Supporting Information

Tuning Supramolecular Rigidity of Peptide Fibers through Molecular Structure

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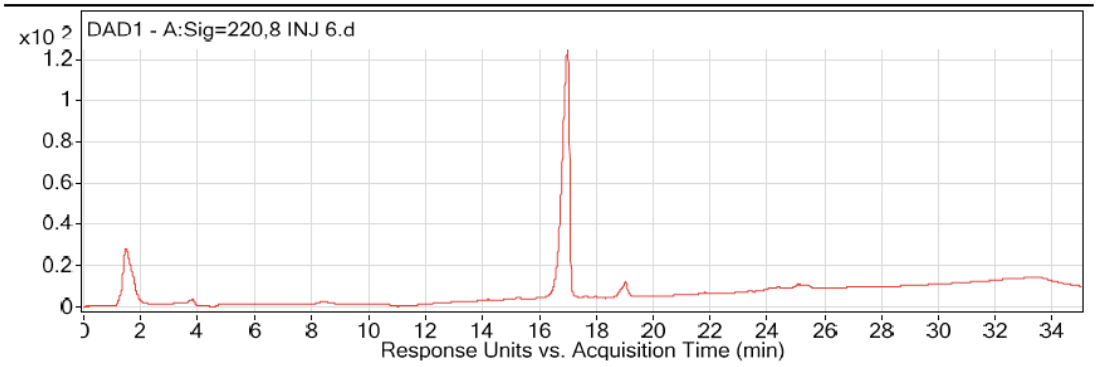
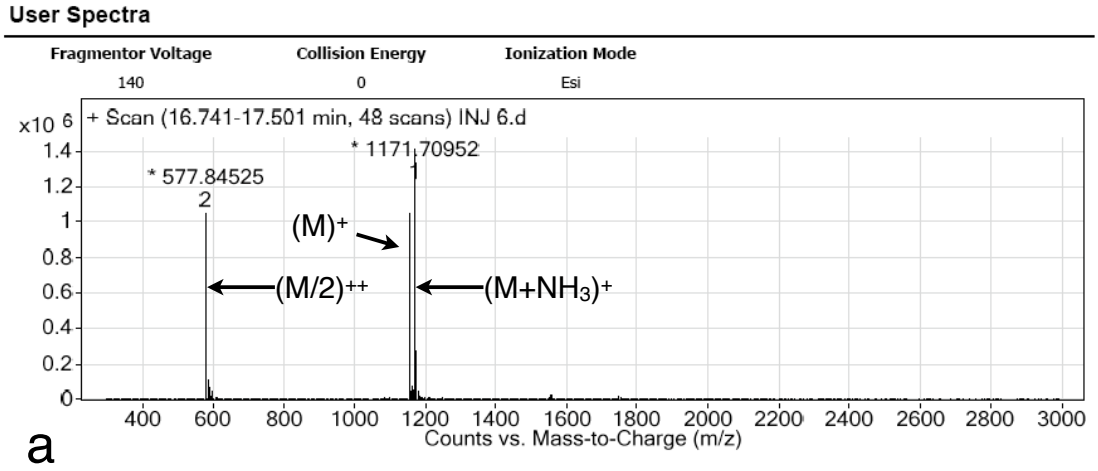
$V_3A_3E_3$





a

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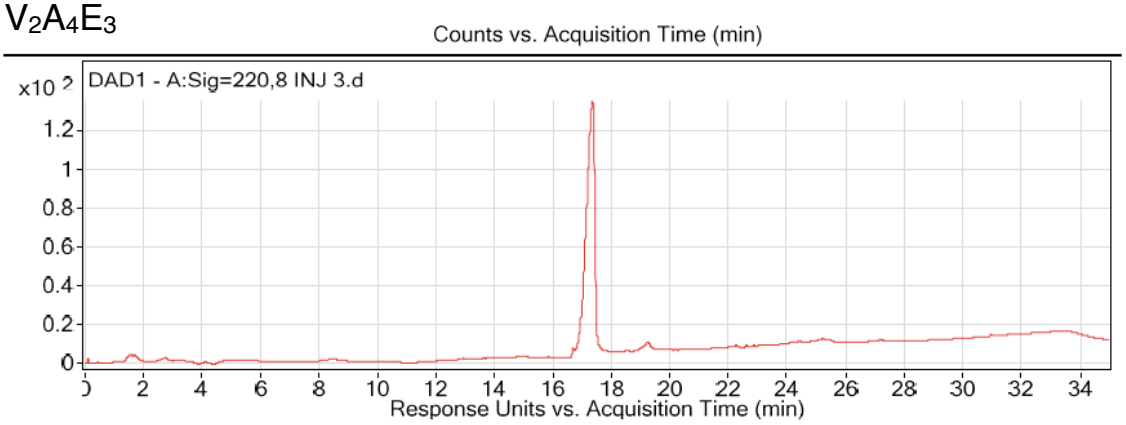

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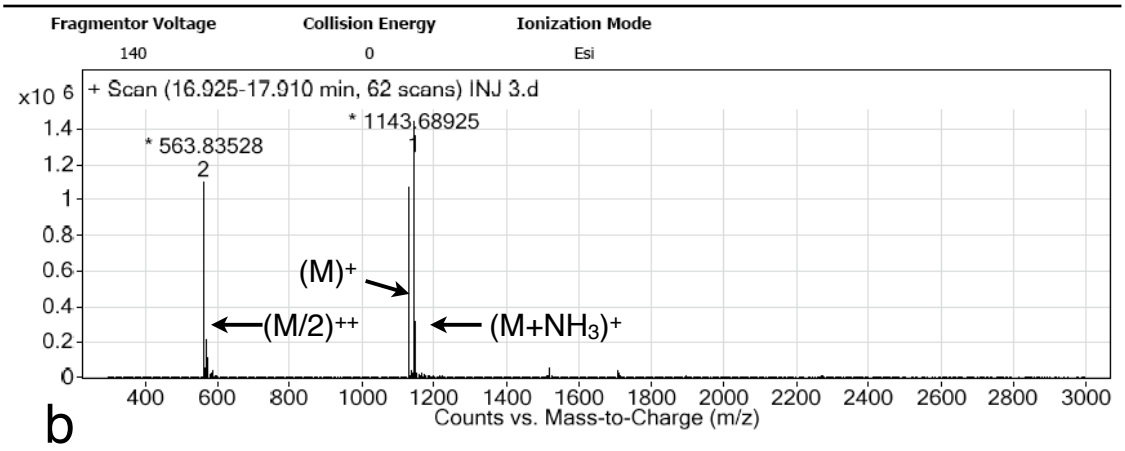

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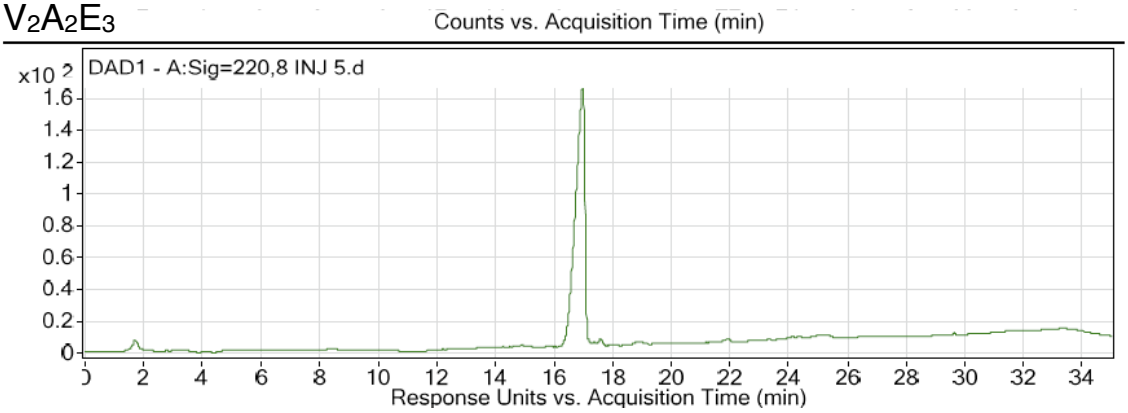

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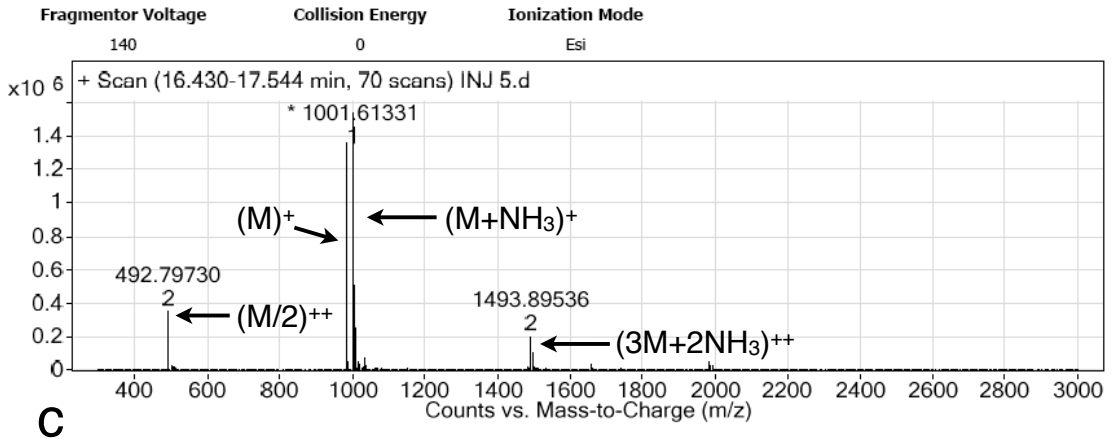
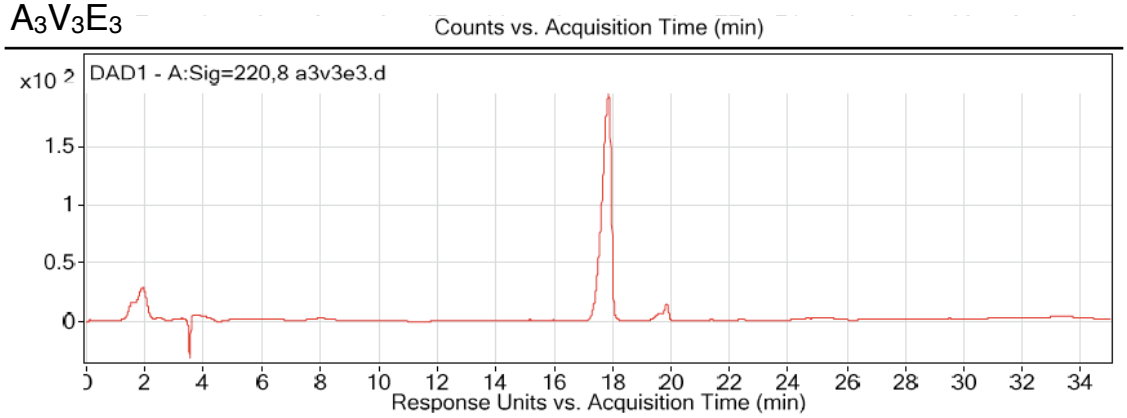


C

$A_3V_3E_3$



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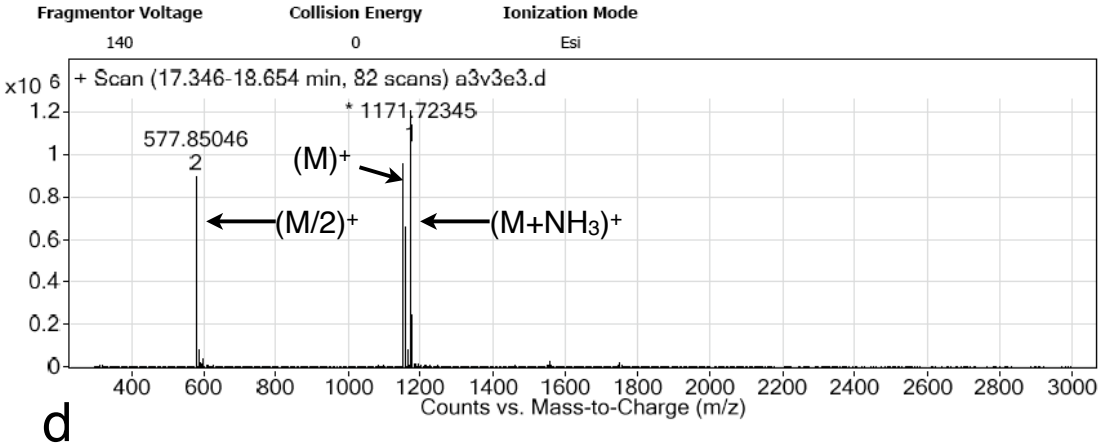

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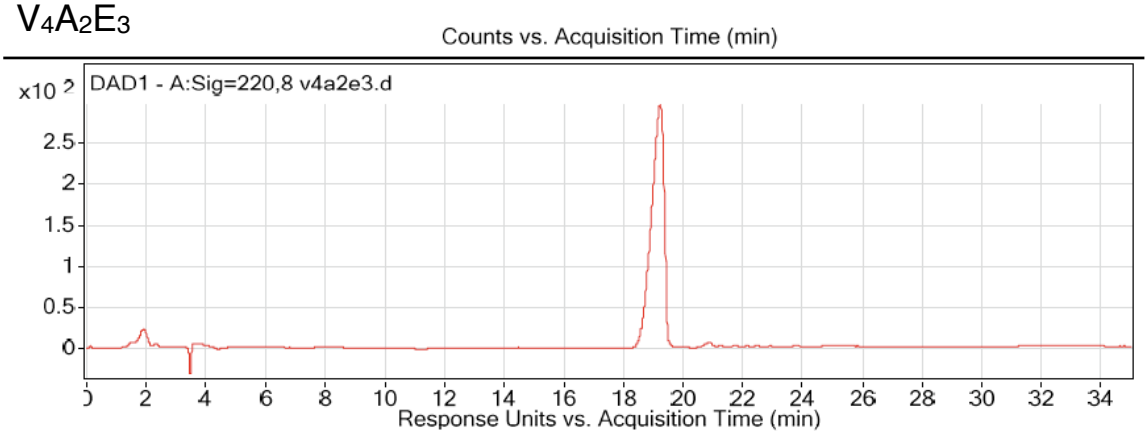

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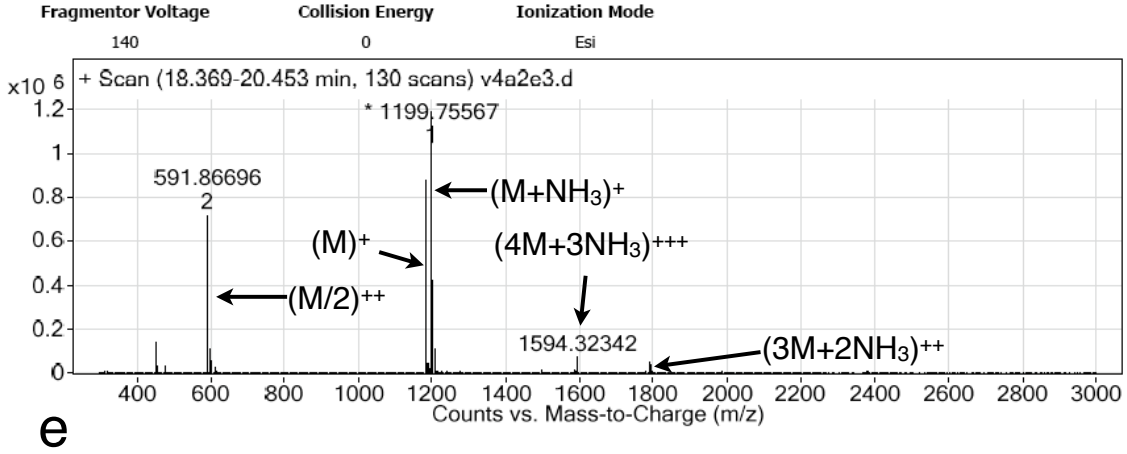

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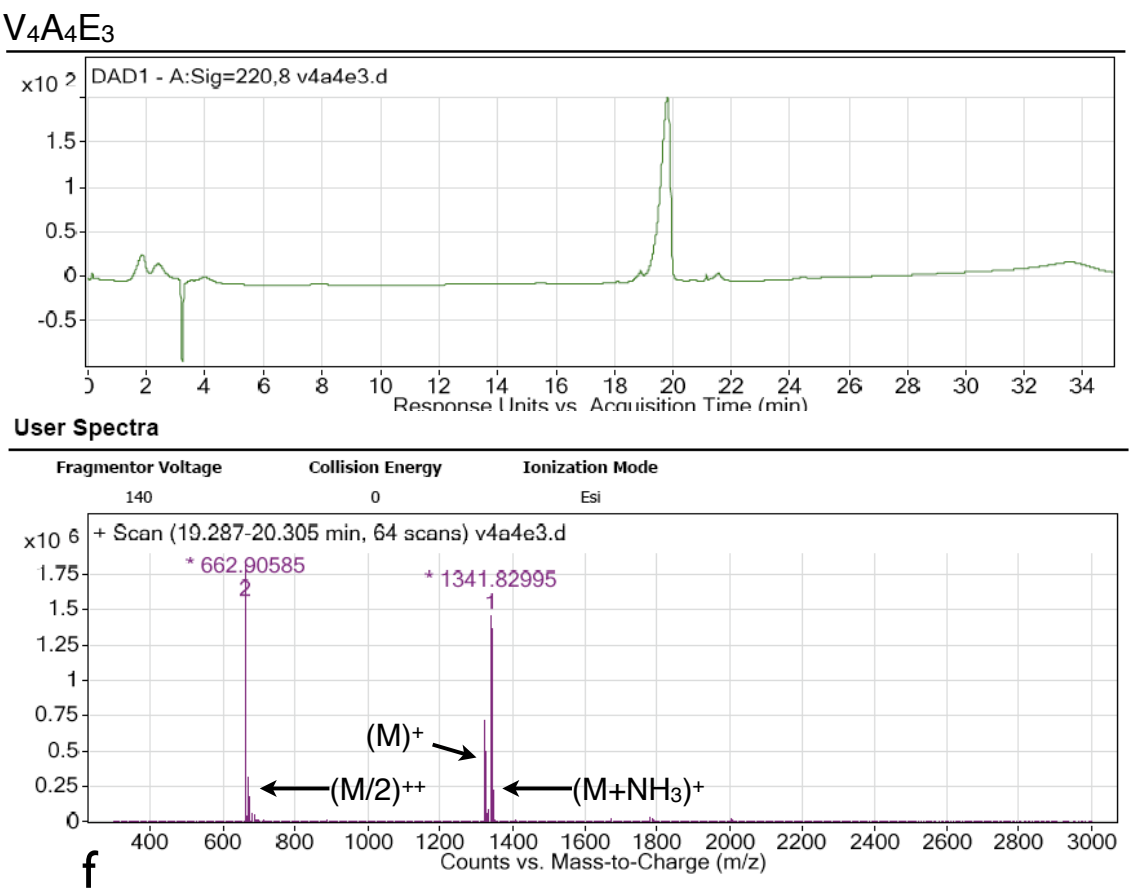

Figure S1. Analytical HPLC and electrospray ionization (ESI) mass spectroscopy of PAs 1-7, (a) $V_3A_3E_3$ (b) $V_4A_2E_3$ (c) $V_2A_2E_3$ (d) $A_3V_3E_3$ (e) $V_2A_4E_3$ (f) $V_4A_4E_3$.

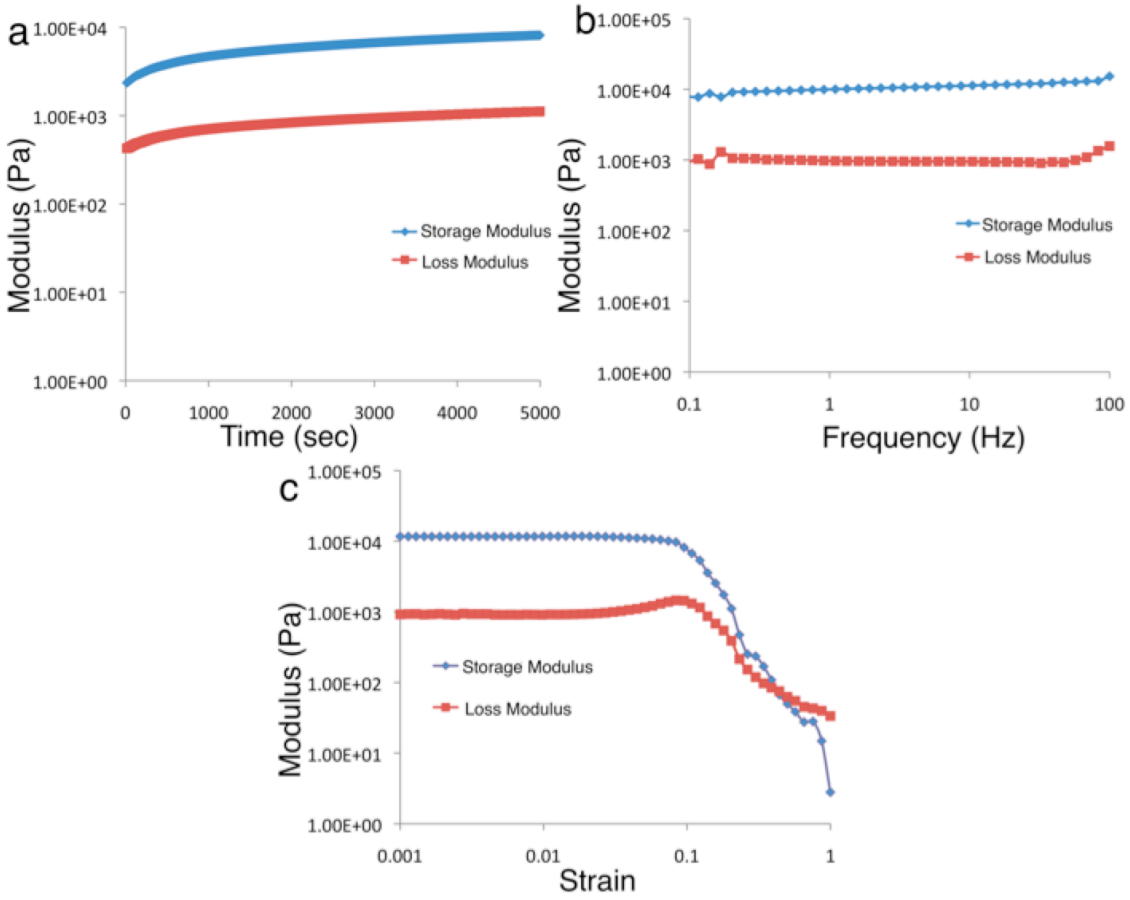

Figure S2. Storage and loss moduli of PA **1** ($V_3A_3E_3$) as a function of a) time, b) frequency and c) strain.

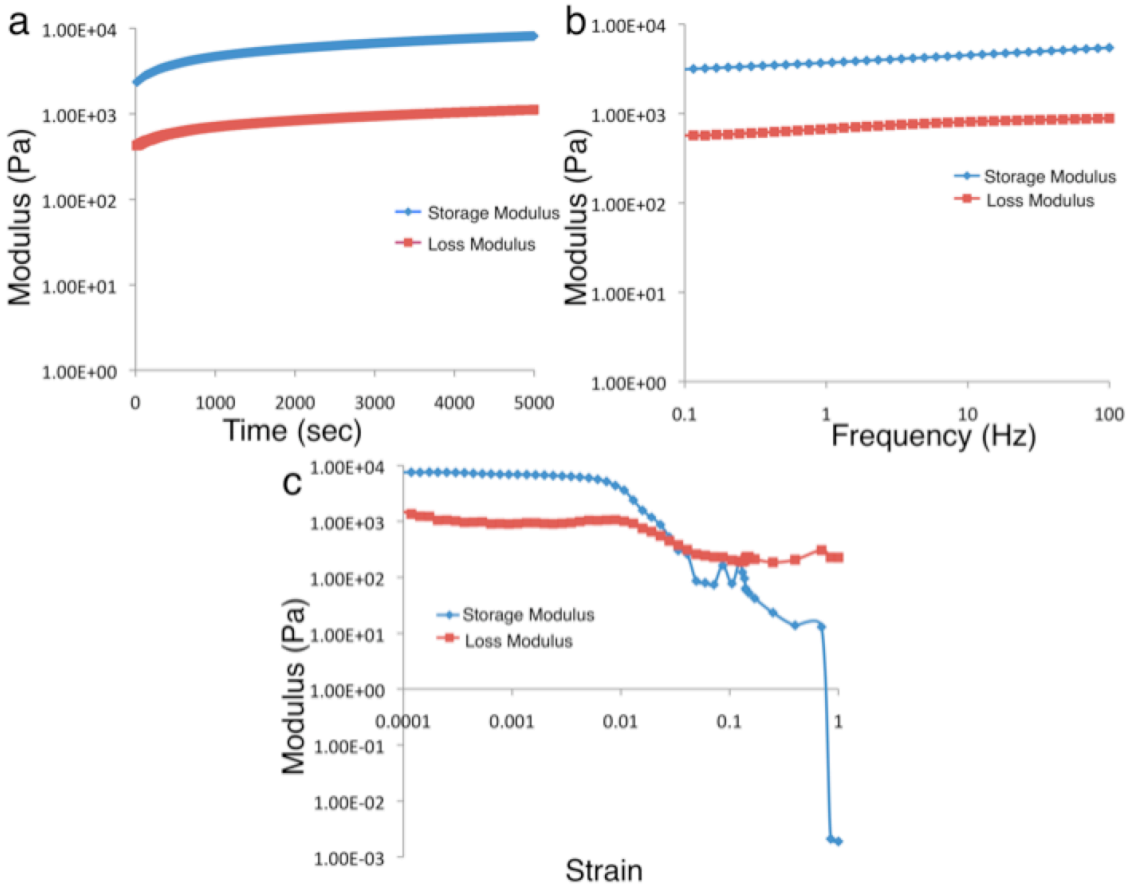

Figure S3. Storage and loss moduli of PA **2** ($V_2A_2E_3$) as a function of a) time, b) frequency and c) strain.

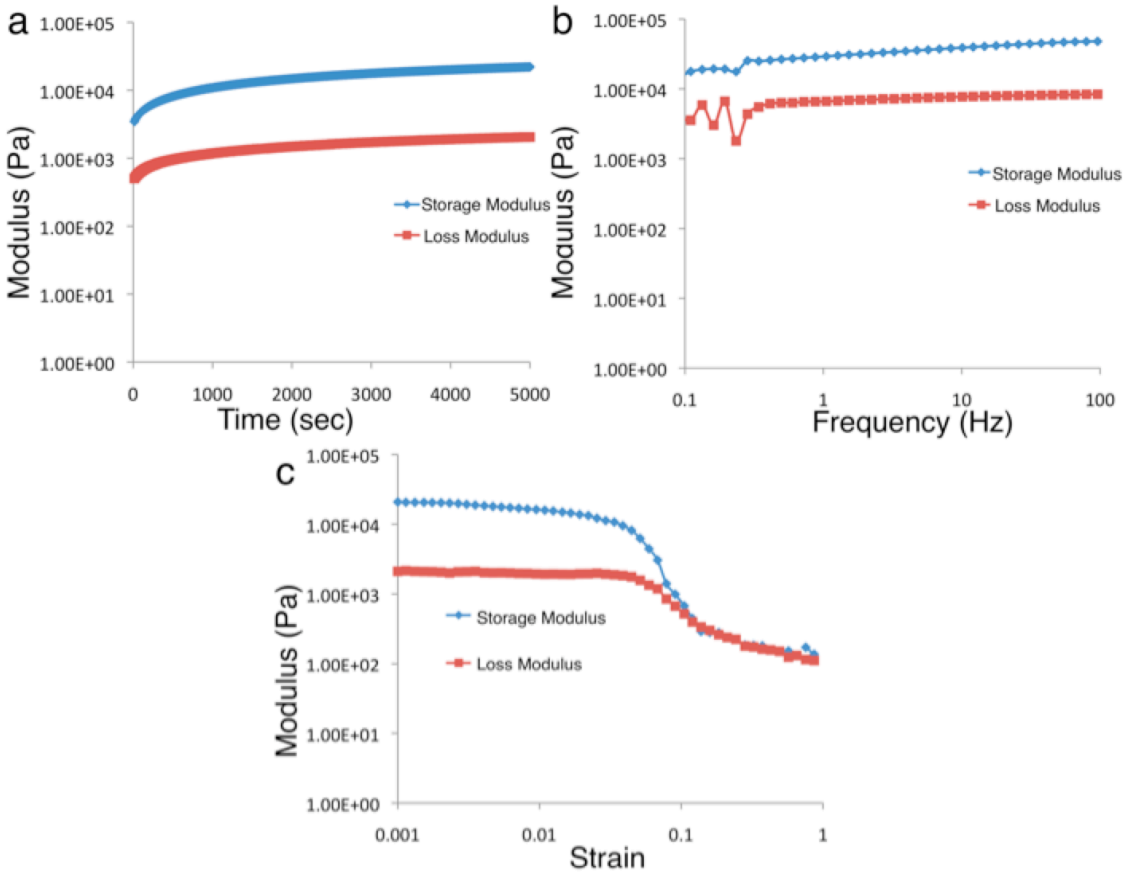

Figure S4. Storage and loss moduli of PA **3** ($V_4A_4E_3$) as a function of a) time, b) frequency and c) strain.

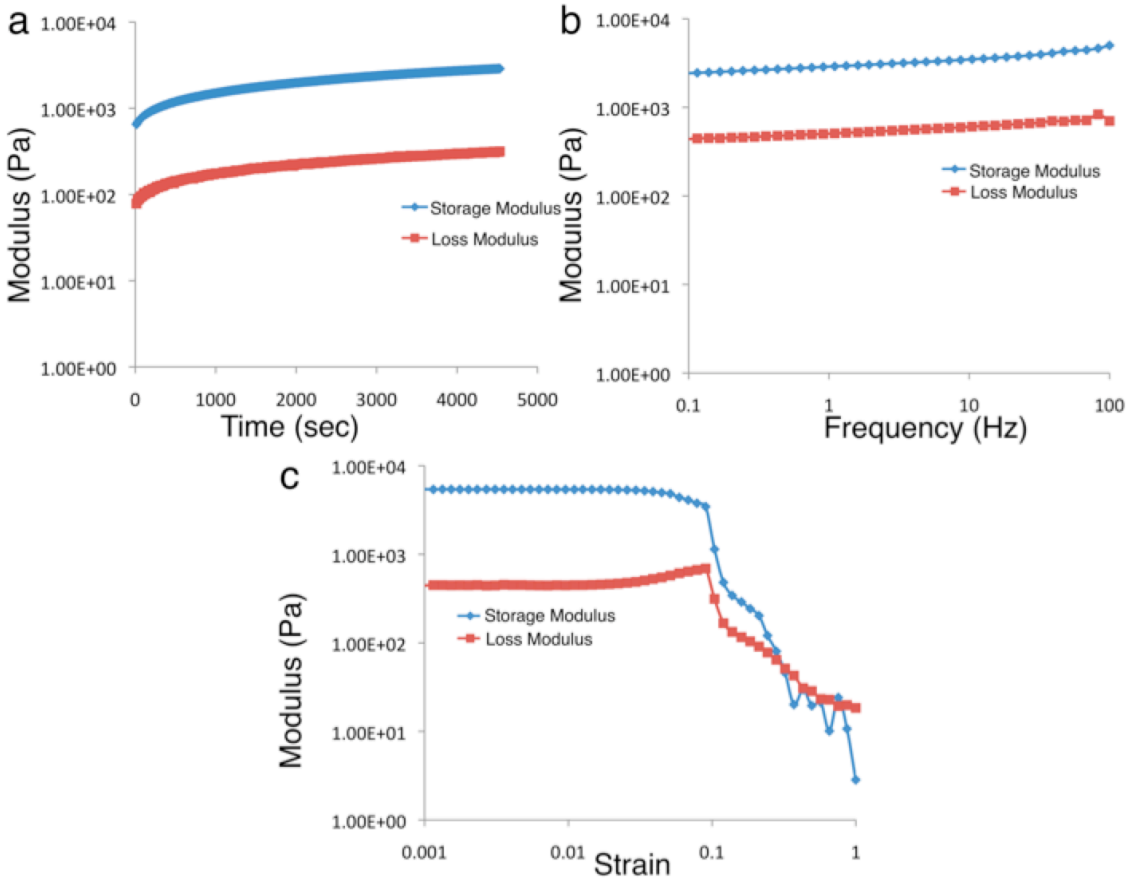

Figure S5. Storage and loss moduli of PA **4** ($V_2A_4E_3$) as a function of a) time, b) frequency and c) strain.

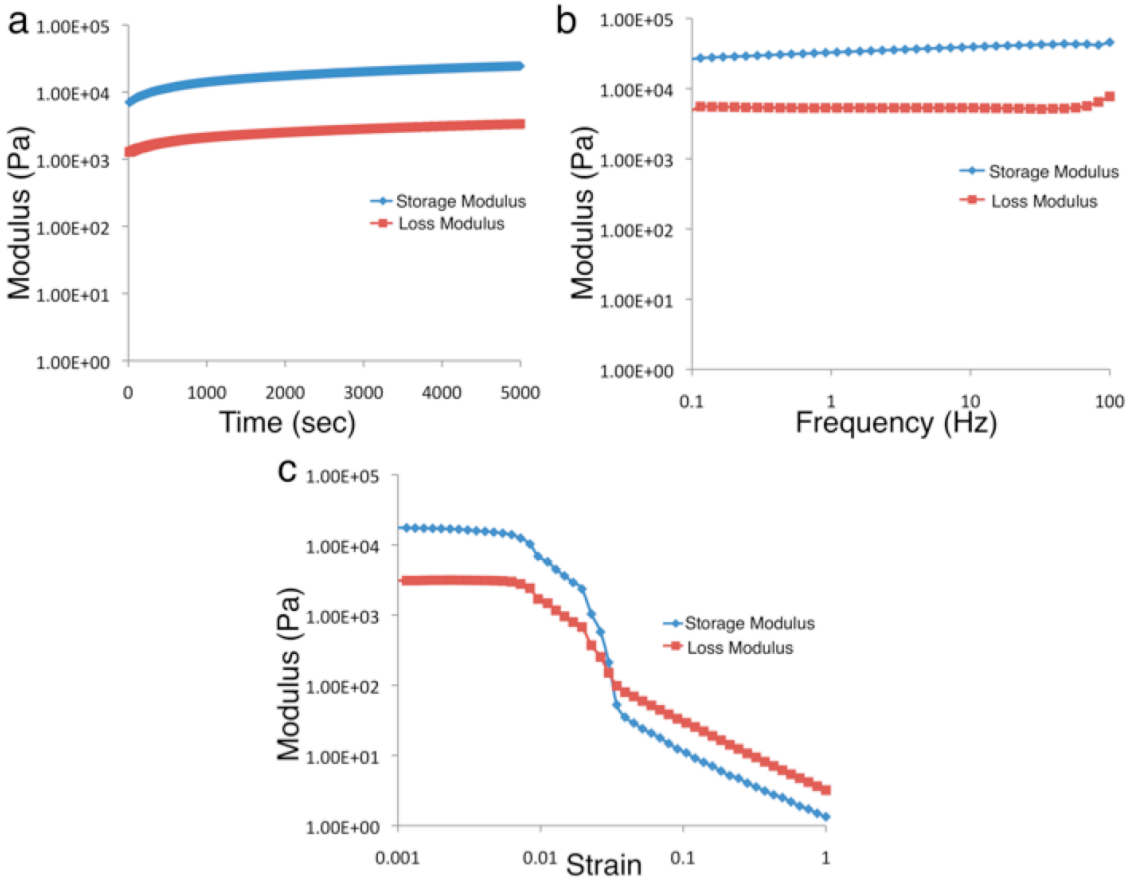

Figure S6. Storage and loss moduli of PA **5** ($V_4A_2E_3$) as a function of a) time, b) frequency and c) strain.

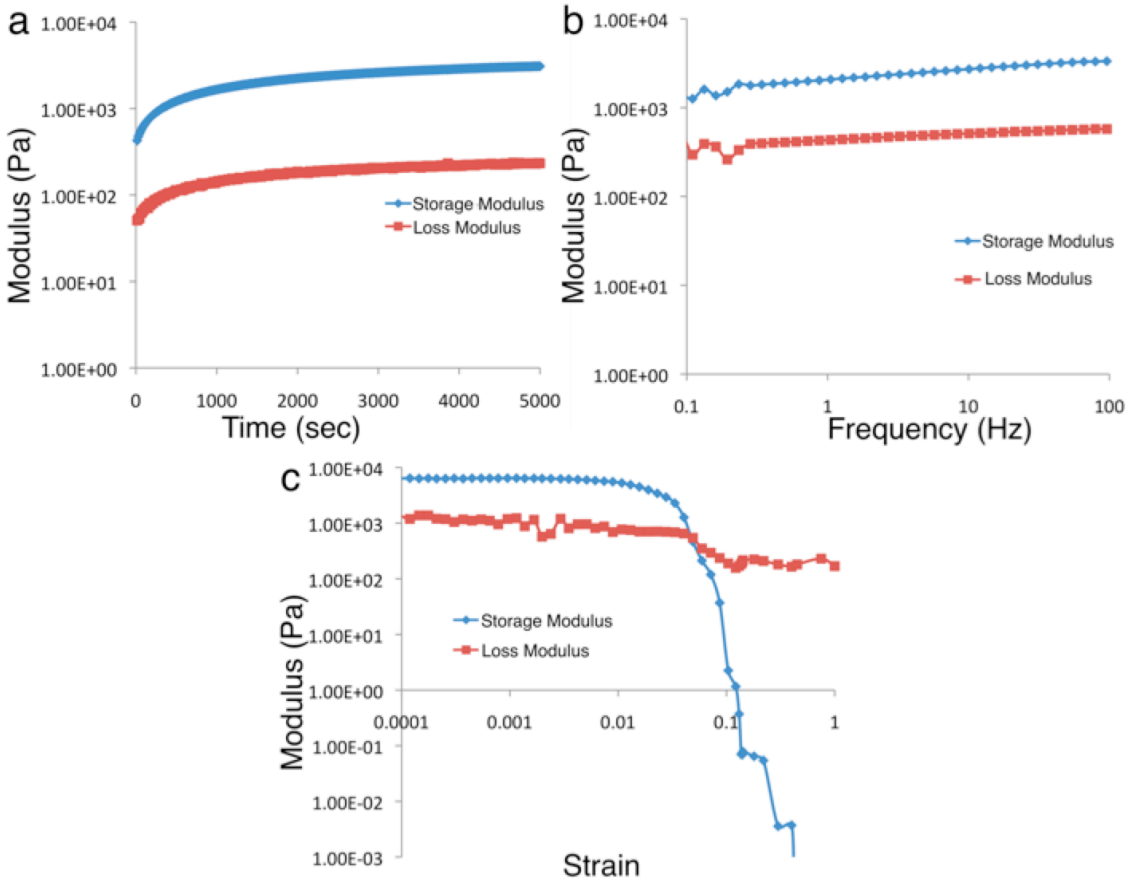

Figure S7. Storage and loss moduli of PA **6** ($A_3V_3E_3$) as a function of a) time, b) frequency and c) strain.

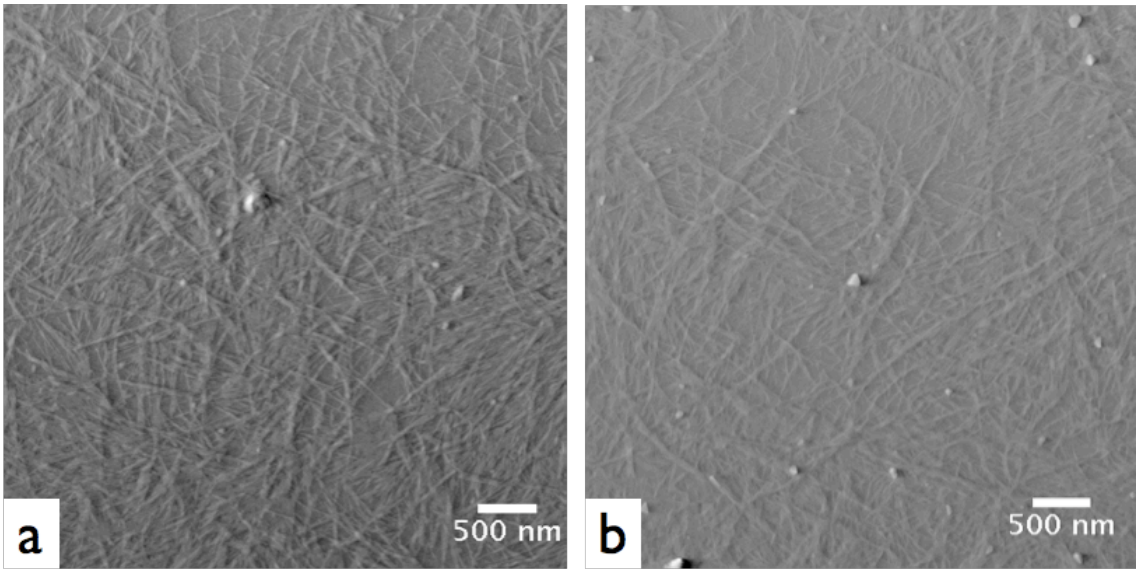

Figure S8. AFM images of PA1 on a (a) gold PM-IRRAS substrate and (b) silicon transmission IR substrate.

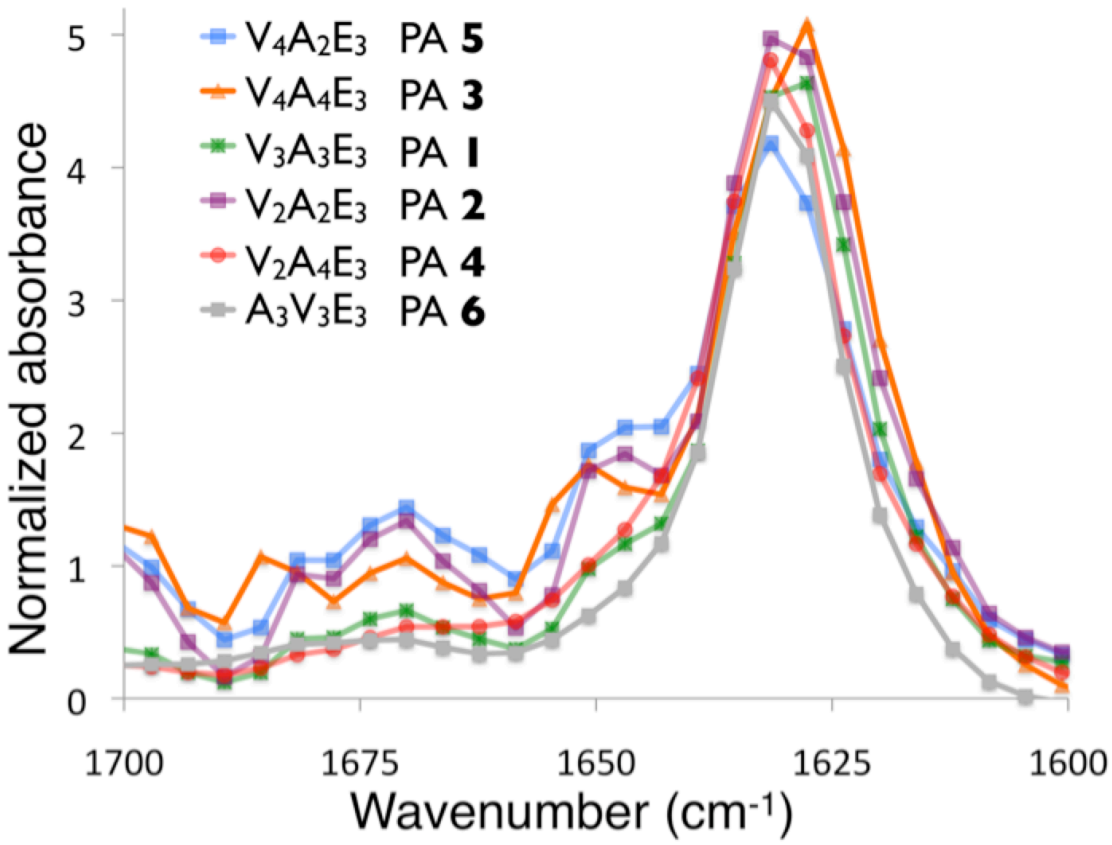

Figure S9. Transmission FTIR or PAs **1-6**. Peaks at 1630 cm^{-1} and 1630 cm^{-1} correspond to β-sheets structures, while the peak at 1647 cm^{-1} peak is indicative of turns or bends.[1]

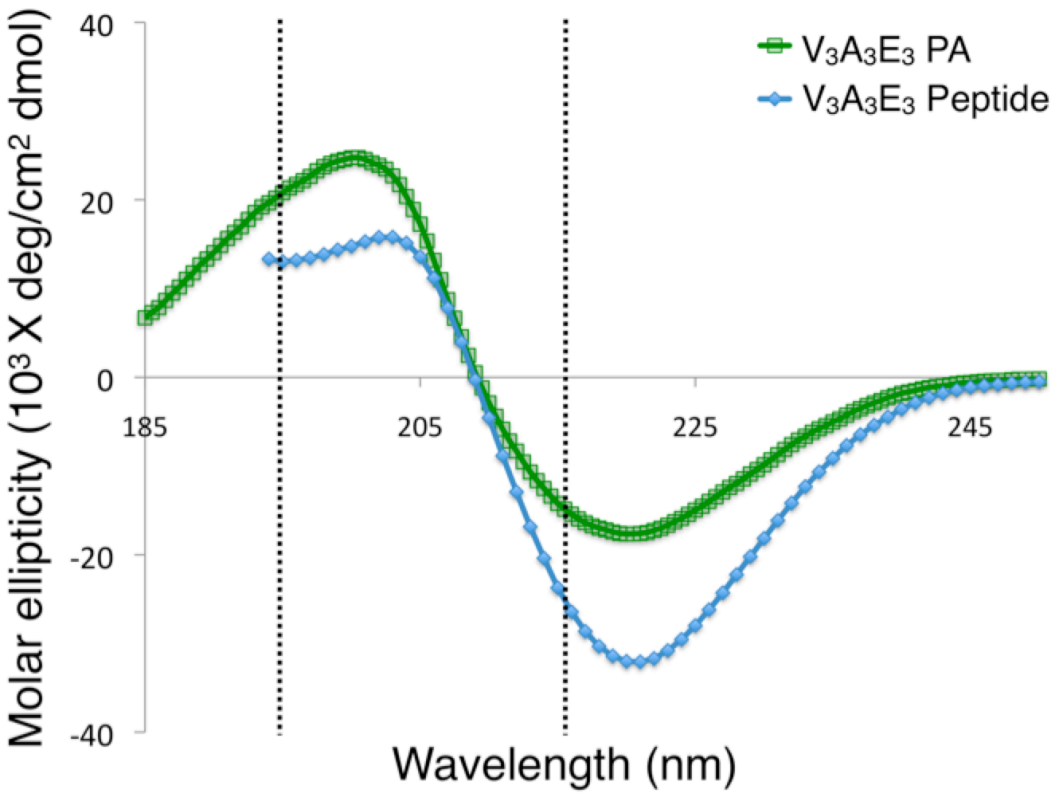

Figure S10. Circular dichroism showing that the $V_3A_3E_3$ peptide (lacking the palmitoyl tail) is more red-shifted than that of the $V_3A_3E_3$ PA (with the alkyl tail), indicating that the β-sheets are more twisted. Dashed lines indicate 195nm and 216 nm, the canonical maximum and minimum of a β-sheet.

Class Level Information		
Class	**Levels**	**Values**
treatment	6	A3V3 V2A2 V2A4 V3A3 V4A2 V4A4

Number of Observations Read	24				
Number of Observations Used	24				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	5	1786569807	357313961	5.22	0.0039
Error	18	1233240538	68513363		
Corrected Total	23	3019810344			

R-Square	Coeff Var	Root MSE	response Mean
0.591617	68.66784	8277.280	12054.09

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	5	1786569807	357313961	5.22	0.0039

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	5	1786569807	357313961	5.22	0.0039

treatment	response LSMEAN	Standard Error	Pr > |t|
A3V3	2273.8900	4138.6400	0.5895
V2A2	6493.3325	4138.6400	0.1341
V2A4	5057.5000	4138.6400	0.2375

V3A3	11233.3325	4138.6400	0.0142
V4A2	24703.1225	4138.6400	<.0001
V4A4	22563.3325	4138.6400	<.0001

Note:	This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	68513363
Critical Value of t	2.10092
Least Significant Difference	12297

Means with the same letter are not significantly different.				
t Grouping		**Mean**	**N**	**treatment**
	A	24703	4	V4A2
	A			
B	A	22563	4	V4A4
B				
B	C	11233	4	V3A3
	C			
	C	6493	4	V2A2
	C			
	C	5058	4	V2A4
	C			
	C	2274	4	A3V3

Figure S11. Statistical analysis of the storage modulus of PAs **1**-**6** using a one-way ANOVA.

Experimental Section

Peptide Amphiphile Synthesis and Purification PAs were synthesized using resins and Fmoc-protected amino acids purchased from Novabiochem Corporation. All other solvents were ACS reagent grade and purchased from Mallinckrodt and reagents were purchased from Aldrich and used as received. Manual solid-phase peptide synthesis was performed on a 0.5 mmole scale using 50mL peptide synthesis vessels (Chemglass) and a wrist-action shaker. In each molecule the first glutamic acid was purchased preloaded as a Fmoc - Glu (OtBu) Wang resin. For each coupling the Fmoc protecting group was removed by shaking the resin in 30% piperidine in N,N dimethyl formamide (DMF) for ten minutes, rinsed and repeated a second time. The resin was washed with dichloromethane (DCM) and DMF, and allowed to swell in DCM for 15 minutes before coupling. Amino acids were activated by adding 4 molar equivalents of the Fmoc-protected amino acids to 4 molar equivalents of O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate(HBTU) and dissolving in 30 ml of DMF. Six molar equivalents of diisopropylethylamine (DIEA) were added to the amino acid solution, which was allowed to sit for a minute before being added to the resin. The coupling reaction was allowed to proceed for three hours, at the end of which the resin was washed in DCM and DMF and ninhydrin tests were done to check for the presence of free amines. If the ninhydrin test yielded a positive result the coupling was repeated. The palmitoyl tail was added using same molar ratio of palmitic acid:HBTU:DIEA of 4:4:6. Alternatively, some PAs were synthesized on a CS Bio Co. automatic peptide synthesizer using a similar methodology, however with 90 minute coupling times.

PAs were cleaved by shaking the resin in a peptide cleavage solution of 95% trifluoroacetic acid (TFA), 2.5% triisopropyl silane (TIS) and 2.5% H_2O for three hours. The cleavage solution was drained into a round bottom flask and the resin was rinsed several times with DCM. All liquid was removed using rotory evaporation, and the PA residue was washed with cold diethyl ether and poured into a fritted filter. After the diethyl ether passed through the filter, the PA flakes were rinsed again with diethyl ether, allowed to dry and then placed in a vacuum dessicator until HPLC purification.

To dissolve the PA after cleavage, ultrapure water was added to make the PA 20 millimolar and sodium hydroxide was added until the pH was raised to 8. The solution was passed through a 0.22 micrometer filter and injected into a prep scale reverse phase HPLC running a mobile phase gradient of 98% H_2O and 2% acetonitrile (spectroscopic grade, Mallinckrodt) to 100% acetonitrile. 0.1% NH_4OH was added to all mobile phases to aid PA solubility. The Phenomenex C_{18} Gemini NX column had a 5 micron pore size, a 110Å particle size and was 150 x 30 mm HPLC fractions were checked for the correct compound using electrospray ionization mass spectroscopy (ESI-MS), rotoary evaporated to remove acetonitrile and lyophilized (Labconco, FreezeZone6) at a pressure 0.015 torr. To remove any excess salts, PAs were dissolved in water and dialyzed in 500 molecular weight cutoff dialysis tubing (Spectrum Laboratories). After dialysis the pH was adjusted to 7.4 using NaOH and the samples were lyophilized.

Rheological Measurements All rheological studies used a Paar Physica MCR - 300 rheometer. All time test were performed at 100 Hz with 0.5% strain at 25 ˚C, using a 25 mm parallel plate with a 0.5 mm gap distance. 25 μL of 0.2M $CaCl_2$ were added to gel 250 μLs of freshly solubilized 10 mM PA solution on the rheometer. The storage moduli

used were taken from the time test at 5000 seconds. Each value represents the average of four time tests. Frequency sweeps were done at a constant strain of 0.5%, and strain sweeps were done at a frequency of 100hz.

Electron Microscopy Samples for vitreous ice cryo-transmission electron microscopy (cryo-TEM) were prepared by pipetting 10 μL of 10 mM PA solution onto a plasma cleaned holey carbon TEM grid (Electron Microscopy Sciences), blotted and plunged into liquid ethane using a FEI Vitrobot Mark IV. Samples were kept at -180°C and imaged using a JEOL 1230 TEM.

Circular Dichroism CD was done using a model J-715 Jasco Circular Dichroism Spectrometer. 30 μL of 10 mM PA were gelled with 3 μL of 0.2 M $CaCl_2$ to make gels. After sitting for 15 minutes to allow the gel to mature, 30 μL of each gel was diluted with water to 1 mL to yield a final PA concentration of 0.3 mM. Each trace represents the average of five scans. Red-shifting was calculated by taking the average of the positive CD peak minus 195 nm and the negative CD peak minus 216 nm.

FTIR: Borosilicate float glass (Fisher Scientific) was cut into 14×25 mm sections and ultrasonicated for 20 minutes in water (Millipore filtered, resistivity 18.2 MΩ-cm) and 20 minutes in isopropanol (Mallindrokt). A 50 nm layer of gold was then sputter coated (Desk III, Denton Vacuum Corp) onto the cleaned glass. Undoped silicon wafers (University Wafer, 3,000-3,500 ohm-cm resistivity) were cut into 10×20 mm sections and sonicated in the same conditions as the float glass. All substrates were plasma cleaned for five minutes before being dipped into a 1 millimolar solution of PA in water (Millipore filtered, resistivity 18.2 MΩ cm) and drawn out at a rate of 15 mm/min. In some cases

the substrates had to be dipped several times to get a usable FTIR signal. FTIR spectra were collected using a Thermo Nicolet, Nexus 870 FT-IR, baseline corrected using the Omnic FTIR software, and the area under the peaks were fit using a Lorentzian function in Origin. AFM was done on representative FTIR samples to verify that the PA fibers formed a flat layer less than 50nm thick on the gold substrate surface (supporting information). The samples were imaged on a JEOL 5200 scanning probe microscope using Asylum Research AC240TS cantilever tips.

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(1) Byler, D. M.; Susi, H. *Biopolymers* **1986**, *25*, 469-487.