

Supplementary Information

Comparing variants of the cell-penetrating peptide sC18 to design peptide-drug conjugates

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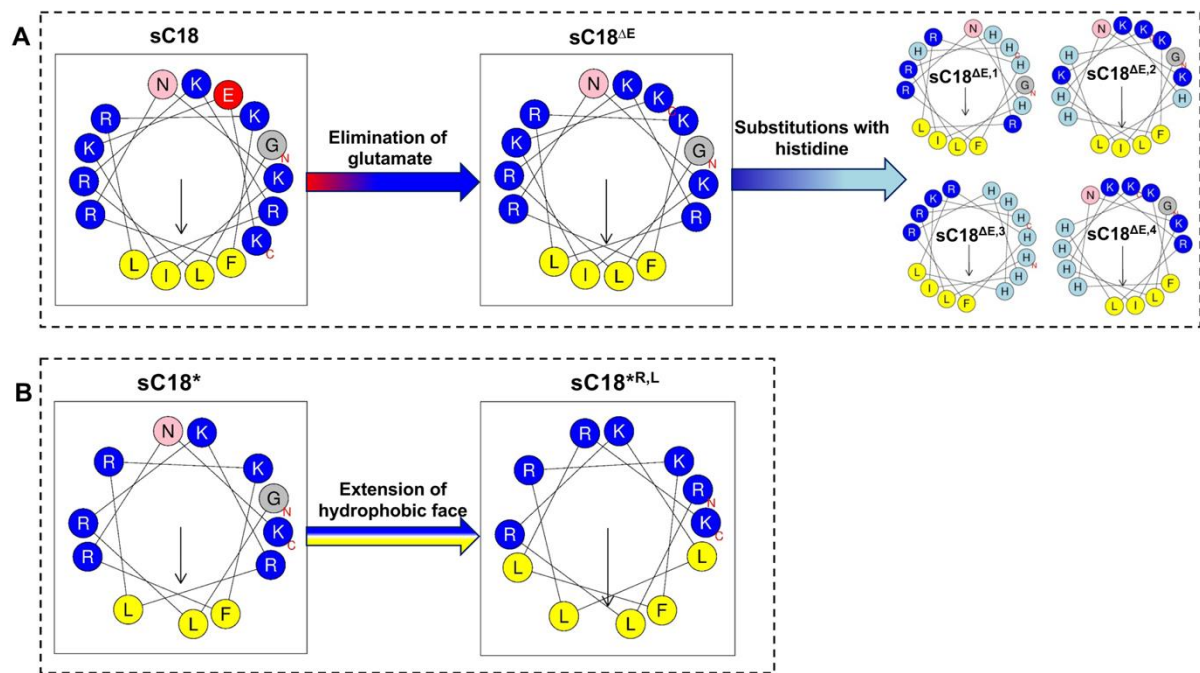


Figure S1 Peptides investigated within this work were based on two different sC18 variants **(A)** sC18^{ΔE} lacks glutamate and is, thus, more hydrophilic. To increase pH-responsiveness, variants sC18^{ΔE,1-4} were generated having different substitutions with histidine included. **(B)** sC18* lacks four amino acids at the C-terminal part. Within this study, two more arginines were introduced by substituting glycine and asparagine. This leads to a nearly perfect amphipathic helix.

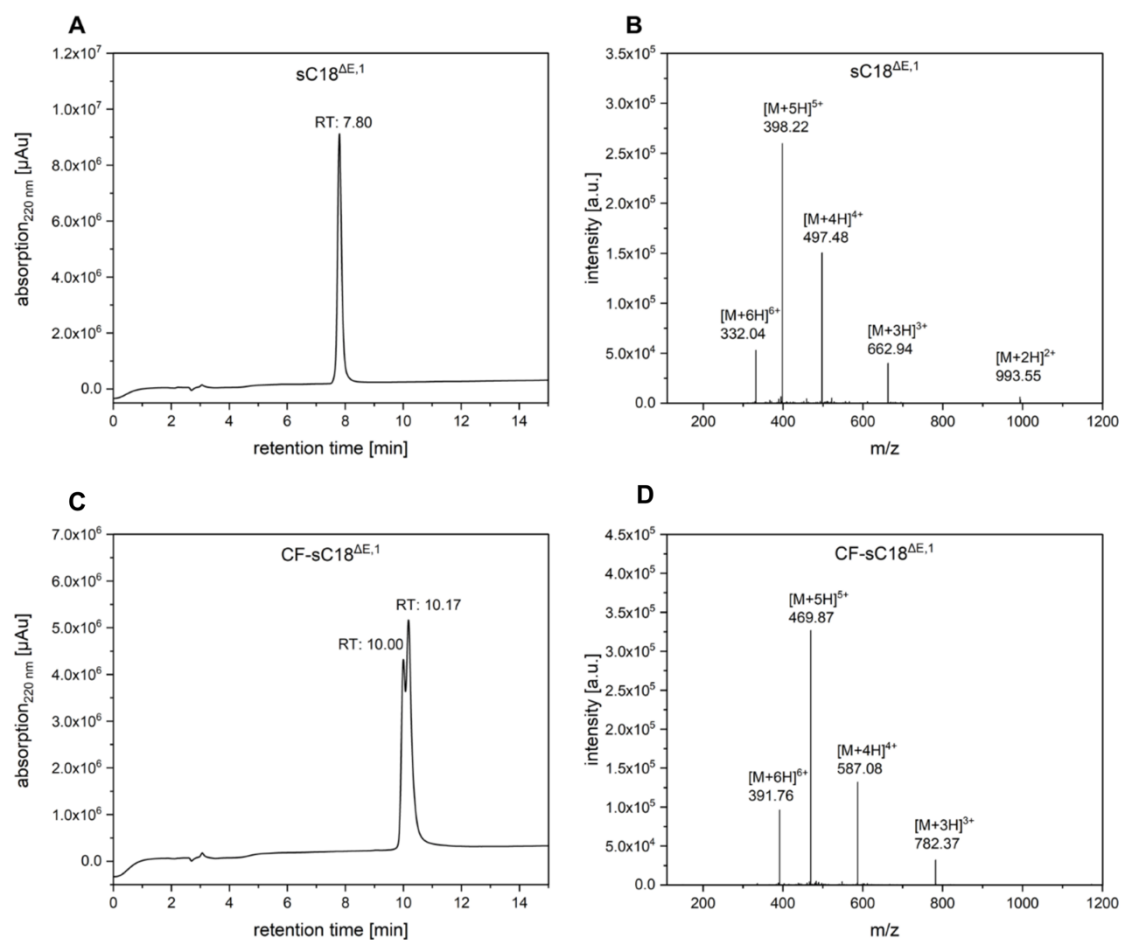


Figure S2 LC-MS analysis showing UV chromatographs was recorded using a linear gradient from 10–60% ACN in water (incl. 0.1% TFA) over 15 min (A, B). Mass spectra of sC18 $\Delta E,1$ and CF-sC18 $\Delta E,1$ (C, D), respectively.

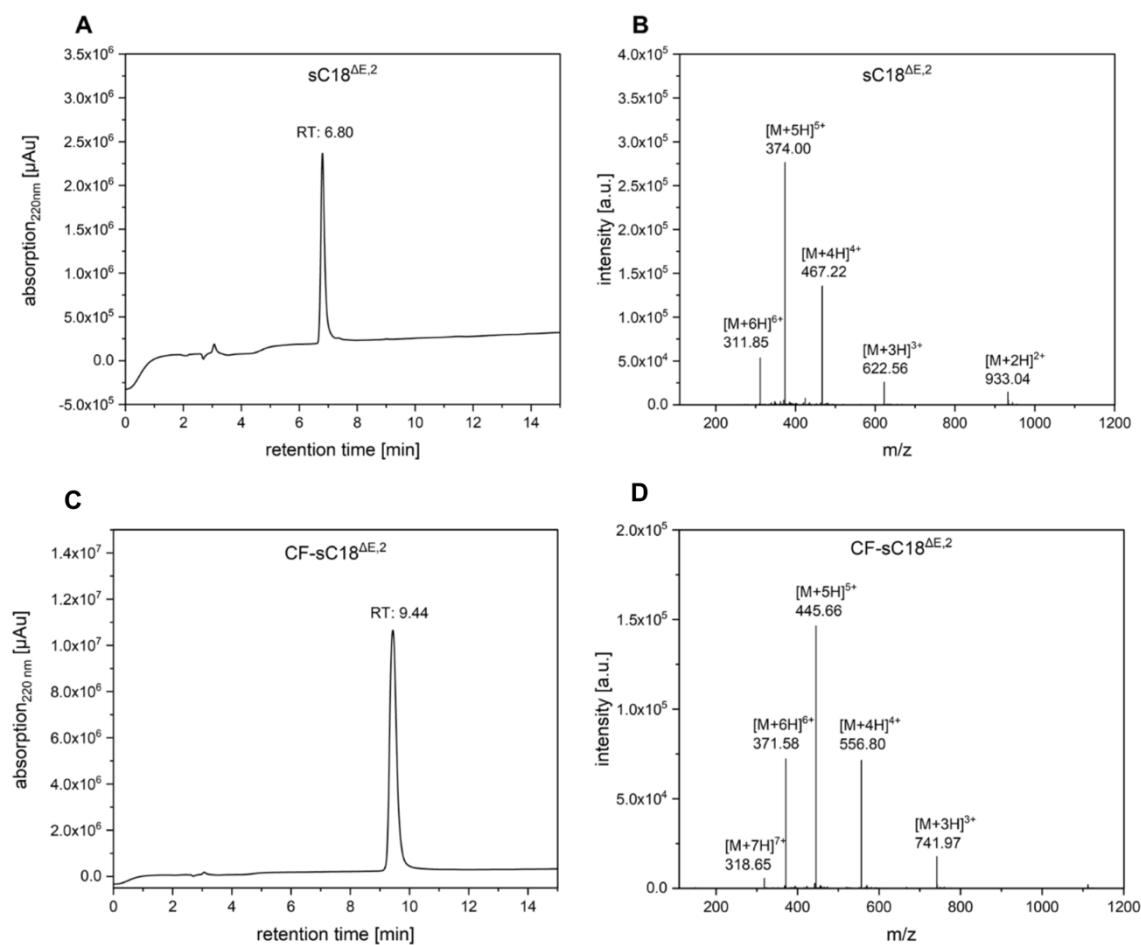


Figure S3 LC-MS analysis showing UV chromatographs was recorded using a linear gradient from 10–60% ACN in water (incl. 0.1% TFA) over 15 min (A, B). Mass spectra of sC18 Δ E₂ and CF-sC18 Δ E₂ (C, D), respectively.

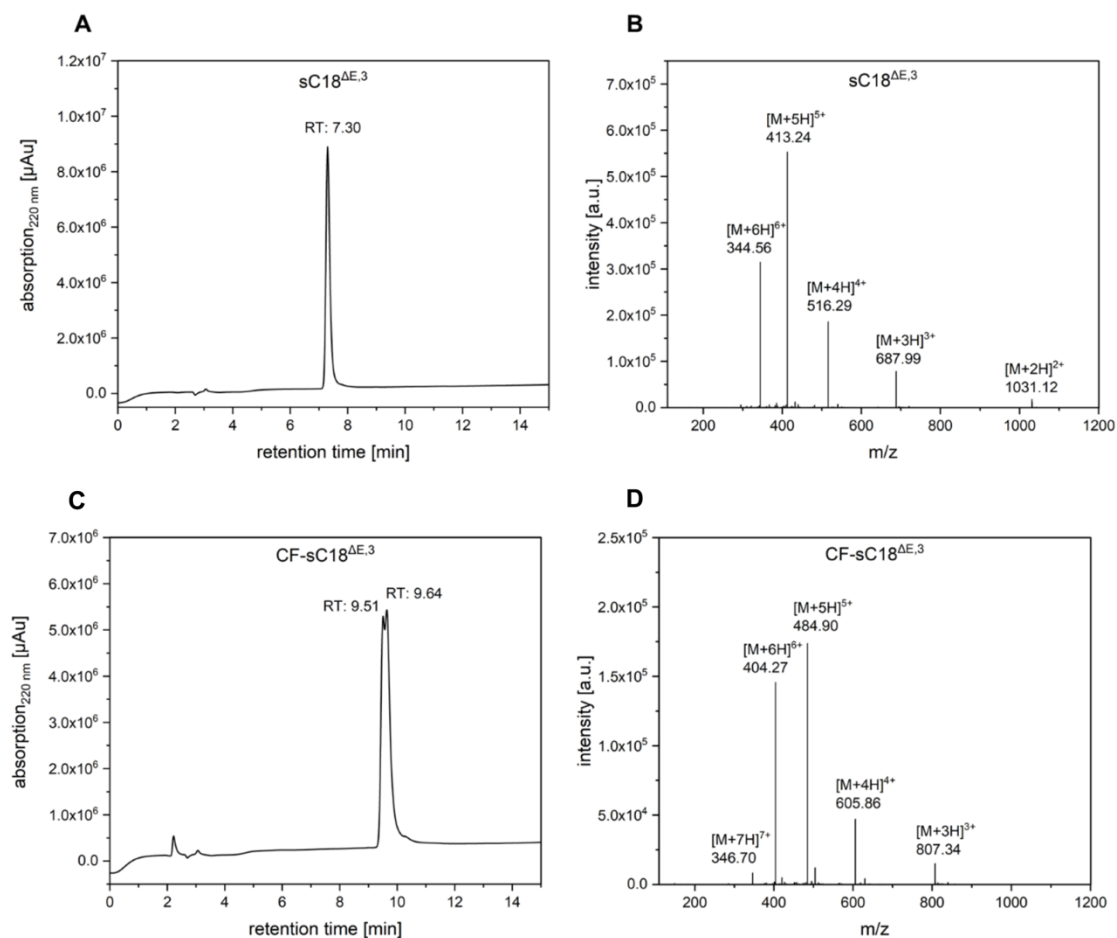


Figure S4 LC-MS analysis showing UV chromatographs was recorded using a linear gradient from 10–60% ACN in water (incl. 0.1% TFA) over 15 min (A, B). Mass spectra of sC18 $\Delta E,3$ and CF-sC18 $\Delta E,3$ (C, D), respectively.

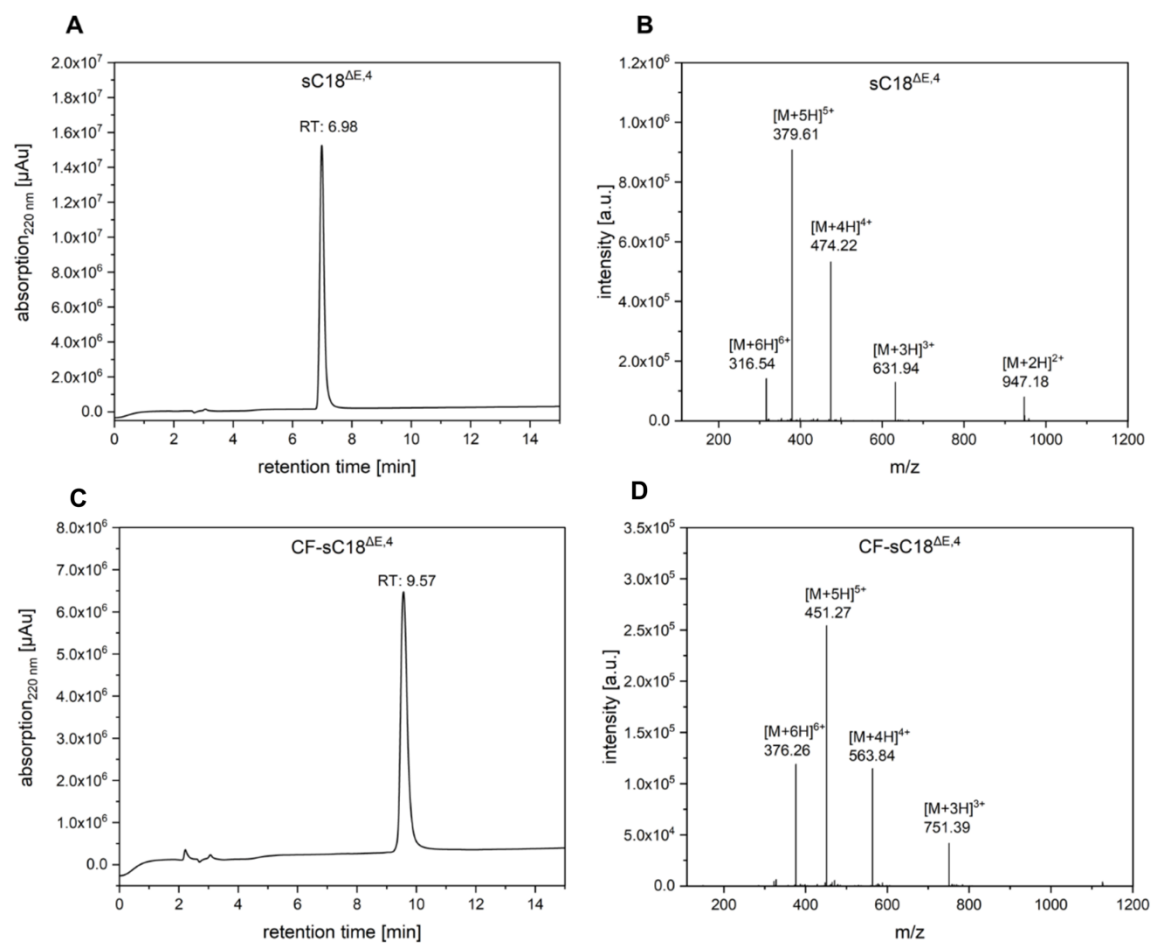


Figure S5 LC-MS analysis showing UV chromatographs was recorded using a linear gradient from 10–60% ACN in water (incl. 0.1% TFA) over 15 min (A, B). Mass spectra of sC18 $\Delta E,4$ and CF-sC18 $\Delta E,4$ (C, D), respectively.

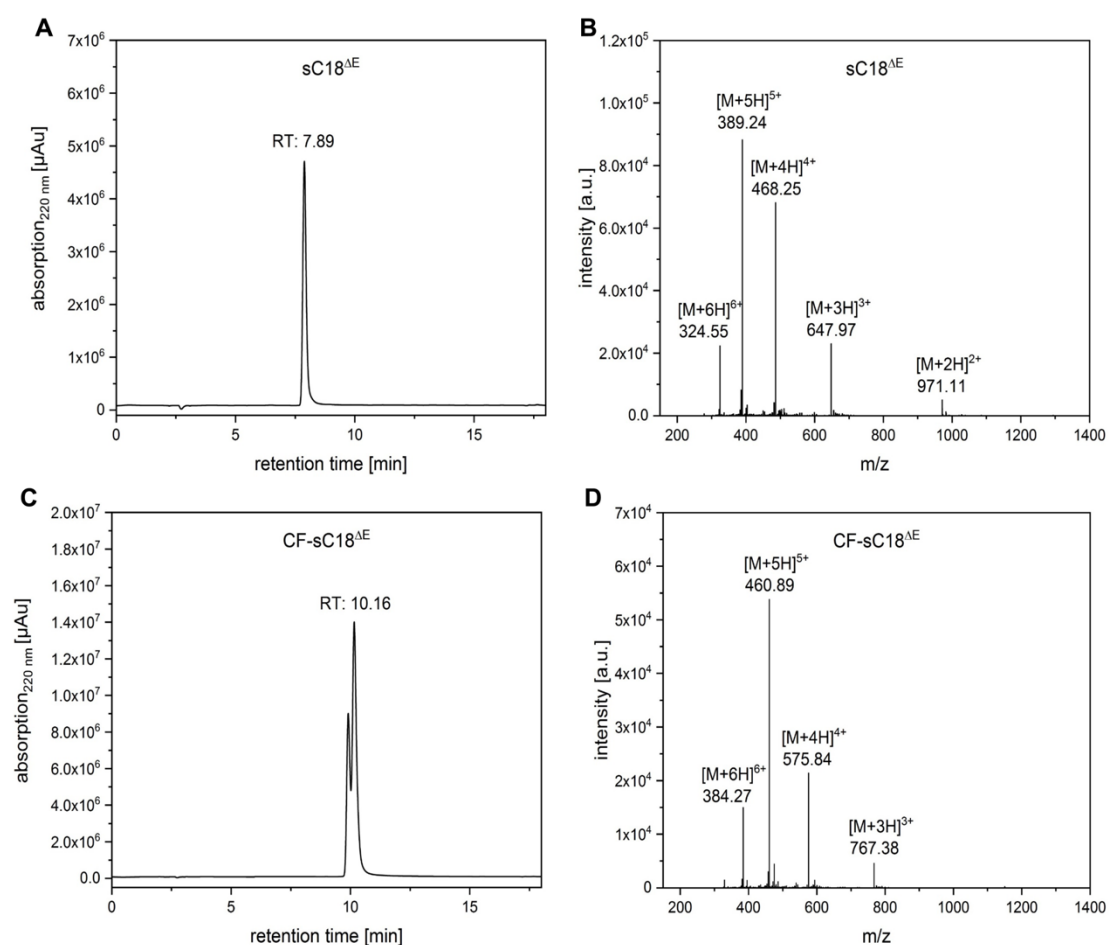


Figure S6 LC-MS analysis showing UV chromatographs was recorded using a linear gradient from 10–60% ACN in water (incl. 0.1% TFA) over 15 min (A, B). Mass spectra of sC18^{ΔE} and CF-sC18^{ΔE} (C, D), respectively.

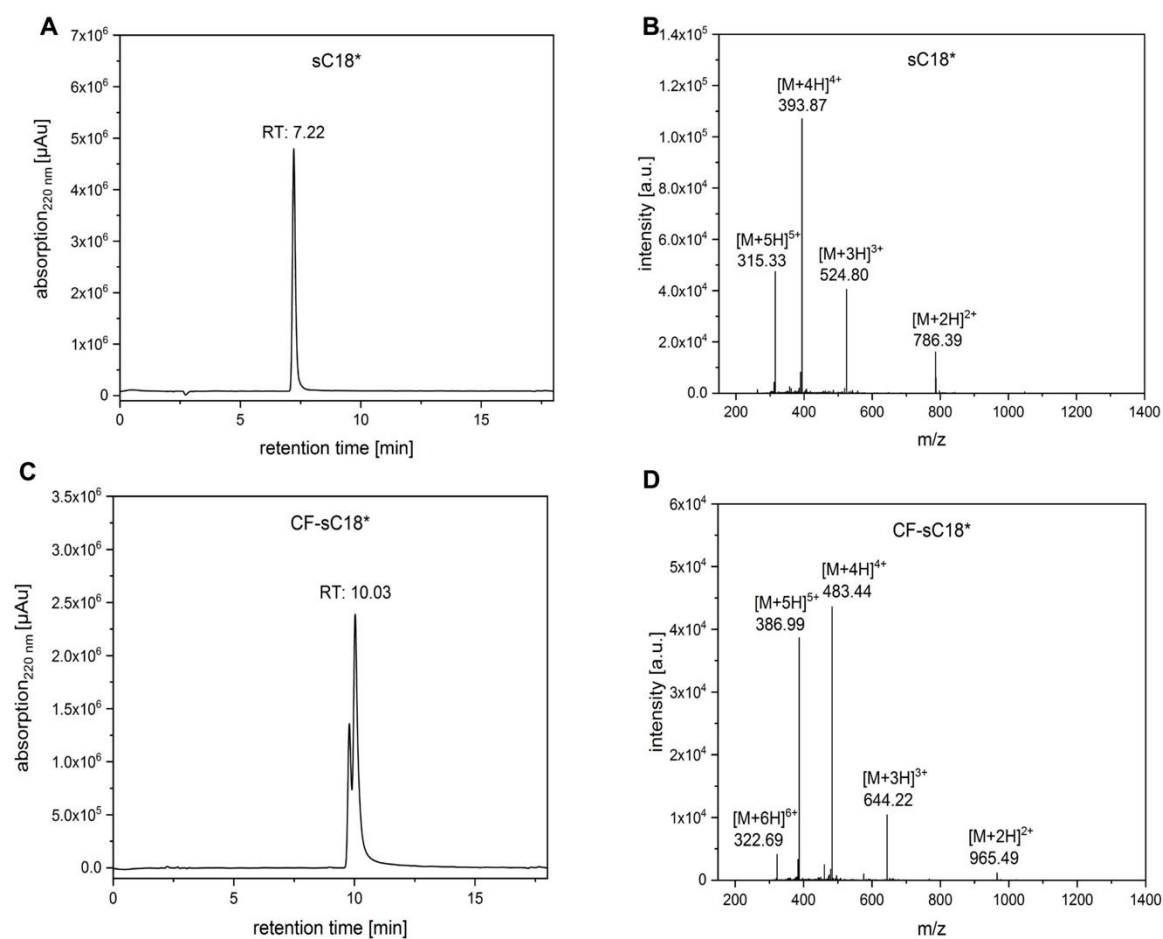


Figure S7 LC-MS analysis showing UV chromatographs was recorded using a linear gradient from 10–60% ACN in water (incl. 0.1% TFA) over 15 min (A, B). Mass spectra of sC18* and CF-sC18* (C, D), respectively.

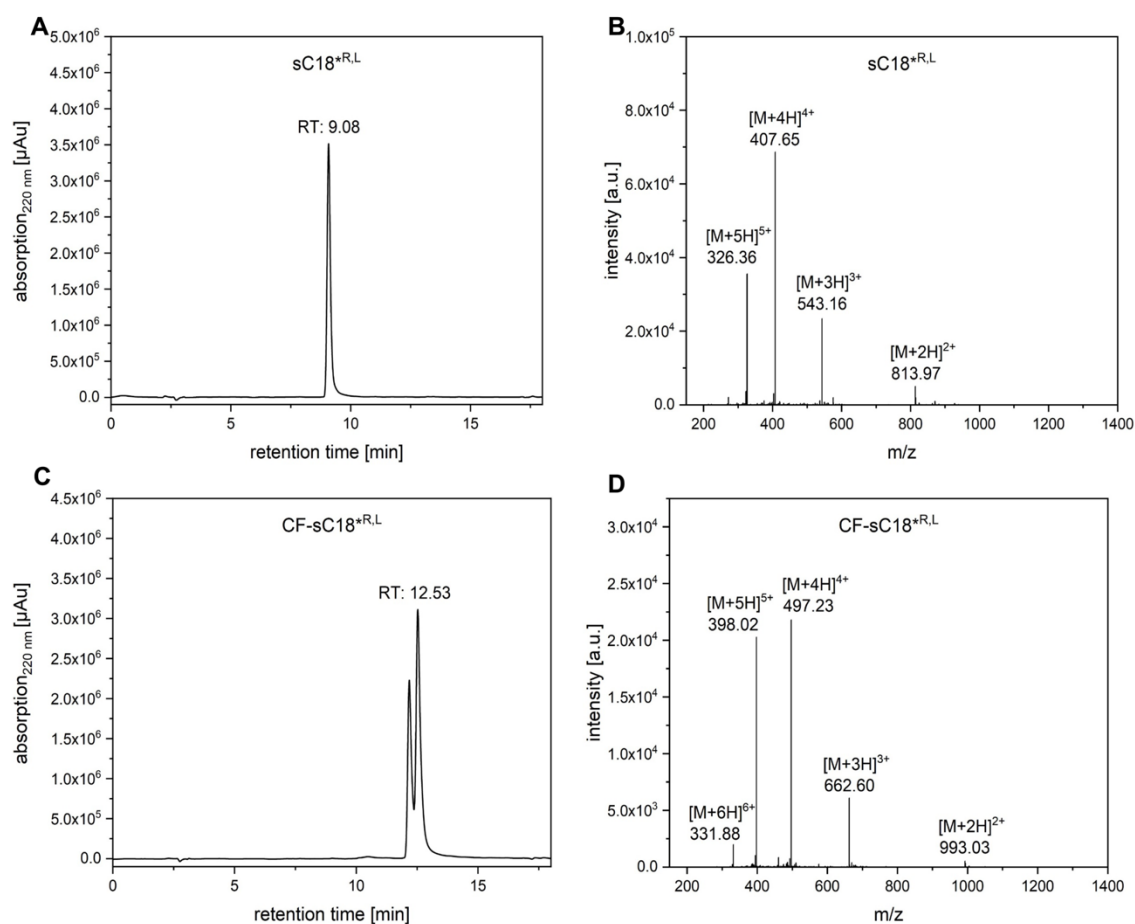


Figure S8 LC-MS analysis showing UV chromatographs was recorded using a linear gradient from 10–60% ACN in water (incl. 0.1% TFA) over 15 min (A, B). Mass spectra of $sC18^{*RL}$ and $CF-sC18^{*RL}$ (C, D), respectively.

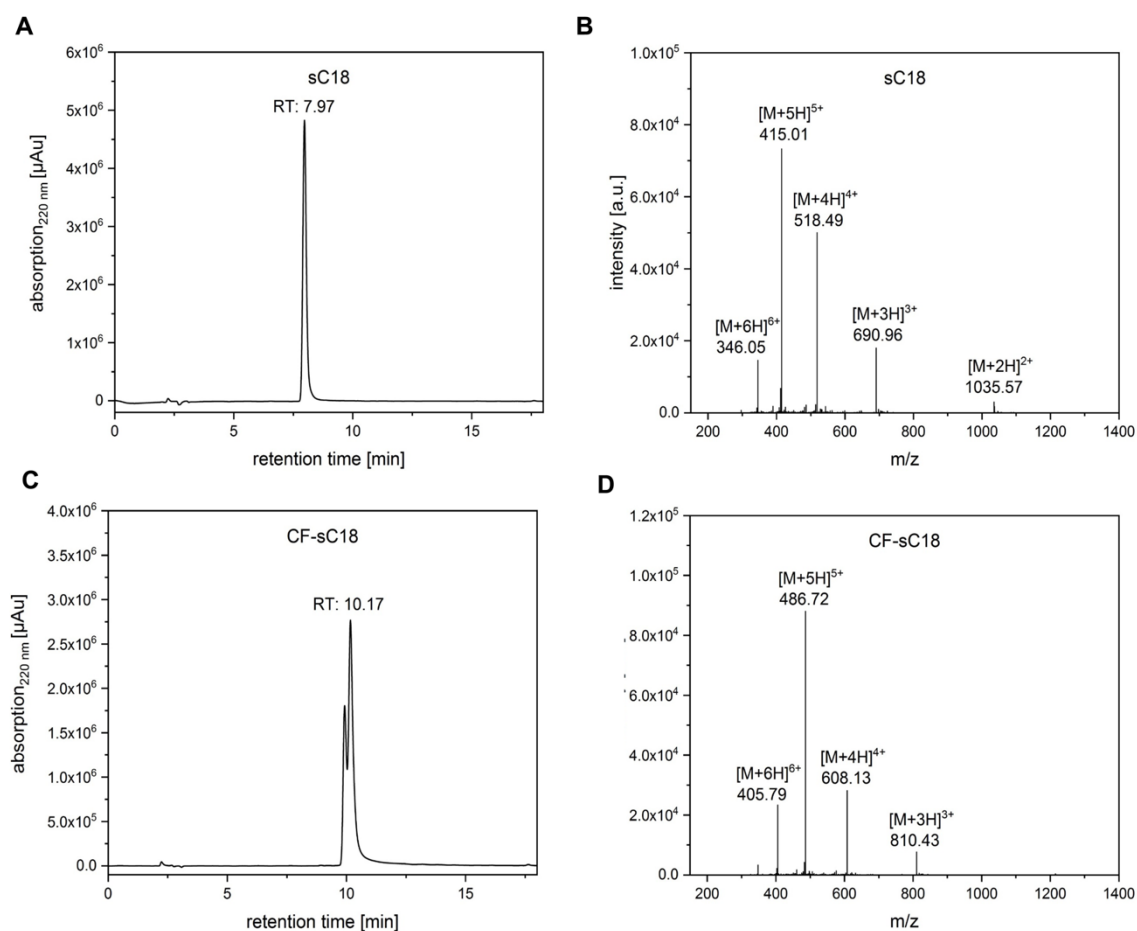


Figure S9 LC-MS analysis showing UV chromatographs was recorded using a linear gradient from 10–60% ACN in water (incl. 0.1% TFA) over 15 min (A, B). Mass spectra of sC18 and CF-sC18 (C, D), respectively.

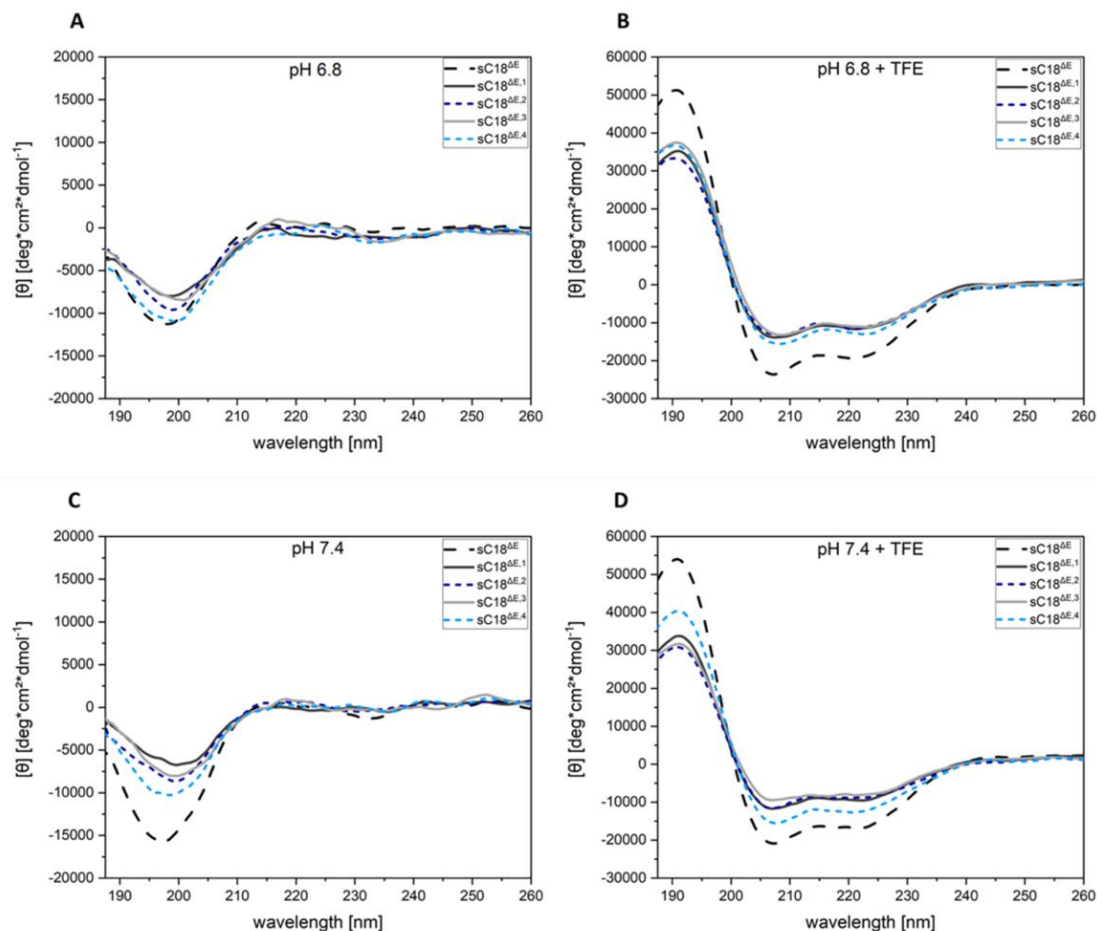


Figure S10 CD spectra of sC18^{ΔE} and all histidine variants in different media (potassium phosphate buffer: A, C and phosphate buffer with the addition of 50 % TFE: B, D) and at different pH values (pH 6.8: A, B; pH 7.4: C, D).

Table S1 Calculated R-values $\left[\frac{[\theta]_{220 \text{ nm}}}{[\theta]_{207 \text{ nm}}} \right]$ of the synthesized peptides in potassium phosphate buffer with 50 % TFE at pH 6.8 and 7.4.

Peptide	R-value	
	pH 6.8 +TFE	pH 7.4 +TFE
sC18 ^{ΔE}	0.81	0.81
sC18 ^{ΔE,1}	0.84	0.82
sC18 ^{ΔE,2}	0.88	0.76
sC18 ^{ΔE,3}	0.87	0.86
sC18 ^{ΔE,4}	0.85	0.81

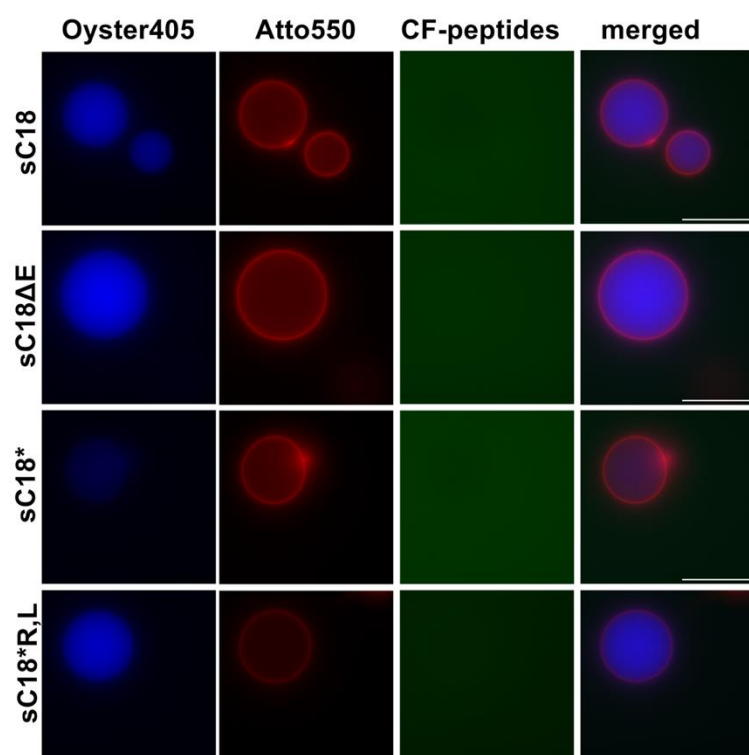


Figure S11 Peptide interaction with giant lamellar vesicles (GUVs) composed of DOPC/DOPE (50:50). 1 μ M solutions of sC18, sC18^{ΔE}, sC18^{*} and sC18^{*R,L} were incubated with GUVs for 30 min and inspected using a fluorescence microscope (Keyence). Red: Atto550; green: CF-labeled peptides, blue: Oyster 405. Scale bar: 50 μ m.

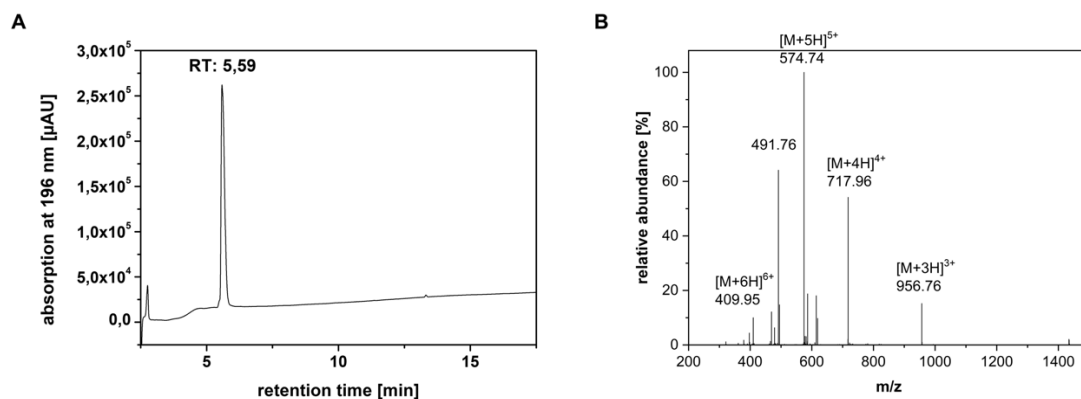


Figure S12 LC-MS analysis showing UV chromatograph (A) and corresponding mass spectra (B) of PDC-1. UV-chromatograph was recorded using a linear gradient from 10–60% ACN in water (incl. 0.1% FA) over 15 min.

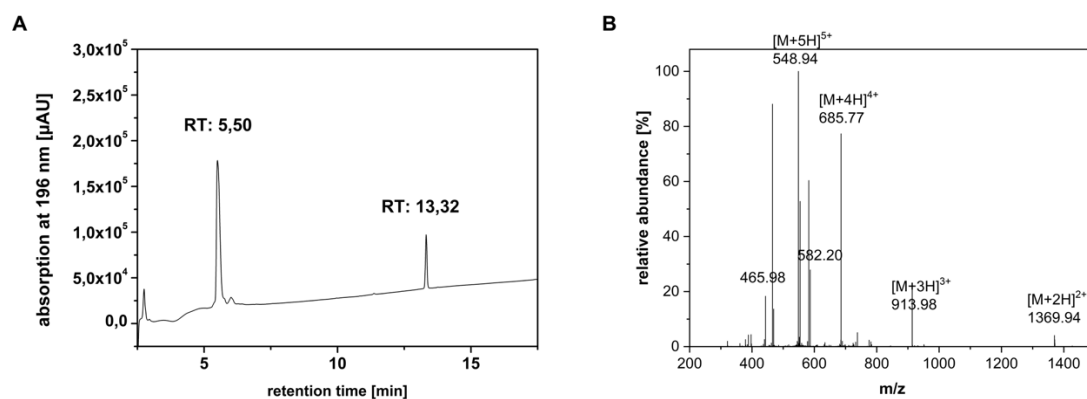


Figure S13 LC-MS analysis showing UV chromatograph (A) and corresponding mass spectra (B) of PDC-2. UV-chromatograph was recorded using a linear gradient from 10–60% ACN in water (incl. 0.1% FA) over 15 min.

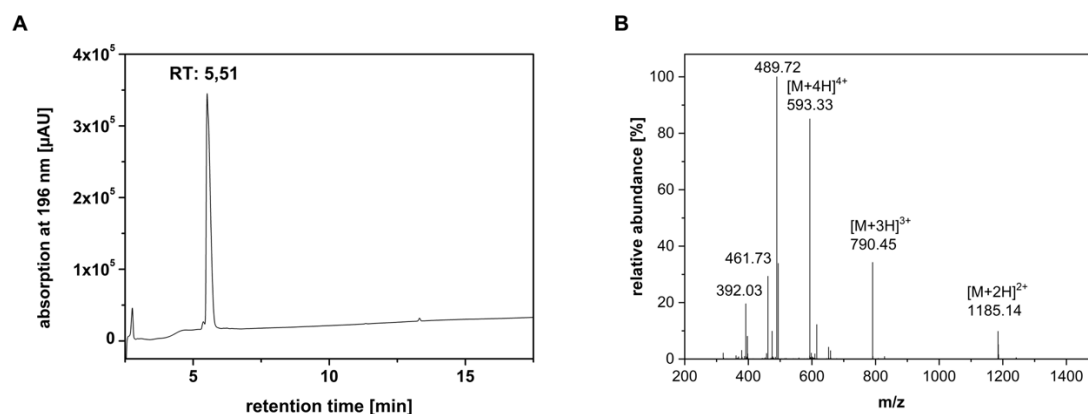


Figure S14 LC-MS analysis showing UV chromatograph (A) and corresponding mass spectra (B) of PDC-3. UV-chromatograph was recorded using a linear gradient from 10–60% ACN in water (incl. 0.1% FA) over 15 min.

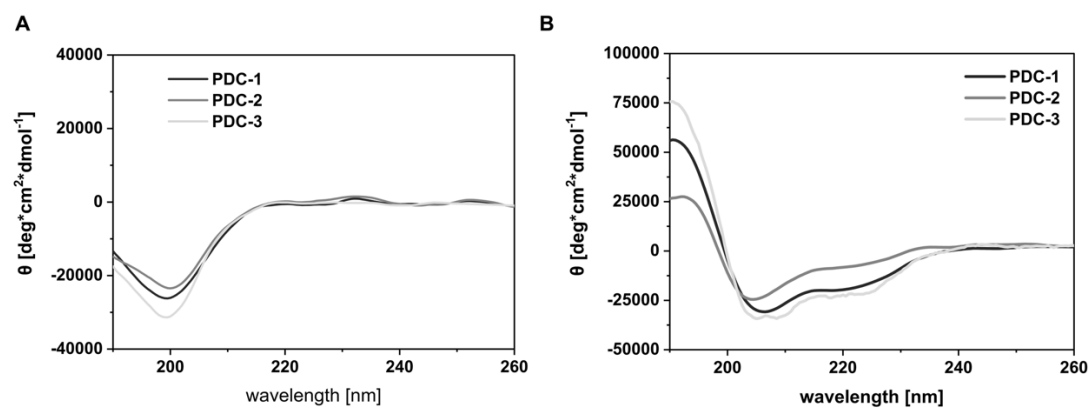


Figure S15 CD spectra of all PDCs in different media (potassium phosphate buffer (pH: 7): A and potassium phosphate buffer (pH: 7) with the addition of 50 % TFE: B)