Online Supporting Information for:

Amino Acid Sequence in Constitutionally Isomeric Tetrapeptide Amphiphiles Dictates Architecture of One-Dimensional Nanostructures

Honggang Cui[†,┤], *Andrew G. Cheetham*[§], *E. Thomas Pashuck*[†], *and Samuel I. Stupp*[†,‡,§,╣,¶,*]

Department of Materials Science and Engineering, Department of Chemistry, Department of Biomedical Engineering, and Department of Medicine, Northwestern University, 2220 Campus Drive, Evanston, Illinois 60208, Simpson Querrey Institute, Northwestern University, Chicago, IL 60611

E-mail: s-stupp@northwestern.edu

* Corresponding author

† Department of Materials Science and Engineering

‡ Department of Chemistry

╣ Department of Biomedical Engineering

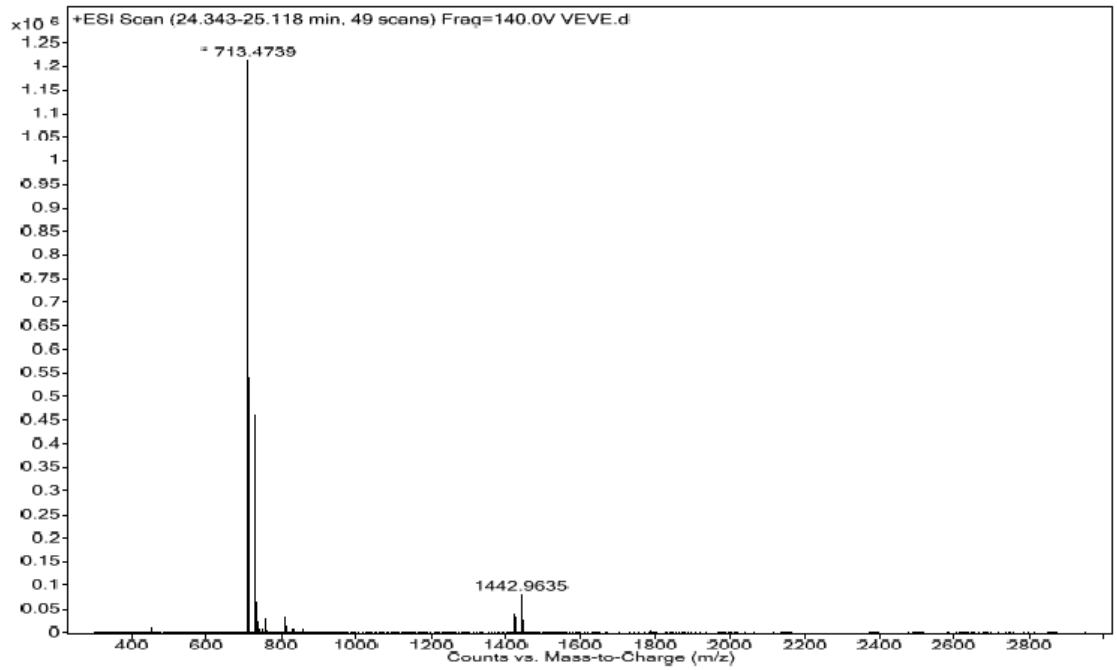
§ Simpson Querrey Institute

¶ Department of Medicine

┤ Current address: Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD 21218, United States.

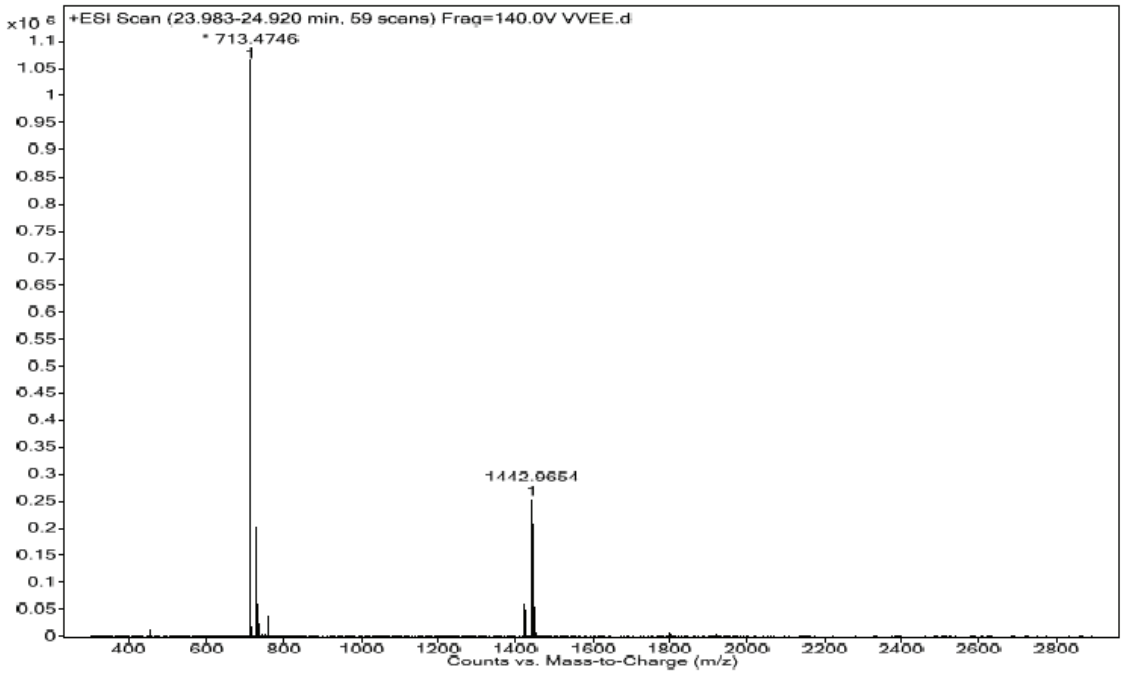
S1. Molecular characterization of the studied peptides

S1a. Electrospray ionization mass spectrometry of $C_{16}H_{31}OVEVE$



Full mass spectrum of $C_{16}H_{31}OVEVE$. The protonated adduct of $C_{16}H_{31}OVEVE$ was observed at 713.47. Also observed was the formation of an ammoniated dimer, $[2M+NH_4]^+$, at 1442.91.

S1b. Electrospray ionization mass spectrometry of $C_{16}H_{31}OVVEE$



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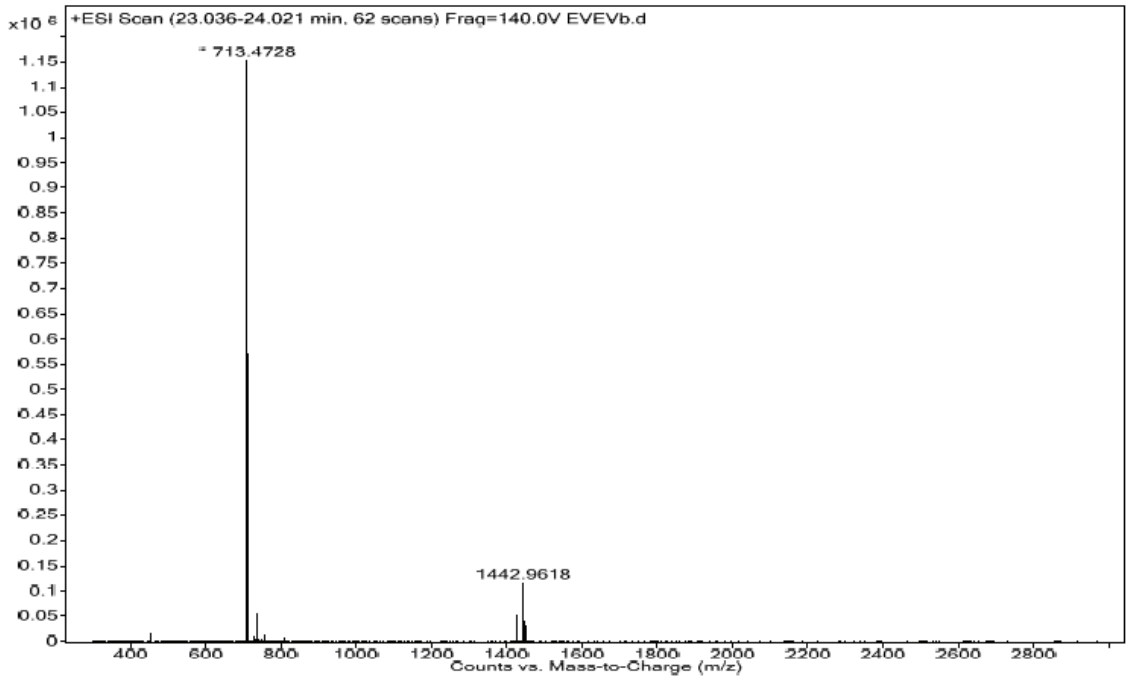
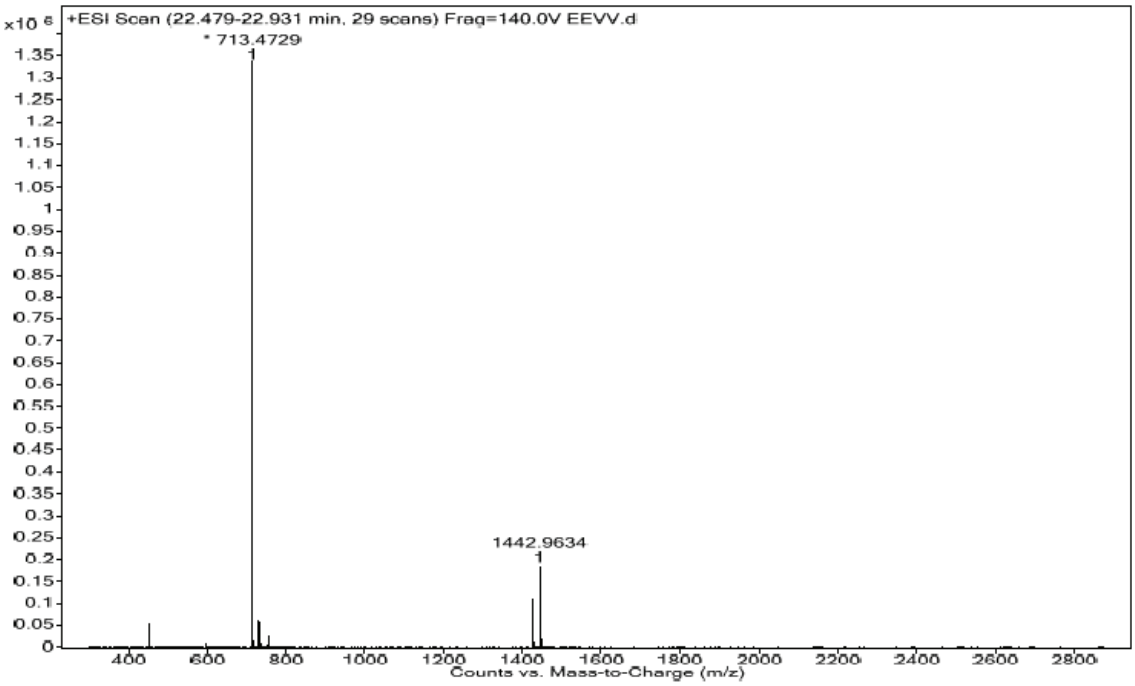
S1c. Electrospray ionization mass spectrometry of $C_{16}H_{31}OEVEV$



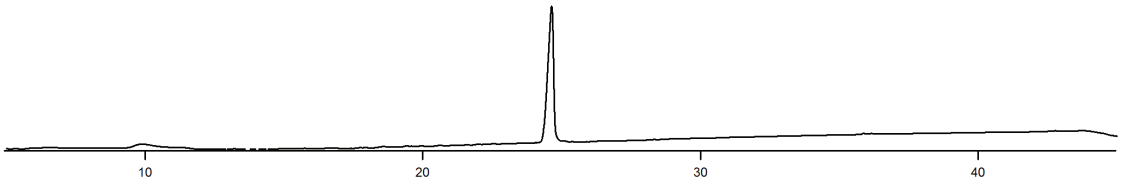
Full mass spectrum of $C_{16}H_{31}OEVEV$. The protonated adduct of $C_{16}H_{31}OVEVE$ was observed at 713.47. Also observed was the formation of an ammoniated dimer, $[2M+NH_4]^+$, at 1442.96.

S1d. Electrospray ionization mass spectrometry of $C_{16}H_{31}OEEVV$

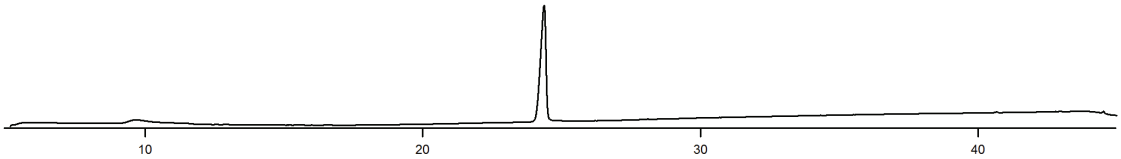


Full mass spectrum of $C_{16}H_{31}OEVEV$. The protonated adduct of $C_{16}H_{31}OVEVE$ was observed at 713.47. Also observed was the formation of an ammoniated dimer, $[2M+NH_4]^+$, at 1442.96.

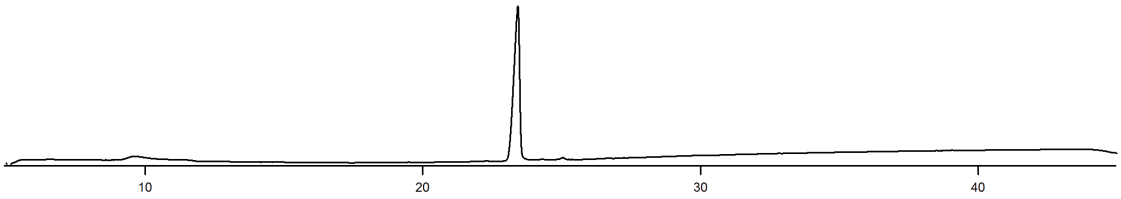
S1e. Analytical Reverse-Phase HPLC of $C_{16}H_{31}OVEVE$



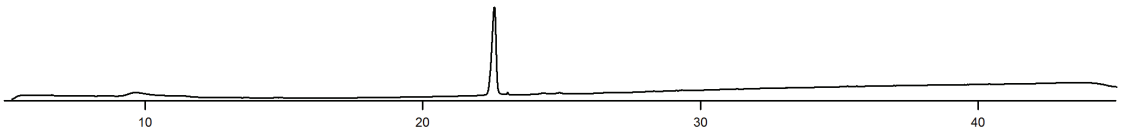
S1f. Analytical Reverse-Phase HPLC of $C_{16}H_{31}OVVEE$



S1g. Analytical Reverse-Phase HPLC of $C_{16}H_{31}OEVEV$



S1h. Analytical Reverse-Phase HPLC of $C_{16}H_{31}OEEVV$



S2. Circular dichroism spectra of the studied peptide solutions

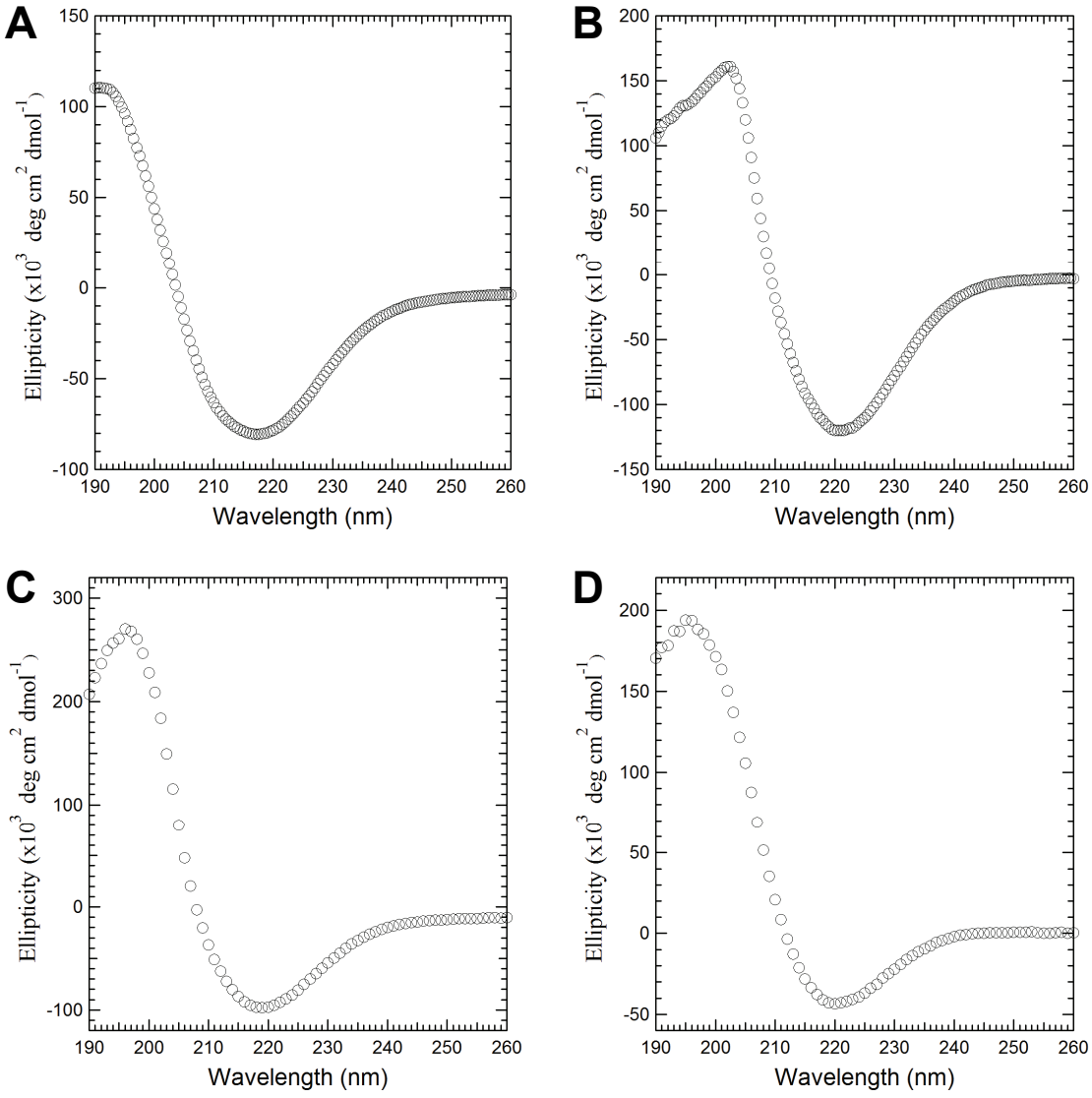
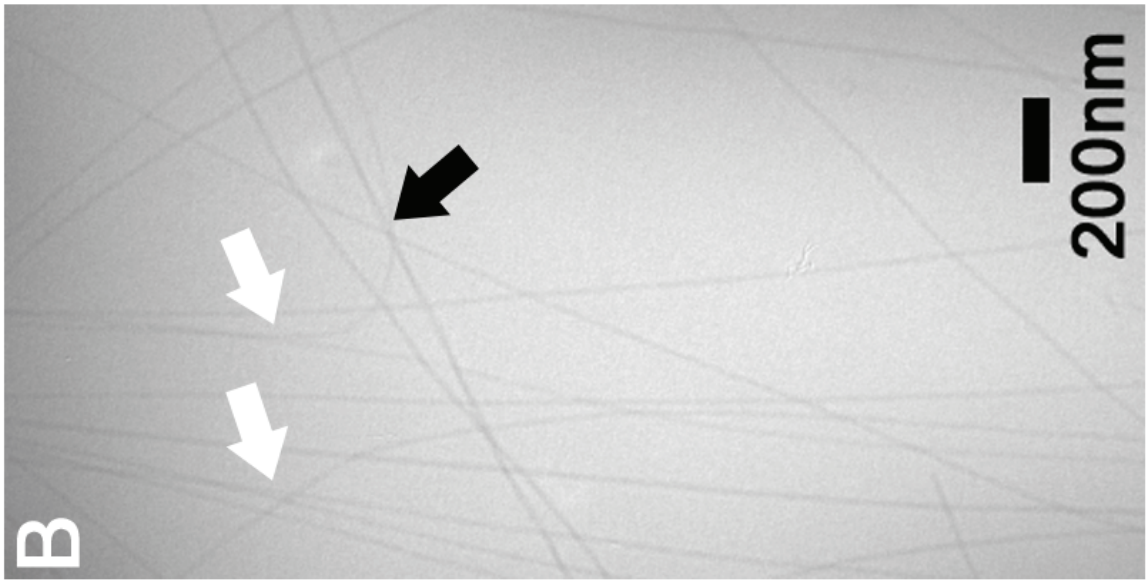


Figure S2 Circular dichroism spectra of **VEVE** (**A**), **VVEE**, **EVEV** (**C**) and **EEVV** (**D**). All four peptides show the β-sheet secondary structure, as indicated by the negative peak between 216 nm and 222 nm. The β-sheet secondary structure was also confirmed by wide angle X-ray scattering (WAXS) experiments.

S3 High Resolution cryo-TEM images of bundled nanofibers of **EEVV**



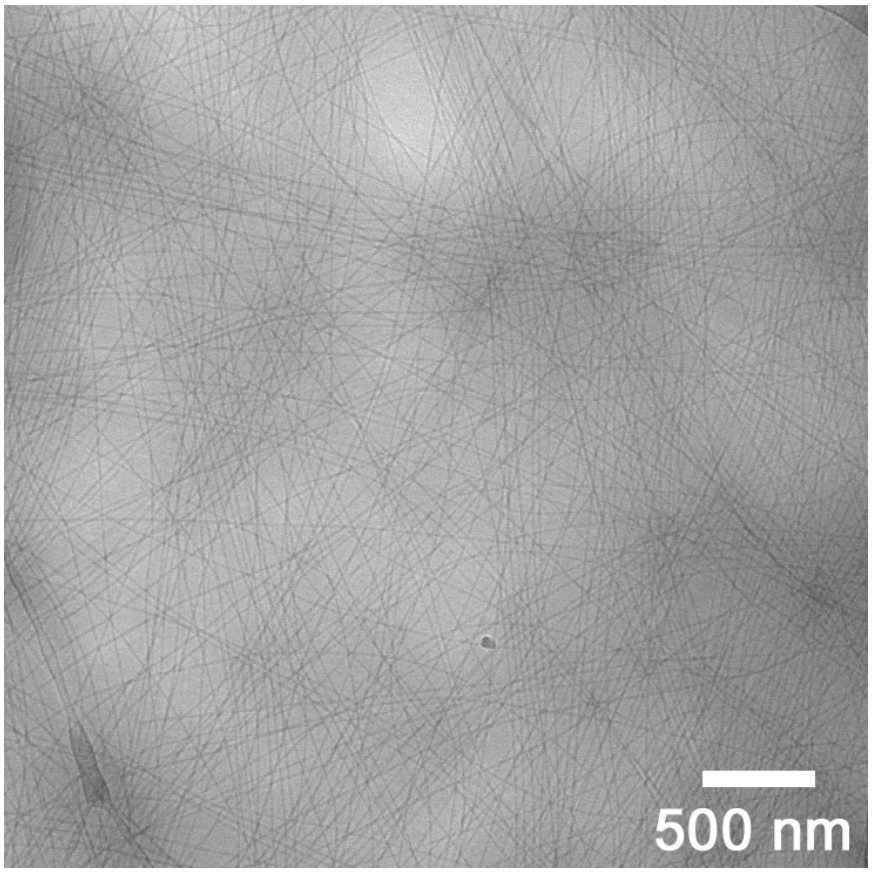


Figure S3 Top, the high resolution picture of figure 5B. Two or three narrower nanofibers can be clearly seen to intertwine and bundle into larger nanofibers. White arrows mark the locations where bundled nanofibers split into two separate narrower nanofibers. The black arrow marks a bundled nanofiber of a larger diameter. **Bottom**, cryo-TEM micrograph of **EEVV** nanofibers in a large area. Bundling of nanofibers can be seen in a number of areas.

S4 Rheology measurement of 1 wt % **EEVV** and **VVEE** solutions

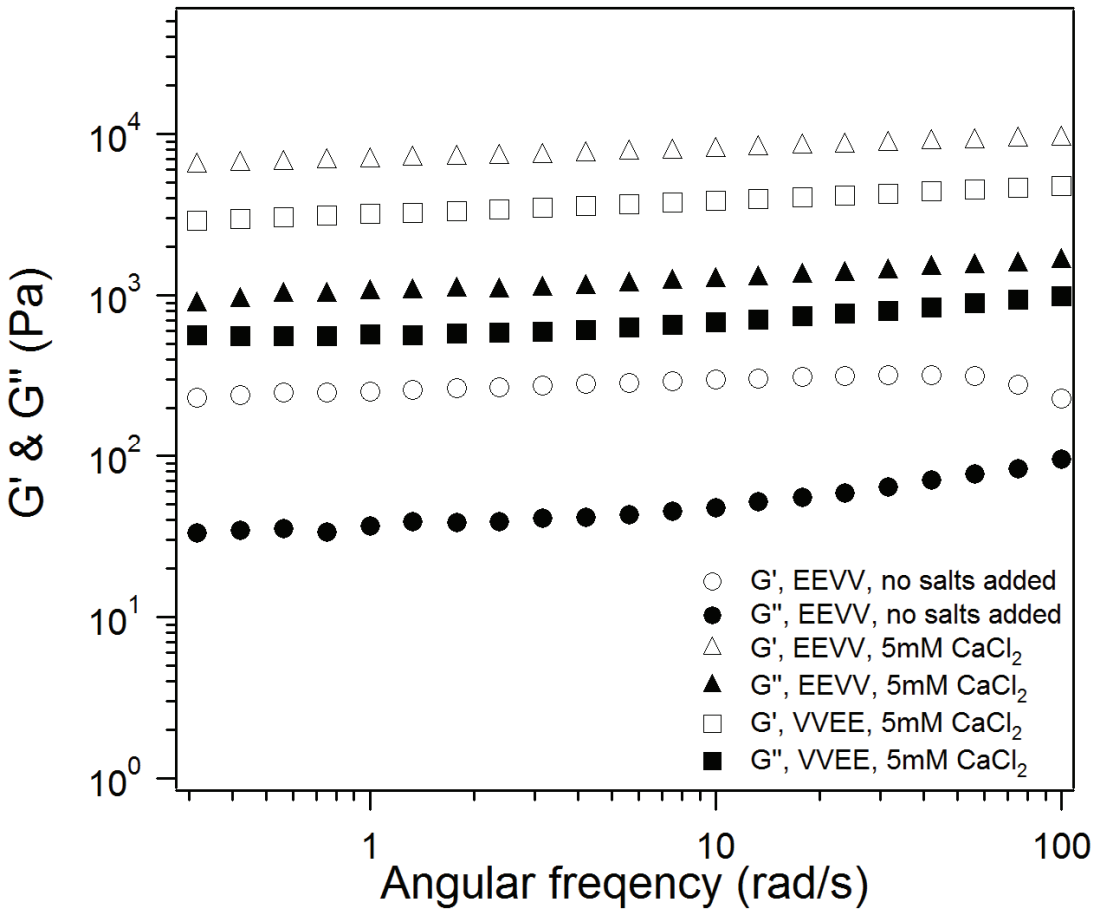


Figure S4 Frequency sweep (0.5 % strain) rheological measurements for 1 wt % **VVEE** and **EEVV** aqueous solutions with and without the addition of $CaCl_2$. Without the addition of any salts, the storage modulus (G') of the **EEVV** hydrogels is an order of magnitude greater than the corresponding loss modulus (G"). In contrast, the **VVEE** nanofibers only form a viscous aqueous solution in the absence of multivalent salts. We were not able to obtain any reliable data using the 25 mm parallel plates due to the detection limit of the instrument. When 5 mM $CaCl_2$ was added, both **EEVV** and **VVEE** nanofibers form stiffening hydrogels. As shown in the figure, it is evident that the **EEVV** hydrogel is stronger than that of the **VVEE** hydrogel, with a G' value of 7920 Pa at the frequency of 10 rad/s in comparison to the G' value of 3850 Pa of the **VVEE** gel.

S5 Experimental details of CD and rheological measurements

Circular Dichroism CD spectra were collected on a Jasco 715 instrument. 0.05 wt % sample solutions were loaded into a quartz cell of 1 mm path length. All CD spectra were collected between 190 nm and 260 nm. The mean molar ellipticity per molecule [θ] was calculated using the equation $[\theta]= \theta_b/10lc$, where θ_b is the measured ellipticity in millidegrees, l is the length of the cell (centimeters), and c is the concentration (molar).

Rheology Rheological measurements were performed on a Paar Physica MCR-300 rheometer using the 25 mm diameter parallel plate with a 0.5 mm gap. Gel samples were carefully loaded on the bottom plate. After standing for 2 hours, frequency sweep experiments were conducted within the linear viscoelastic regime. The evaporation of water from the hydrogel was avoided by covering the sides of the plate with Kimwipe papers soaked with water.