IRMPD Spectroscopy: Evidence of Hydrogen Bonding in the Gas Phase Conformations of Lasso Peptides and their Branched-Cyclic Topoisomers

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EXPERIMENTAL METHODS

Purification of lasso peptides. MccJ25 and capistruin were extracted from the culture supernatants by solid phase extraction using SepPak C_8 35 cc cartridges (Waters). Upon loading of the supernatant, the resin was washed with water/0.1% formic acid and eluted with 20%, 30% and 40% acetonitrile/0.1% formic acid in water. The eluted fractions containing the peptide of interest were then evaporated and purified by semi-preparative reversed-phase

Ultimate 3000 HPLC system (Dionex) on a Luna C_{18} column (250 mm × 4.6 mm × 5 µm). Elution was performed using the following gradient of water/0.1% formic acid (solvent A) and acetonitrile (solvent B) at 40°C and 1 mL/min: linear increase from 10% B to 40% B within 30 min followed by a linear increase to 100% B in 5 min.

ESI source parameters. Multiprotonated charged ions of peptides were generated with an electrospray source ionization (ESI) operated in the positive ion mode. Solutions were introduced in the source using direct infusion with a syringe pump with a flow rate of 120 µL/h. The instrument was operated at a capillary voltage of 4000 V using N_2 as nebulizer gas at 1.5 bar and as dry gas at 4.5 L/min and a dry temperature of 150 °C. All mass spectra were acquired in the broadband mode from *m/z* 97.2 to *m/z* 1200. The image signal was amplified and digitized using 256 K data point resulting in the recording of a 0.12 s time domain signal, which was transformed into the corresponding frequency domain by Fourier transform. No apodization was used here.

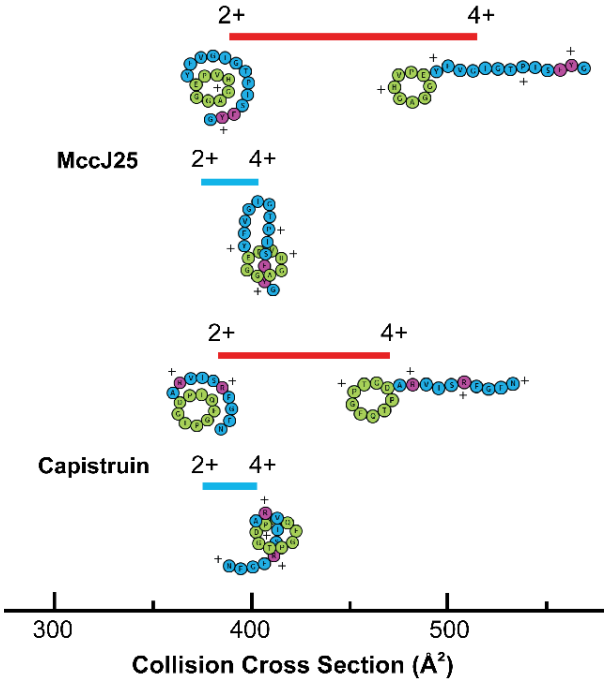

Figure S1. CCS range of different charge states of the lasso peptides (blue traces) and their corresponding branched-cyclic (red traces) topoisomers.

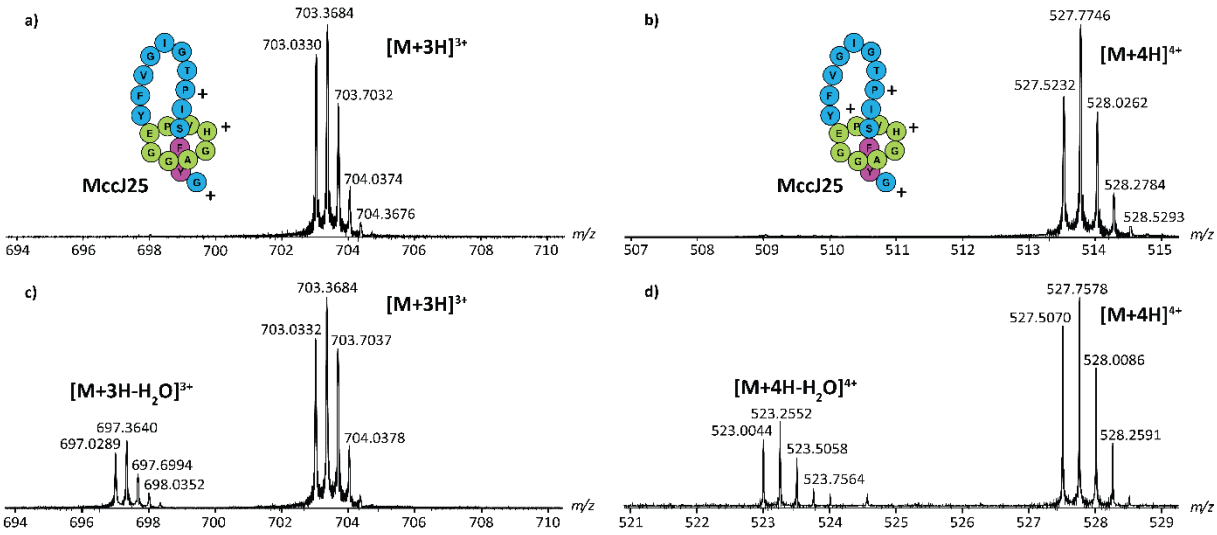

Figure S2. Mass spectra of mass-selected a) $[M+3H]^{3+}$ and b) $[M+4H]^{4+}$ ions and IRMPD spectra of c) $[M+3H]^{3+}$ and d) $[M+4H]^{4+}$ ions of MccJ25. IRMPD spectra were obtained at 3475 cm^{-1} with an irradiation time of 1 ms and a CO_2 pulse of 500 µs.

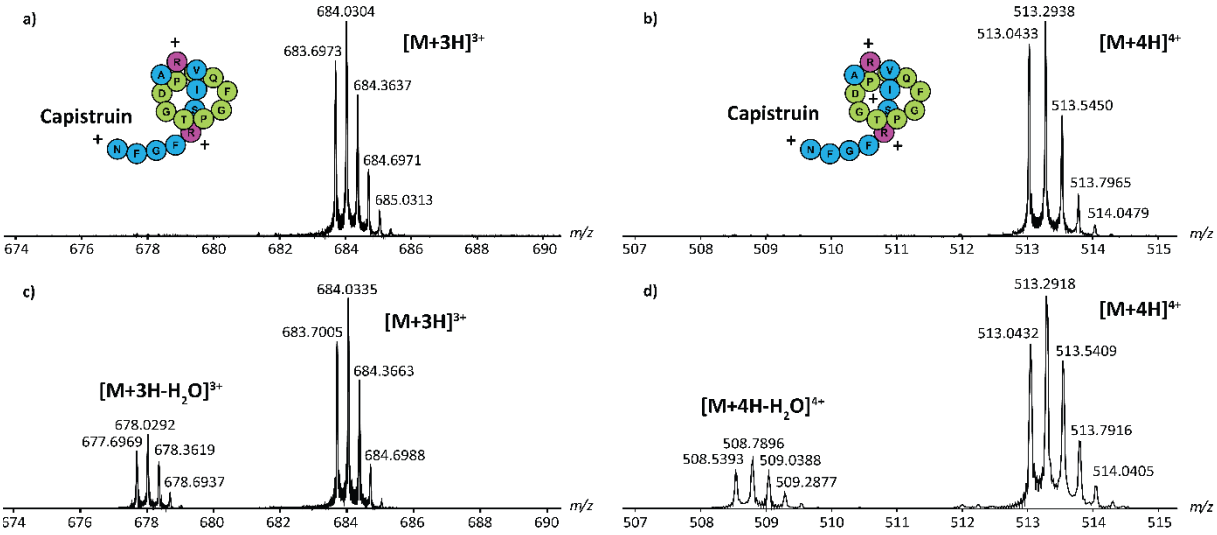

Figure S3. Mass spectra of mass-selected a) $[M+3H]^{3+}$ and b) $[M+4H]^{4+}$ ions and IRMPD spectra of c) $[M+3H]^{3+}$ and d) $[M+4H]^{4+}$ ions of capistruin. IRMPD spectra were obtained at 3360 cm^{-1} with an irradiation time of 1 ms and a CO_2 pulse of 1 ms.

Table S1. Experimental vibrational frequencies (cm^{-1}) of MccJ25 and its branched-cyclic topoisomer in the 1400-1800 and 2800-3700 cm^{-1} spectral regions.

	Lasso		Br. cycl.		
	$[M+3H]^{3+}$	$[M+4H]^{4+}$	$[M+3H]^{3+}$	$[M+4H]^{4+}$	Tentative assignment
CCS	383 Å²	404 Å²	437 Å²	514 Å²	
IRMPD (cm^{-1})	1505	1505	n.d.	1495	Amide NH bend
	n.d.	n.d.	1540	n.d.	H-bonded amide NH bend
	1610-1640	1610-1640	1610-1640	n.d.	H-bonded C=O stretch
	1650-1710	1650-1710	1650-1710	1650-1710	Carbonyl C=O stretch
	2930-3000	2930-3000	2930-3000	2930-3000	Aliphatic/aromatic CH stretch
	3200-3450	3200-3450	3200-3450	n.d.	H-bonded amide NH stretch
	3475	3475	3480	3475	Amide NH stretch
	3570	3570	3570	3570	Acid carboxylic OH stretch
	3640	3640	3640	3640	Alcohol OH stretch

n.d.: no signal detected; Br. cycl.: branched-cyclic peptide

Table S2. Experimental vibrational frequencies (cm^{-1}) of capistruin and its branched-cyclic topoisomer in the 1400-1800 and 2800-3700 cm^{-1} spectral regions.

	Lasso		Br. cycl.		
	$[M+3H]^{3+}$	$[M+4H]^{4+}$	$[M+3H]^{3+}$	$[M+4H]^{4+}$	Tentative assignment
CCS	373 Å²	403 Å²	388 Å²	471 Å²	
IRMPD (cm^{-1})	1505	1505	n.d.	1500	Amide NH bend
	n.d.	n.d.	1525	n.d.	H-bonded amide NH bend
	1610-1640	1610-1640	1610-1640	n.d.	H-bonded C=O stretch
	1655-1710	1655-1710	1655-1710	1655-1710	Carbonyl C=O stretch
	2930-3000	2930-3000	2930-3000	2930-3000	Aliphatic/aromatic CH stretch
	3200-3410	3200-3410	3200-3410	n.d.	H-bonded amide NH stretch
	3440	3440	3440	3440	Amide NH_2 sym. stretch
	3470	3470	3470	3470	Amide NH stretch
	3520 (sh.)	3520 (sh.)	3520 (sh.)	3520 (sh.)	H-bonded OH stretch
	3550	3550	3550	3555	Amide NH_2 asym. stretch
	n.d.	n.d.	n.d.	3650	Alcohol OH stretch

n.d.: no peak detected; Br. cycl.: branched-cyclic peptide; sh.: shoulder