Supplementary Information for:

Negatively-Charged Helices in the Gas-Phase

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General materials and methods

Reagents were purchased from Sigma-Aldrich, NovaBiochem, Advanced Chemtech, and Chem-Impex and were used without further purification. Synthesis of the peptides was performed with Fmoc-protected amino acids on solid phase using microwave-assisted coupling[1]. Due to the hydrophobic nature and secondary structure-forming propensity of polyalanine-based peptides, coupling efficiencies at later steps of the synthesis were poor. Microwave irradiation provided both increased efficiency and reduced reaction times over simple room temperature agitation. During each deprotection step, the reaction vessel was cooled to 0 °C before being irradiated (40 W, 45 s). During coupling, the reaction vessel was cooled to 0 °C and irradiated (40 W, 45 s, 5x), again being cooled to 0 °C between irradiations.

Aad-Ala$_{19}$-Am. Rink amide resin (47.8 mg, 0.033 mmol, 1 equiv.) was pre-swollen in *N*-methylpyrrolidone (NMP), used for its lower propensity to degrade under microwave irradiation. The resin was deprotected with piperidine (10 mL, 20% v/v in NMP; irradiated as above). Resin was rinsed with NMP and dichloromethane (DCM) (3 x 10 mL). Fmoc-Ala-OH (41.6 mg, 0.134 mmol, 4 equiv.), *N,N,N′,N′*-tetramethyl-*O*-(benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (50.7 mg, 0.134 mmol, 4 equiv.), hydroxybenzotriazole (HOBt) (20.0 mg, 0.130 mmol, 3.9 equiv.), diisopropylethylamine (DIPEA) (46.5 μL, 0.267 mmol, 8 equiv.) in NMP (4 mL) were agitated for 10 min before being added to the deprotected resin. Coupling was performed as described above and the resin was washed with NMP and DCM (3 x 10 mL). Deprotection and coupling reactions were repeated 18 additional times, to give the 19mer on resin. The final coupling step was performed with Fmoc-Aad(OtBu)-OH (58.7 mg, 0.134 mmol, 4 equiv.) HBTU (50.7 mg, 0.134 mmol, 4 equiv.), HOBt (20.0 mg, 0.130 mmol, 3.9 equiv.), DIPEA (46.5 μL, 0.267 mmol, 8 equiv.) in NMP (4 mL). Coupling was performed as described above and the resin was washed with NMP and DCM (3 x 10 mL) and deprotection was performed with piperidine

(10 mL, 20% v/v in NMP; irradiated as above). Cleavage was performed with 95:2.5:2.5 trifluoroacetic acid/DCM/triethylsilane, which was agitated for 1 hr at room temperature. The solvent was removed under a stream of nitrogen and the crude peptide was precipitated twice from diethyl ether (~10 mL). The peptide was stored as a dry powder at -20 °C. The reverse peptide, Ala_{19}-Aad-Am was synthesized as above, except that Fmoc-Aad(OtBu)-OH was coupled directly to the resin, followed by the addition of 19 alanine residues.

Ac-Ala$_{19}$-Lys. Wang resin (45.5 mg, 0.050 mmol, 1 equiv.) was pre-swollen in dry dimethylformamide (DMF) (1 mL, 20 min). Fmoc-Lys(Boc)-OH (58.6 mg, 0.125 mmol, 2.5 equiv.) and HOBt (19.2 mg, 0.125 mmol, 2.5 equiv.) were dissolved in dry DMF (2 mL) and added to the swollen resin. *N,N′*-diisopropylcarbodiimide (DIC) (6.3 mg, 0.05 mmol, 1 equiv.) and 4-dimethylaminopyridine (DMAP) (0.6 mg, 5.0 μmol, 0.1 equiv.) were added to the resin and the mixture agitated for 3 hr at room temperature. Acetic anhydride (10 mg, 0.10 mmol, 2 equiv.) and pyridine (8.0 mg, 0.10 mmol, 2 equiv.) were added, and the mixture agitated for an additional 30 min at room temperature. The resin was deprotected with piperidine (10 mL, 20% v/v in NMP; irradiated as above). Resin was rinsed with NMP and DCM (3 x 10 mL). Fmoc-Ala-OH (41.6 mg, 0.134 mmol, 4 equiv.) HBTU (50.7 mg, 0.134 mmol, 4 equiv.), HOBt (20.0 mg, 0.130 mmol, 3.9 equiv.), DIPEA (46.5 μL, 0.267 mmol, 8 equiv.) in NMP (4 mL) were allowed to activate for 10 min before being added to the deprotected resin. Coupling was performed as above and the resin was washed with NMP and DCM (3 x 10 mL). Deprotection and coupling reactions were repeated 18 additional times resulting in the 20mer on resin. Deprotection was completed with piperidine (10 mL, 20% v/v in NMP; irradiated as above) and acetylation was carried out by the addition of 20% v/v acetic anhydride in DMF (~10mL) and DIPEA (19.5 μL, 1.5 equiv.), agitated for 30 min at room temperature. Cleavage was performed with 95:2.5:2.5 trifluoroacetic acid/DCM/triethylsilane, which was agitated for 1 hr at room temperature. The solvent was removed under a stream of nitrogen and the crude peptide was precipitated twice from diethyl ether (~10 mL). The peptide was stored as a dry powder at -20 °C. The reverse peptide, Ac-Lys-Ala_{19} was synthesized as above, except that an alanine residue was loaded on the resin first, followed by coupling of 18 additional alanine units before the final lysine residue.

Ion Mobility Measurements

Measurements were performed on a Synapt G2 (Waters) ion mobility mass spectrometer. Peptides were dissolved in a 50:50 mixture of acetonitrile/water with 0.1% formic acid. Samples were directly infused at 20 µL/min. Ion mobility separations were performed in N_2 at 100 mL/min (~3.6 bar). Travelling wave velocity was held at 400 m/s and wave height of 40 V. Data analysis was performed in Driftscope 2.4.

Characterization of the Elongated Conformer of Ala_{19}-Aad-Am

Fragmentation of the elongated conformers observed for $[Ala_{19}\text{-Aad-Am-H}]^-$ indicate that they are identical in sequence to the desired species, and not a contaminating peptide (data not shown). Drift time distributions for Ala_n-Aad-Am for n = 15-19 are shown in Figure S1. The elongated structure becomes more populated with increasing peptide length suggesting that the propensity of polyalanine to form a helix is strong enough to overcome the potentially destabilizing forces mentioned in the text at sufficient peptide length (*i.e.* >15 Ala residues). In addition, it is possible that a different charge configuration occurs in a subset of the ions, leading to this structure, which would also be favored in a longer peptide.

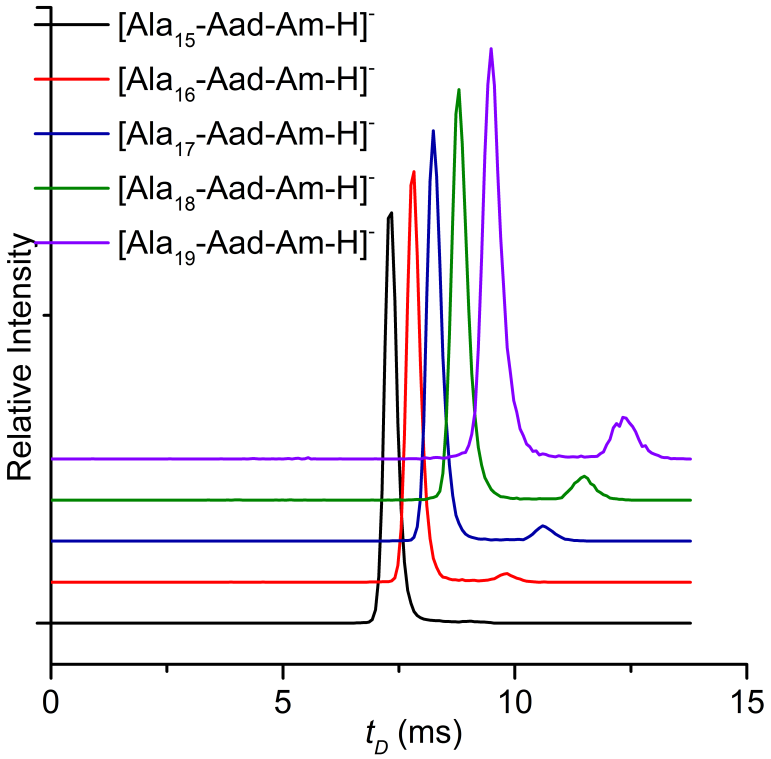

Figure S1. Drift time distributions of $[Ala_n\text{-Aad-Am-H}]^-$. All intensities are normalized.

Measurements of Acidic Peptides in ESI(+)

The helical peptide, $[Ala_n\text{-Aad-Am-H}]^-$is expected to lose the stabilizing contributions to a helical structure under positive ionization, as the terminal amine would likely be positively charged, the acidic side chain likely neutral, or perhaps adopting a zwitterionic form. In order to further validate our predictions, we measured the acidic peptides under both ESI(+) and ESI(-). The drift times are presented in Figure S2. Both acidic peptides in positive mode adopt a confirmation consistent with a globular form, with only the designed helical peptide, $[Ala_n\text{-Aad-Am-H}]^-$, adopting a predominantly elongated conformation. In these studies, a minor population of elongated species were observed for both $[Ala_n\text{-Aad-Am+H}]^+$ and $[Aad\text{-}Ala_n\text{-Am+H}]^+$. The populations increase with peptide length just as in Figure S1. As such, we attribute these minor elongated structures to the helical propensity overcoming the potential destabilizing forces at sufficient peptide length.

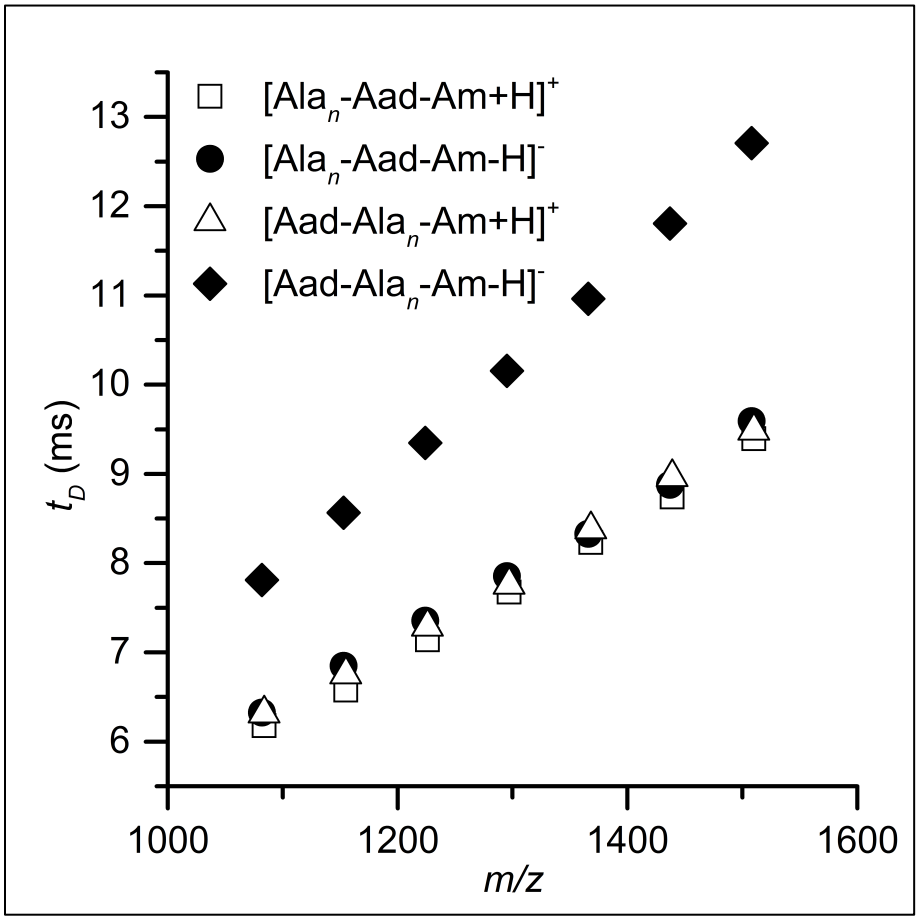

Figure S2. Drift times for acidic peptides measured in ESI(+) and ESI(-).

Molecular Dynamics simulations

MD simulations were performed in InsightII. During initial helix design, candidate structures were heated from 300 to 500 K over 10 ps, cooled to 300 K over 5 ps, and allowed to equilibrate for 30 ps. 100 iterations were performed and the lowest energy structures were manually assessed for helical propensity. For determination of stable CCS for the four series of peptides in the text, annealing was similarly performed, with melting temperatures of 350 K for globular structures and 310 K for helical structures, in order to obtain more uniform results. Higher temperature experiments were performed with no effective difference in outcome. CCS's were calculated using the trajectory method in mobcal.

References

1. B. Bacsa, B. Desai, G. Dibo and C. O. Kappe, *J. Pept. Sci.*, 2006, **12**, 633-638.