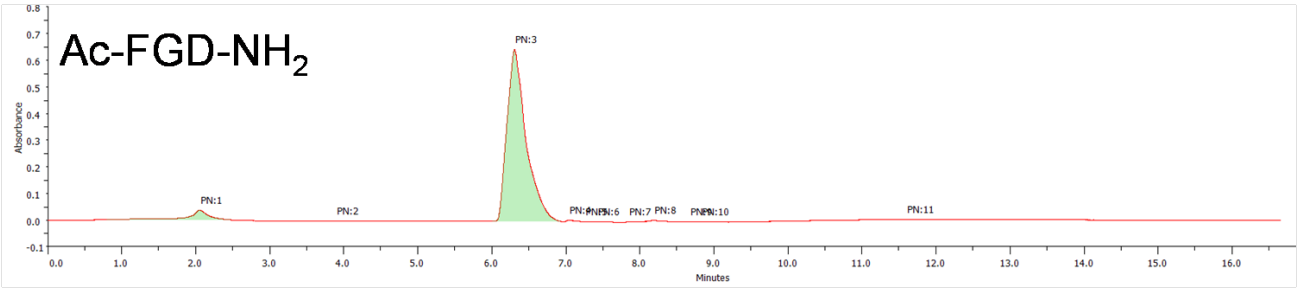

Figure S2: Analytical HPLC chromatogram of Ac-FGD-NH_2(Purity = 93%).

Ac-FAD-NH$_2$

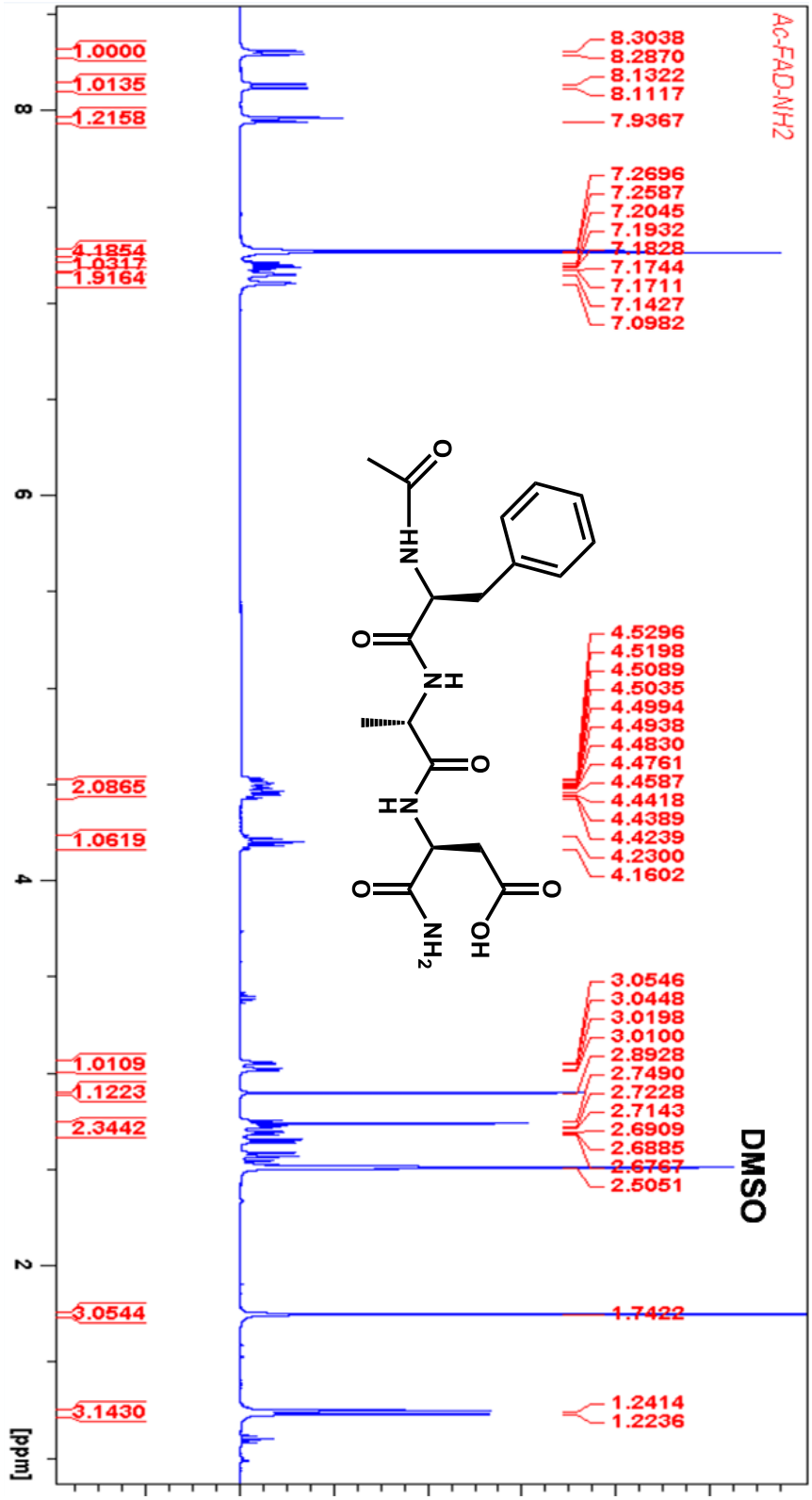

Figure S3: ^{1}H NMR spectroscopy of Ac-FAD-NH$_2$

ESI-MS: Calculated- 392.41 g/mol; Found- 392.8 g/mol

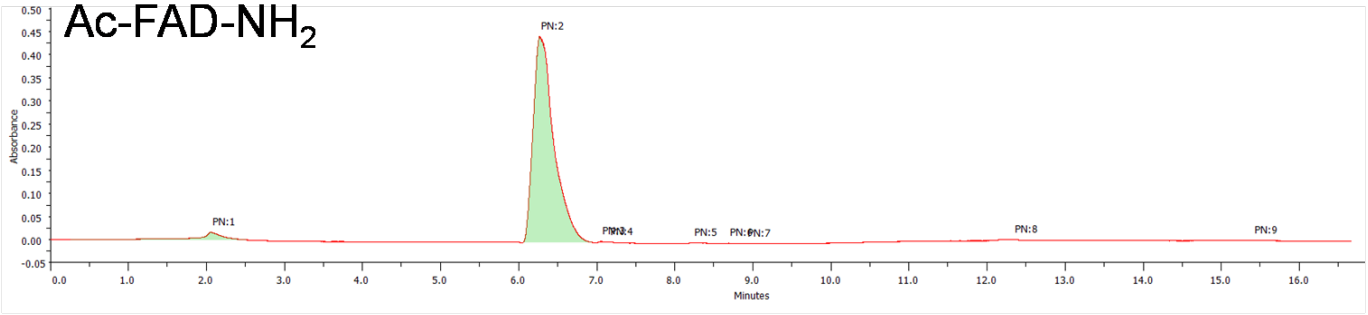

Figure S4: Analytical HPLC chromatogram of Ac-FAD-NH_2(Purity = 94.6%).

Ac-FVD-NH_2

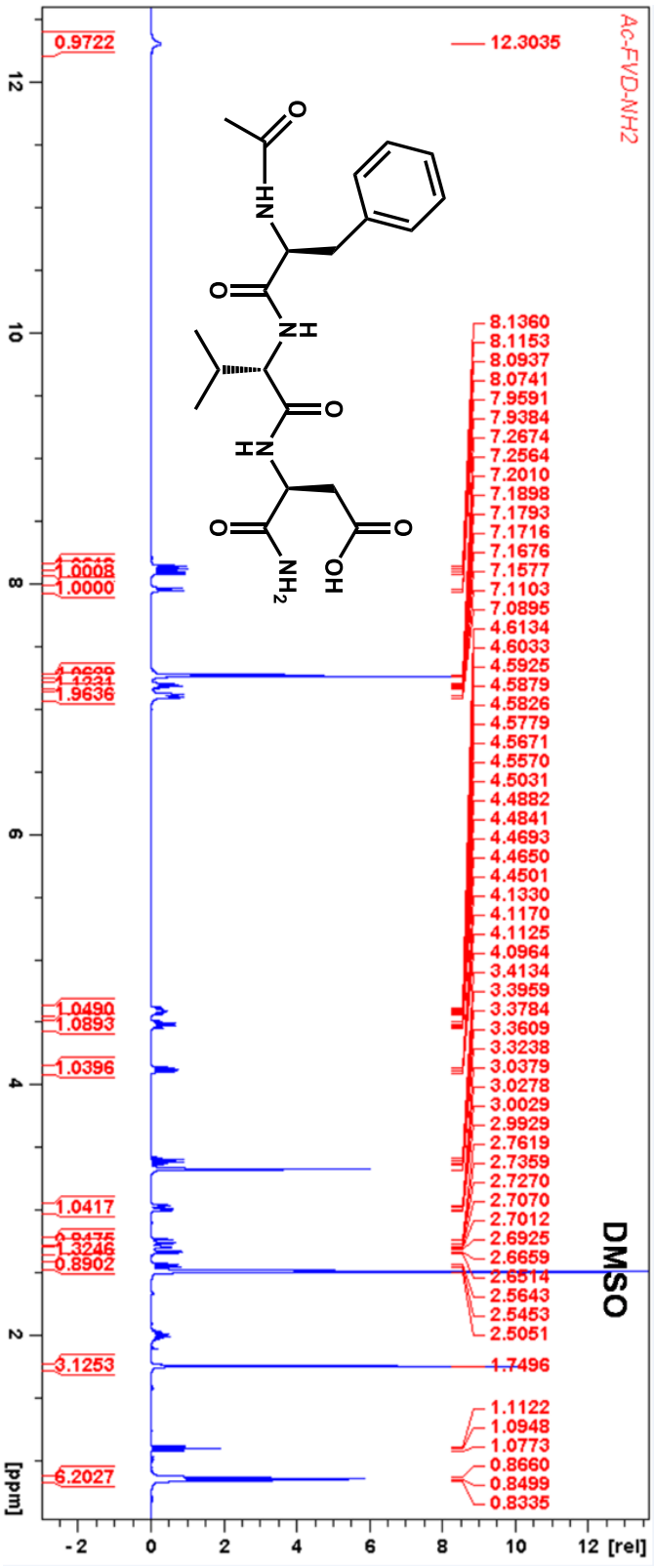

Figure S5: ^{1}H NMR spectroscopy of Ac-FVD-NH_2

ESI-MS: Calculated- 420.47 g/mol; Found- 420.9 g/mol

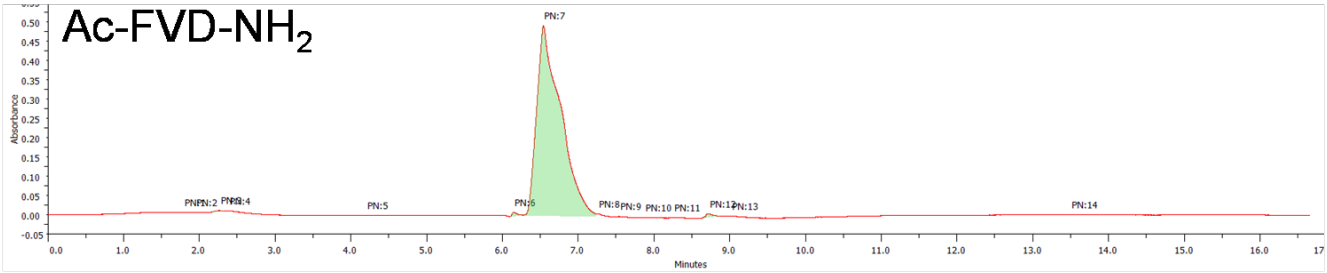

Figure S6: Analytical HPLC chromatogram of Ac-FVD-NH_2(Purity = 99%).

Ac-FLD-NH$_2$

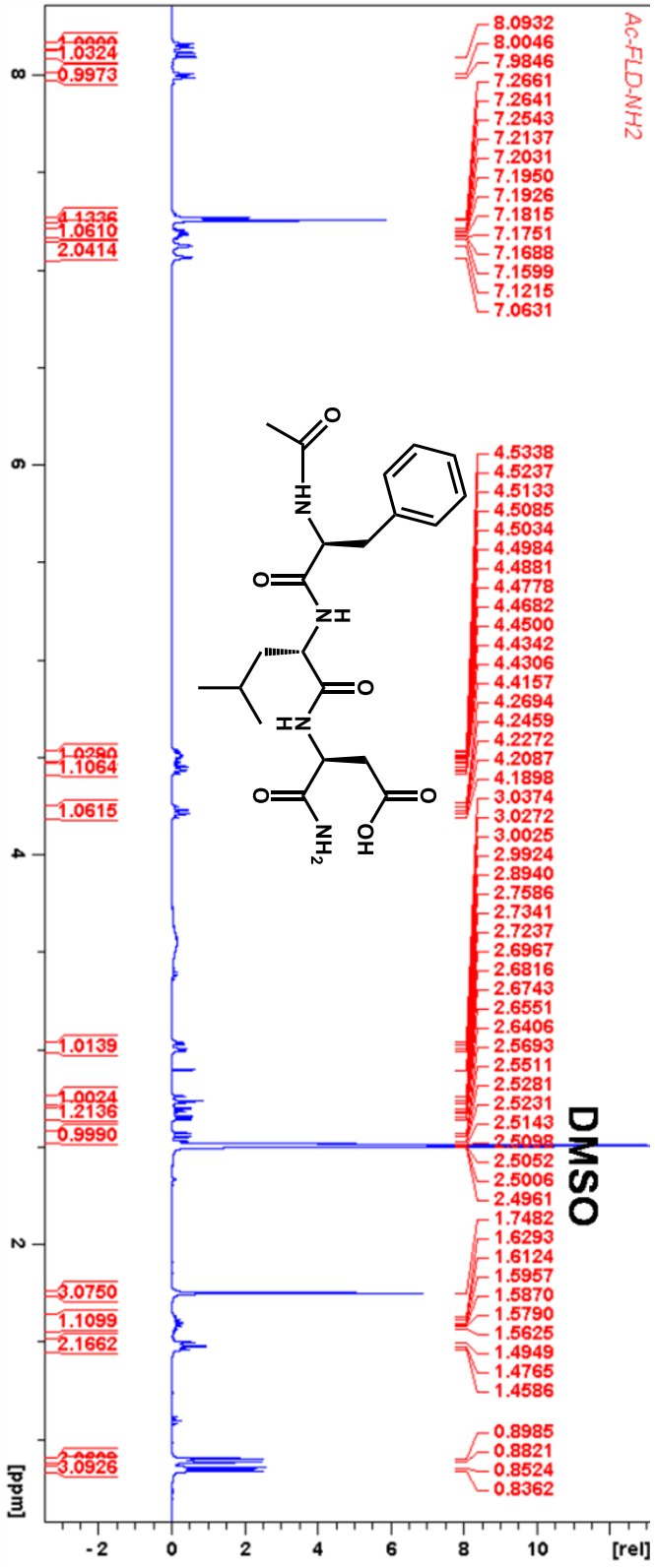

Figure S7: ^{1}H NMR spectroscopy of Ac-FLD-NH$_2$

ESI-MS: Calculated- 434.49 g/mol; Found- 434.9 g/mol

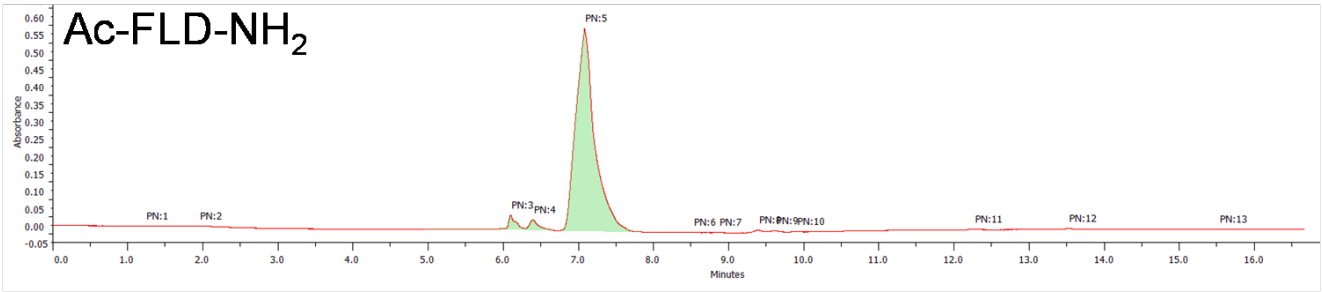

Figure S8: Analytical HPLC chromatogram of Ac-FLD-NH_2 (Purity = 94 %).

Ac-FID-NH$_2$

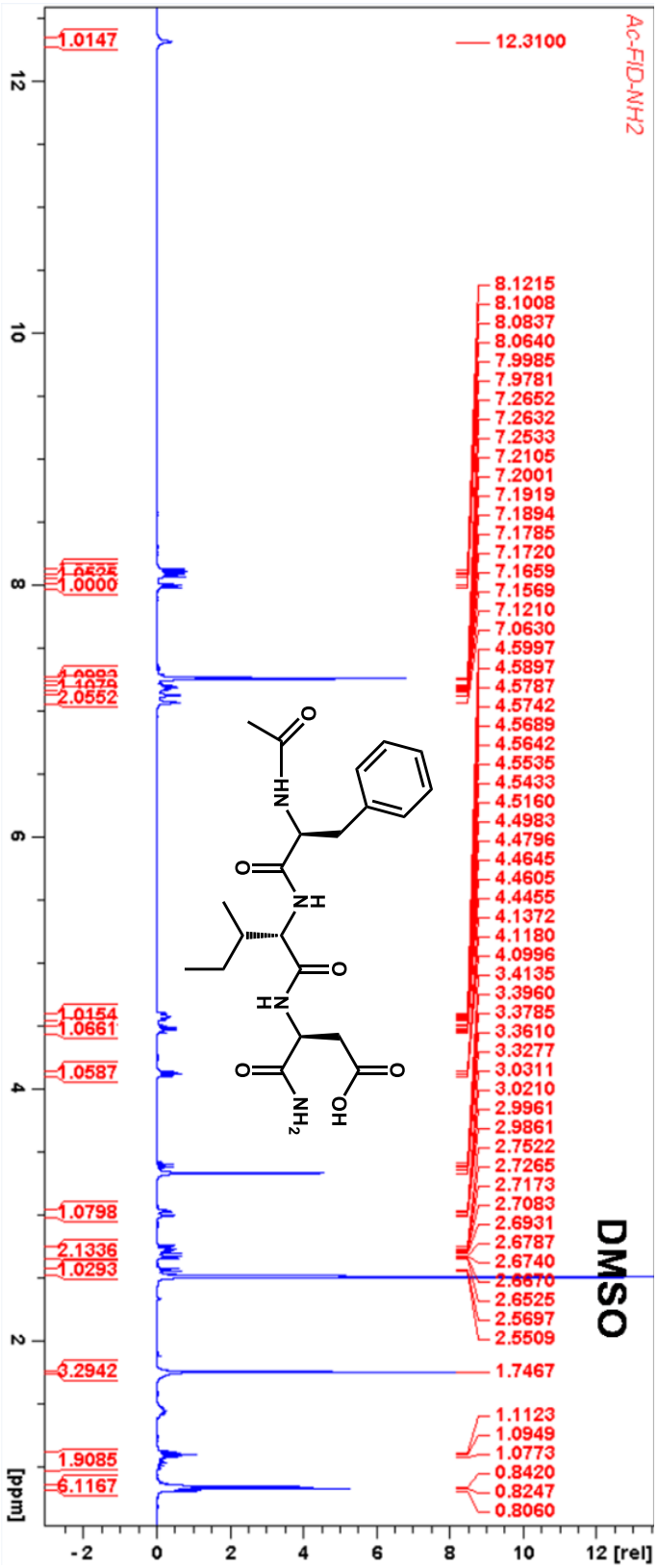

Figure S9: ^{1}H NMR spectroscopy of Ac-FID-NH$_2$

ESI-MS: Calculated- 434.49 g/mol; Found- 434.9 g/mol

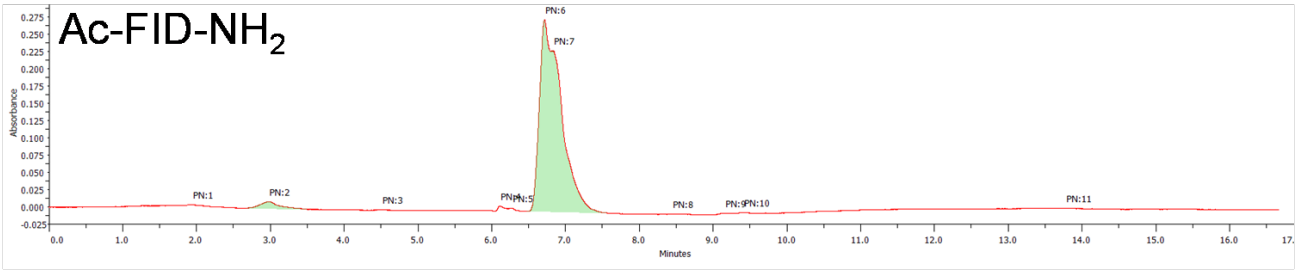

Figure S10: Analytical HPLC chromatogram of Ac-FID-NH_2 (96.9 %).

4. Additional Supporting Data

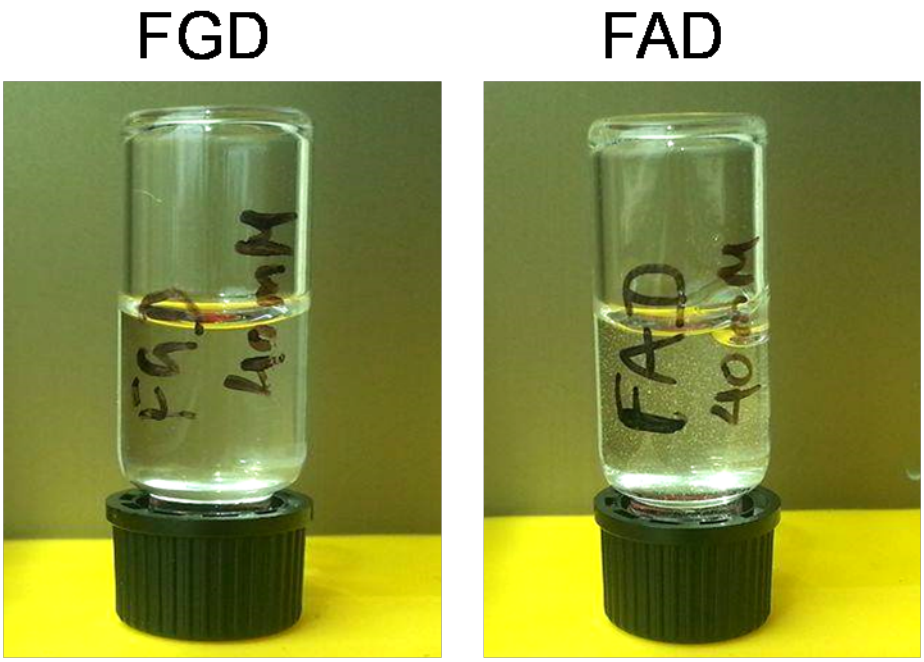

Figure S11: FGD and FAD tripeptides do not gel even at 40 mM concentration

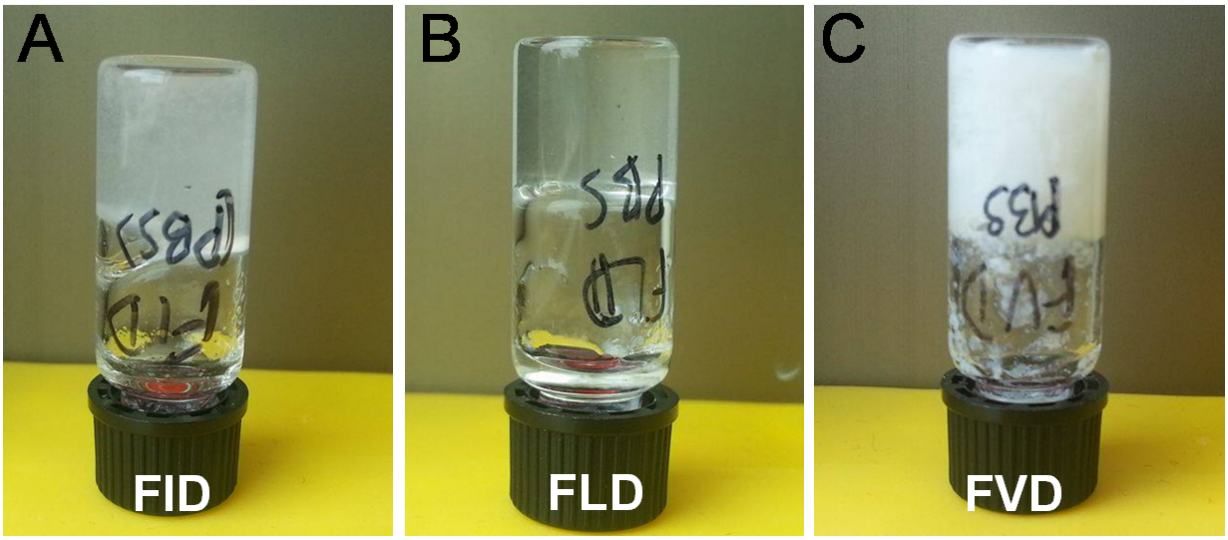

Figure S12: Formation of hydrogels in Phosphate Buffered Saline (PBS) at pH 7.4

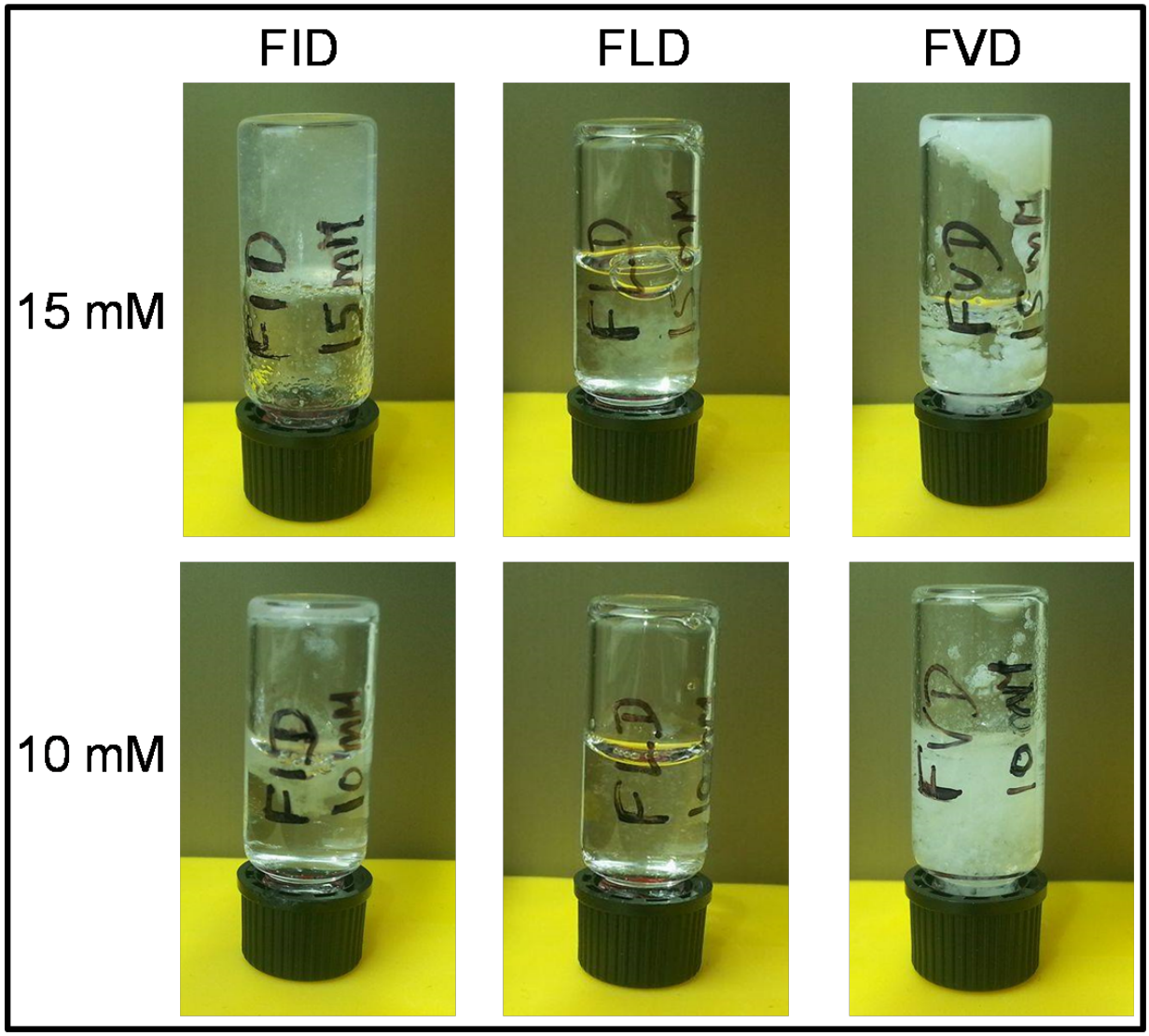

Figure S13: Gelation experiments at different tripeptide concentrations: Samples of gelling tripeptides were prepared at 15 mM (top) and 10 mM (bottom) and inverted to assess formation of self-supporting hydrogels.

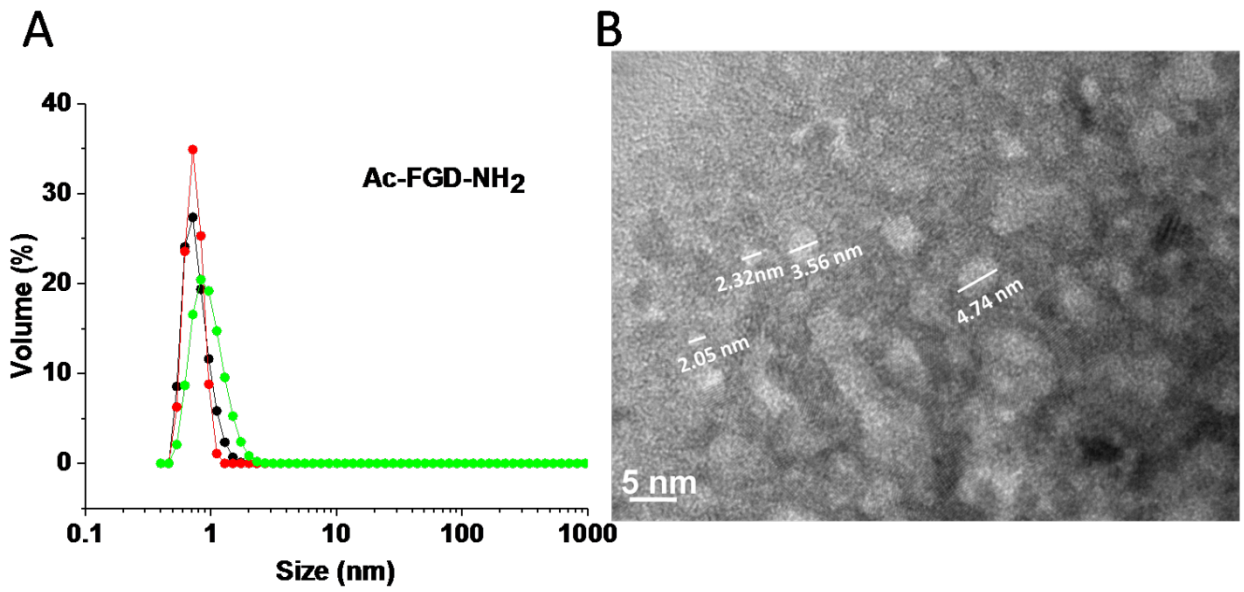

Figure S14: (A) Dynamic Light Scattering (DLS) of FGD which shows the size distribution of spherical aggreagtes by volume percent, with three different measurements shown. (B) Transmission electron microscopy image of FGD at high magnification showing spherical aggregates with sample measurements overlayed.

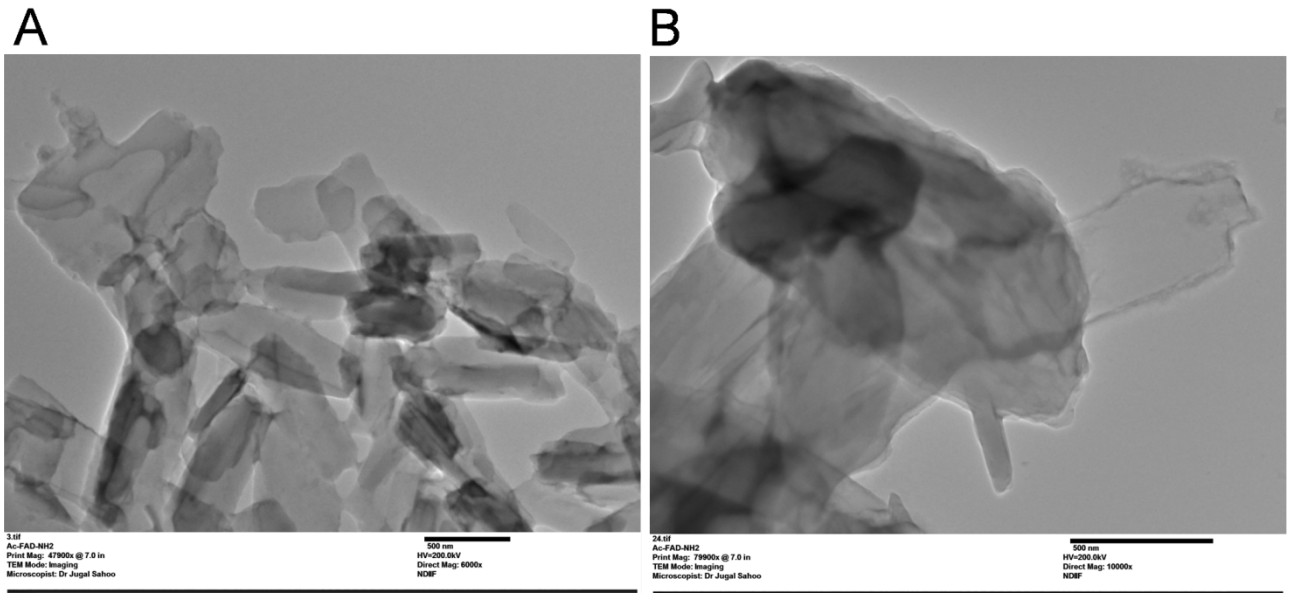

Figure S15: Additional TEM images of Ac-FAD-NH_2 depicting observed 2D-plaque structures.

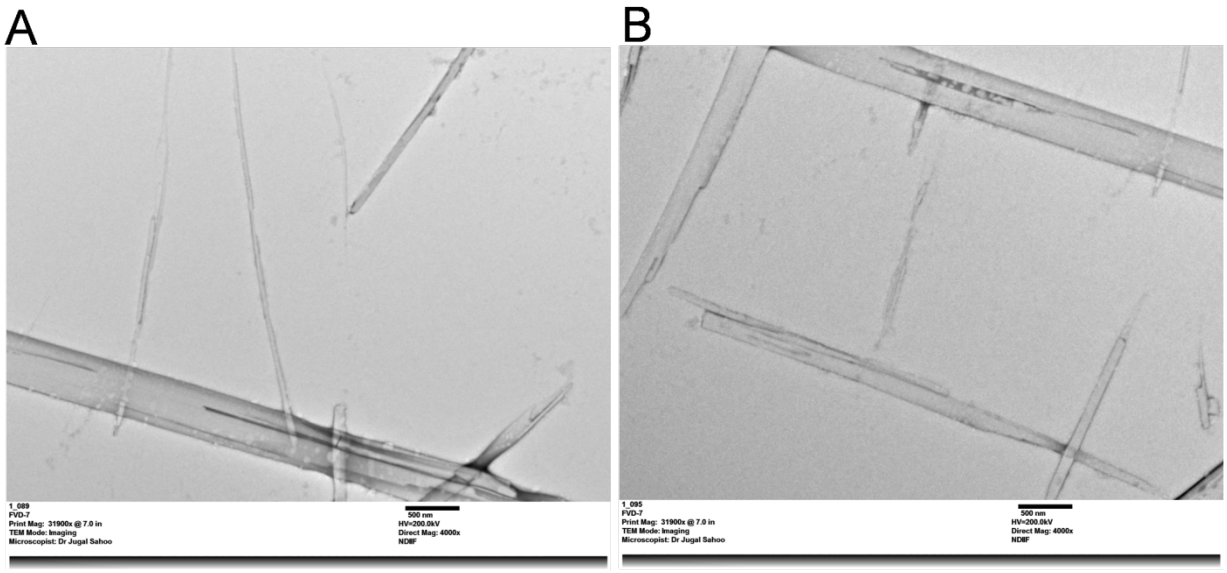

Figure S16: Additional TEM images of Ac-FVD-NH_2 depicting elongated sheets (or tube) nanostructures.

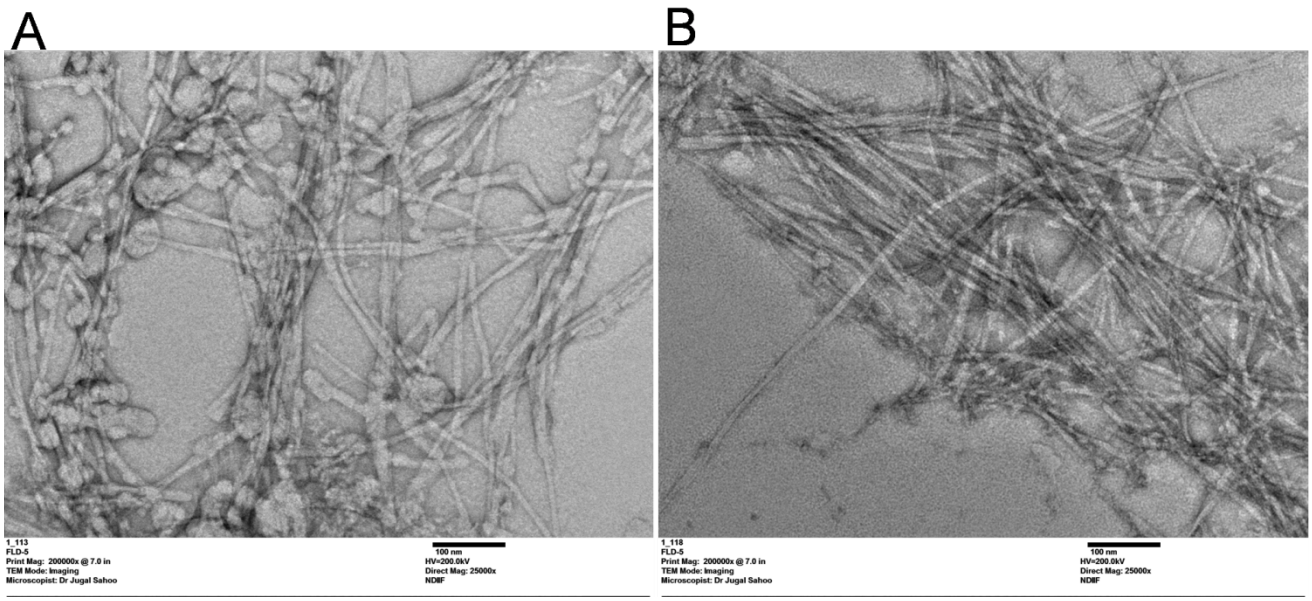

Figure S17: Additional electron microscopy images of Ac-FLD-NH_2 depicting fiber-like morphology.

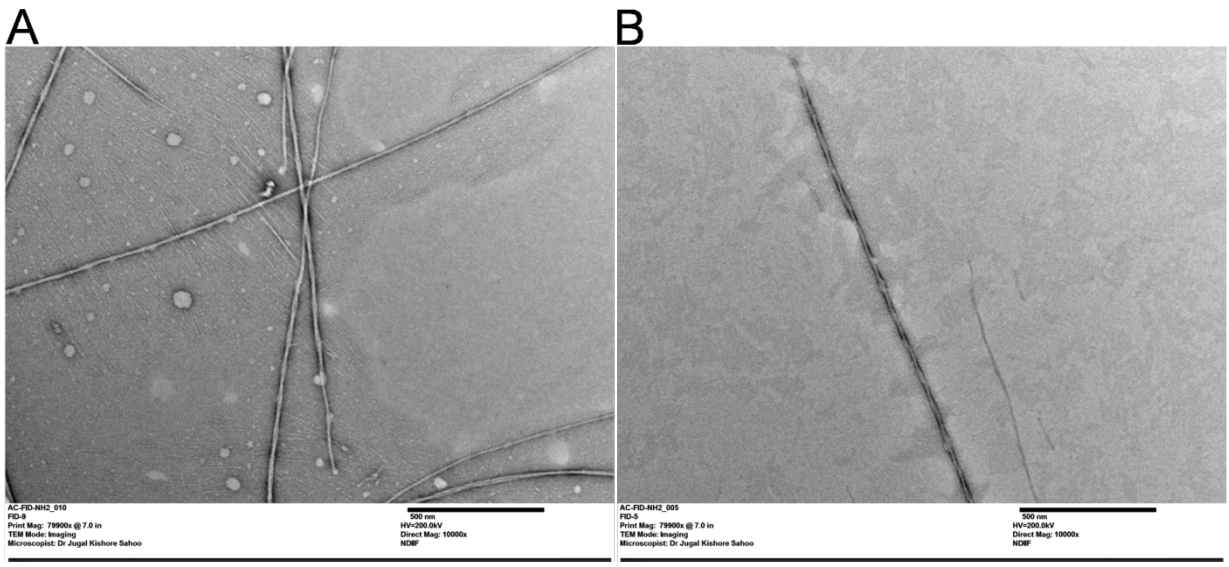

Figure S18: Additional electron microscopy images of Ac-FID-NH_2 depicting twisted nanofibers.

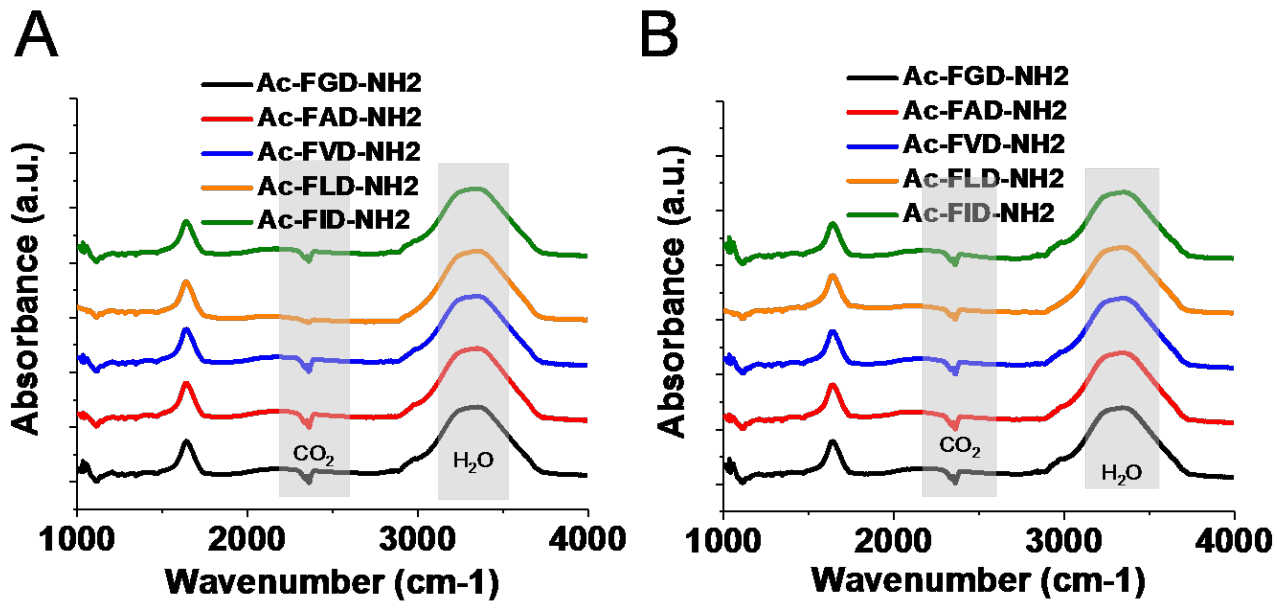

Figure S19: Full FTIR spectra for all tripeptides at (A) 20 mM and (B) 5 mM.