

Supplementary Information

Fast and Effective Ion Mobility – Mass Spectrometry Separation of D-Amino Acid Containing Peptides

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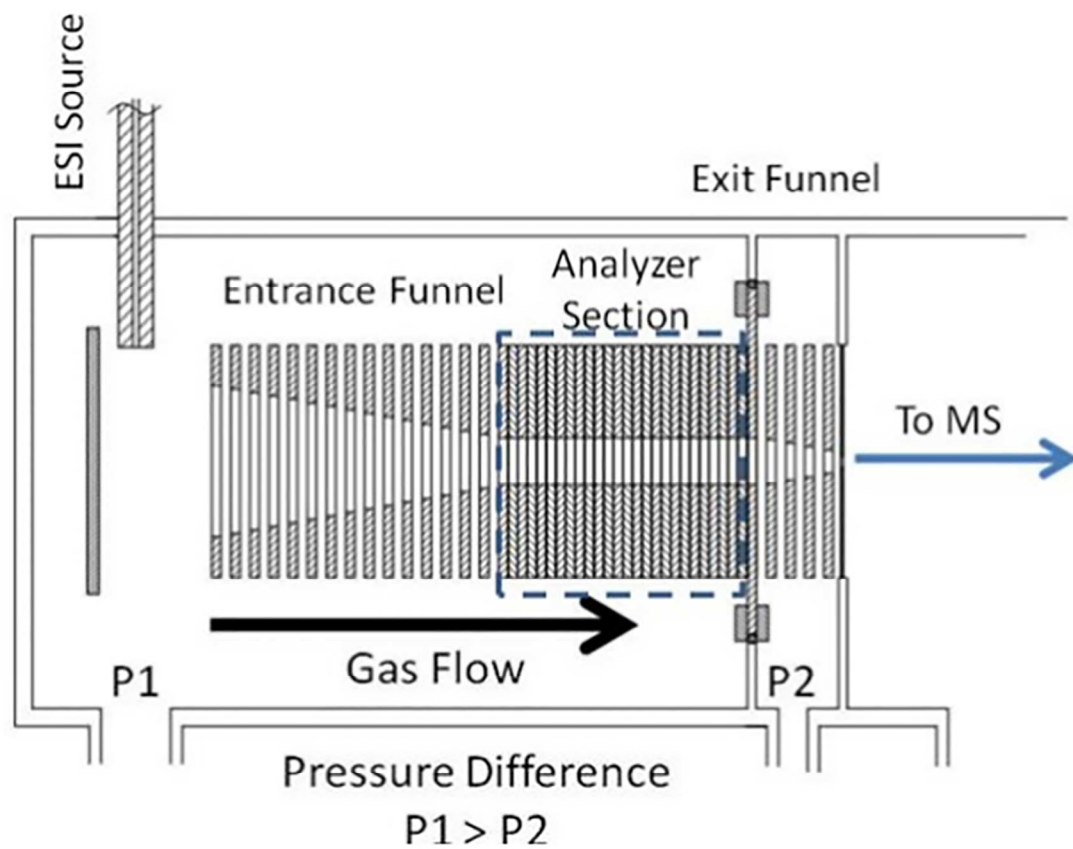


Figure S1. Scheme of the TIMS cell

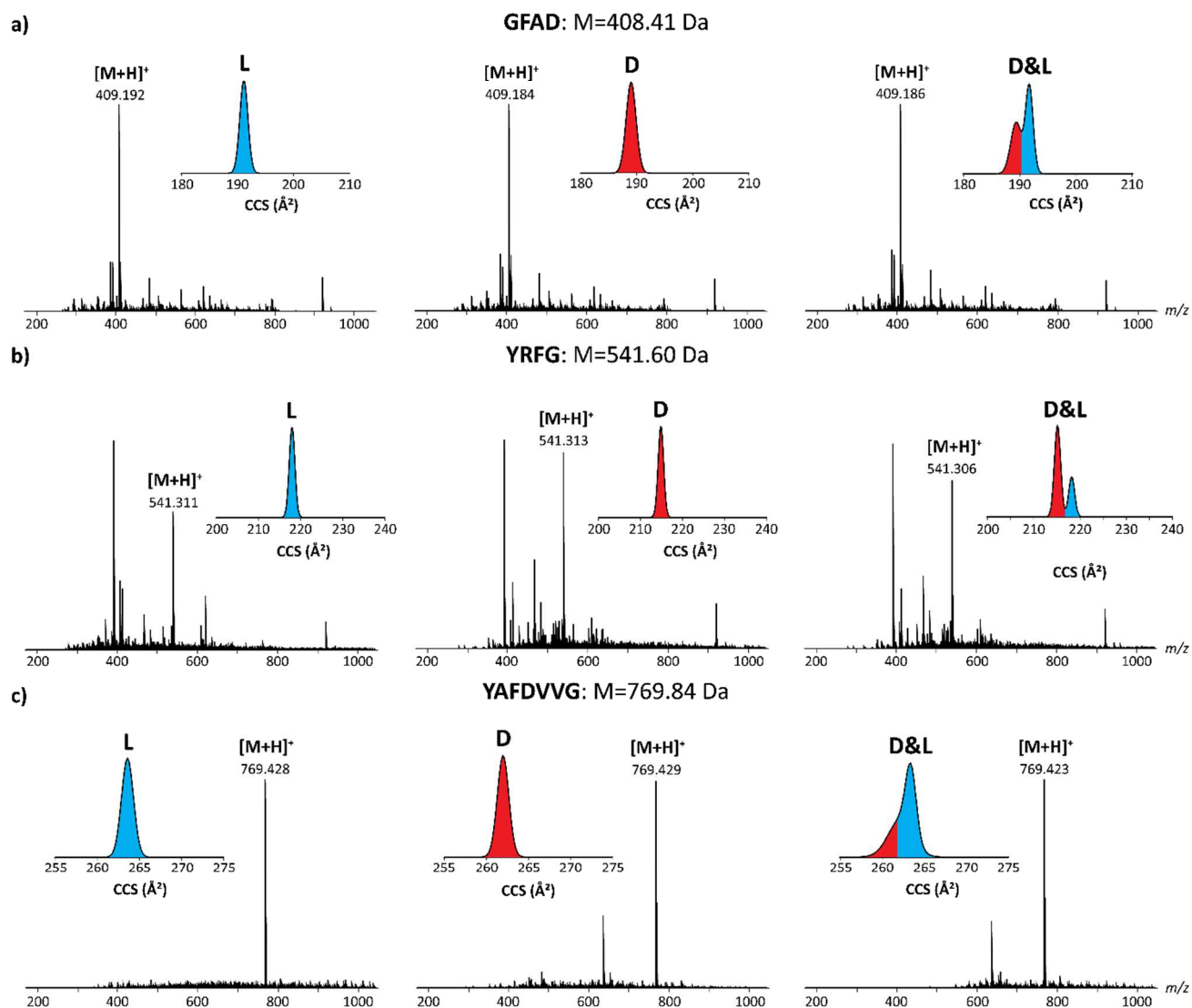


Figure S2. Mass spectra and ion mobility profiles using TIMS of the smallest peptides a) GFAD, b) YRFG and c) YAFDVVG. The D- and L-epimers are colored in red and blue, respectively.

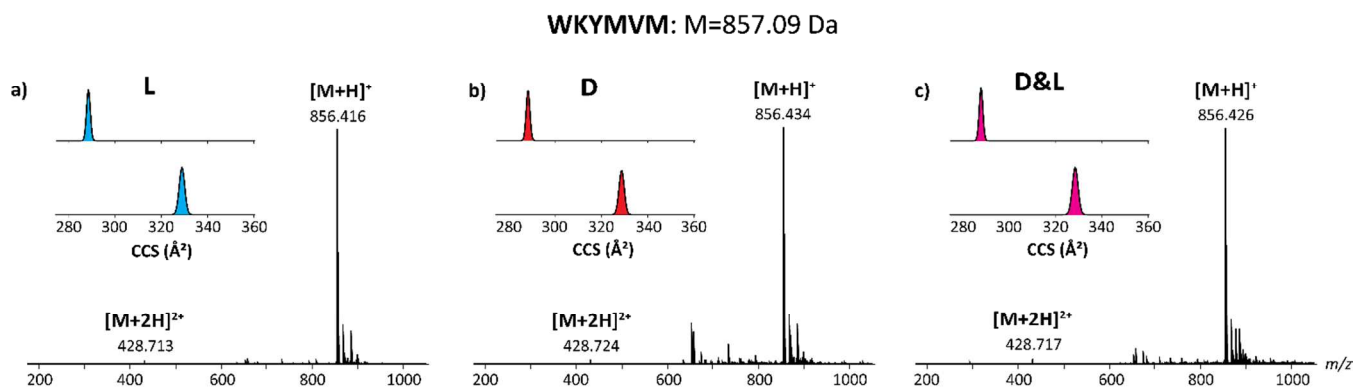


Figure S3. Mass spectra and ion mobility profiles using TIMS of a) WKYMVM (blue trace), b) WKYMVdM (red trace) and c) in mixture (magenta trace).

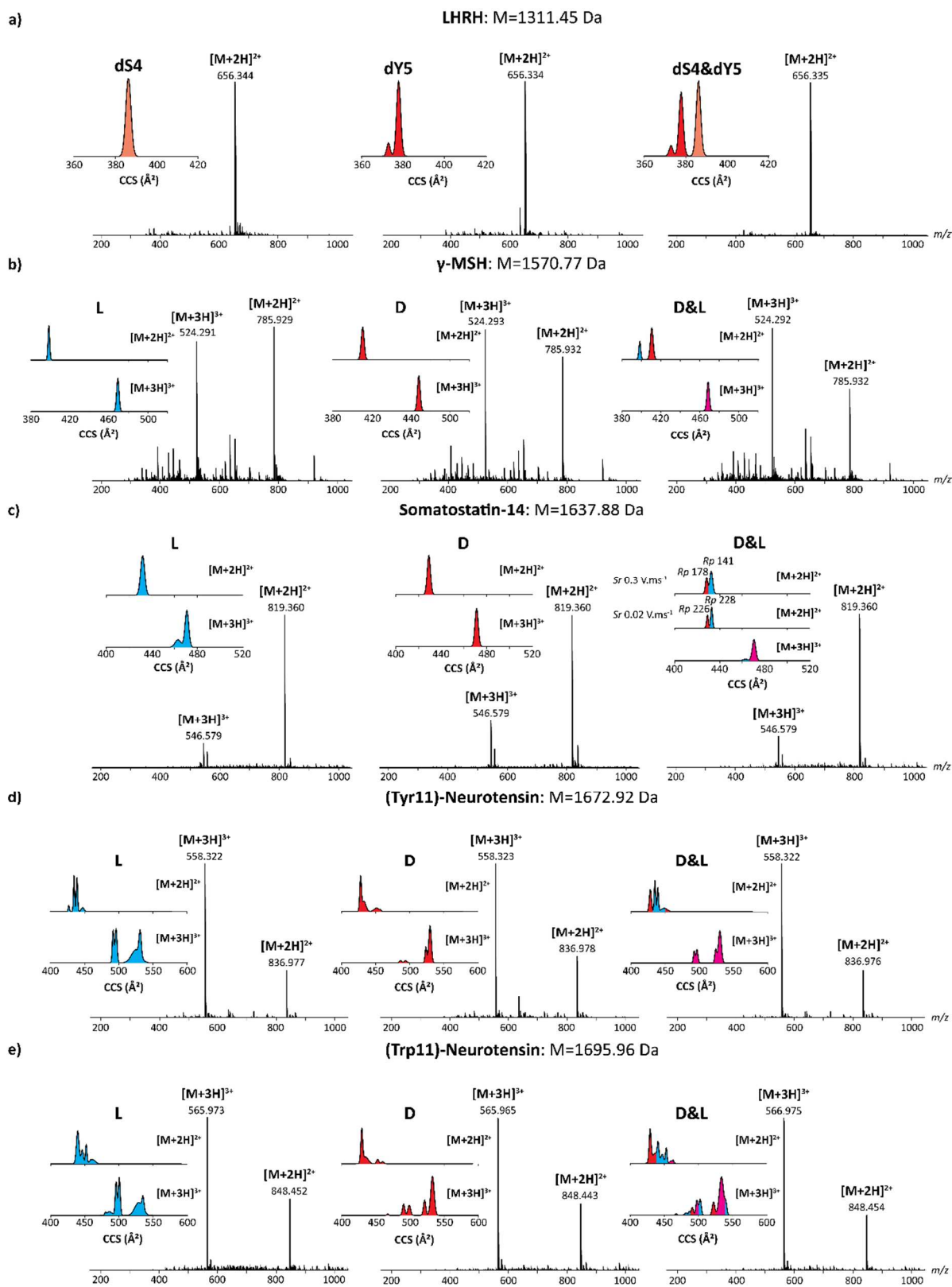


Figure S4. Mass spectra and ion mobility profiles using TIMS of the larger peptides a) LHRH, b) γ -MSH, c) somatostatin-14, d) Tyr¹¹-neurotensin and e) Trp¹¹-neurotensin. The D- and L-epimers are colored in red and blue, respectively.

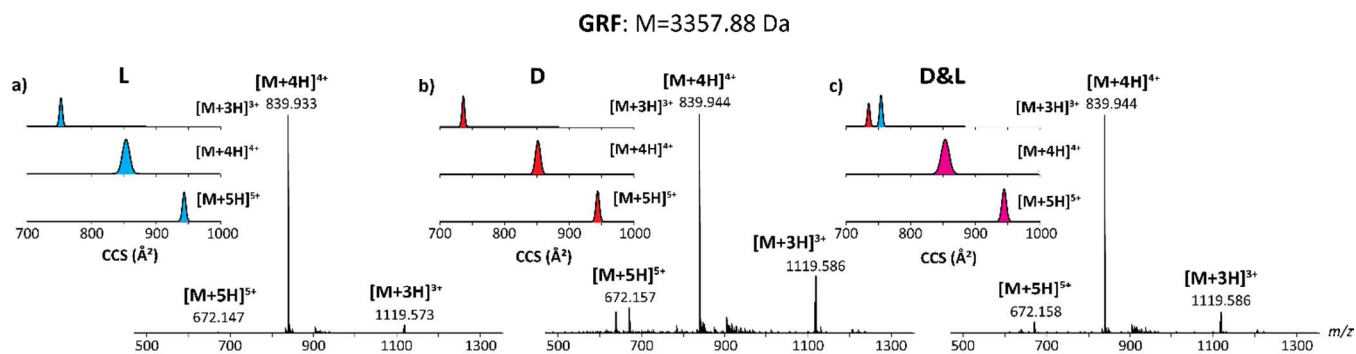


Figure S5. Mass spectra and ion mobility profiles using TIMS of a) GRF (blue trace), b) (D-Ala₂)-GRF (red trace) and c) in mixture (magenta trace).

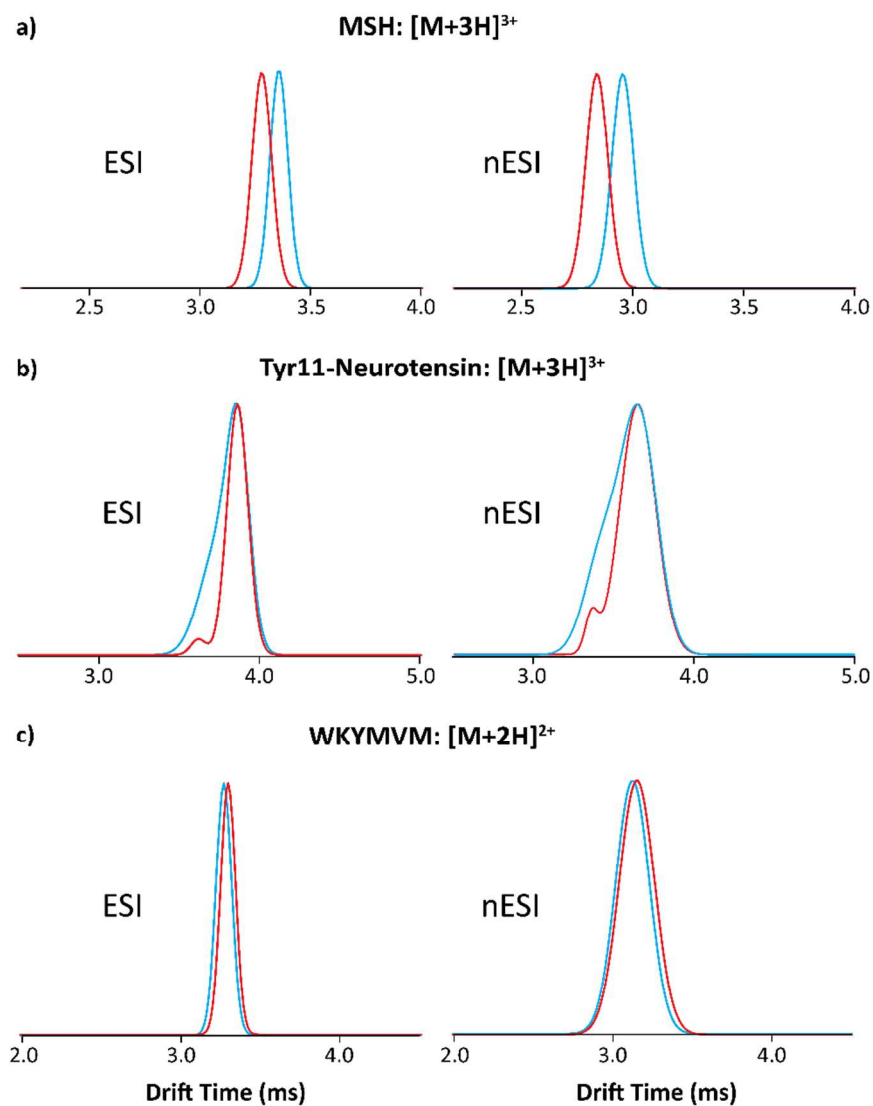


Figure S6. Synapt profiles of the a) triply protonated γ -MSH, b) triply protonated Tyr₁₁-neurotensin, and c) doubly protonated WKYMVM. L- and D-stereoisomers are colored in blue and red, respectively.

Table S1. TIMS experimental ion-neutral collision cross sections (CCS, Å²), resolving power (*R*) and resolution (*r*) for the multiply protonated species of the studied DAACPs.

Peptide	Ion	CCS (Å ²), std. error of mean: ± 0.04%	CCS $\pm$ (%)	<i>R</i>	<i>r</i>
Achatin-I	[M+H] ⁺	191.7	1.1	190	1.20
(D-Phe2)-Achatin-I	[M+H] ⁺	189.6		181	
Dermorphin (1-4)	[M+H] ⁺	218.2	1.4	235	1.86
(D-Arg2)-Dermorphin (1-4)	[M+H] ⁺	215.2		222	
Deltorphan I	[M+H] ⁺	263.5	0.6	223	0.90
(D-Ala2)-Deltorphan I	[M+H] ⁺	262.0		336	
WKYMVM	[M+H] ⁺	288.9	0.03 (1+)	301	0.07 (1+)
	[M+K] ⁺	293.7		237	
	[M+2H] ²⁺	329.0	0.8 (K+)	117	1.04 (K+)
WKYMVdM	[M+H] ⁺	288.8		301	
	[M+K] ⁺	291.4	0.06 (2+)	256	0.04 (2+)
	[M+2H] ²⁺	328.8		124	
(D-Ser4-D-Trp6)-LHRH	[M+2H] ²⁺	386.3	2.2	149	2.03
(D-Tyr5-D-Trp6)-LHRH	[M+2H] ²⁺	372.9/377.8		186/157	
γ -MSH	[M+2H] ²⁺	398.4	3.0 (2+)	173	2.54 (2+)
	[M+3H] ³⁺	469.2		156	
(D-Trp8)- γ -MSH	[M+2H] ²⁺	410.7	0.2 (3+)	121	0.17 (3+)
	[M+3H] ³⁺	468.3		140	
	[M+2H] ²⁺	432.0	0.7 (2+)	228	1.25 (2+)
Somatostatin-14	[M+3H] ³⁺	463.2/470.9		179/124	
	[M+2H] ²⁺	429.1	0.04 (3+)	226	0.04 (3+)
	[M+3H] ³⁺	471.1		137	
(L-Tyr11)-Neurotensin	[M+2H] ²⁺	426.7/434.4/438.8/447.0	1.5 (2+)	195/208/184/95	1.51 (2+)
	[M+3H] ³⁺	491.8/496.1/525.2/531.4		175/145/66/139	
(D-Tyr11)-Neurotensin	[M+2H] ²⁺	427.9/432.8/451.2	0.3 (3+)	144/62/58	0.23 (3+)
	[M+3H] ³⁺	490.7/494.9/523.7/529.8		123/104/179/121	
(L-Trp11)-Neurotensin	[M+2H] ²⁺	439.5/446.2/452.0/458.3/462.3	2.3 (2+)	96/114/168/249/251	1.58 (2+)
	[M+3H] ³⁺	480.0/485.2/495.9/500.5/529.1/535.3		214/217/162/148/138/156	
(D-Trp11)-Neurotensin	[M+2H] ²⁺	429.2/434.6/452.4/459.7	0.6 (3+)	131/107/115/102	0.44 (3+)
	[M+3H] ³⁺	467.2/490.6/498.8/521.4/532.9		187/122/106/128/90	
GRF	[M+3H] ³⁺	752.2	2.2 (3+)	184	2.40 (3+)
	[M+4H] ⁴⁺	852.6		67	
	[M+5H] ⁵⁺	943.3	0.11 (4+)	147	0.05 (4+)
	[M+3H] ³⁺	736.0		189	
(D-Ala2)-GRF	[M+4H] ⁴⁺	851.7	0.02 (5+)	96	0.02 (5+)
	[M+5H] ⁵⁺	943.5		144	

Table S2. Synapt (ESI) experimental drift times (t_D , ms), resolving power (R) and resolution (r) for the multiply protonated species of the studied DAACPs.

Peptide	Ion	Drift Time (ms)	$t_D \neq$ (%)	R	r
Achatin-I	$[M+H]^+$	4.10	0.2	22	0.03
(D-Phe2)-Achatin-I	$[M+H]^+$	4.09		26	
Deltorphan I	$[M+H]^+$	7.45	1.7	27	0.28
(D-Ala2)-Deltorphan I	$[M+H]^+$	7.32		28	
WKYMVM	$[M+H]^+$	8.86	0.2 (1+)	27	0.04 (1+)
	$[M+2H]^{2+}$	3.27		28	
WKYMVdM	$[M+H]^+$	8.88	0.6 (2+)	26	0.10 (2+)
	$[M+2H]^{2+}$	3.29		30	
(D-Ser4-D-Trp6)-LHRH	$[M+2H]^{2+}$	4.41	3.6	29	0.65
(D-Tyr5-D-Trp6)-LHRH	$[M+2H]^{2+}$	4.25		30	
γ -MSH	$[M+2H]^{2+}$	5.17	0.8 (2+)	22	0.12 (2+)
	$[M+3H]^{3+}$	3.36		35	
(D-Trp8)- γ -MSH	$[M+2H]^{2+}$	5.21	2.4 (3+)	33	0.47 (3+)
	$[M+3H]^{3+}$	3.28		31	
(L-Tyr11)-Neurotensin	$[M+2H]^{2+}$	5.58	2.0 (2+)	22	0.53 (2+)
	$[M+3H]^{3+}$	3.84		16	
(D-Tyr11)-Neurotensin	$[M+2H]^{2+}$	5.46/5.82	0.8 (3+)	29/21	0.06 (3+)
	$[M+3H]^{3+}$	3.62/3.86		33/26	

Table S3. TIMS scanning rates ($\Delta V/t_{trap}$, V.ms⁻¹) used for the multiply protonated species of the studied L/DAACPs.

Peptide	Ion	t_{trap} (ms)	V_{ramp} (V)	ΔV (V)	$\Delta V/t_{trap}$ (V.ms ⁻¹)	V_{ramp} (V)	ΔV (V)	$\Delta V/t_{trap}$ (V.ms ⁻¹)
Achatin-I (D-Phe2)-Achatin-I	[M+H] ⁺	500	-200 to -50	150	0.3	-130 to -100	30	0.06
Dermorphin (1-4) (D-Arg2)-Dermorphin (1-4)	[M+H] ⁺	500	-200 to -50	150	0.3	-150 to -125	25	0.05
Deltorphan I (D-Ala2)-Deltorphan I	[M+H] ⁺	500	-200 to -50	150	0.3	-183 to -175	8	0.016
WKYMVM	[M+H] ⁺	500	-280 to -0	280	0.56	-210 to -195	15	0.03
WKYMVdM	[M+K] ⁺	500	-280 to -0	280	0.56	-210 to -195	15	0.03
(D-Ser4-D-Trp6)-LHRH (D-Tyr5-D-Trp6)-LHRH	[M+2H] ²⁺	500	-200 to -50	150	0.3	-	-	-
γ -MSH	[M+2H] ²⁺	500	-200 to -50	150	0.3	-	-	-
(D-Trp8)- γ -MSH	[M+3H] ³⁺	500	-200 to -50	150	0.3	-	-	-
Somatostatin-14	[M+2H] ²⁺	500	-200 to -50	150	0.3	-137 to -127	10	0.02
(D-Trp8)-Somatostatin-14	[M+3H] ³⁺	500	-200 to -50	150	0.3	-	-	-
(L-Tyr11)-Neurotensin	[M+2H] ²⁺	500	-200 to -50	150	0.3	-	-	-
(D-Tyr11)-Neurotensin	[M+3H] ³⁺	500	-200 to -50	150	0.3	-	-	-
(L-Trp11)-Neurotensin	[M+2H] ²⁺	500	-200 to -50	150	0.3	-	-	-
(D-Trp11)-Neurotensin	[M+3H] ³⁺	500	-200 to -50	150	0.3	-	-	-
GRF	[M+3H] ³⁺	500	-200 to -50	150	0.3	-168 to -150	18	0.036
(D-Ala2)-GRF	[M+4H] ⁵⁺	500	-200 to -50	150	0.3	-	-	-
	[M+5H] ⁵⁺	500	-200 to -50	150	0.3	-	-	-