Supporting Information for

Programming Two-component Peptides Self-assembly by Tuning the Hydrophobic Linker

Sangshuang Li, Huayang Liu, Yu Fang, Yaoting Li, Laicheng Zhou, Dinghao Chen, Juan Liang, Huaimin Wang*

Department of Chemistry, Westlake University; Institute of Natural Sciences, Westlake Institute for Advanced Study, 18 Shilongshan Road, Hangzhou 310024, Zhejiang Province, China

Content

S1. Supplemental figures

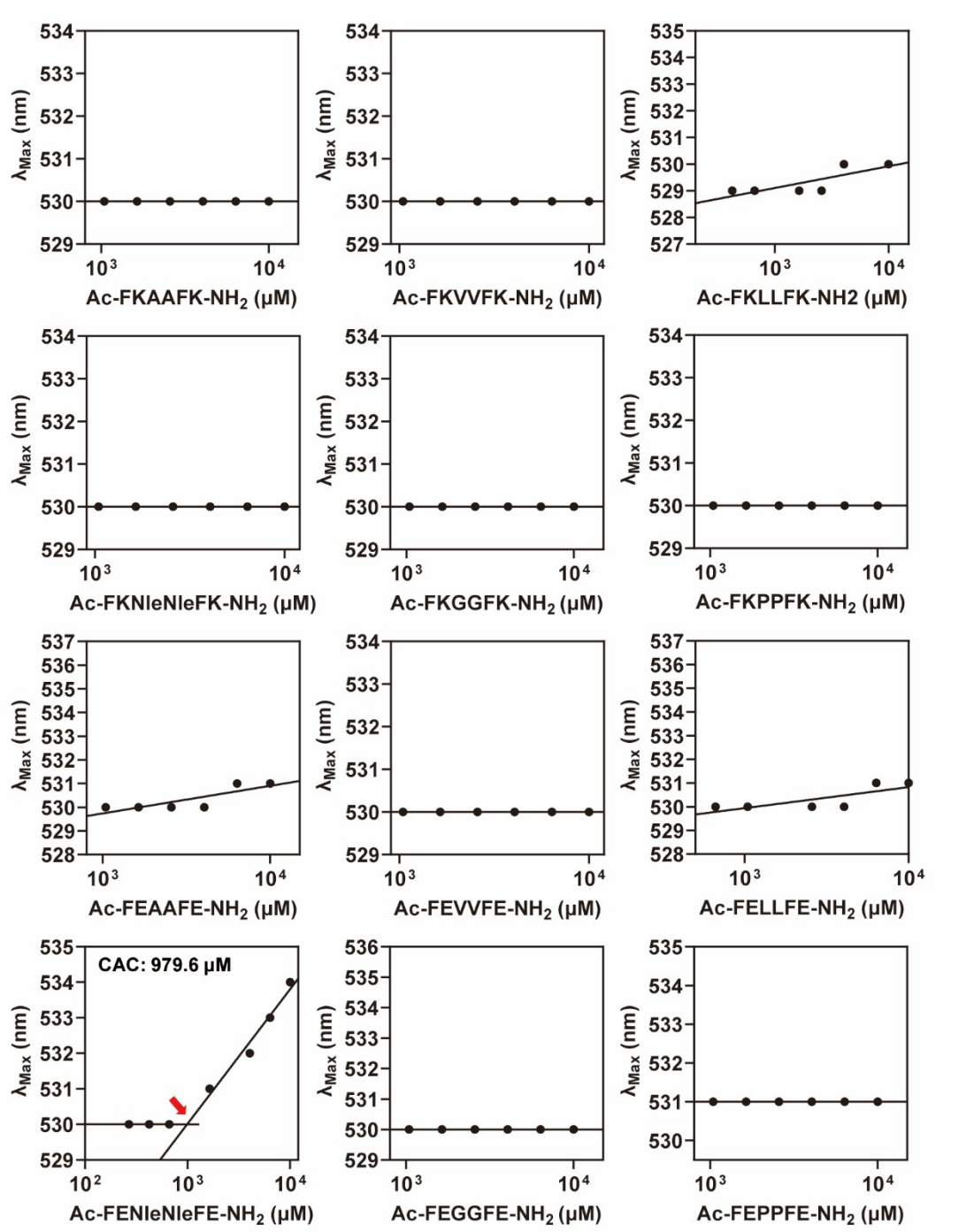

Figure S1. CAC results of the single peptides in an aqueous solution (pH=7.4) after 24 hours of incubation.

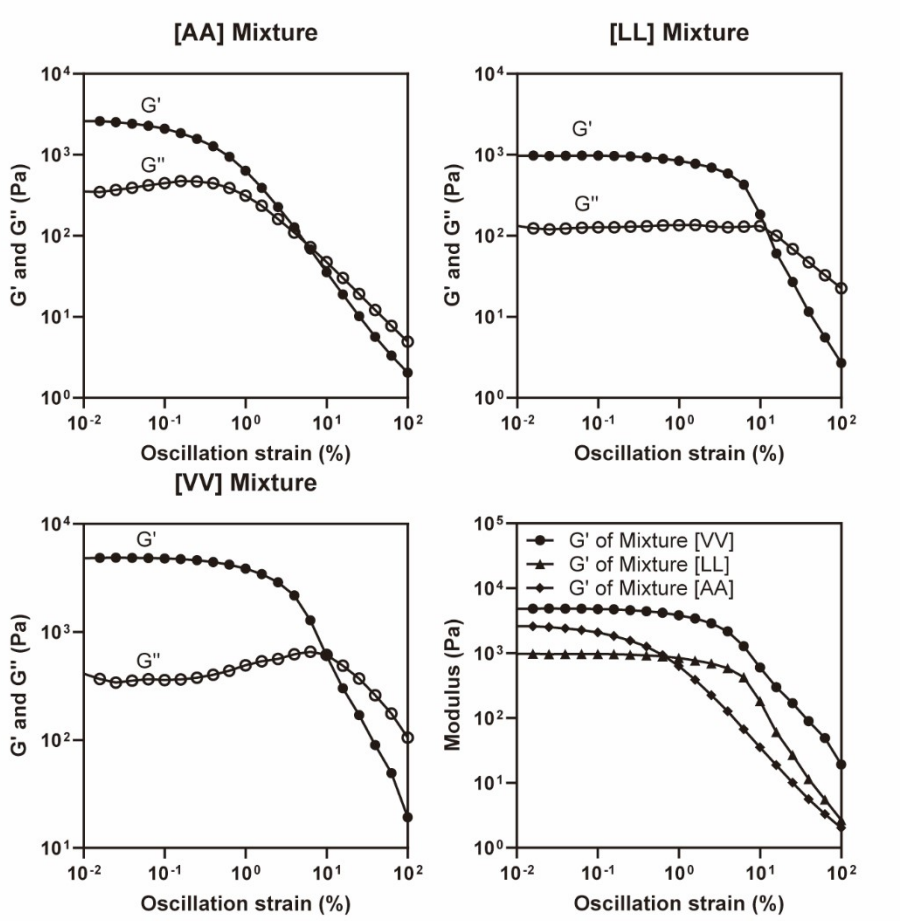

Figure S2. Strain sweep of the hydrogel (10.0 mM) formed by the [AA] mixture, [LL] mixture and [VV] mixture after stabilization in aqueous solution (pH 7.4).

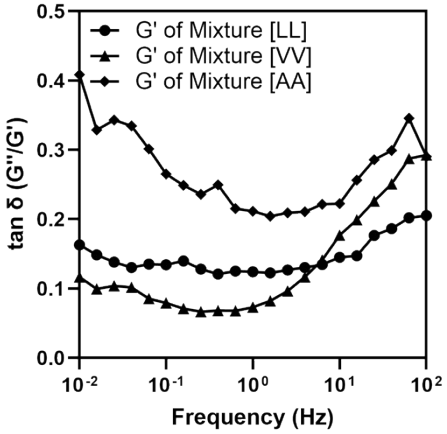

Figure S3. Loss factor (Tan δ) across the frequency range of the hydrogel (10.0 mM) formed by the [AA] mixture, [LL] mixture and [VV] mixture after stabilization in aqueous solution (pH 7.4) under the strain of 0.1%, and the G' of 1 Hz.

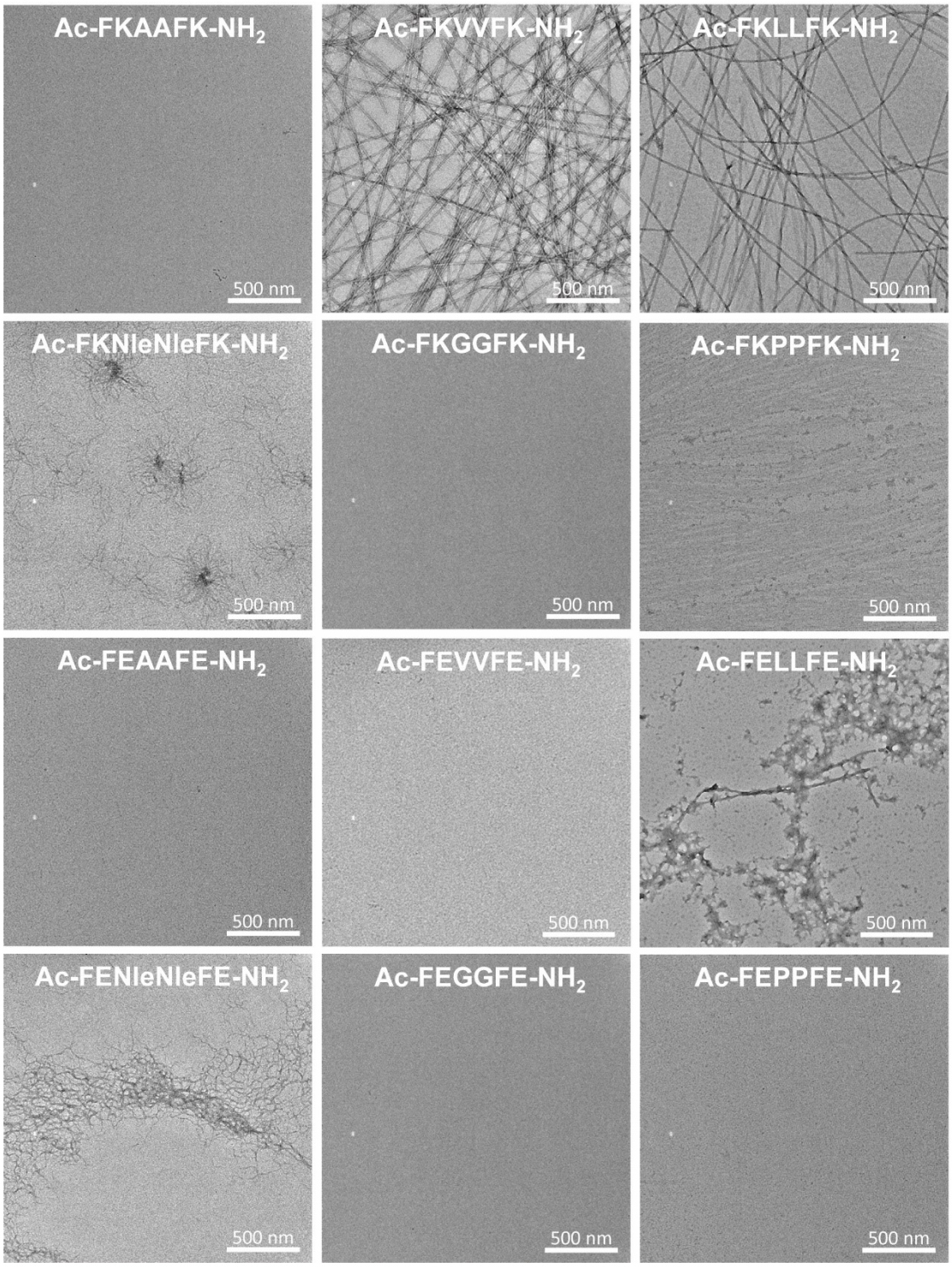

Figure S4. TEM image of the single peptides (10.0 mM) in an aqueous solution (pH 7.4).

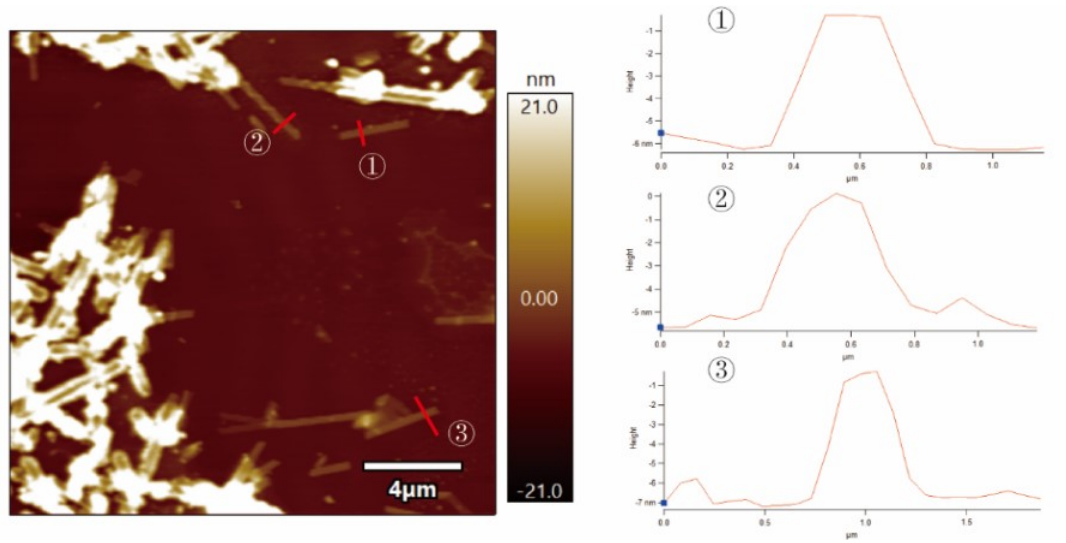

Figure S5. AFM image of the [AA] mixture (10.0 mM) in an aqueous solution (pH 7.4) and height measurement of a typical assembly at three locations 1, 2 and 3.

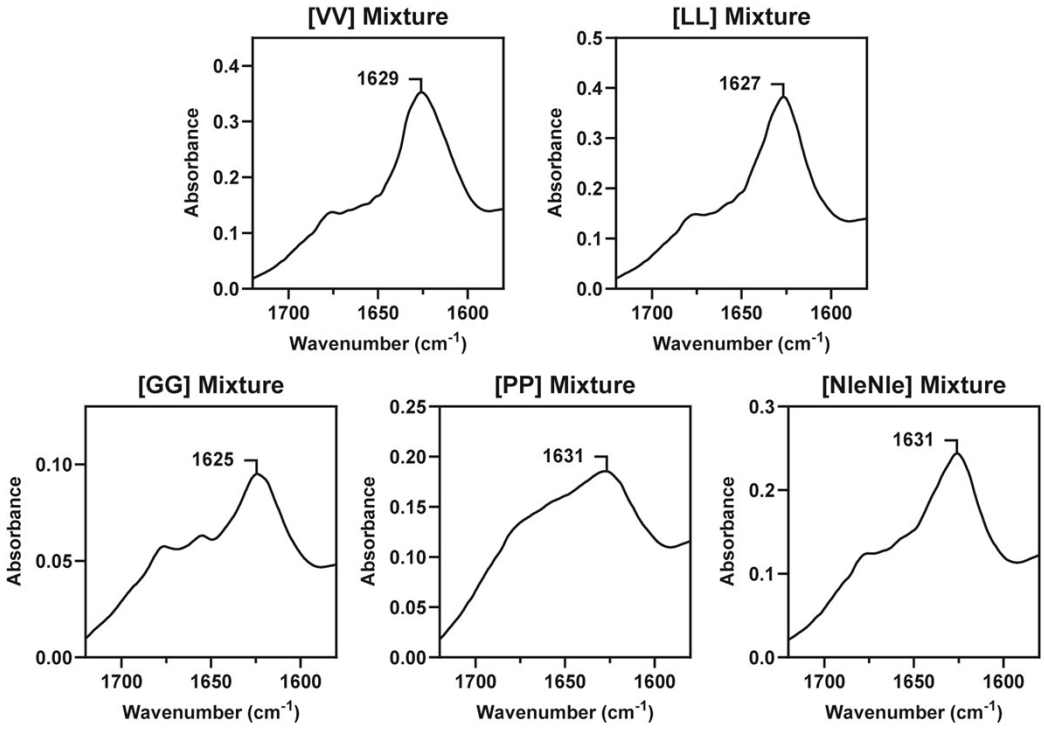

Figure S6. FTIR spectra of the two-component system (10.0 mM) in an aqueous solution (pH 7.4).

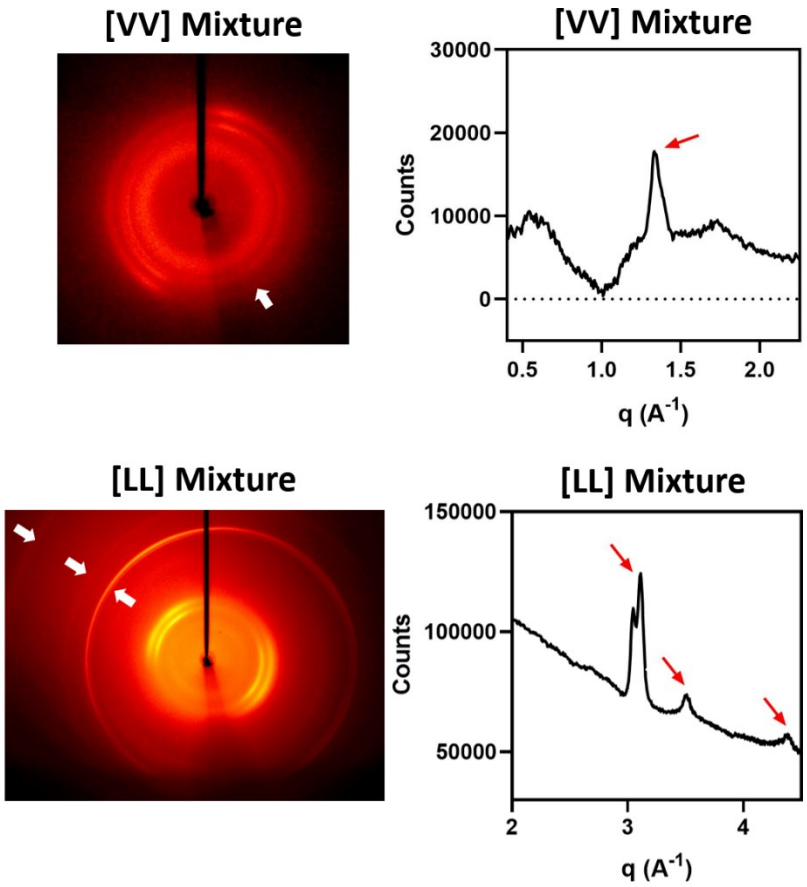

Figure S7. WAXS debye rings and integrated WAXS spectra of the hydrogel (10.0 mM) formed by the [LL] mixture and [VV] mixture after stabilization in aqueous solution (pH 7.4).

S2. Characterization and purity data for peptides

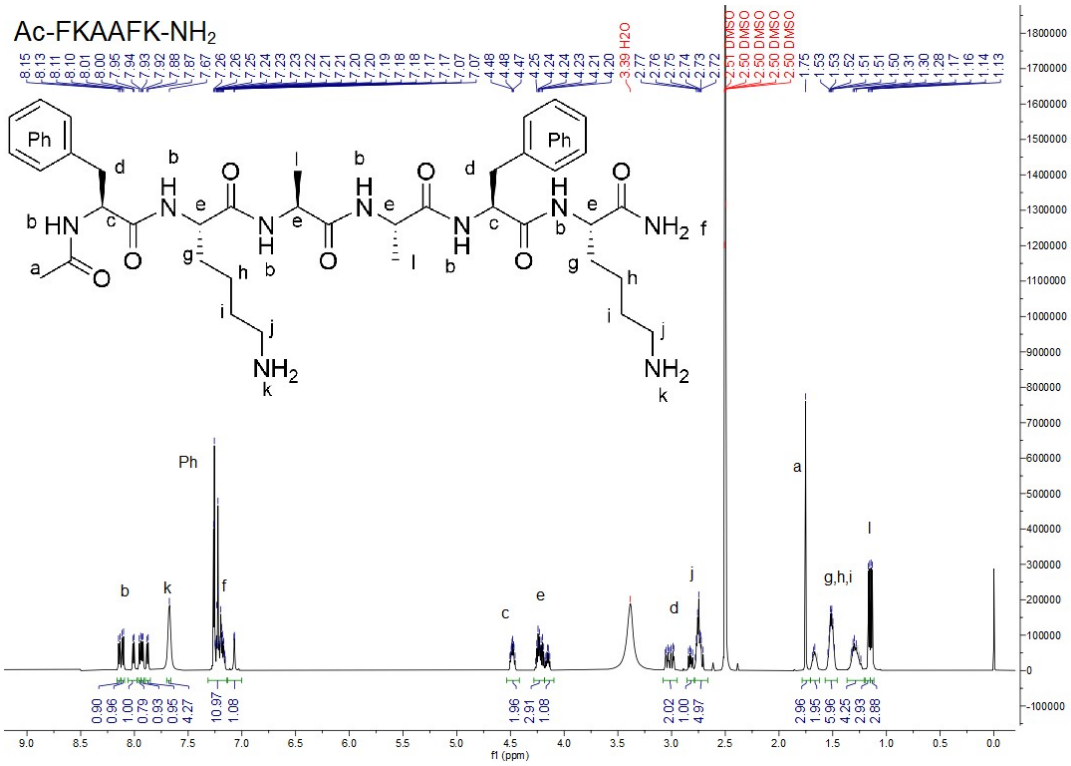

Figure S8. ^{1}H NMR of Ac-FKAAFK-NH_2 in DMSO-d_6.

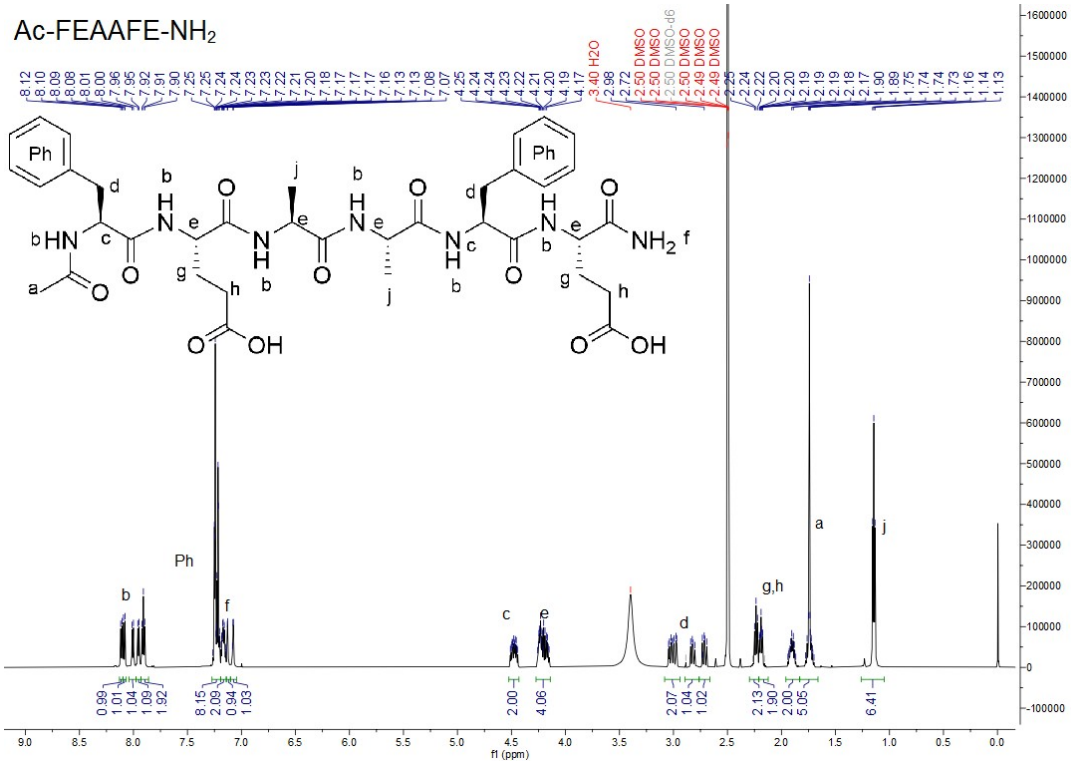

Figure S9. ^{1}H NMR of Ac-FEAAFE-NH_2 in DMSO-d_6.

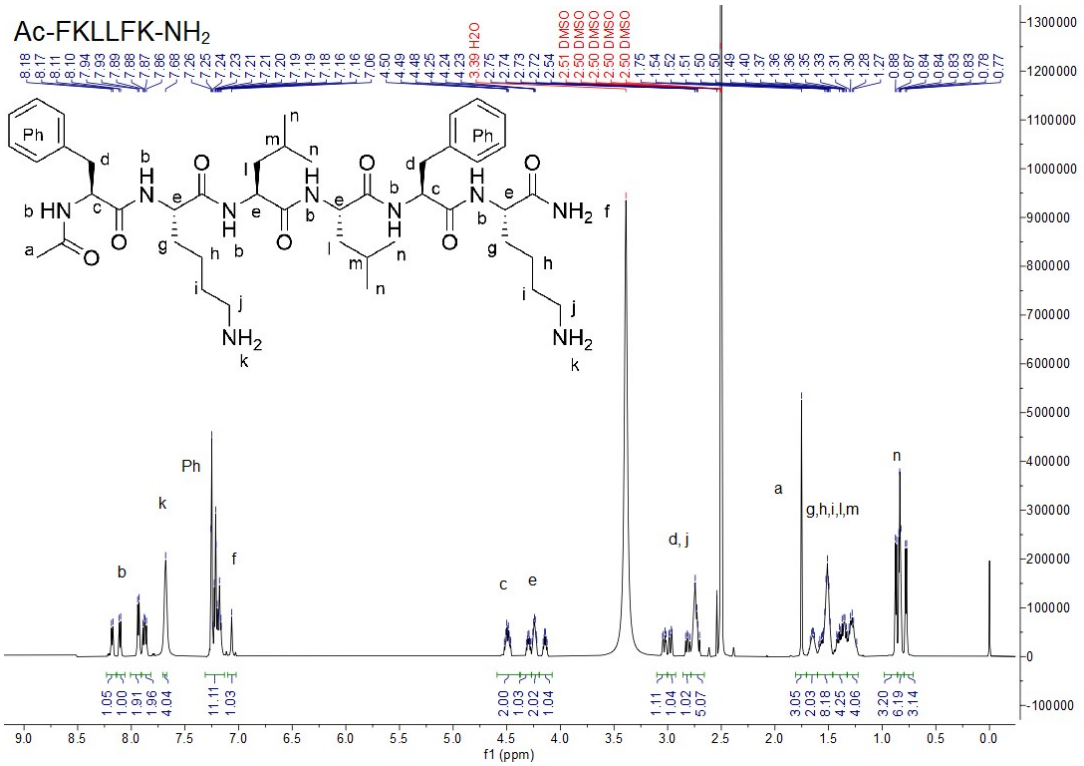

Figure S10. ^{1}H NMR of Ac-FKLLFK-NH_2 in DMSO-d_6.

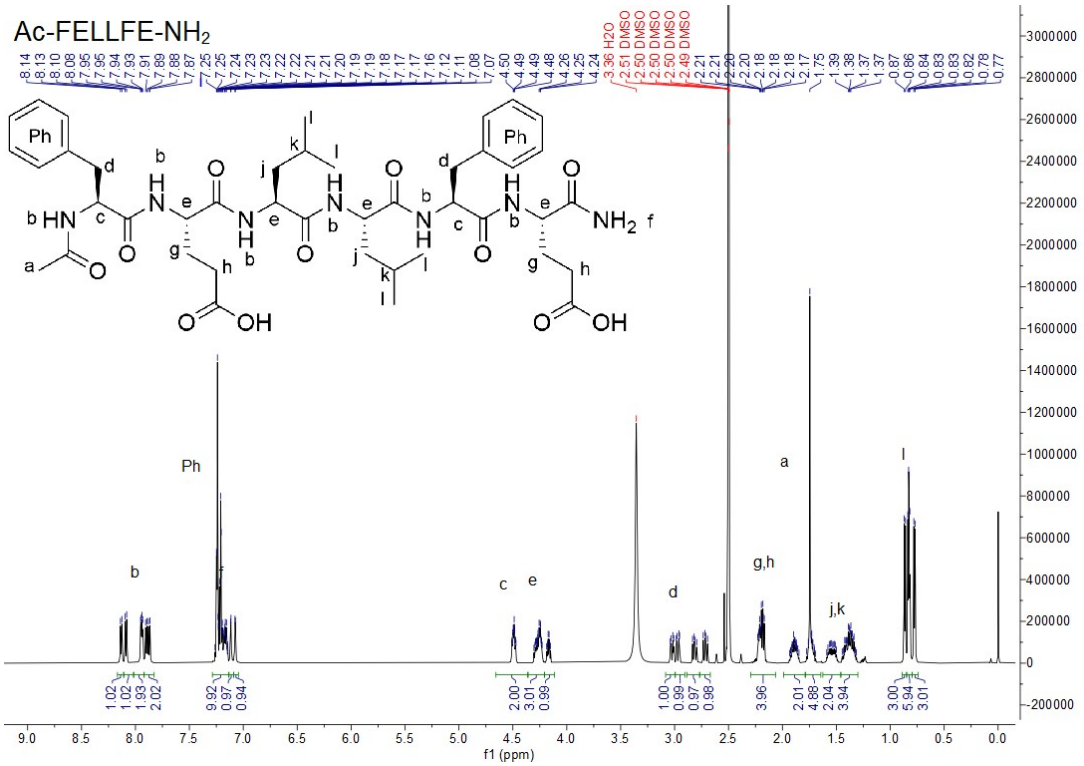

Figure S11. ^{1}H NMR of Ac-FELLFE-NH_2 in DMSO-d_6.

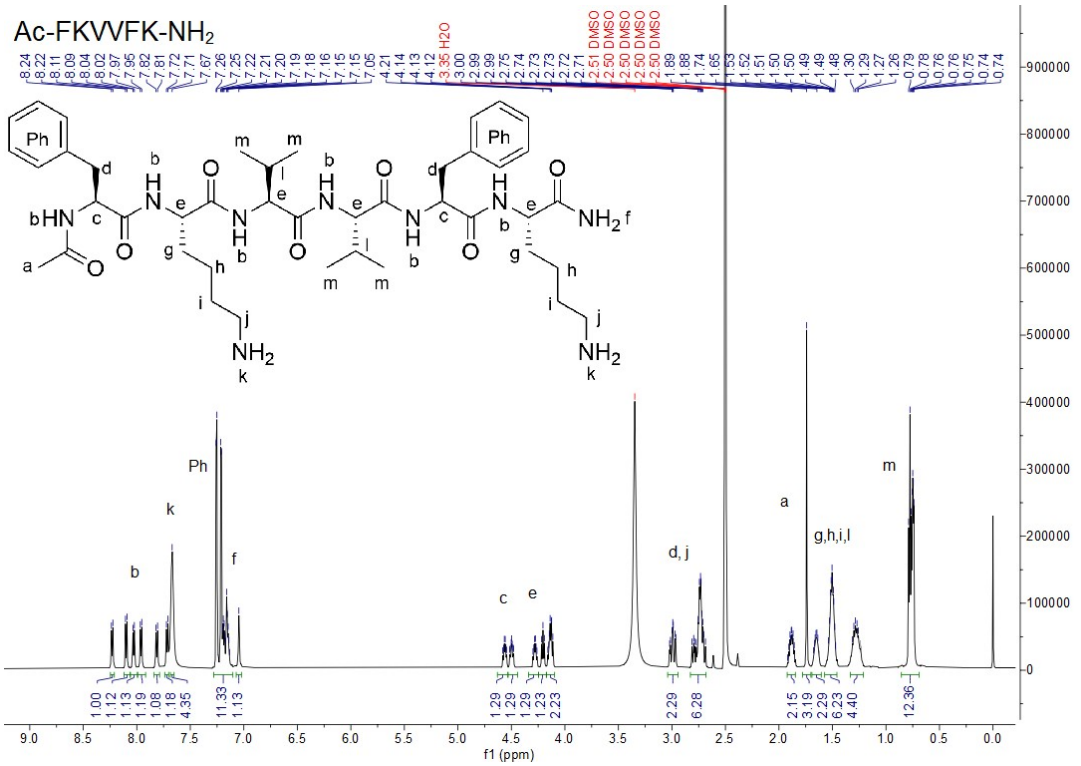

Figure S12. ^{1}H NMR of Ac-FKVVFK-NH_2 in DMSO-d_6.

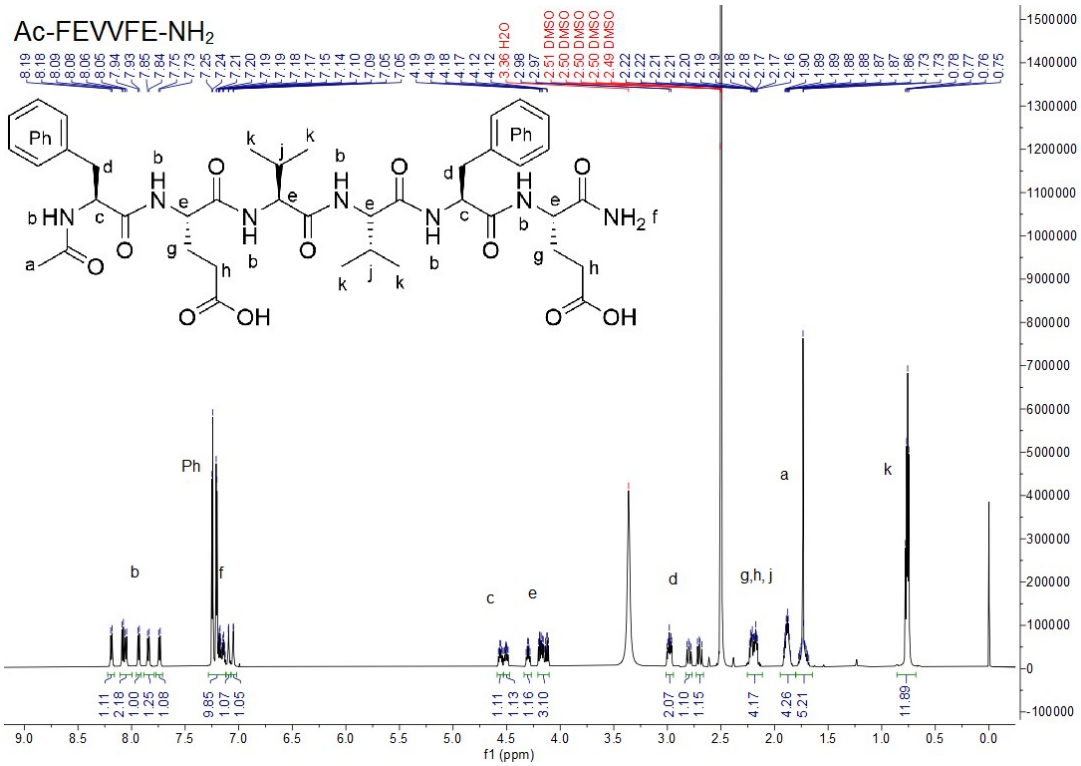

Figure S13. ^{1}H NMR of Ac-FEVVFE-NH_2 in DMSO-d_6.

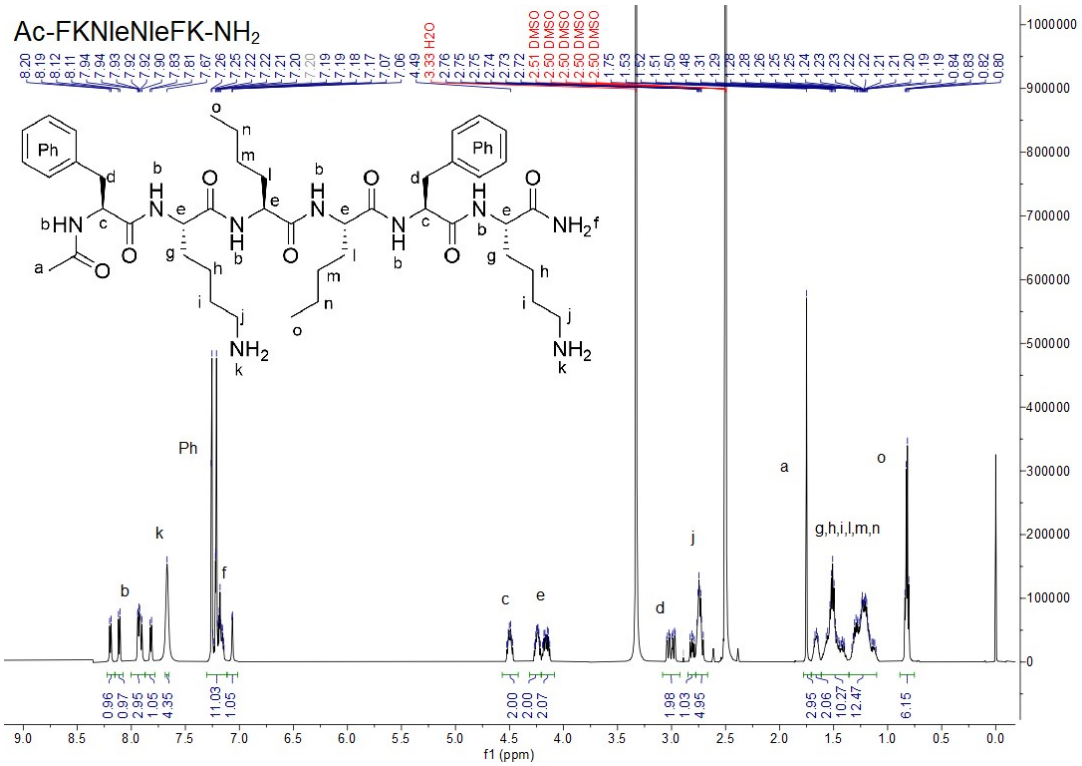

Figure S14. ^{1}H NMR of Ac-FKNleNleFK-NH_2 in DMSO-d_6.

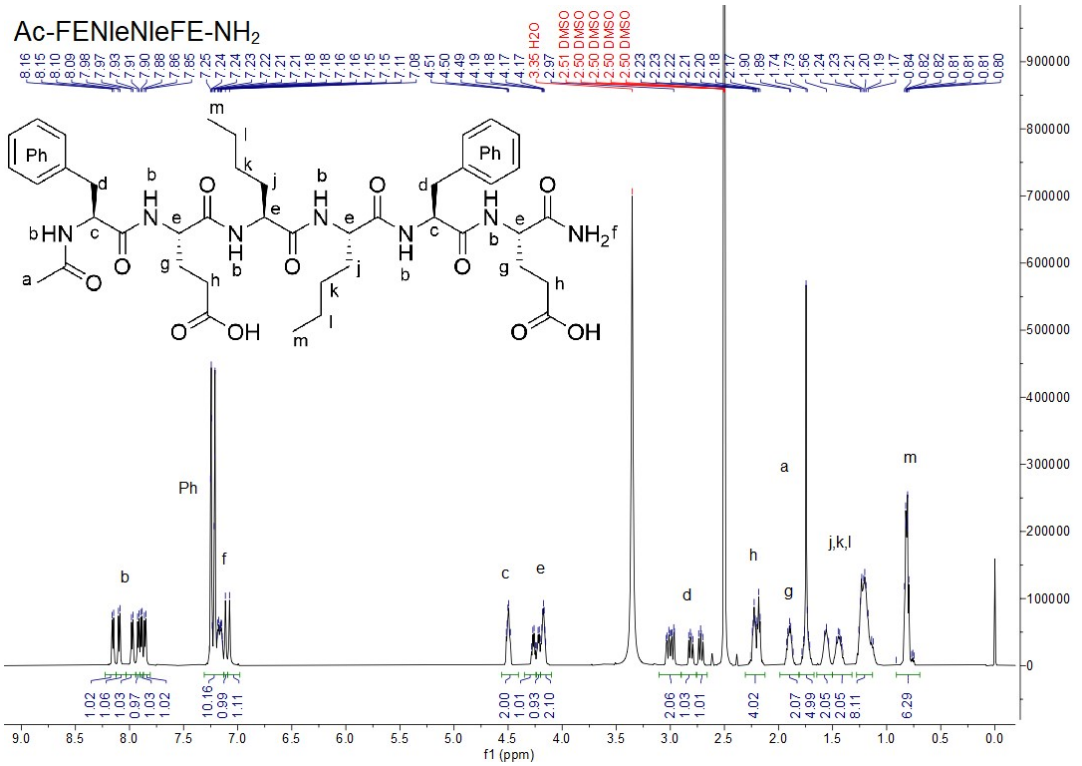

Figure S15. ^{1}H NMR of Ac-FENleNleFE-NH_2 in DMSO-d_6.

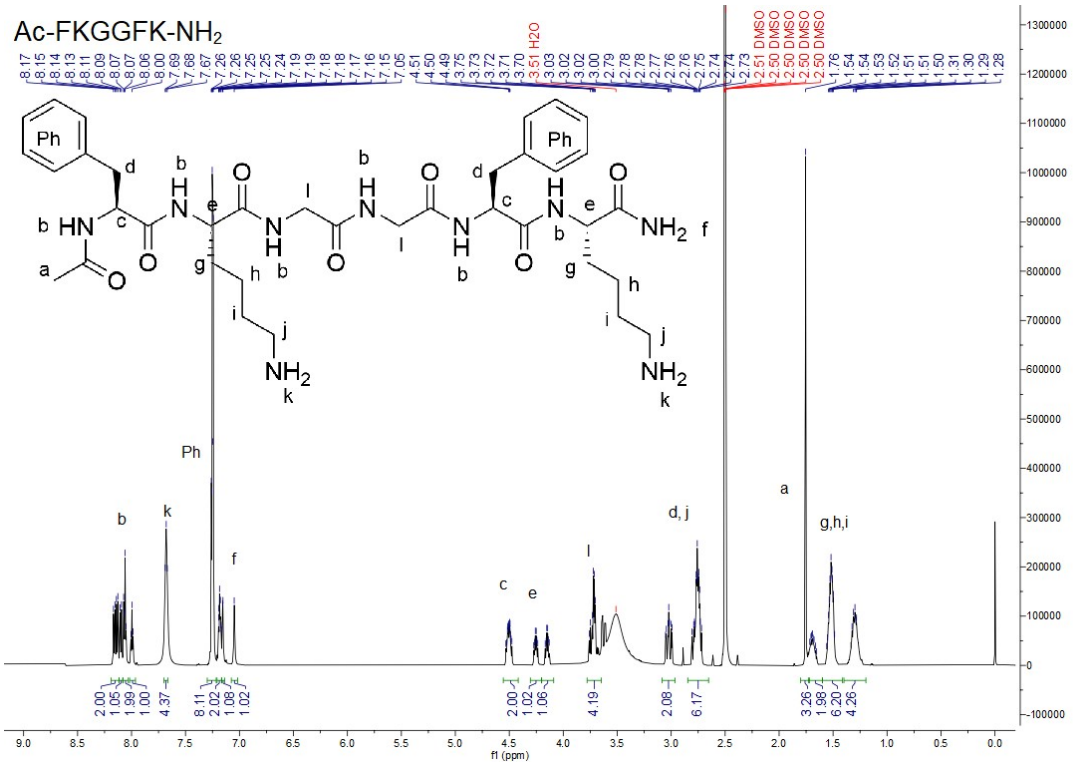

Figure S16. ^{1}H NMR of Ac-FKGGFK-NH_2 in DMSO-d_6.

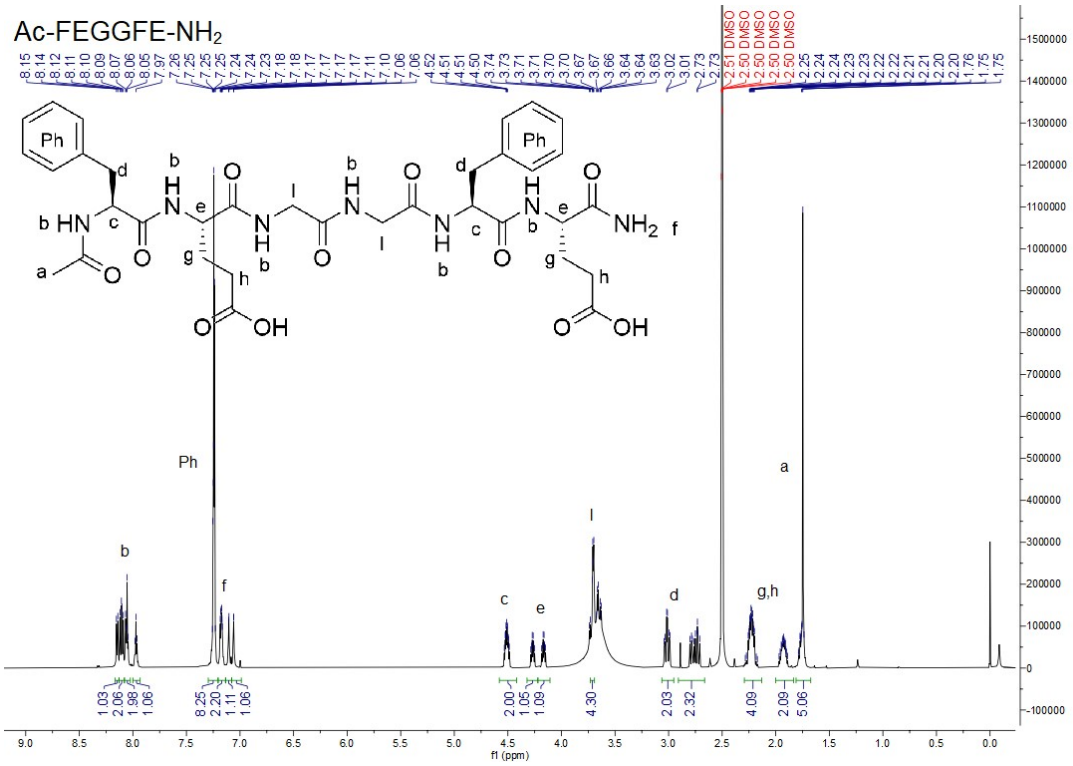

Figure S17. ^{1}H NMR of Ac-FEGGFE-NH_2 in DMSO-d_6.

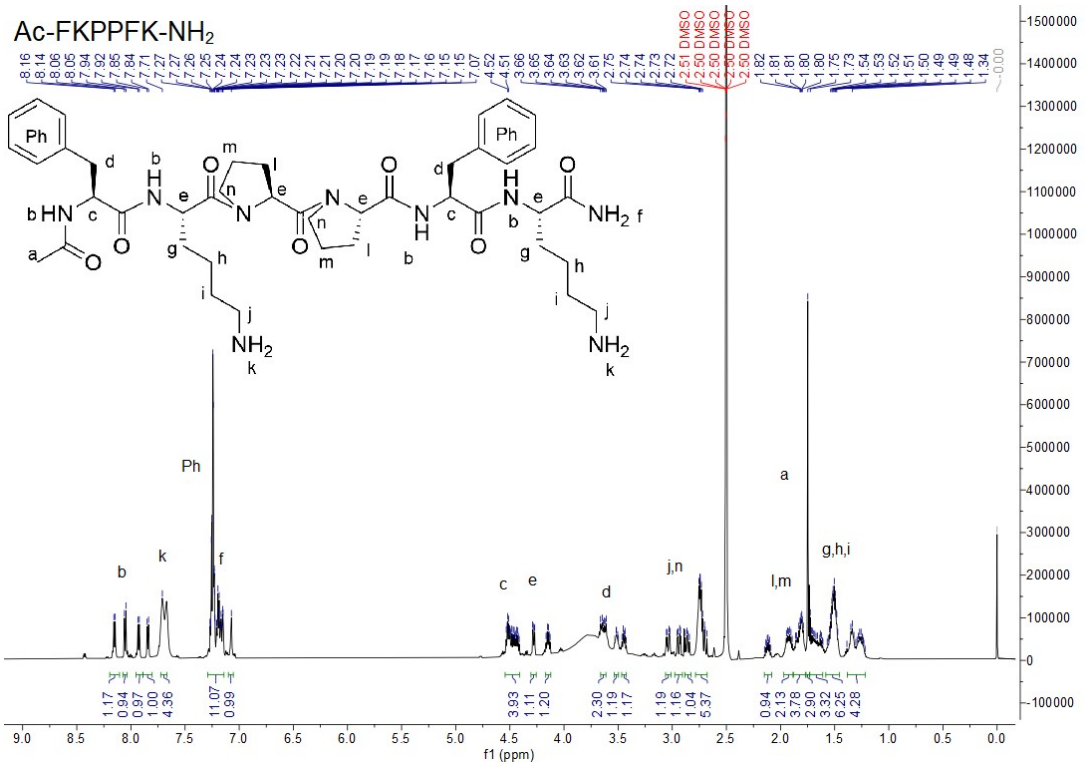

Figure S18. ^{1}H NMR of Ac-FKPPFK-NH_2 in DMSO-d_6.

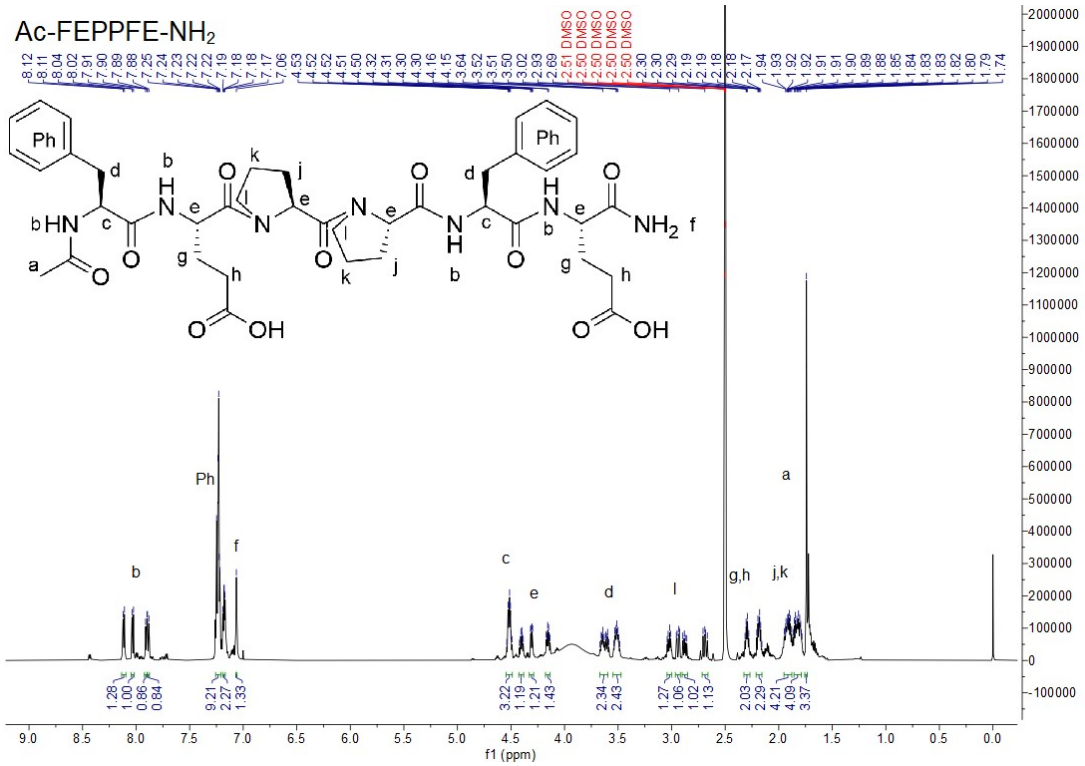

Figure S19. ^{1}H NMR of Ac-FEPPFE-NH_2 in DMSO-d_6.

Ac-FKAAFK-NH_2: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.14 (d, *J* = 8.0 Hz, 1H), 8.11 (d, *J* = 8.1 Hz, 1H), 8.01 (d, *J*=7.4 Hz, 1H), 7.95 (d, *J* = 8.1 Hz, 1H) , 7.93 (d, *J* = 7.1 Hz, 1H), 7.88 (d, *J* = 7.9 Hz, 1H), 7.67 (s, 4H), 7.31 – 7.14 (m, 11H), 7.07 (d, *J* = 2.1 Hz, 1H), 4.52 – 4.44 (m, 2H), 4.28 – 4.12 (m, 4H), 3.02 (m, 2H), 2.82 (dd, *J* =13.9, 8.9 Hz, 1H), 2.79-2.66 (m, 5H), 1.75 (s, 3H), 1.67 (d, *J* = 7.9 Hz, 2H), 1.54 – 1.49 (m, 6H), 1.30 (m, 4H), 1.16 (d, *J* = 7.1 Hz, 3H), 1.13 (d, *J* = 7.1 Hz, 3H).

Ac-FEAAFE-NH_2: ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.09 (s, 2H), 8.11 (d, *J* = 7.8 Hz, 1H), 8.09 (d, *J* = 8.2 Hz, 1H), 8.01 (d, *J* = 7.3 Hz, 1H), 7.95 (d, *J* = 7.0 Hz, 1H), 7.91 (t, *J* = 7.6 Hz, 2H), 7.27 – 7.19 (m, 8H), 7.19 – 7.14 (m, 2H), 7.13 (d, *J* = 2.2 Hz, 1H), 7.08 (d, *J* = 2.1 Hz, 1H), 4.52 – 4.43 (m, 2H), 4.27 – 4.14 (m, 4H), 3.01 (m, 2H), 2.82 (dd, *J* = 13.9, 8.9 Hz, 1H), 2.71 (dd, *J* = 13.9, 10.2 Hz, 1H), 2.24 (t, *J* = 8.2 Hz, 2H), 2.19 (ddd, *J* = 8.9, 6.6, 2.2 Hz, 2H), 1.96 – 1.83 (m, 2H), 1.83 – 1.66 (m, 5H), 1.14 (t, *J* = 6.7 Hz, 6H).

Ac-FKLLFK-NH_2: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.18 (d, *J* = 8.0 Hz, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 8.2 Hz, 2H), 7.88 (dd, *J* = 12.1, 8.0 Hz, 2H), 7.68 (s, 4H), 7.31 – 7.13 (m, 11H), 7.06 (s, 1H), 4.49 (dtd, *J* = 13.1, 8.6, 4.5 Hz, 1H), 4.30 (td, *J* = 8.8, 5.3 Hz, 1H), 4.24 (tt, *J* = 8.7, 4.0 Hz, 2H), 4.14 (td, *J* = 8.3, 5.3 Hz, 1H), 3.03 (dd, *J* = 14.1, 4.8 Hz, 1H), 2.97 (dd, *J* = 14.0, 4.0 Hz, 1H), 2.81 (dd, *J* = 14.1, 9.0 Hz, 1H), 2.78-2.66 (m, 5H), 1.75 (s, 3H), 1.65 (dp, *J* = 15.0, 5.7 Hz, 2H), 1.60 – 1.46 (m, 8H), 1.46 – 1.32 (m, 4H), 1.31 – 1.24 (m, 4H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.86 – 0.80 (m, 6H), 0.78 (d, *J* = 6.4 Hz, 3H).

Ac-FELLFE-NH_2: ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.08 (s, 2H), 8.13 (d, *J* = 7.8 Hz, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.94 (dd, *J* = 8.0, 3.9 Hz, 2H), 7.89 (dd, *J* = 15.8, 8.0 Hz, 2H), 7.29 – 7.13 (m, 10H), 7.12 (d, *J* = 2.1 Hz, 1H), 7.08 (d, *J* = 2.1 Hz, 1H), 4.49 (dtd, *J* = 10.3, 4.8, 1.9 Hz, 2H), 4.36 – 4.21 (m, 3H), 4.17 (td, *J* = 8.3, 5.1 Hz, 1H), 3.02 (dd, *J* = 14.0, 5.0 Hz, 1H), 2.97 (dd, *J* = 13.9, 4.1 Hz, 1H), 2.81 (dd, *J* = 14.0, 8.9 Hz, 1H), 2.71 (dd, *J* = 13.9, 10.1 Hz, 1H), 2.29 – 2.06 (m, 4H), 1.99 – 1.78 (m, 2H), 1.75 (s, 5H), 1.63 – 1.46 (m, 2H), 1.46 – 1.19 (m, 4H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.83 (dd, *J* = 6.6, 4.8 Hz, 6H), 0.78 (d, *J* = 6.5 Hz, 3H).

Ac-FKVVFK-NH_2: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.23 (d, *J* = 8.1 Hz, 1H), 8.10 (d, *J* = 8.2 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.81 (d, *J* = 9.0 Hz, 1H), 7.72 (d, *J* = 8.7 Hz, 1H), 7.67 (s, 4H), 7.28 – 7.10 (m, 11H), 7.05 (s, 1H), 4.56 (td, *J* = 8.6, 4.9 Hz, 1H), 4.50 (ddd, *J* = 10.9, 8.4, 3.8 Hz, 1H), 4.28 (td, *J* = 8.4, 5.1 Hz, 1H), 4.20 (t, *J* = 7.8 Hz, 1H), 4.14 (ddd, *J* = 15.3, 8.6, 6.0 Hz, 2H), 2.99 (ddd, *J* = 17.2, 14.2, 4.4 Hz, 2H), 2.83 – 2.68 (m, 6H), 1.88 (dq, *J* = 9.9, 6.7 Hz, 2H), 1.74 (s, 3H), 1.65 (tt, *J* = 9.9, 4.7 Hz, 2H), 1.54 – 1.46 (m, 6H), 1.31 – 1.23 (m, 4H), 0.86 – 0.59 (m, 12H).

Ac-FEVVFE-NH_2: ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.07 (s, 2H), 8.19 (d, *J* = 7.9 Hz, 1H), 8.07

(dd, *J* = 18.1, 8.2 Hz, 2H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.84 (d, *J* = 8.9 Hz, 1H), 7.74 (d, *J* = 8.6 Hz, 1H), 7.28 – 7.12 (m, 10H), 7.10 (d, *J* = 2.2 Hz, 1H), 7.05 (s, 1H), 4.56 (td, *J* = 8.6, 5.1 Hz, 1H), 4.51 (ddd, *J* = 10.3, 8.3, 3.9 Hz, 1H), 4.30 (td, *J* = 8.1, 5.4 Hz, 1H), 4.21 – 4.04 (m, 3H), 2.98 (ddd, *J* = 13.3, 8.3, 4.5 Hz, 2H), 2.80 (dd, *J* = 14.1, 9.1 Hz, 1H), 2.70 (dd, *J* = 13.9, 10.3 Hz, 1H), 2.30 – 2.09 (m, 4H), 1.91 – 1.85 (m, 4H), 1.80 – 1.65 (m, 5H), 0.77 (m, 12H).

Ac-FKGGFK-NH$_2$: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.15 (dd, *J* = 16.2, 8.0 Hz, 2H), 8.10 (d, *J* = 8.0 Hz, 1H), 8.08 – 8.03 (m, 2H), 8.00 (t, *J* = 5.8 Hz, 1H), 7.68 (t, *J* = 5.8 Hz, 4H), 7.25 (dd, *J* = 6.8, 4.6 Hz, 7H), 7.21 – 7.17 (m, 2H), 7.15 (d, *J* = 2.1 Hz, 1H), 7.05 (s, 1H), 4.50 (tdd, *J* = 10.4, 8.3, 4.4 Hz, 2H), 4.25 (td, *J* = 8.5, 5.0 Hz, 1H), 4.15 (td, *J* = 8.6, 5.1 Hz, 1H), 3.78 – 3.65 (m, 4H), 3.02 (ddd, *J* = 17.9, 13.9, 4.5 Hz, 2H), 2.85 – 2.65 (m, 6H), 1.76 (s, 3H), 1.69 (m, 2H), 1.52 (m, 6H), 1.31 (m, 4H).

Ac-FEGGFE-NH$_2$: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.15 (d, *J* = 7.8 Hz, 1H), 8.11 (dd, *J* = 10.6, 8.1 Hz, 2H), 8.08 – 8.02 (m, 2H), 7.97 (t, *J* = 5.7 Hz, 1H), 7.25 (m, 8H), 7.20 – 7.14 (m, 2H), 7.10 (d, *J* = 2.1 Hz, 1H), 7.04 (s, 1H), 4.58 – 4.42 (m, 2H), 4.27 (td, *J* = 8.2, 5.3 Hz, 1H), 4.17 (td, *J* = 8.4, 5.2 Hz, 1H), 3.70 (m, 4H), 3.01 (td, *J* = 13.7, 4.5 Hz, 2H), 2.91 – 2.66 (m, 2H), 2.23 (m, 4H), 2.00 – 1.83 (m, 2H), 1.81 – 1.67 (m, 5H).

Ac-FKPPFK-NH$_2$: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.15 (d, *J* = 7.7 Hz, 1H), 8.05 (t, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 8.2 Hz, 1H), 7.84 (d, *J* = 7.6 Hz, 1H), 7.71 (m, 4H), 7.29 – 7.14 (m, 11H), 7.07 (s, 1H), 4.59 – 4.34 (m, 4H), 4.28 (dd, *J* = 8.3, 3.0 Hz, 1H), 4.15 (td, *J* = 8.5, 5.2 Hz, 2H), 3.63 (dd, *J* = 10.5, 4.5 Hz, 2H), 3.53 – 3.50 (m, 1H), 3.44 (dt, *J* = 9.6, 7.1 Hz, 1H), 3.04 (dd, *J* = 14.1, 5.2 Hz, 1H), 2.94 (dd, *J* = 13.9, 4.3 Hz, 1H), 2.86 (dd, *J* = 14.0, 8.8 Hz, 1H), 2.80 – 2.66 (m, 5H), 2.12 (dq, *J* = 12.5, 7.6 Hz, 1H), 1.98 – 1.88 (m, 2H), 1.88 – 1.76 (m, 4H), 1.75 (s, 3H), 1.73 – 1.61 (m, 3H), 1.52 (m, 6H), 1.38 – 1.22 (m, 4H).

Ac-FEPPFE-NH$_2$: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.03 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 7.5 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.26 – 7.21 (m, 9H), 7.19 – 7.17 (m, 2H), 7.06 (s, 1H), 4.51 (td, *J* = 8.3, 4.9 Hz, 3H), 4.40 (td, *J* = 8.0, 5.5 Hz, 1H), 4.31 (dd, *J* = 8.6, 3.2 Hz, 1H), 4.15 (td, *J* = 8.4, 5.0 Hz, 1H), 3.67 – 3.59 (m, 7.2 Hz, 2H), 3.56 – 3.42 (m, 2H), 3.03 (dd, *J* = 14.0, 5.4 Hz, 1H), 2.94 (dd, *J* = 13.9, 4.3 Hz, 1H), 2.91 – 2.85 (m, 1H), 2.70 (td, *J* = 14.1, 9.3 Hz, 1H), 2.32 – 2.27 (m, 2H), 2.19 (dt, *J* = 9.3, 6.2 Hz, 2H), 1.92 (m, 4H), 1.87 – 1.76 (m, 4H), 1.74 (m, 3H).

LC-MS

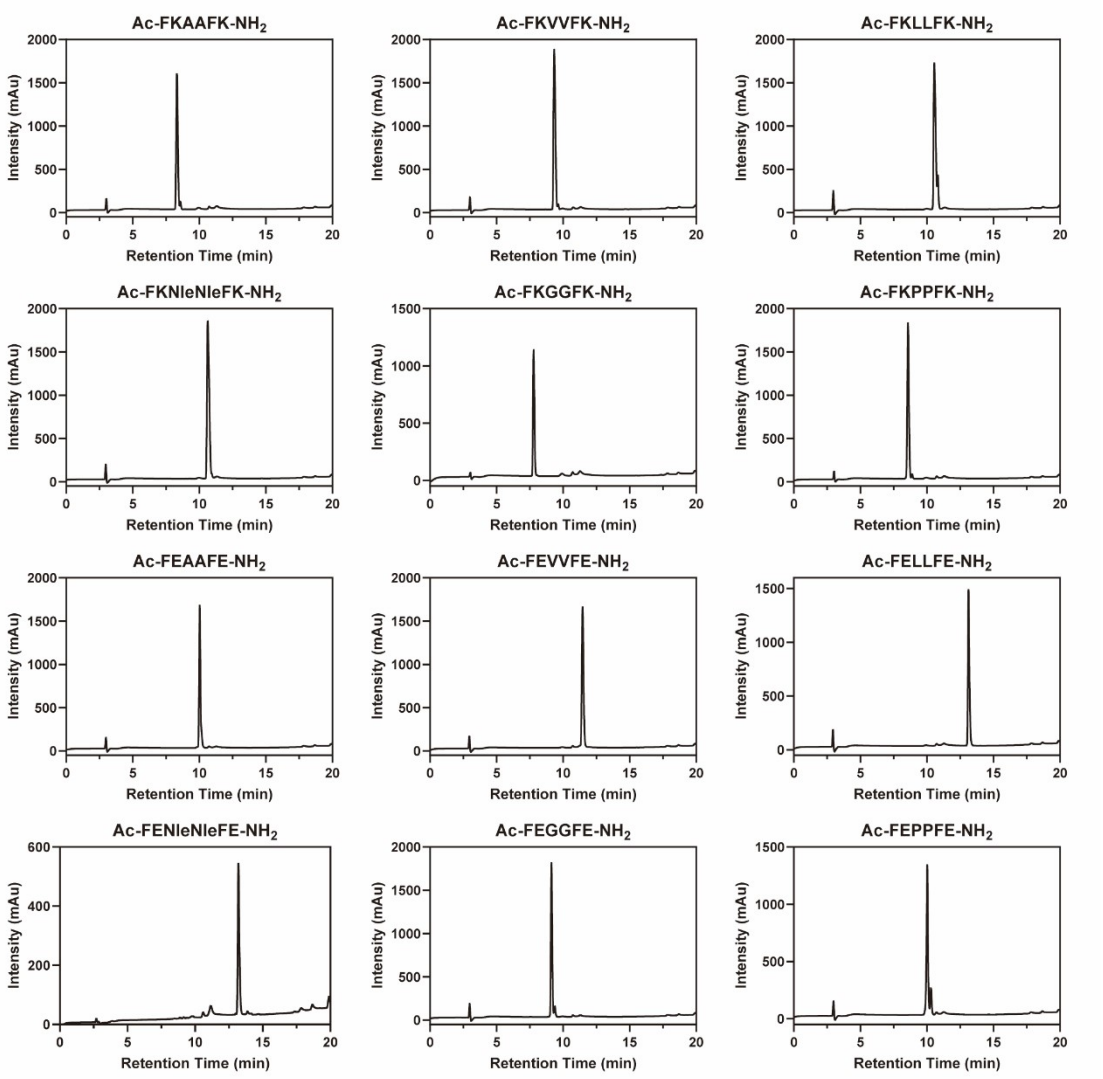

Figure S20. HPLC spectra of peptides.

Ac-FKAAFK-NH$_2$: calc. M = 752.0, obsvd. (M+H$^+$) = 752.2.
Ac-FEAAFE-NH$_2$: calc. M = 753.9, obsvd. (M+H$^+$) = 754.1.
Ac-FKLLFK-NH$_2$: calc. M = 836.2, obsvd. (M+H$^+$) = 836.3.
Ac-FELLFE-NH$_2$: calc. M = 838.1, obsvd. (M+H$^+$) = 838.2.
Ac-FKVVFK-NH$_2$: calc. M = 808.1, obsvd. (M+H$^+$) = 809.6.
Ac-FEVVFE-NH$_2$: calc. M = 810.0, obsvd. (M+H$^+$) = 810.3.
Ac-FKNleNleFK-NH$_2$: calc. M = 836.2, obsvd. (M+H$^+$) = 837.5.
Ac-FENleNleFE-NH$_2$: calc. M = 838.1, obsvd. (M+H$^+$) = 838.4.
Ac-FKGGFK-NH$_2$: calc. M = 724.0, obsvd. (M+H$^+$) = 724.1.
Ac-FEGGFE-NH$_2$: calc. M = 725.9, obsvd. (M+H$^+$) = 726.0.
Ac-FKPPFK-NH$_2$: calc. M = 804.1, obsvd. (M+H$^+$) = 804.2.
Ac-FEPPFE-NH$_2$: calc. M = 806.0, obsvd. (M+H$^+$) = 806.1.