Adjusting heterodimeric coiled-coils (K/E zipper) to connect autophagy-inducing peptide with cell-penetrating peptide

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Table S1. Synthesized peptides used in this study: Nomenclature and structure[a]

Peptide	Sequence	Exact mass Calculated	Observed	Purity (%)
AIP-K1	Tmr-VWNATFHIWHD- KIAALKE	2591.30	2591.27	98
AIP-K2	Tmr-VWNATFHIWHD-(KIAALKE)$_2$	3344.78	3343.72	98
AIP-K3	Tmr-VWNATFHIWHD-(KIAALKE)$_3$	4098.25	4099.07	98
AIP-K4	Tmr-VWNATFHIWHD-(KIAALKE)$_4$	4851.73	4850.63	98
E1-CPP	Fam- EIAALEK -RRRRRRRR	2379.31	2379.31	98
E2-CPP	Fam-(EIAALEK)$_2$-RRRRRRRR	3133.73	3135.42	98
E3-CPP	Fam-(EIAALEK)$_3$-RRRRRRRR	3887.17	3889.92	98
E4-CPP	Fam-(EIAALEK)$_4$-RRRRRRRR	4641.59	4642.71	98

[a] The calculated masses and the masses observed by MALDI-TOF mass spectrometry and the purity found by RP-HPLC are listed for each of the peptides.
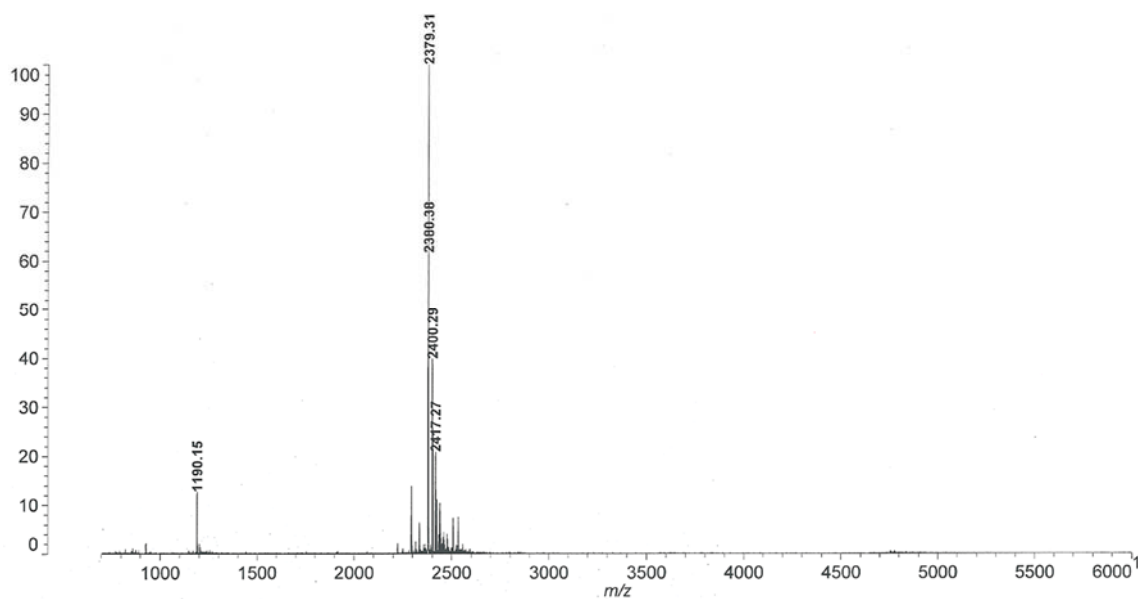
Figure S1. MALDI-Tof Mass spectrum of **E1-CPP**. An α-CHCA was used as a matrix. calcd. [M+H]$^+$ = 2379.31 and obsd. [M+H]$^+$ = 2379.31.
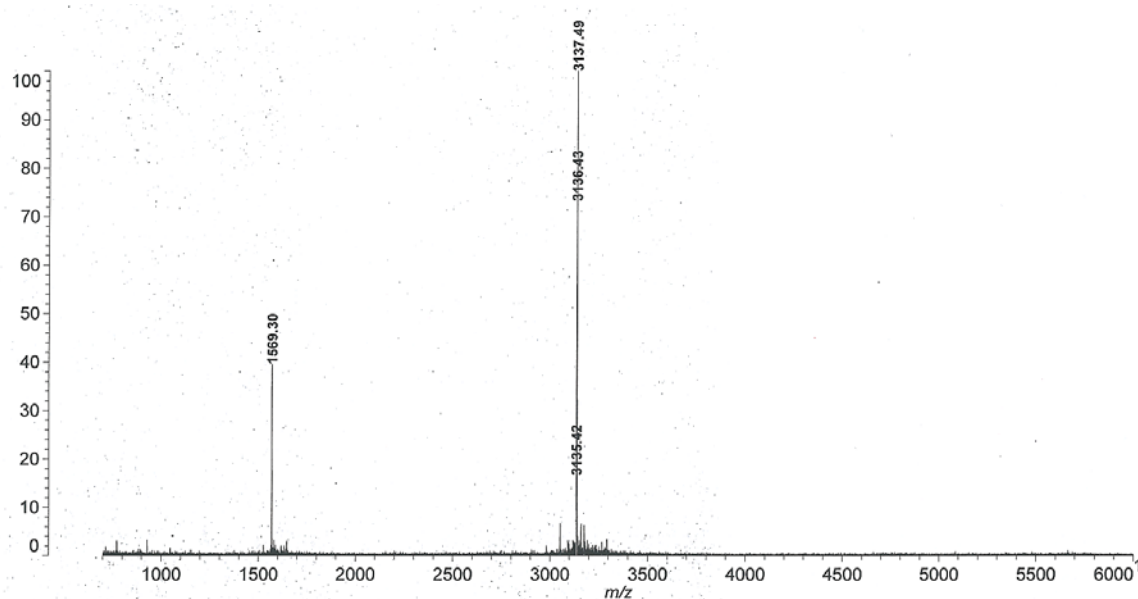

Figure S2. MALDI-Tof Mass spectrum of **E2-CPP**. An α-CHCA was used as a matrix. calcd. [M+H]$^+$ = 3133.73 and obsd. [M+H]$^+$ = 3135.42.
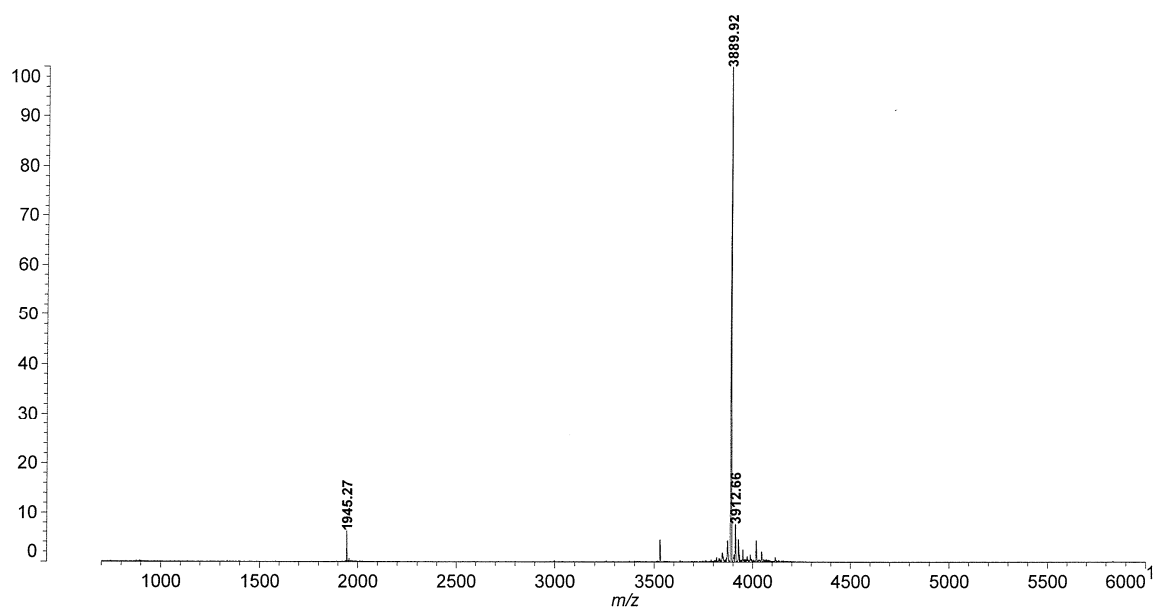
Figure S3. MALDI-Tof Mass spectrum of **E3-CPP**. An α-CHCA was used as a matrix. calcd. [M+H]$^+$ = 3887.17 and obsd. [M+H]$^+$ = 3889.92.
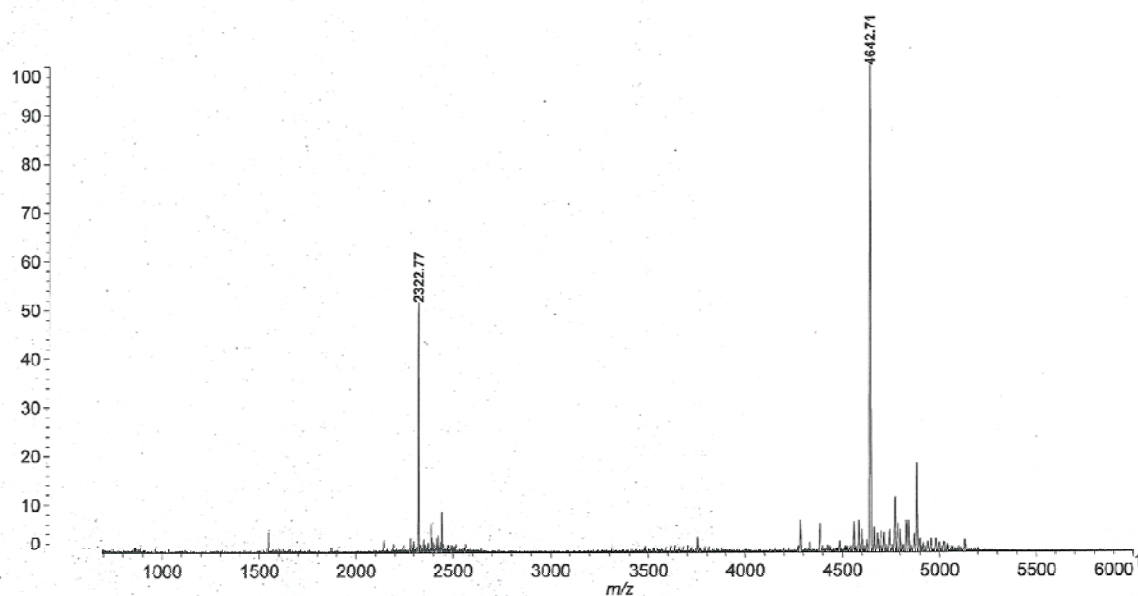

Figure S4. MALDI-Tof Mass spectrum of **E4-CPP**. An α-CHCA was used as a matrix. calcd. [M+H]$^+$ = 4641.59 and obsd. [M+H]$^+$ = 4642.71.
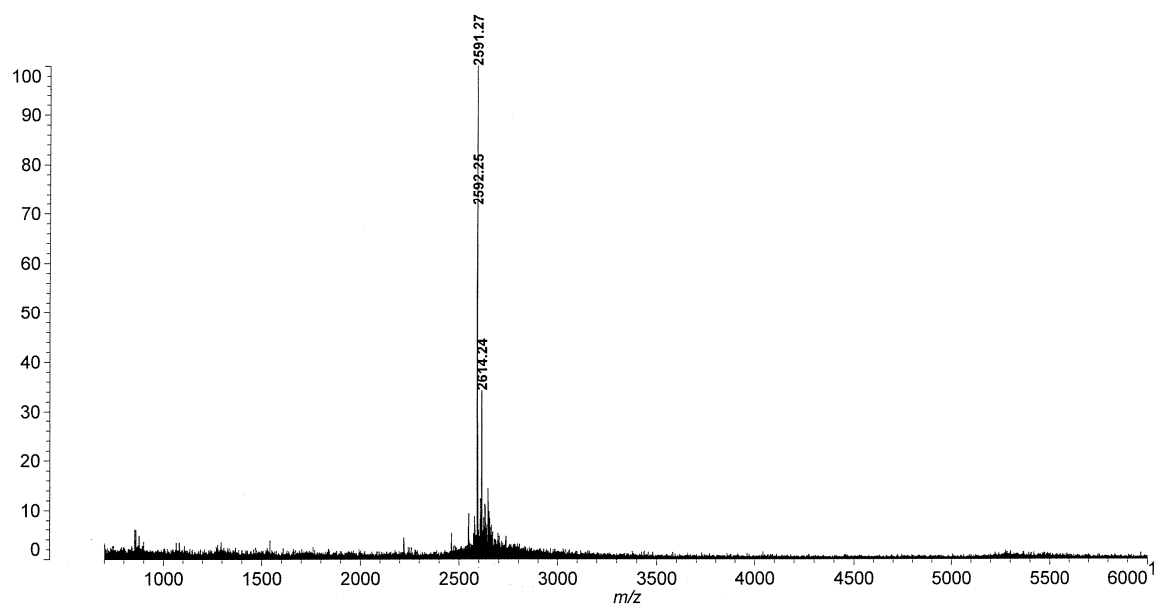
Figure S5. MALDI-Tof Mass spectrum of **AIP-K1**. An α-CHCA was used as a matrix. calcd. [M+H]$^+$ = 2591.30 and obsd. [M+H]$^+$ = 2591.27.
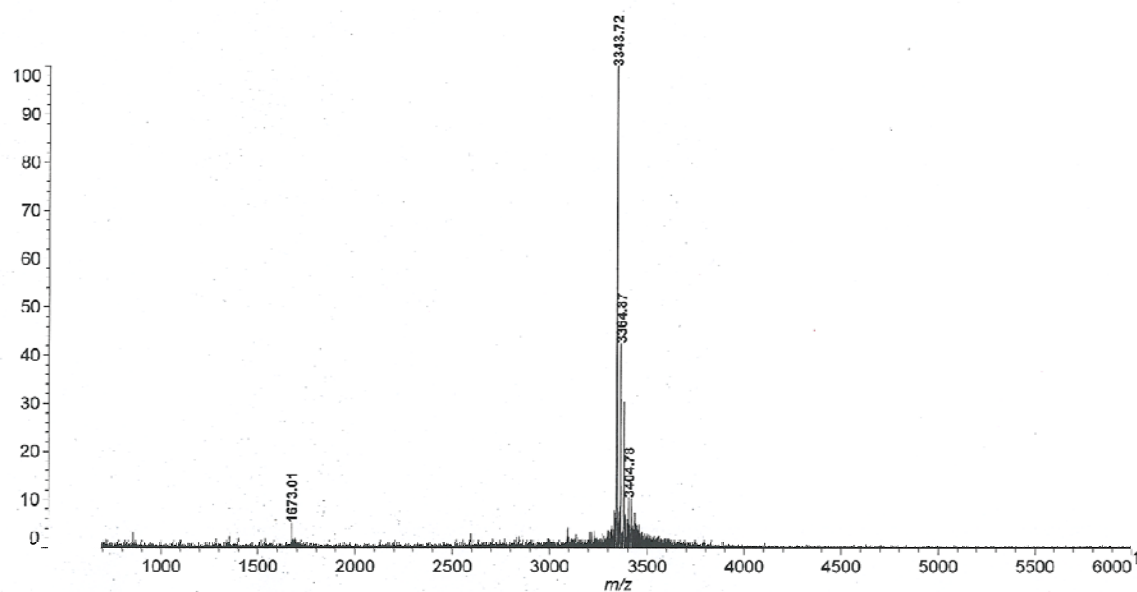

Figure S6. MALDI-Tof Mass spectrum of **AIP-K2**. An α-CHCA was used as a matrix. calcd. [M+H]$^+$ = 3344.78 and obsd. [M+H]$^+$ = 3343.72.
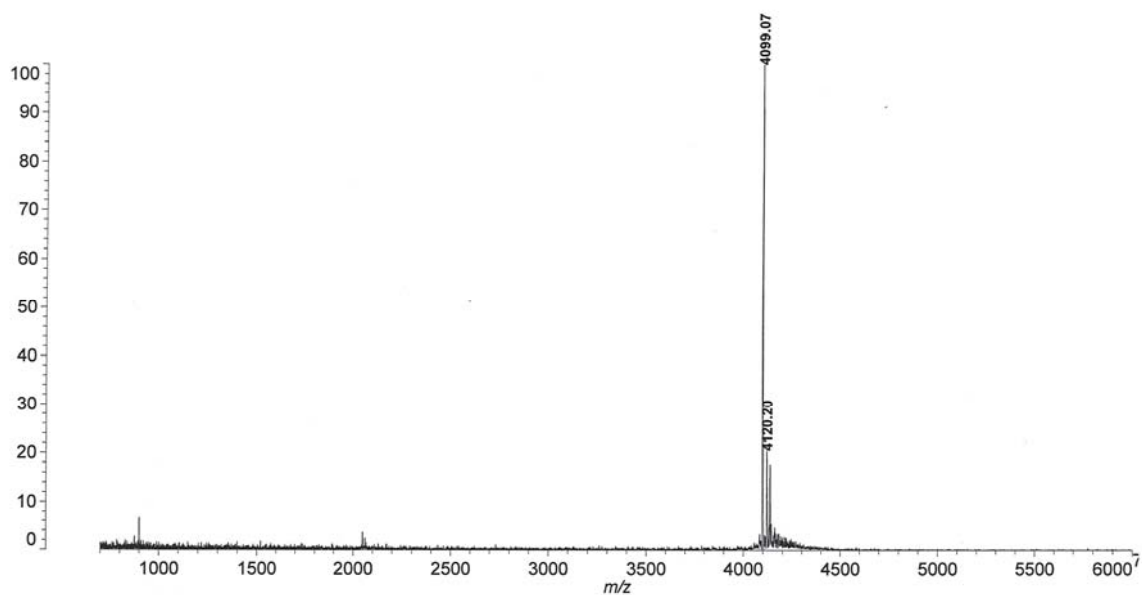
Figure S7. MALDI-Tof Mass spectrum of **AIP-K3**. An α-CHCA was used as a matrix. calcd. $[M+H]^+$ = 4098.25 and obsd. $[M+H]^+$ = 4099.07.
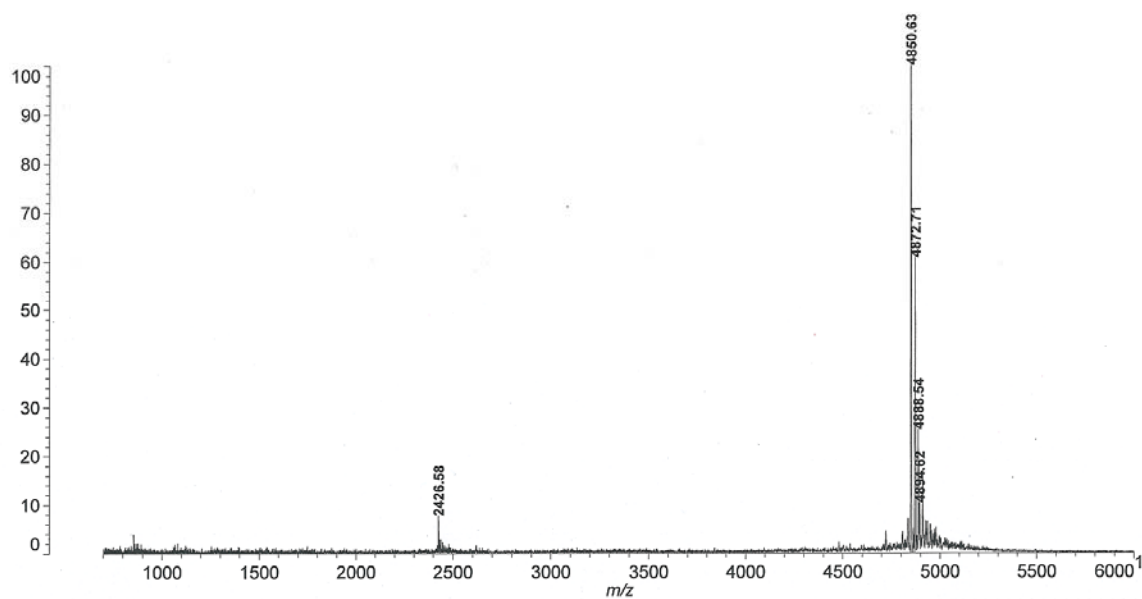

Figure S8. MALDI-Tof Mass spectrum of **AIP-K4**. An α-CHCA was used as a matrix. calcd. $[M+H]^+$ = 4851.73 and obsd. $[M+H]^+$ = 4850.63.
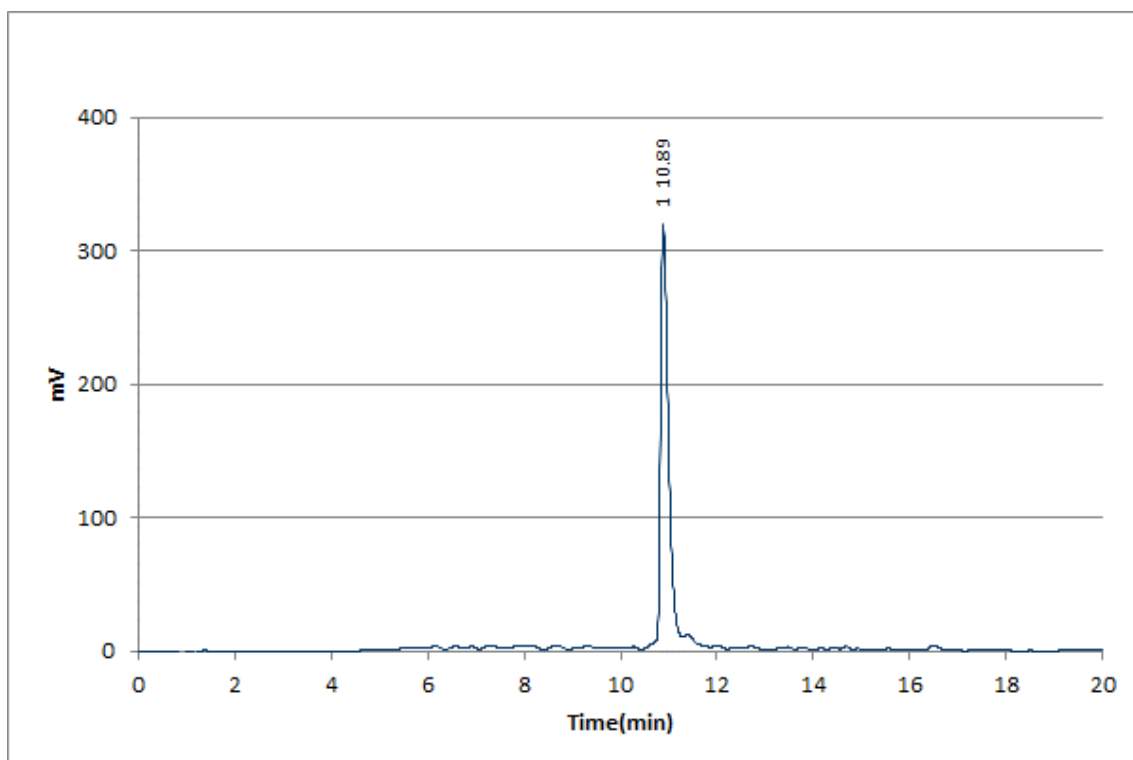
Figure S9. RP-HPLC chart of **E1-CPP** on C18 column. Buffer A. 0.1% TFA in water; buffer B, acetonitrile and monitoring at 230 nm with a gradient of 10-80% for 20 min.
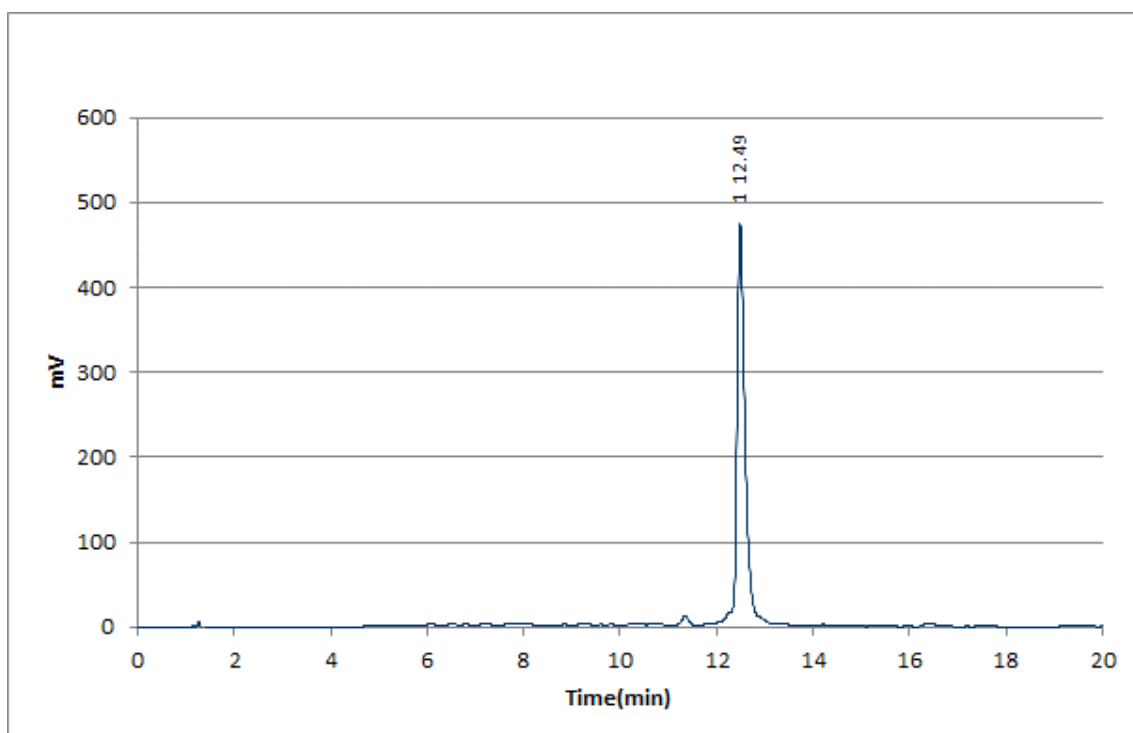

Figure S10. RP-HPLC chart of **E2-CPP** on C18 column. Buffer A. 0.1% TFA in water; buffer B, acetonitrile and monitoring at 230 nm with a gradient of 10-80% for 20 min.
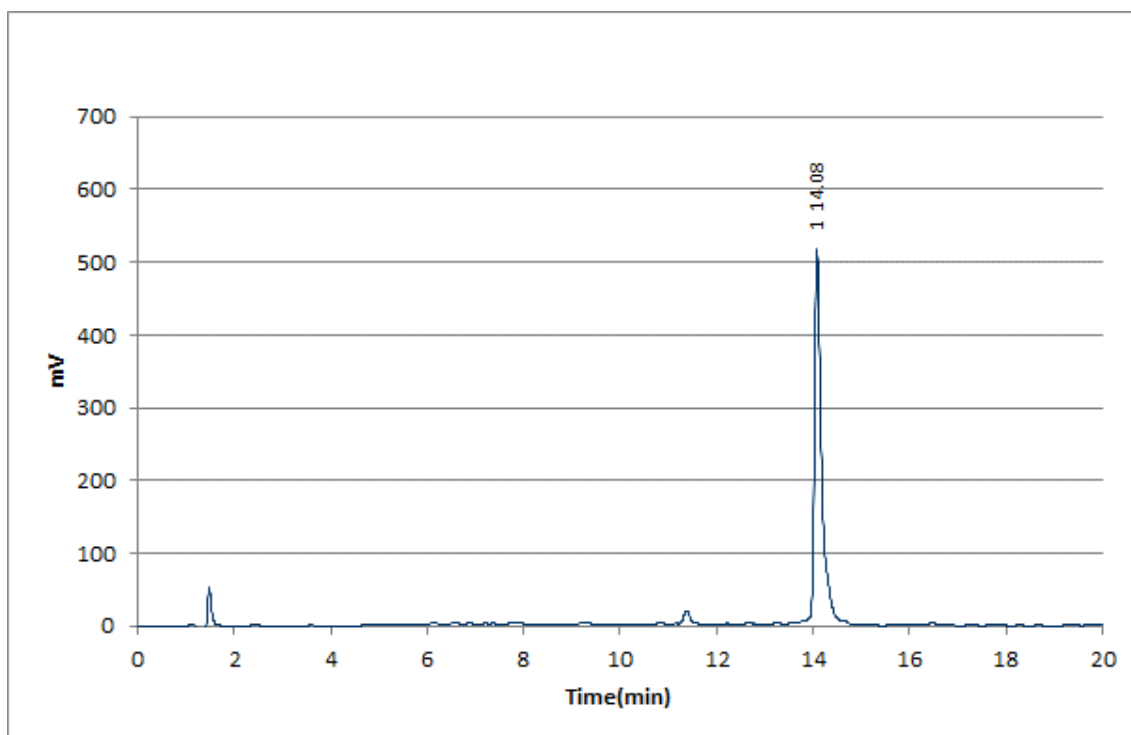
Figure S11. RP-HPLC chart of **E3-CPP** on C18 column. Buffer A. 0.1% TFA in water; buffer B, acetonitrile and monitoring at 230 nm with a gradient of 10-80% for 20 min.
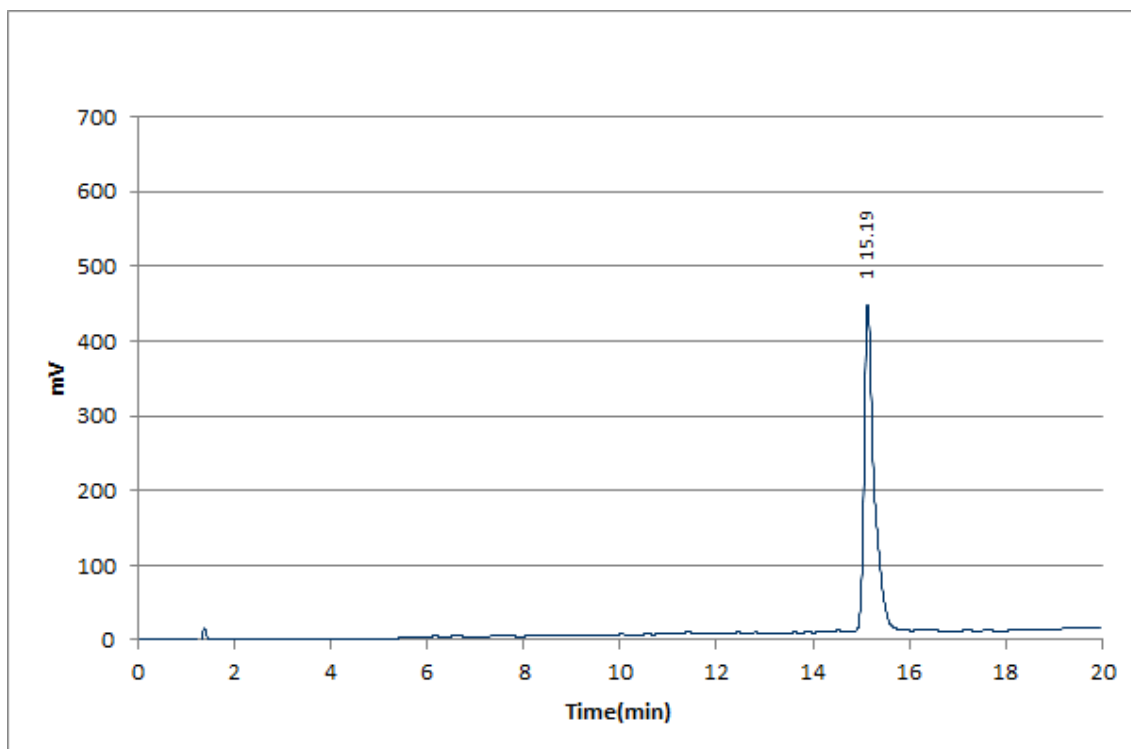

Figure S12. RP-HPLC chart of **E4-CPP** on C18 column. Buffer A. 0.1% TFA in water; buffer B, acetonitrile and monitoring at 230 nm with a gradient of 10-80% for 20 min.
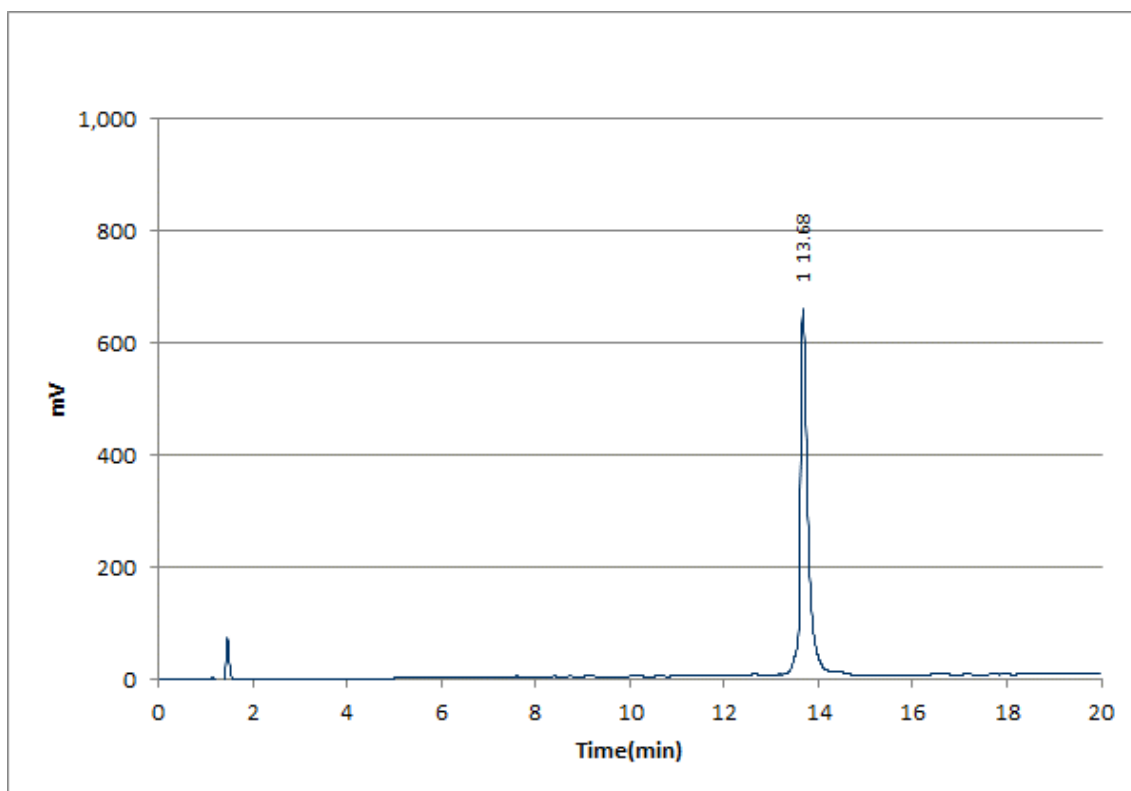
Figure S13. RP-HPLC chart of **AIP-K1** on C18 column. Buffer A. 0.1% TFA in water; buffer B, acetonitrile and monitoring at 230 nm with a gradient of 10-80% for 20 min.
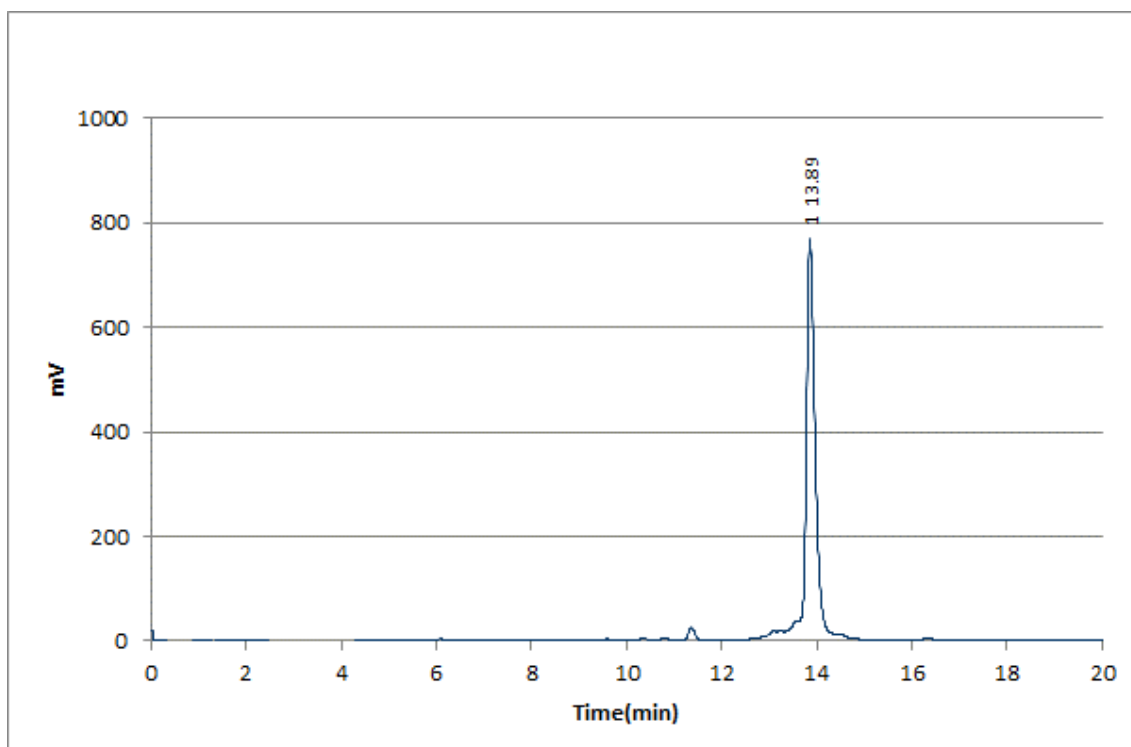

Figure S14. RP-HPLC chart of **AIP-K2** on C18 column. Buffer A. 0.1% TFA in water; buffer B, acetonitrile and monitoring at 230 nm with a gradient of 10-80% for 20 min.
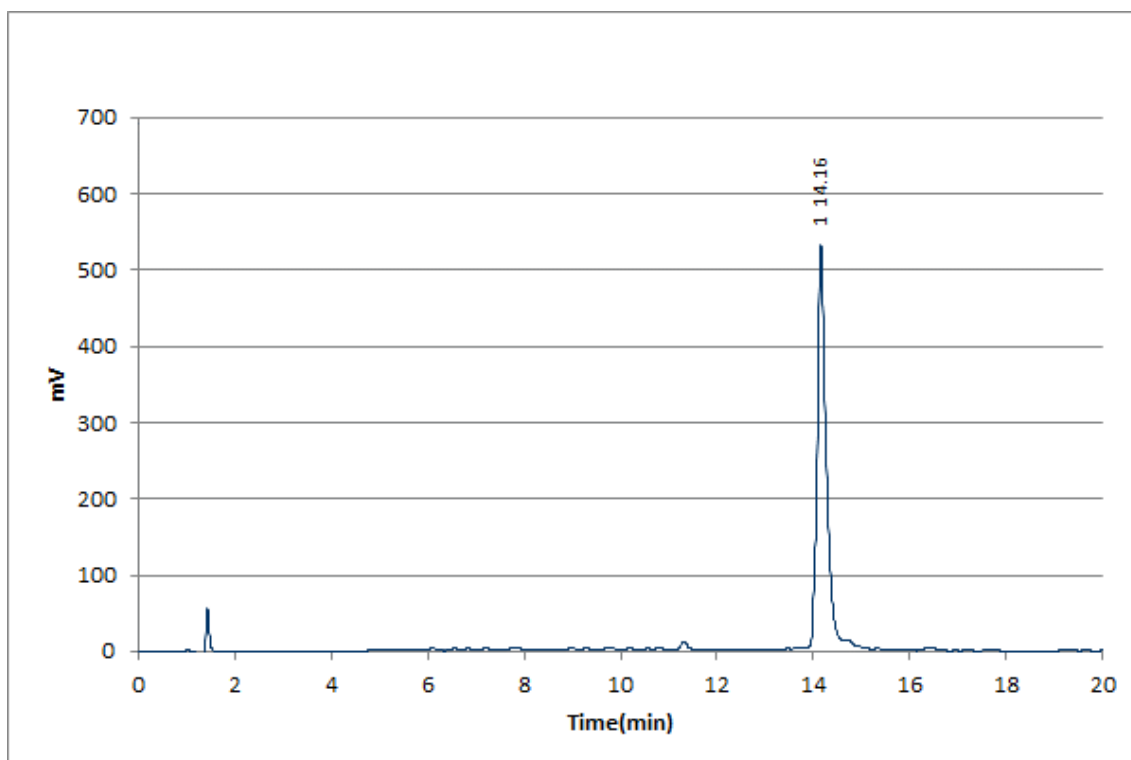
Figure S15. RP-HPLC chart of **AIP-K3** on C18 column. Buffer A. 0.1% TFA in water; buffer B, acetonitrile and monitoring at 230 nm with a gradient of 10-80% for 20 min.
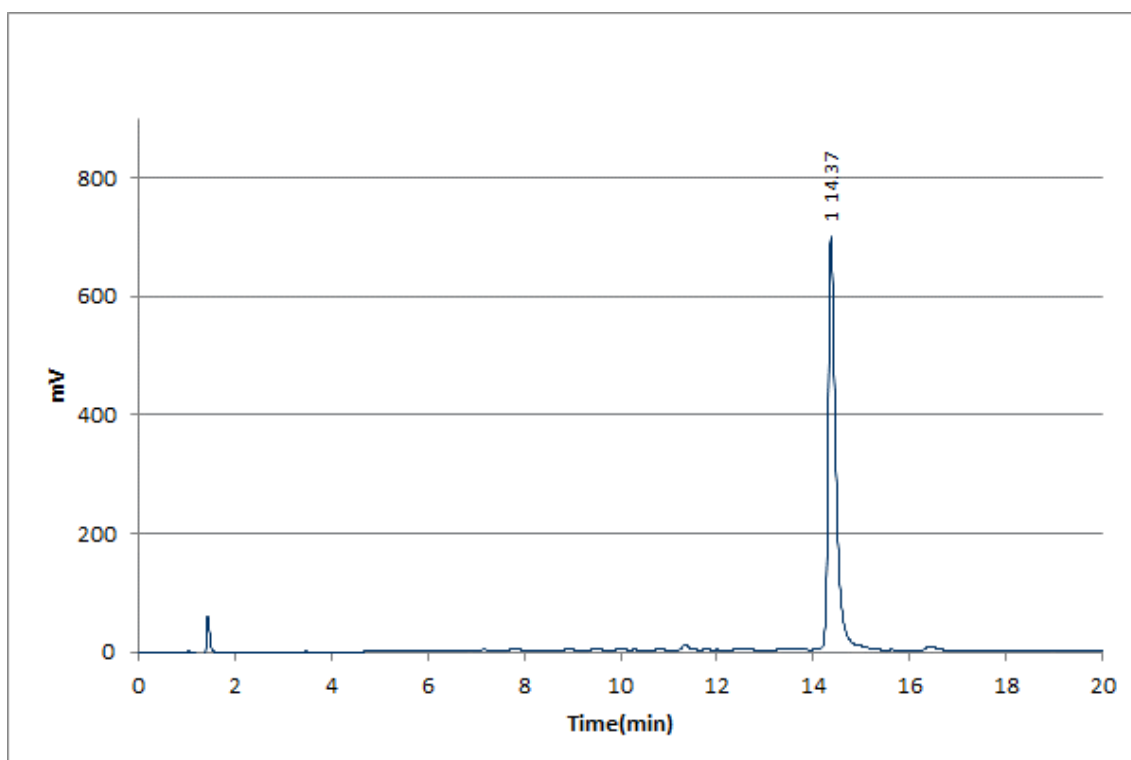

Figure S16. RP-HPLC chart of **AIP-K4** on C18 column. Buffer A. 0.1% TFA in water; buffer B, acetonitrile and monitoring at 230 nm with a gradient of 10-80% for 20 min.
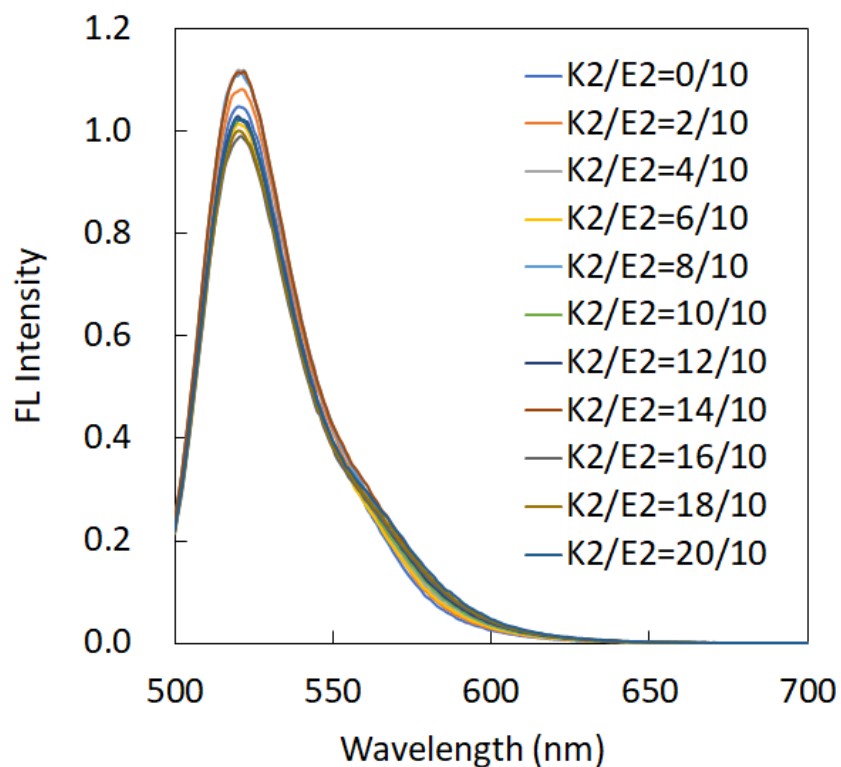
Figure S17. Fluorescence spectra of 50 nM **E2-CPP** with various concentrations of **AIP-K2** in aqueous solution.
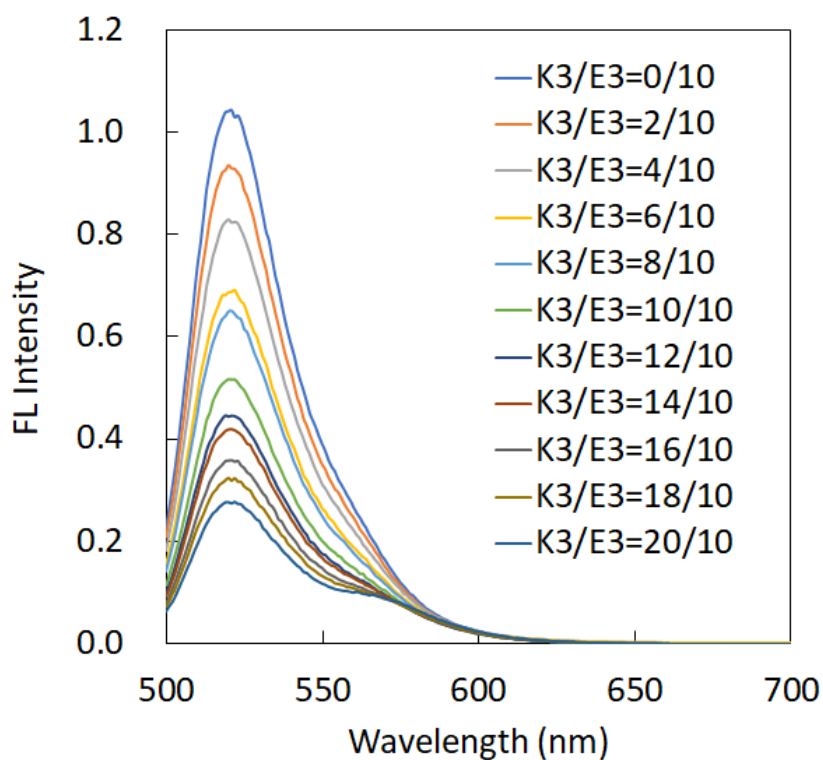

Figure S18. Fluorescence spectra of 50 nM **E3-CPP** with various concentrations of **AIP-K3** in aqueous solution.
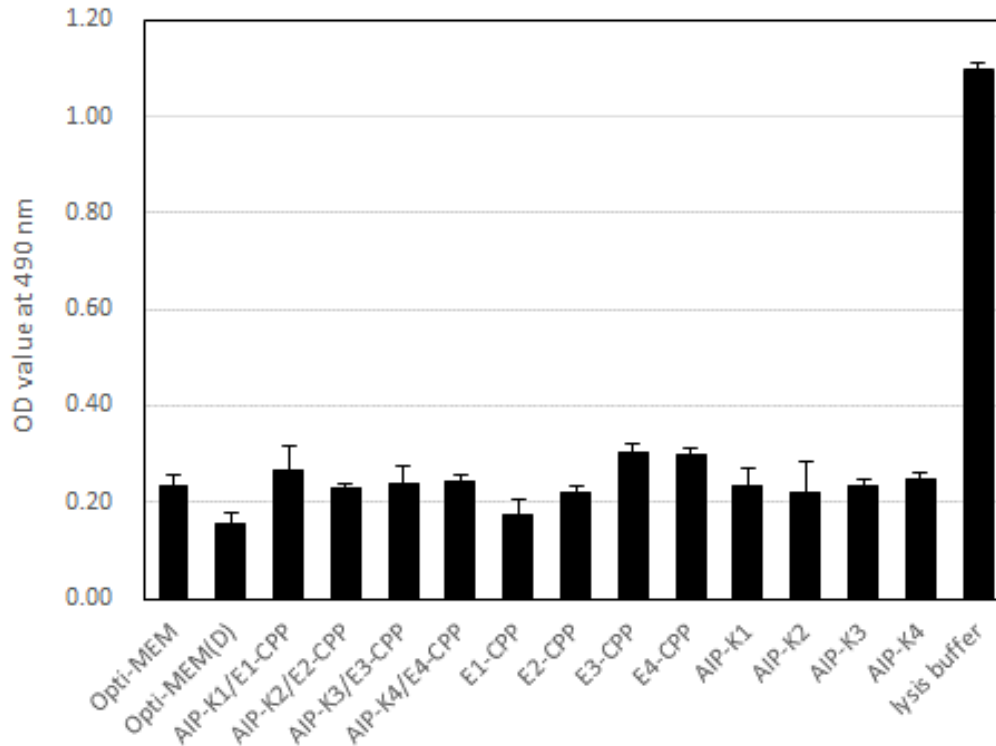
Figure S19. Cytotoxicity of **Kn** peptides, **En** peptides, and **Kn/En** hybrids. HeLa cells were treated with 10 μM of the indicated **En**, **Kn** peptides, or **Kn/En** hybrids. The cytotoxicity was evaluated with measurement of lactate dehydrogenase in the culture supernatant, which is a stable cytosolic protein and released from cells into the supernatant upon cell lysis. The data represent means with standard errors from three independent experiments.