Ion mobility - mass spectrometry of lasso peptides: signature of a rotaxane topology

Kevin Jeanne Dit Fouque,[†] Carlos Afonso,[†*] Séverine Zirah,[‡] Julian D. Hegemann,[§] Marcel Zimmermann,[§] Mohamed A. Marahiel,[§] Sylvie Rebuffat,[‡] Hélène Lavanant [†]

[†] Normandie Univ, COBRA, UMR 6014 and FR 3038; Université de Rouen; INSA Rouen; CNRS, IRCOF, 1 Rue Tesnière, 76821 Mont-Saint-Aignan Cedex, France

[‡] Muséum national d'Histoire naturelle, Sorbonne Universités, Centre national de la Recherche scientifique, Laboratoire Molécules de Communication et Adaptation des Microorganismes, UMR 7245 CNRS-MNHN, CP 54, 57 rue Cuvier, 75005 Paris, France.

[§] Philipps-Universität Marburg, Fachbereich Chemie-Biochemie, Hans-Meerwein-Strasse 4 *and LOEWE-Center for Synthetic Microbiology* , 35032 Marburg, Germany.

Supporting Information

Table S1. Experimental parameters used for the ion mobility mass spectrometry measurements on the SYNAPT G2™ (Waters)

ESI + V Resolution mode		DC potentials (V)		Traveling wave parameters	
m/z range	50 -2000	Trap Collision Energy	5	Source Wave Velocity (m/s)	300
Capillary (kV)	2.7	Transfer Collision Energy	0	Source Wave Height (V)	0.2
Source Temperature (°C)	70	Trap DC Entrance	3	Trap Wave Velocity (m/s)	300
Sampling Cone (V)	20	Trap DC Bias	40	Trap Wave Height (V)	5
Extraction Cone (V)	5	Trap DC	-1	IMS Wave Velocity (m/s)	350
Source Gas Flow (mL/min)	20	Trap DC Exit	0	IMS Wave Height (V)	20
Desolvation Temperature (°C)	250	IMS DC Entrance	25	Transfer Wave Velocity (m/s)	350
Cone Gas Flow (L/hr)	0	Helium Cell DC	35	Transfer Wave Height (V)	5
Desolvation Gas Flow (L/hr)	500	Helium Exit	-5		
Trap Gas Flow (mL/min)	0.4	IMSBias	0		
Helium Cell Gas Flow	180	IMS DC Exit	0		
IMS Gas Flow (mL/min)	70	Transfer DC Entrance	4		
Sample Infusion Flow Rate (µL/min)	30	Transfer DC Exit	15		

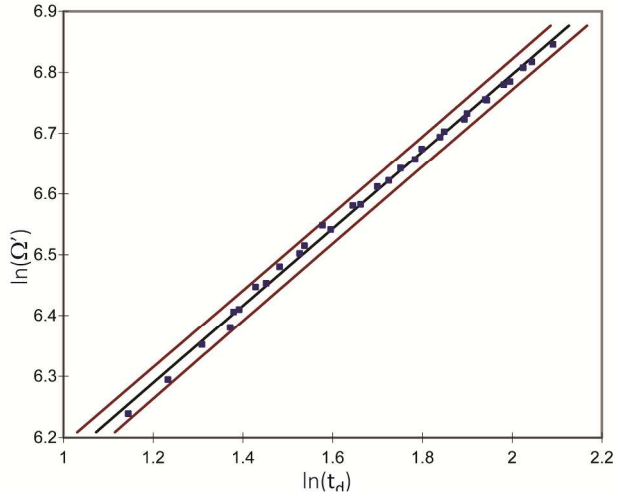

Figure S1. Correlation used for the estimation of collision cross section from TWIM drift times. Determination coefficient was 0.9973. The red lines are the error of prediction with a confidence level of 95%.

The reference data used for the calibration of TWIM drift times to estimate collision cross sections are the drift-tube derived collisional cross sections of doubly and protonated polyalanine determined in helium gas by Bush *et al.*. Using the method proposed by Smith *et al*., the cross sections were converted to reduce cross sections Ω´ using nitrogen gas and correlated with experimental TWIM drift times. Natural logarithm was used to linearize the equation. The equation then used with the measured drift times of the compounds in this study and the resulting Ω´ were converted back to collision cross section in helium. Very little change was observed on the determination coefficient, when nitrogen collision cross sections instead of helium cross sections and when nitrogen gas or helium was used to calculate reduced mass and cross sections.

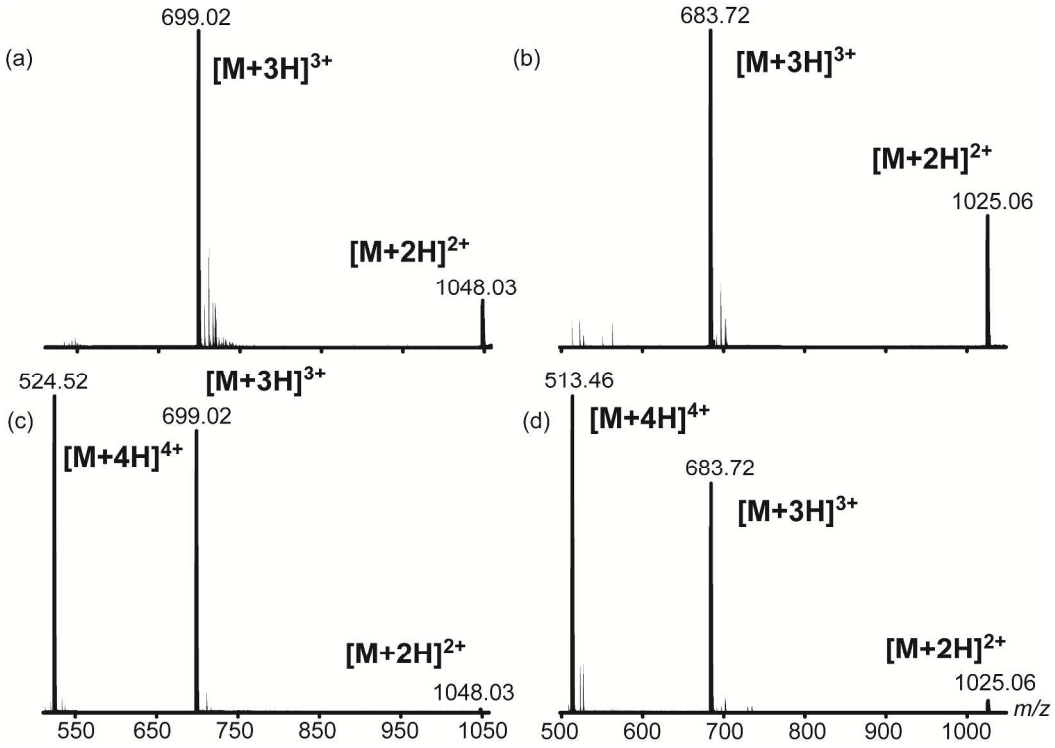

Figure S2. Mass spectra of astexin-1 (a) and (c) and capistruin (b) and (d) lasso without sulfolane (a) and (b) and with sulfolane (c) and (d).

Table S2. Drift time, FWHM and relative intensities from the ion mobility spectra of five class II lasso peptides and their synthetic branched-cyclic topoisomers obtained from 10^{-5} mol/L peptide solutions with and without sulfolane

		$[M+2H]^{2+}$			$[M+3H]^{3+}$			$[M+4H]^{4+}$		
Peptides	Sulfolane (mM)	Drift Time (ms)	FWHM (ms)	Relative Intensity (%)	Drift Time (ms)	FWHM (ms)	Relative Intensity (%)	Drift Time (ms)	FWHM (ms)	Relative Intensity (%)
Astexin-1 lasso	50	8.51	0.53	1	4.49	0.19	46	3.39	0.17	53
	0	8.49	0.53	8	4.50	0.19	92	n. d.	n. d.	n. d.
Astexin-1 br. cycl.	50	8.75	0.61	1	4.76	0.26	32	4.34	0.19	67
	0	8.76	0.63	16	4.77	0.27	84	n. d.	n. d.	n. d.
Capistruin lasso	50	8.65	0.36	2	4.51	0.31	46	3.20	0.21	52
	0	8.66	0.37	24	4.51	0.34	76	n. d.	n. d.	n. d.
Capistruin br. cycl.	50	8.93	0.53	0	4.74	0.42	21	3.88 / 4.10 / 4.37	0.14 / 0.18 / 0.23	79
	0	8.93	0.53	4	4.72	0.42	86	3.90 / 4.11 / 4.39	0.15 / 0.21 / 0.22	10
Caulosegnin I lasso	50	8.35	0.37	2	4.21	0.18	94	2.84	0.14	4
	0	8.36	0.38	35	4.22	0.18	65	n. d.	n. d.	n. d.
Caulosegnin I br. cycl.	50	8.18	1.03	1	5.31	0.66	29	4.12	0.31	70
	0	8.17	1.00	30	5.29	0.66	70	n. d.	n. d.	n. d.
Microcin J25 lasso	50	8.66	0.65	1	4.67	0.22	91	3.21	0.23	8
	0	8.67	0.61	11	4.67	0.23	89	n. d.	n. d.	n. d.
Microcin J25 br. cycl.	50	9.16	0.54	1	5.14 / 5.71	0.66 / 0.35	42	4.72	0.17	57
	0	9.16	0.54	18	5.15 / 5.70	0.66 / 0.36	82	n. d.	n. d.	n. d.
Syanodin I lasso	50	5.70	0.31	36	3.04	0.16	64	n. d.	n. d.	n. d.
	0	5.71	0.32	100	n. d.	n. d.	0	n. d.	n. d.	n. d.
Syanodin I br. cycl.	50	5.92	0.37	7	3.79	0.62	93	n. d.	n. d.	n. d.
	0	5.91	0.37	99	3.77	0.63	1	n. d.	n. d.	n. d.

n. d.: no peak detected; br. cycl.: synthetic branched-cyclic peptide

The determination coefficient and slopes were very close to 1, even when only doubly and triply charged ions were correlated.

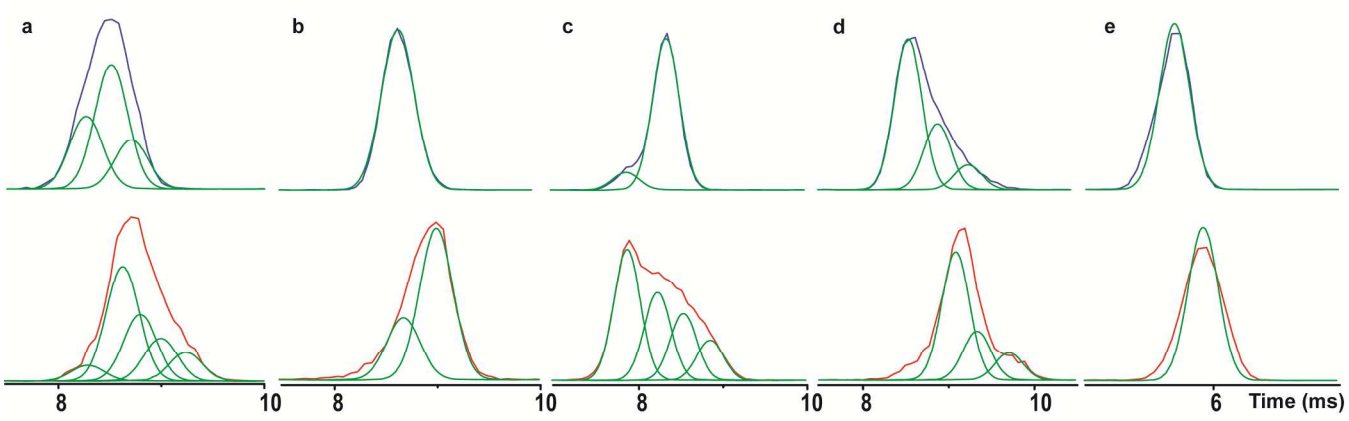

Figure S3. Drift time profiles and fitted peaks (green trace) of doubly protonated ion $[M+2H]^{2+}$ of lasso (top blue trace) and branched-cyclic (bottom red trace) peptides of (a) astexin-1, (b) capistruin, (c) caulosegnin I, (d) microcin J25, and (e) syanodin

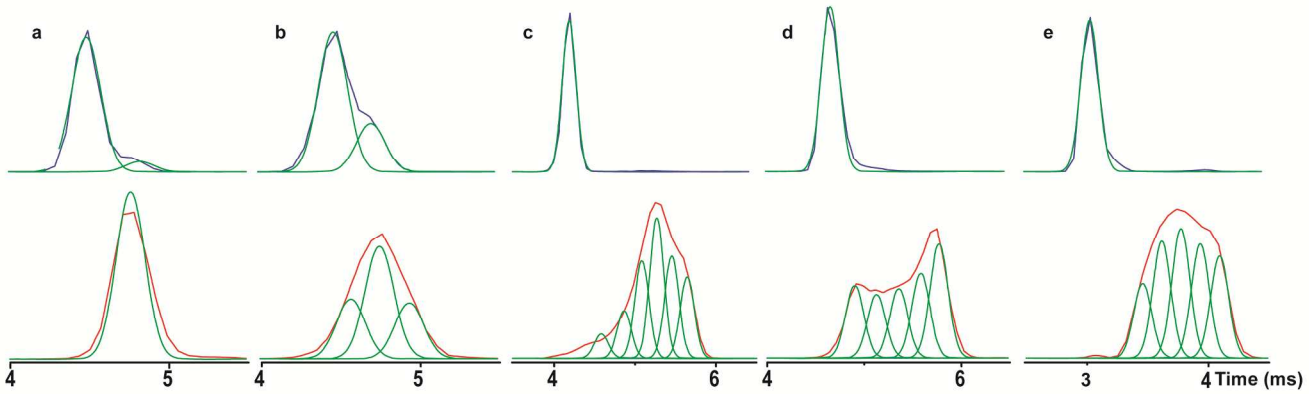

Figure S4. Drift time profiles and fitted peaks (green trace) of triply protonated ion $[M+3H]^{3+}$ of lasso (top blue trace) and branched-cyclic (bottom red trace) peptides of (a) astexin-1, (b) capistruin, (c) caulosegnin I, (d) microcin J25, and (e) syanodin

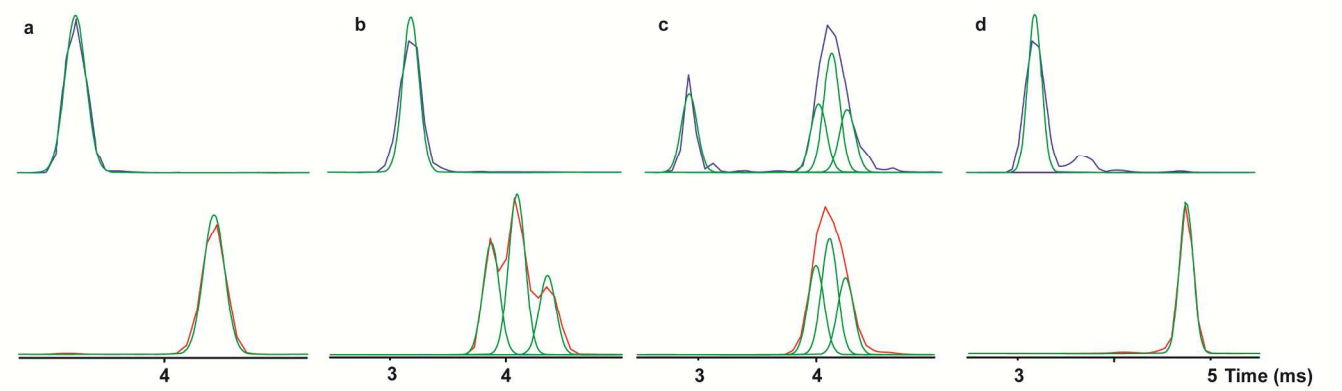

Figure S5. Drift time profiles and fitted peaks (green trace) of quadruply protonated ion $[M+4H]^{4+}$ of lasso (top blue trace) and branched-cyclic (bottom red trace) peptides of (a) astexin-1, (b) capistruin, (c) caulosegnin I, (d) microcin J25. The caulosegnin I lasso sample (c) contained significant amount of cyclic branched peptide (as verified by LC-MS). **The microcin J25 lasso (d) contained a second peak which correspond to a fragment of this peptide (as verified by MS-MS/IM-MS).**

The search for hidden peaks was carried out with Origin Pro 9.1 using the peak analyzer fit tool. No baseline treatment was needed and applied, gaussian peak shapes were assumed. The peak filtering was set to 10% by height, and hidden peaks were searched using the second derivative except for the triply charged protonated molecules of capistruin, caulosegnin I, microcin J25 and syanodin I branched-cyclic peptides (Figure S4 bottom red traces: b, c, d, and e) and $[M+4H]^{4+}$ of caulosegnin I (Figure S5 bottom red trace: c) branched-cyclic peptide for which the residual of the first derivative gave higher R^2 values. In all case, the R^2 values were higher than 0.95. Each experiment was repeated five times, and the drift time values obtained after peak fitting were found to vary less than 0.5%.