Supporting Information to Accompany:

Understanding self-assembled amphiphilic peptide supramolecular structures from primary sequence helix propensity.

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The lyophilized peptide samples were obtained from Protein and Chemistry Facility, Institute of Biochemistry, University of Lausanne. We were able to obtain consistent results with different peptide batches obtained from the company mentioned above over time.

Peptide Synthesis and Purification (as provided by Protein and Chemistry Facility, Institute of Biochemistry, University of Lausanne).

Reagents and solvents. All amino acid derivatives were obtained from Merck, Novabiochem (Läufelfingen Switzerland) and other reagents from Fluka and Sigma Chemie (Buchs, Switzerland).

Peptide synthesis. The peptide was chemically synthesized using solid phase Fmoc chemistry on an Applied Biosystems 433 A Synthesizer. The peptide was prepared using the Rink Amide MBHA Resin (Merck, Novabiochem, Switzerland). The synthesis was performed using a five-fold excess of Fmoc amino acid derivatives, DCCI and HOBt as activating agents and a 60 min coupling time. Side chain protecting groups included: t-butyloxycarbonyl group for Lys. The peptide was deprotected and cleaved from the resin by treatment with 2.5% H2O, 5% triethylsilan in TFA for 2h at room temperature. After removal of the resin by filtration, the peptide was precipitated with tert-butyl methyl ether, centrifuged and the pellet resuspended in 50% acetic acid/ H20 (v/v) and lyophilized. The lyophilized material was then subjected to analytical HPLC and MALDI-TOF MS analysis.

Peptide purification. Crude peptide was reconstituted in 5 ml 50% acetic acid in H2O and low molecular weight contaminants were removed by gel filtration on Sephadex G-25. The materials eluted in the void volume were lyophilized, reconstituted in 5 ml 50% acetic acid in H2O and subjected to RP-HPLC on a Vydac column (250 x 22 mm, 10-15 µm). The column was eluted at a flow rate of 9 ml/min by a linear gradient of 0.1%TFA/acetonitrile on 0.1%TFA/H2O, rising within 60 min from 10% to 100%. The optical density of the eluate was monitored at 220 or 280nm. Fractions were collected and analyzed by mass spectrometry. Fractions containing the peptide of the expected molecular weight were pooled and lyophilized. The purified material was then subjected to MALDI-TOF MS and analytical HPLC on a C18 nucleosil column (250 x 4mm, 5 µm).

Peptide analysis by analytical HPLC (as provided by Protein and Chemistry Facility, Institute of Biochemistry, University of Lausanne).

The lyophilized peptide was dissolved in 50% acetic acid/ H20 (v/v) at a concentration of 1 mg/ml and 50 µl of this peptide solution was subjected to analytical HPLC on a C18 nucleosil column (250 x 4mm, 5 µm). The column was eluted at 1ml/min by a linear gradient of acetonitrile on 0.1%TFA/H2O, rising within 30 min from 0 to 100%. The detection was performed at 220 and 280 nm using a Waters 991 photodiode array detector (Supplemental Figures 1a, 2a and 3a).

Mass Spectrometry (as provided by Protein and Chemistry Facility, Institute of Biochemistry, University of Lausanne).

Materials. α-cyano-4-hydroxycinnamic acid was purchased from Sigma (Sigma Chemical Co., St. Louis, Mo, USA). High performance liquid chromatography (HPLC)-grade trifluoroacetic acid was purchased from Fluka (Buchs, Switzerland), HPLC-grade H_2O from Romil Ltd (Amman Technik SA, Kölliken, Switzerland) and acetonitrile was purchased from Biosolve Ltd (Chemie Brunschwig, Basel). All other chemicals were of highest purity and were used without further purification. MS analyses were performed using a Perseptive Biosystems MALDI-TOF Voyager DE-RP Mass Spectrometer (Framingham, MA, USA) operated in the delayed extraction and linear mode. The lyophilized peptide was dissolved in 50% acetic acid/ H_20 (v/v) at a concentration of 1 mg/ml , mixed with the matrix (α-cyano-4-hydroxycinnamic acid) at 1:20 (v/v) and was then subjected to analysis by MALDI-TOF (Supplemental Figures 1b, 2b and 3b).

Sample Preparation. From the lyophilized peptides stock solutions (I6K2 1 mM, L6K2 0.03 mM, V6K2 0.5 mM) were prepared in 2 mM NaCl salt solution and diluted to the desired concentration series to study the concentration dependence of the aggregation. For I6K2 and V6K2 peptide concentrations 0.5 mM, 0.25 mM, 0.1 mM, 0.05 mM, 0.025 mM, 0.01 mM and 0.005 mM were examined and for L6K2 0.03 mM, 0.01 mM, 0.005 mM, 0.001 mM. Samples were then incubated for 24 hours at 4 °C prior to characterization.

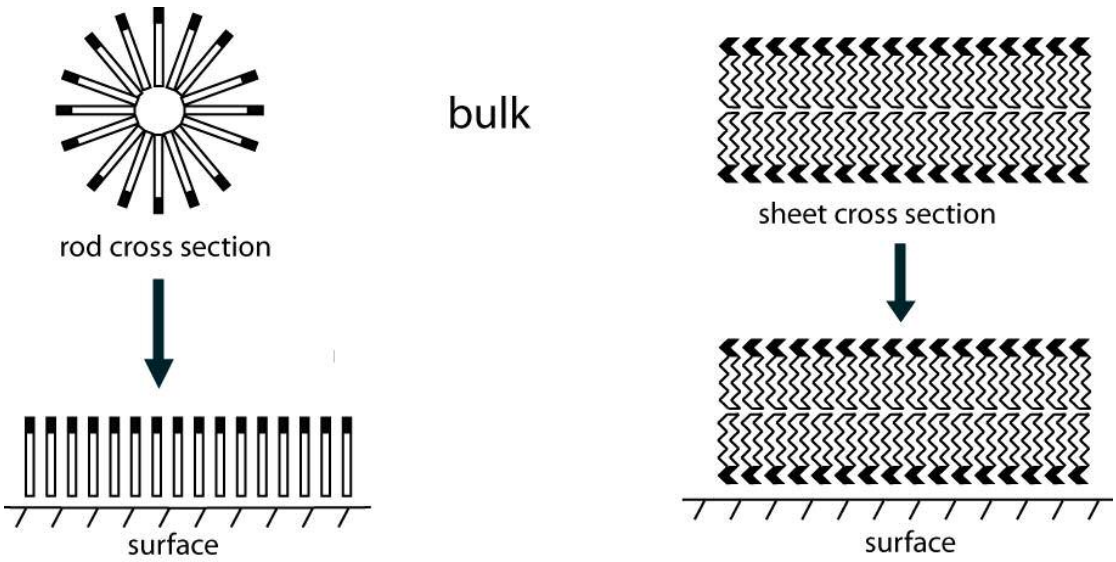
Circular dichroism spectroscopy (CD). CD spectra of the peptide solutions were recorded at 20° Celsius on a Jasco Spectropolarimeter J715 in a 1 cm cell. Spectra were obtained from 190-250 nm and all spectra were solvent subtracted. Spectra were then converted to mean residue ellipticity by using the following equation:

$$\theta_M = \frac{\theta_{obs}}{10} \cdot \frac{MRW}{c \cdot l}$$

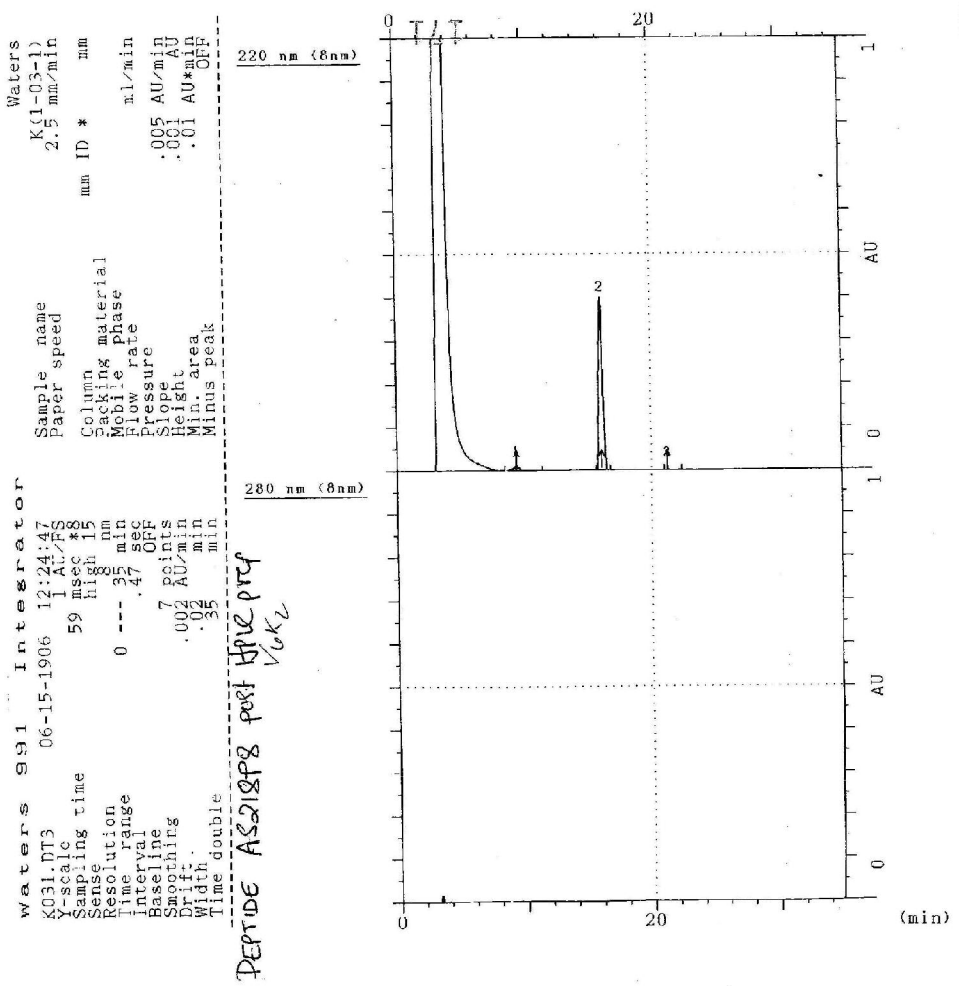
Θ_M: Mean residue ellipticity [deg cm^2/dmol]
Θ_{obs}: Q_{obs} is the observed ellipticity corrected for the buffer at a given wavelength [mdeg]
MRW: Mean residue molecular weight (Mw / number amino acids)
c: Peptide concentration [mg/mL]
l: Pathlength [cm]

Transmission electron microcopy (TEM). The specimens were prepared from the aqueous solutions prior to CD spectroscopy experiments. 20 µL of the peptide solutions were adsorbed for 1 minute on a glow-discharged carbon coated 400 mesh copper TEM grid, washed twice with a droplet of deionized ultra pure water and stained with 20 µL 1% uranyl acetate solution for 30 seconds. The specimens were examined using a FEI Morgagni 268 transmission electron microscope equipped with a tungsten emitter and operated at a voltage of 100kV. Images were recorded via CCD. As control, samples were also stained with phosphoric tungsten acid using the same protocol as above. No differences in supramolecular structures were observed.

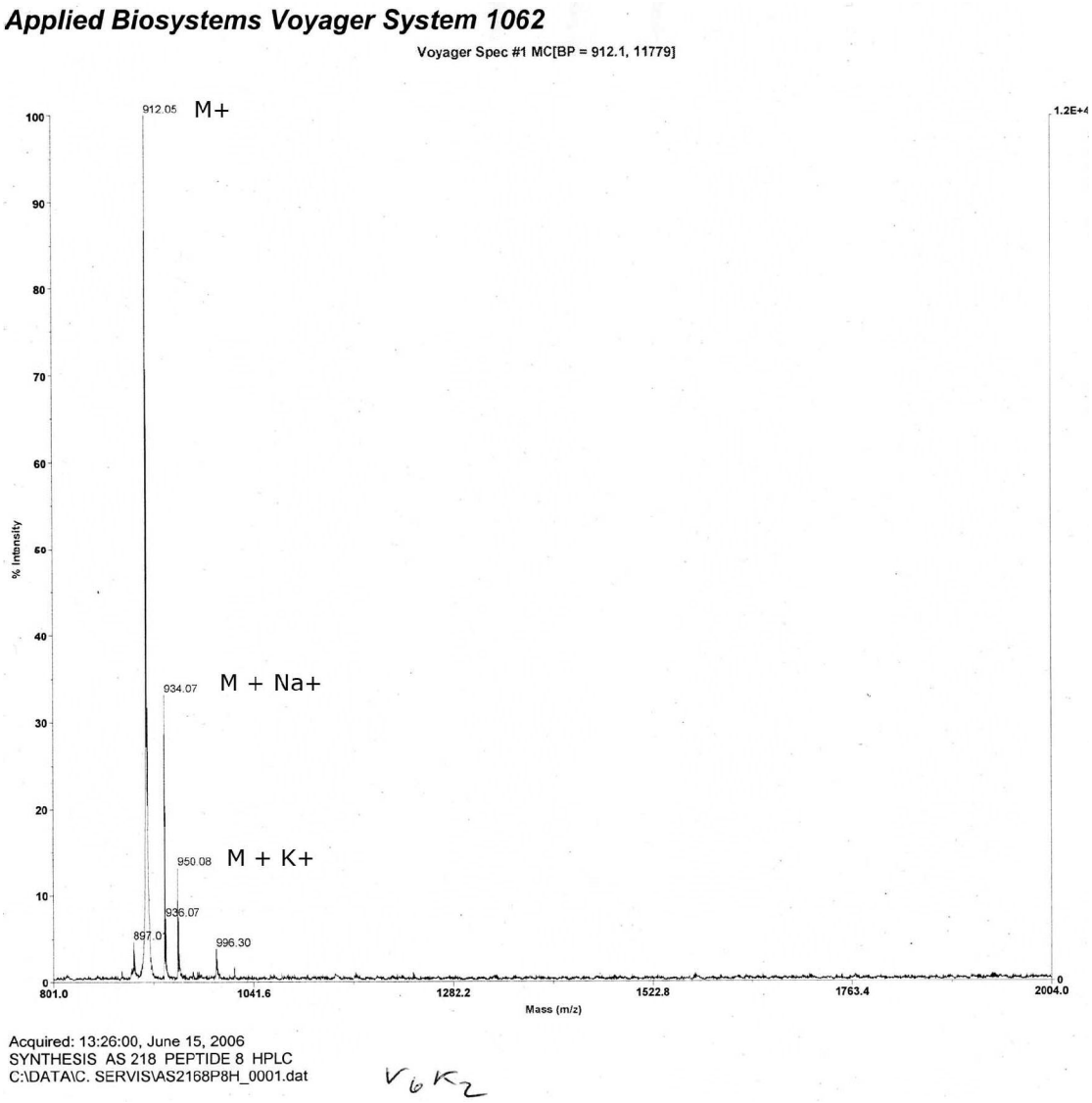
Atomic force microscopy (AFM). AFM operated in tapping mode was used as complementary technique to characterize the supramolecular structures found with TEM. Peptide solutions were characterized using NanoScope IIIa scanning probe work station equipped with MultiMode head using a JV Series piezoceramic scanner (Digital Instruments, Santa Barbara). AFM probes were silicon micro cantilevers with 42 N/m spring constant model OMCL-AC160TS (Olympus Corporation, Japan), a resonance frequency of 300 kHz and a tetrahedral tip shape with Al reflex coating. Samples were imaged dry in air. Micrographs were acquired at 1Hz scan rate. 100µL peptide solution was adsorbed for 30 min on freshly cleaved mica, rinsed with deionized MilliQ water and air dried under laminar flow prior to examination. V6K2 had to be adsorbed on a carbon coated mica leaflet, since no adsorption could be detected on mica alone. Control measurements were done on TEM grids following the same protocol as for TEM specimen preparation but excluding the staining step.



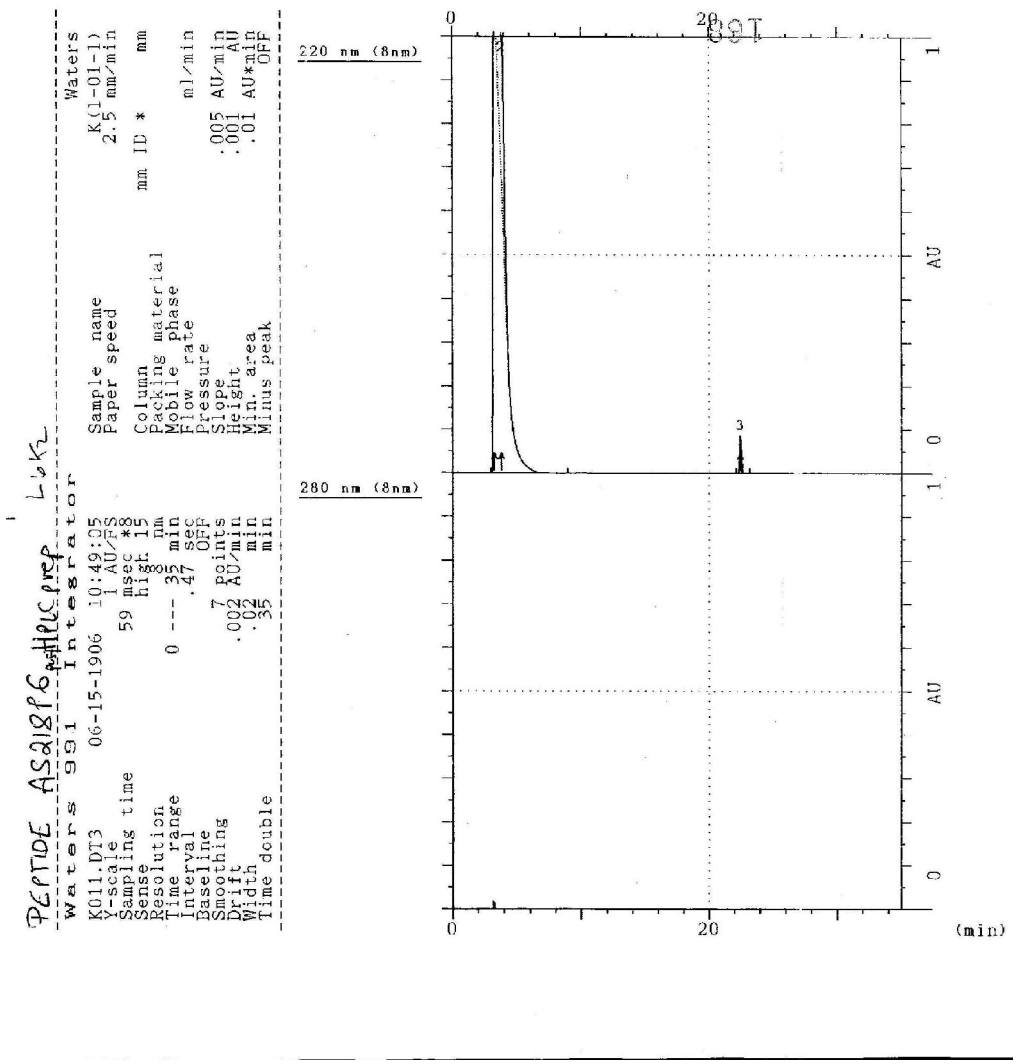
Supporting information Schematic 1. Schematic side-view representation of the adsorption and collapse of structure after adsorption onto surfaces. For rod-like structures where only hydrophobic interactions play a role during self-assembly, opening and adsorption of the rod with their hydrophobic tails exposed to the surface is assumed. This would be consistent with the observed monomer height observed on the surface, which due to the exposed hydrophobic tails would be energetically unfavorable in solution. For the proposed bilayer ribbons where β-sheet interactions add to the stability of the structures adsorption of the intact bilayer is assumed, since this structure allows for conservation both of internal β-sheet hydrogen bonding and low exposure of the aliphatic tail region to the aqueous solution in bulk.



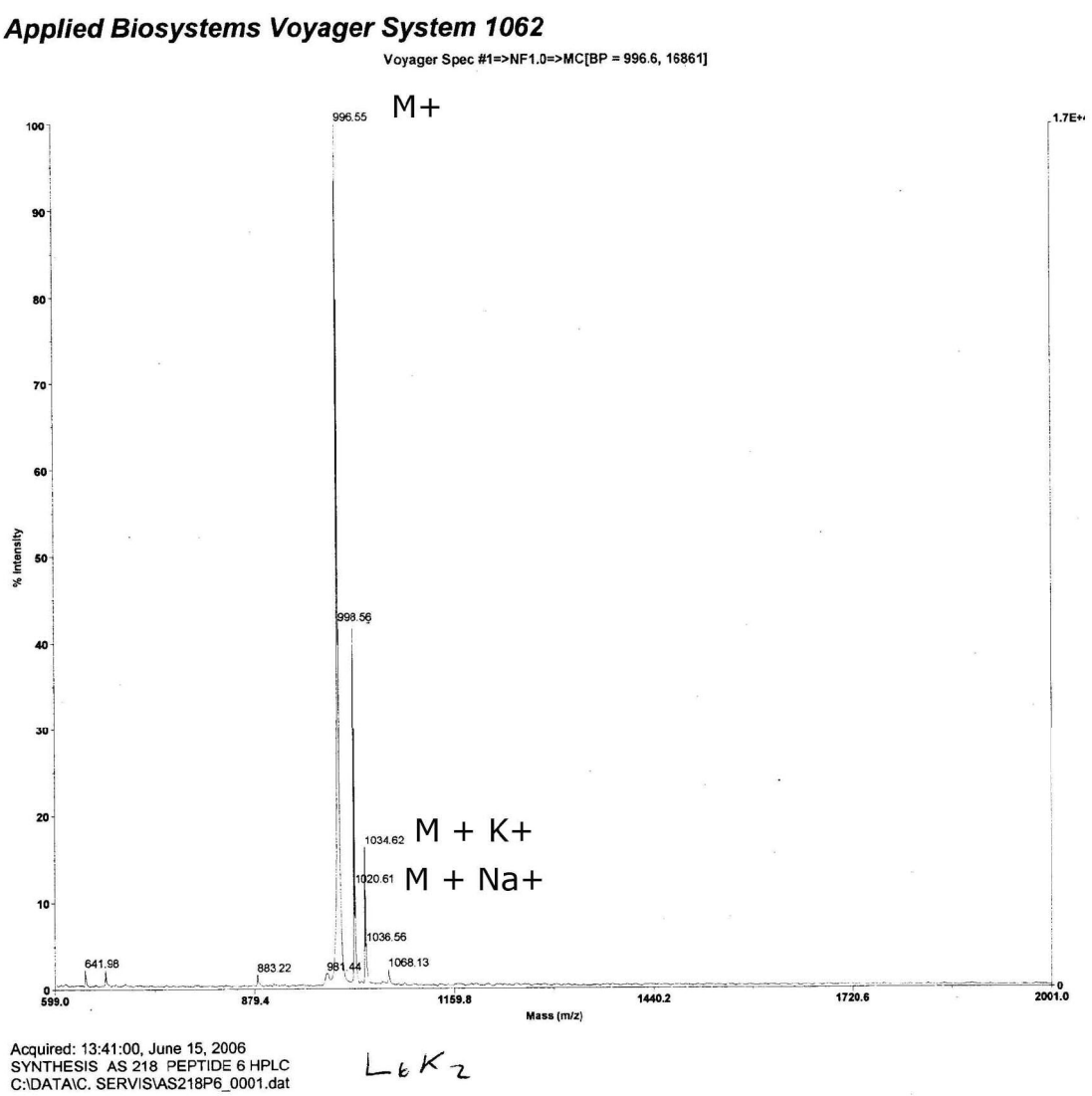
Supplemental Figure 1a Analytical HPLC elution profile of HPLC purified V6K2



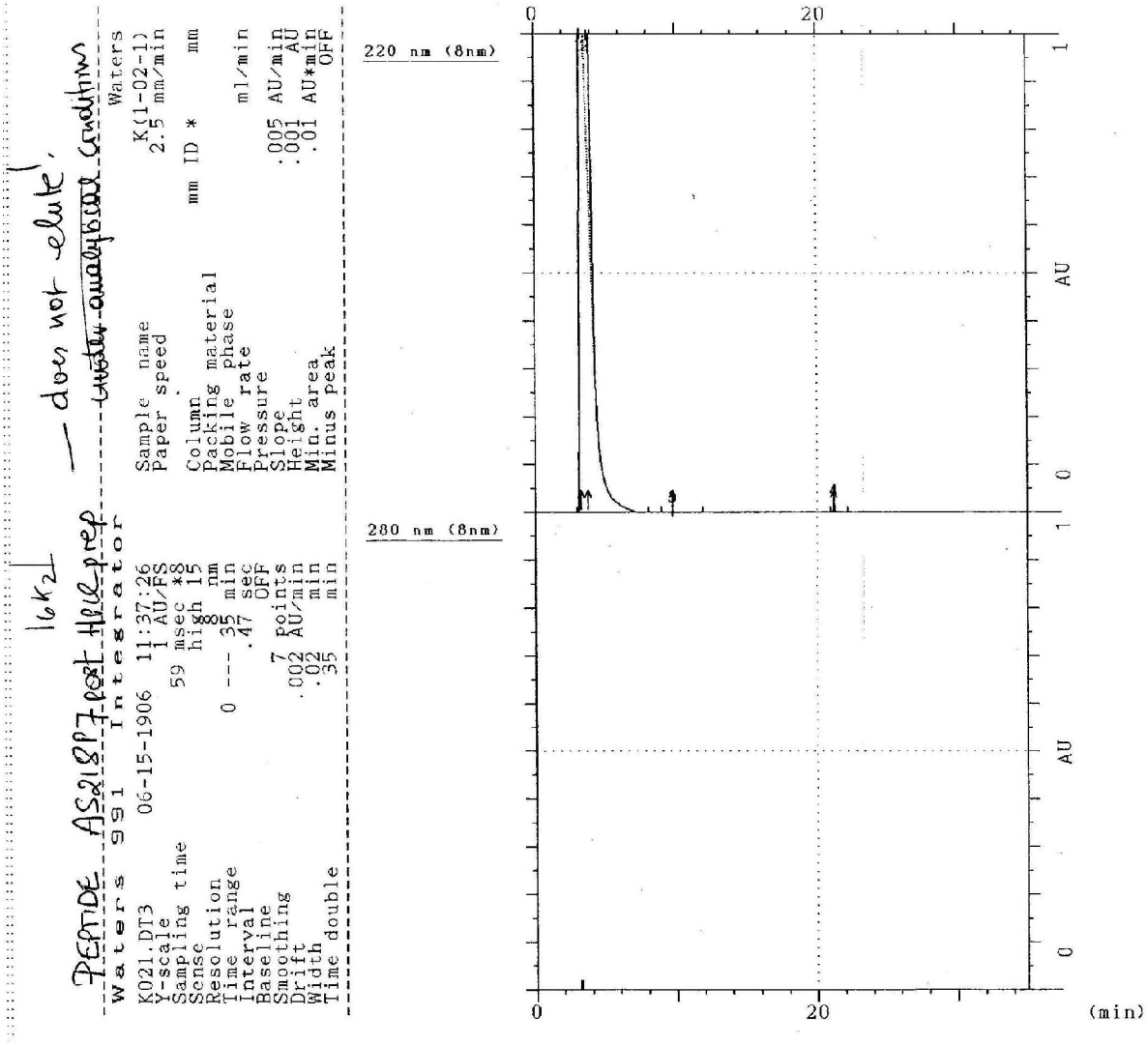
Supplemental Figure 1b. MS of HPLC purified V6K2 (MW 910.2)



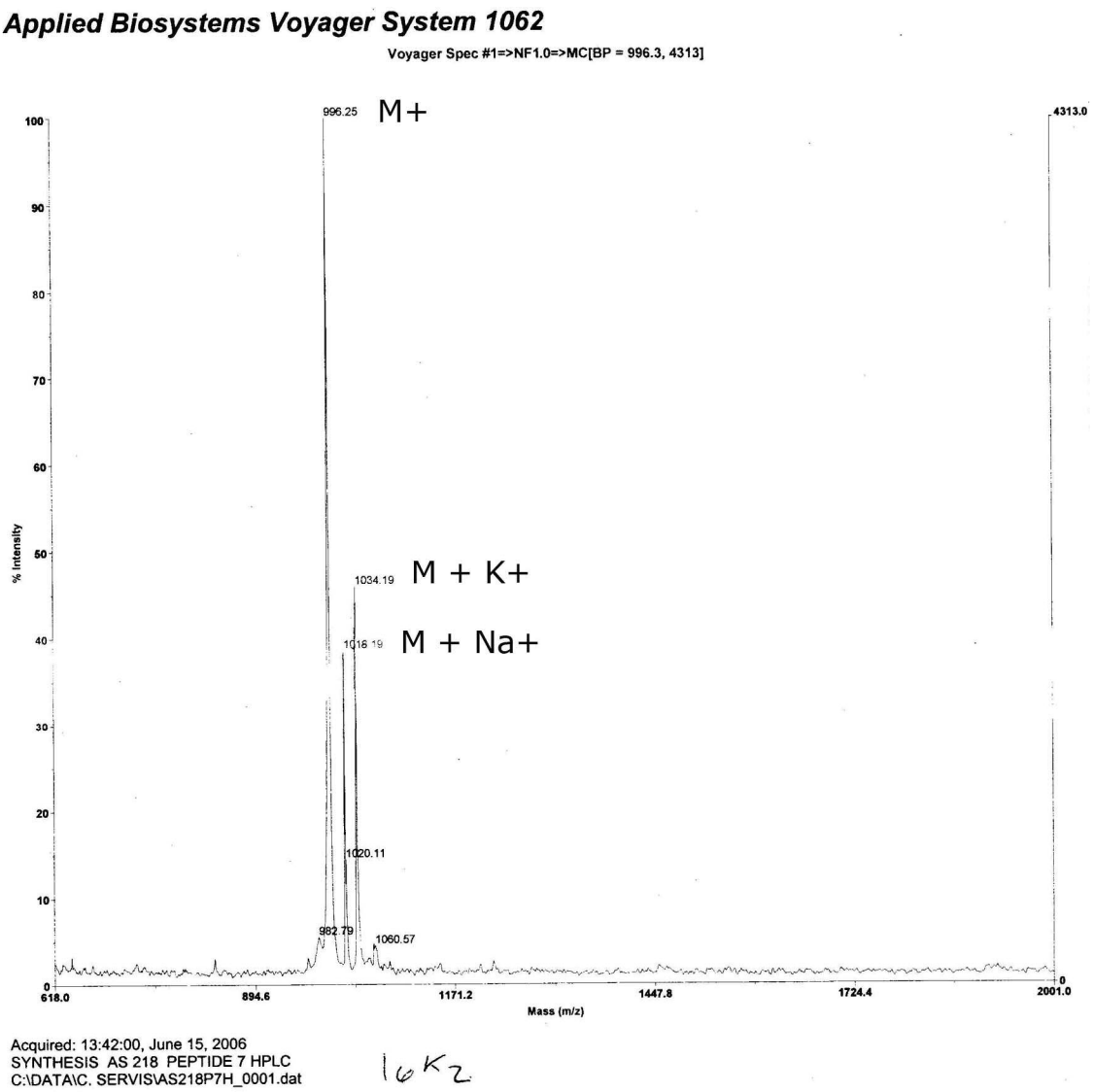
Supplemental Figure 2a. Analytical HPLC elution profile of HPLC purified L6K2.



Supplemental Figure 2b. MS of HPLC purified L6K2 (MW 994.4).



Supplemental Figure 3a. Analytical HPLC elution profile of I6K2. Due to the hydrophobic nature of the peptide amphiphile it was impossible to isolate I6K2 with reverse phase HPLC. Since the MALDI-ToF of the gathered fraction shows the expected mass peaks and no major impurities the authors are confident with the sample quality.



Supplemental Figure 3b. MS of used fraction I6K2 (MW 994.4).