Supplementary document for the manuscript “Effect of Amino Acid Sequence and pH on Nanofiber Formation with the Self-Assembling Peptides EAK16-II and EAK16-IV”

A variety of approaches have been used to verifythe secondary structure of EAK16-II and EAK16-IV including circular dichroism (CD),[1S,2S] and Fourier Transform Infrared (FT-IR) spectroscopy. The peptide solutions were prepared using pure water, and pH was adjusted using 0.1 N hydrochloric acid and sodium hydroxide. A couple of drops of the peptide solution were deposited onto a crystal slide of zinc selenide (ZnSe) and dried at room temperature. The FT-IR spectrum of this thin film was taken using a spectral resolution of 4 cm^{-1}. The baseline was subtracted from the observed absorption intensity and normalized to the maximum intensity in the range of 1600 to 1700 cm^{-1} where the characteristic amide I band appears (see Figure 1S and 2S). Figure 1S shows FT-IR spectra of EAK16s at pH 7. EAK16-IV has a broad peak at ca. 1675 cm^{-1}, which is not observed for EAK16-II. This particular peak is normally attributed to the -turn structure according to the literature.[3S] There may be some concern that this peak is due to the presence of trifluoroacetate (TFA) introduced during the synthesis. However, both of EAK16-II and EAK16-IV should have shown similar peaks if this peak were from TFA, since these peptides were synthesized by the same procedure.

FT-IR spectra of EAK16s at pH 4 in Figure 2S clearly show that there is no significant difference between EAK16-II and EAK16-IV at pH 4. The normalized spectrum was fitted with Gaussian curves to identify the secondary structures. The areas occupied by the Gaussian curves were calculated to show the relative contents of different secondary structures, which is summarized in Table 1S. The FT-IR spectrum of EAK16-IV at pH 7 shows a large portion of -turn structure (28.0 %) compared to EAK16-II at the same pH (0.4 %). When the pH of the solution was lowered to pH 4, the content of -turn structure was significantly reduced (9.4 %) for EAK16-IV, and no significant difference compared to that of EAK16-II at the same pH (5.6 %) was observed. The assignments and the following quantitative analysis of each peak were made according to procedures reported in the literature:[3S] The strong peak between 1620 and 1640 cm^{-1}, and high frequency peak around 1690 cm^{-1} were assigned to those of -sheet structures, and the peaks around 1650 cm^{-1} were assigned to -helix.

The -turn structure is reflected at around 1675 cm^{-1}. It is clear that the -turn peak at 1675 cm^{-1} shown in the FT-IR spectrum of EAK16-IV at pH 7 disappears in comparison to EAK16-II at the same pH. Since this peak is attributed to -turn structure, these FT-IR spectra strongly suggest the possibility of bending of the EAK16-IV molecules. More detailed study on the molecular structure will be performed shortly.

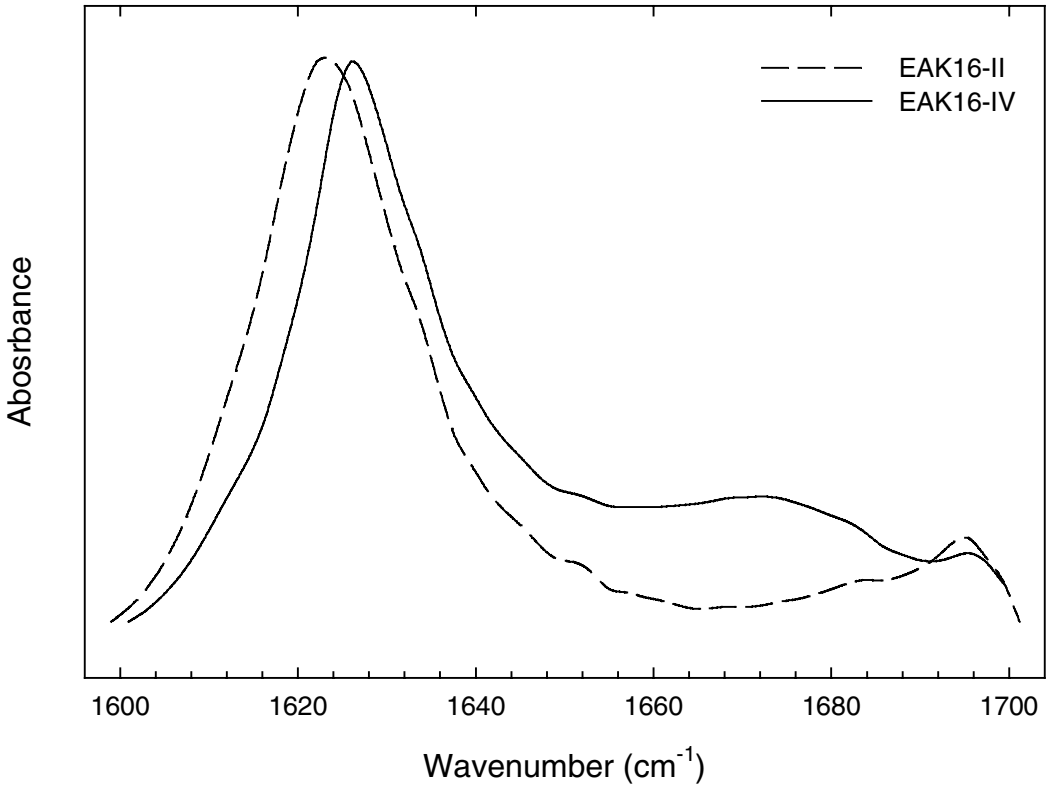

Figure 1S. FT-IR spectra of EAK16s at pH 7, within the amide I bands between 1600 and 1700 cm^{-1}.

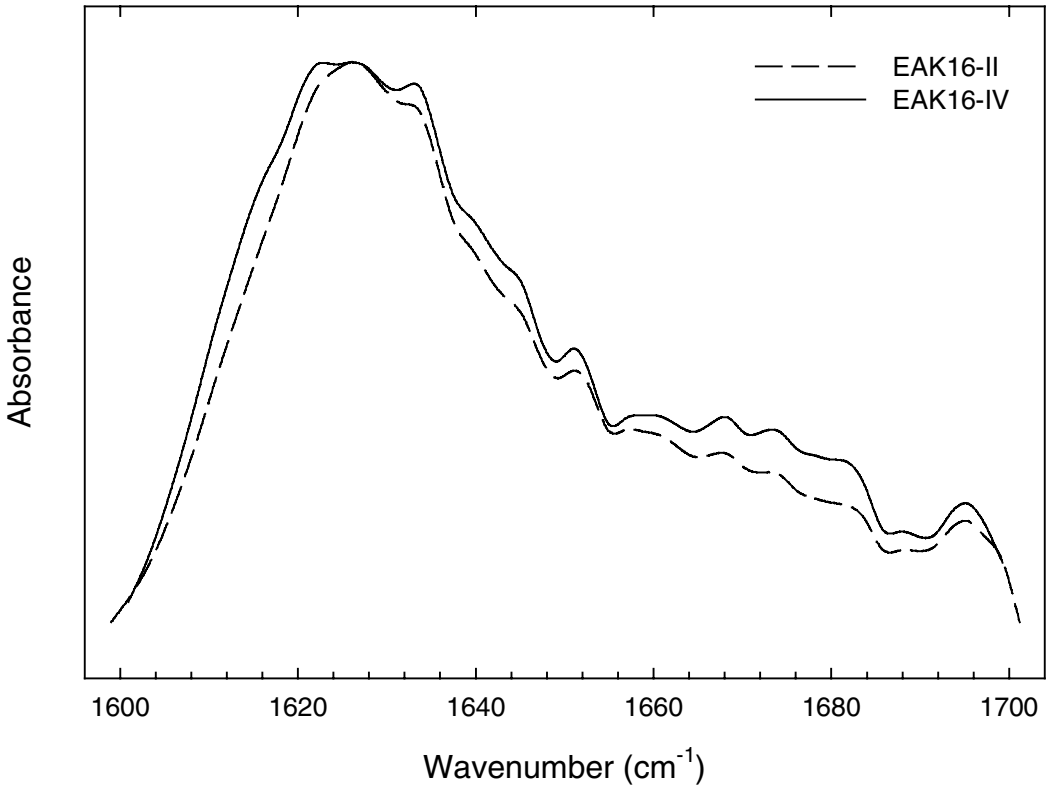

Figure 2S. FT-IR spectra of EAK16s at pH 4, within the amide I bands between 1600 and 1700 cm^{-1}.

		Content of the secondary structures (%)				
		-helix	-sheet	-turn	-turn/ -sheet	undefined
pH 7						
	EAK16-II	1.8	85.4	0.4	3.4	8.9
	EAK16-IV		72.0	28.0		
pH 4						
	EAK16-II	2.0	63.1	5.6	6.0	23.3
	EAK16-IV	4.1	72.6	9.4	8.2	5.7

Table 1S. Summary of the relative contents of secondary structures of EAK16s at pH 4, and 7 from the quantitative analysis of FT-IR spectra.

References

(1S) Altman, M.; Lee, P.; Rich, A.; Zhang, S. Conformational behavior of ionic self-complementary peptides. *Protein Sci*. **2000**, 9, 1095-1105.

(2S) Zhang, S.; Lockshin, C.; Cook, R.; Rich, A. Unusually stable -sheet formation in an ionic self-complementary oligopeptide. *Biopolymers* **1994**, 34, 663-672.

(3S) Benaki, D. C.; Aggeli, A.; Chryssikos, G. D.; Yiannopoulos, Y. D.; Kamitsos, E. I.; Brumley, E.; Case, S. T.; Boden, N.; Hamodrakas, S. J. Laser-Raman and FT-IR spectroscopic studies of peptide-analogues of silkmoth chorion protein segment. *Int. J. Biol. Macromol*. **1998**, 23, 49-59. and references therein.