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## Enantiomers differentiation of aromatic amino acids by traveling wave ion mobility and mass spectrometry

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Supplementary material

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	<sup>D</sup> Phe, CCS <sub>exp</sub> (Å <sup>2</sup> )	<sup>L</sup> Phe, $CCS_{exp}(Å^2)$	$\Delta \text{CCS}_{exp}(\text{\AA}^2)$
Arginine			
[ <sup>D</sup> Arg+ <sup>D/L</sup> Phe+Cu <sup>II</sup> -H] <sup>+</sup>	119.7	121.1	1.4
$[(^{D}Arg)_{2}+^{D/L}Phe+Cu^{II}-H]^{+}$	148.7	150.6	1.9
Tryptophan			
[ <sup>D</sup> Trp+ <sup>D/L</sup> Phe+Cu <sup>II</sup> -H] <sup>+</sup>	122.4	123.0	0.6
$[(^{\mathrm{D}}\mathrm{Trp})_{2}^{+\mathrm{D/L}}\mathrm{Phe}^{+}\mathrm{Cu}^{\mathrm{II}}^{-}\mathrm{H}]^{+}$	155.8	157.0	1.2
Glutamic acid			
[ <sup>D</sup> Glu+ <sup>D/L</sup> Phe+Cu <sup>II</sup> -H] <sup>+</sup>	115.4	115.7	0.3
$[(^{D}Glu)_{2}+^{D/L}Phe+Cu^{II}-H]^{+}$	140.4	142.0	1.6
Threonine			
[ <sup>D</sup> Thr+ <sup>D/L</sup> Phe+Cu <sup>II</sup> -H] <sup>+</sup>	104.3	105.0	0.7
$[(^{\mathrm{D}}\mathrm{Thr})_{2}+^{\mathrm{D/L}}\mathrm{Phe}+\mathrm{Cu}^{\mathrm{II}}-\mathrm{H}]^{+}$	146.4	147.7	1.3
Histidine			
[ <sup>D</sup> His+ <sup>D/L</sup> Phe+Cu <sup>II</sup> -H] <sup>+</sup>	110.5	111.2	0.7
[( <sup>D</sup> His) <sub>2</sub> + <sup>D/L</sup> Phe+Cu <sup>II</sup> -H] <sup>+</sup>	147.5	148.8	1.3
Proline			
[ <sup>D</sup> Pro+ <sup>D/L</sup> Phe+Cu <sup>II</sup> -H] <sup>+</sup>	102.5	103.9	1.4
$[(^{D}Pro)_{2}+^{D/L}Phe+Cu^{II}-H]^{+}$	123.5	127.3	3.8
Tyrosine			
[ <sup>D</sup> Tyr+ <sup>D/L</sup> Phe+Cu <sup>II</sup> -H] <sup>+</sup>	117.0	117.3	0.3
$[(^{\mathrm{D}}\mathrm{Tyr})_{2}+^{\mathrm{D}/\mathrm{L}}\mathrm{Phe}+\mathrm{Cu}^{\mathrm{II}}-\mathrm{H}]^{+}$	152.8	154.6	1.8

**Table S1.** Experimental collision cross section obtained for  $[Y^{+D/L}Phe^{+}Cu^{II}-H]^{+}$  and  $[2Y^{+D/L}Phe^{+}Cu^{II}-H]^{+}$  ions.

	$D_{AA} CCS (Å^2)$	$L_{AA} CCS (Å^2)$	$\Lambda CCS (Å^2)$
	TMI, CCDexp(TI)	TM, CCDexp(TV)	$\Delta CCS_{exp}(R)$
Arginine			
[( <sup>D</sup> Pro) <sub>2</sub> + <sup>D/L</sup> Arg+Cu <sup>II</sup> -H] <sup>+</sup>	126.6	127.9	1.3
Tryptophan			
[( <sup>D</sup> Pro) <sub>2</sub> + <sup>D/L</sup> Trp+Cu <sup>II</sup> -H] <sup>+</sup>	127.5	133.7	6.2
Glutamic acid			
[( <sup>D</sup> Pro) <sub>2</sub> + <sup>D/L</sup> Glu+Cu <sup>II</sup> -H] <sup>+</sup>	121.7	122.7	1.0
Threonine			
[( <sup>D</sup> Pro) <sub>2</sub> + <sup>D/L</sup> Thr+Cu <sup>II</sup> -H] <sup>+</sup>	115.3	116.3	1.0
Glutamine			
[( <sup>D</sup> Pro) <sub>2</sub> + <sup>D/L</sup> Gln+Cu <sup>II</sup> -H] <sup>+</sup>	121.7	123.0	1.3
Lysine			
[( <sup>D</sup> Pro) <sub>2</sub> + <sup>D/L</sup> Lys+Cu <sup>II</sup> -H] <sup>+</sup>	123.0	124.3	1.3
Tyrosine			
[( <sup>D</sup> Pro) <sub>2</sub> + <sup>D/L</sup> Tyr+Cu <sup>II</sup> -H] <sup>+</sup>	126.3	130.7	4.8

Table S2. Experimental collision cross section obtained for  $[(^{D}Pro)_{2}+^{D/L}AA+Cu^{II}-H]^{+}$ 



Figure S1. Example of ion mobility peak Gaussian fit.

a) Ion mobility spectra obtained by the IM-MS instrument for the  $[(Pro)_2+Phe+Cu^{II}-H]^+$  ion (8 points per peak). The drift time values correspond to the peak apexes. These two ion mobility spectra are obtained for the same trimeric species on a repeatability study. Due to the low sampling rate, the measured values present random error of about 0.03 ms.

b) After the peak fit with Origin software, the drift times obtained from the peak apexes are identical for the two experiments.





Ion mobility spectra for  $[(^{D/L}Pro)_2+^{D/L}AA+Cu^{II}-H]^+$  ion for tryptophan with a) D-Proline and b) L-Proline and for tyrosine with c) D-Proline and d) L-Proline. In solid line: ion mobility spectra for <sup>D</sup>AA; in dotted line: ion mobility spectra for <sup>L</sup>AA compounds.

Figure S3. TWIM-MS analyses of tryptophan enantiomers with  $CO_2$  in drift cell



Overlaid of ion mobility spectra for  $[(^{D}Pro)_{2}+ ^{D/L}Trp+Cu^{II}-H]^{+} a)$  N<sub>2</sub> as drift gas; b) CO<sub>2</sub> as drift gas. In solid line: ion mobility spectra for <sup>D</sup>Trp; in dotted line: ion mobility spectra for <sup>L</sup>Trp compound.

Figure S4. Analysis of D and L enantiomers of tyrosine in mixture.



a) Drift time plot obtained for each proportion of D and L enantiomers of tyrosine for

 $[(^{D}Pro)_{2}+^{D/L}Tyr+Cu^{II}-H]^{+}$  ion. b) Curve obtained when  $\log \frac{A(L_{Trp})}{A(D_{Trp})}$  is reported in function of proportion of <sup>L</sup>Tyr (A (<sup>L</sup>Tyr) is the area of the pic corresponding to <sup>L</sup>Tyr).

## **Theoretical results**

Density Functional Theory (DFT) calculations were carried out with the Gaussian 09 program<sup>1</sup> in order to get insight into the structures of such complexes in gas phase. Consistently with our previous study,<sup>2</sup> the  $\omega$ B97X-D exchange-correlation functional<sup>3</sup> was used in conjunction with the 6-31++G(d,p) basis set for H, C, O, N atoms, and the quasi-relativistic Stuttgart-Dresden (SDD, MDF10) pseudopotential with the associated optimized valence basis sets for Cu. Only doublet spin states were considered. Importantly, no spin contamination was observed.

As a test molecule,  $[(^{D}Pro)_{2}^{+L}Trp+Cu^{II}-H]^{+}$  was firstly investigated. A dozen of possible coordination modes were considered. The related detailed discussion will be reported in a forthcoming paper. Figure S5 presents four of these structures. In **C1** (which is the most stable), Trp coordinates in a bidentate manner, as well as the first Pro, which positions *trans* to it, forming an approximate square plane. The second Pro is bound to the metal through oxygen. Noteworthy, the ligands also interact between them via hydrogen bonds involving atoms of the first coordination sphere (O...H-O (1.70 Å) between Try and Pro, N...H-N (2.01 Å) between the two Pro).

In C2, Trp and the first Pro coordinate in the same way, but the second Pro now binds by the nitrogen atom. C2 displays a third type of intramolecular hydrogen bond, involving Pro and Trp (O...H-N, 2.14 Å). In C3, one Pro is still bidentate, with oxygen in the Trp-Cu plane, but with N in axial position. The second Pro then coordinates in a monodentate mode, but also forms one O...H-N hydrogen bond (2.28 Å) with the other Pro. In C4, the two Pro are monodentate ligands, binding by their nitrogen atoms. Once more, they also interact through one O...H-N hydrogen bond (2.20 Å), while another O...H-N (2.38 Å) is found between Trp and one Pro. It can be stressed that all reported connectivities (with the metal and the hydrogen bonds) are fully confirmed by the existence of the corresponding bond paths and bond critical points (as defined by Bader<sup>4</sup>) in the electron density topological graph.

The CCSs were then calculated using the MOBCAL software and the trajectories (TM) method.<sup>5-7</sup> As suggested in MOBCAL's manual, the *imp* and *inum* numbers were raised respectively by factors 100 and 20. The Lennard-Jones parameters for copper were taken from those for silicon. We first explored the use of three atomic charges distributions:  $\{q(Cu)=+1, q(all other atoms)=0\}$ , NPA and HLYGAt. For C1, the CCS values are respectively equal to

146.5 Å<sup>2</sup>, 146 Å<sup>2</sup>, and 145.9 Å<sup>2</sup>, confirming that the choice of atomic charges definition is not crucial. Only the first scheme was thus subsequently used. For **C2**, **C3** and **C4**, the following values were obtained: 144.6, 142.3, 143.8 Å<sup>2</sup>.

**C1-4** were then optimized with <sup>D</sup>Trp instead of <sup>L</sup>Trp. The corresponding CCS were found to be equal to 140.7 Å<sup>2</sup>, 131.5 Å<sup>2</sup>, 136.7 Å<sup>2</sup>, 132.6 Å<sup>2</sup>, which are all lower than those found for <sup>L</sup>Trp, accounting for the observed enantiomers separation. Besides, the calculated CCS(**C1**-<sup>L</sup>Trp) – CCS(**C1**-<sup>D</sup>Trp) = 5.8 Å<sup>2</sup> is very close to the experimental value (6.2 Å<sup>2</sup>).

As a final test, we reoptimized the C1-type structure, replacing Trp by Phe. The same computational protocol provides:  $CCS(C1-^{L}Ph) - CCS(C1-^{D}Ph) = 4.4 \text{ Å}^2$ , once more in good agreement with the experimental value (3.8 Å<sup>2</sup>).

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**Figure S5.** Selected energy minima for the  $[(^{D}Pro)_{2}^{+L}Trp+Cu^{II}-H]^{+}$  compound. H in white, C in grey, N in blue, O in red, Cu in pink. Intramolecular hydrogen bonds are represented in dashed lines.





Figure S6. Analyses of tryptophan and tyrosine enantiomers with other transition metals.

Ion mobility spectra of  $[(^{D}Pro)_{2}+^{D/L}AA+X^{II}-H]^{+}$  ion for a) tryptophan, left to right: Cu<sup>II</sup>, Ni<sup>II</sup>, Co<sup>II</sup> and b) tyrosine, left to right: Cu<sup>II</sup>, Ni<sup>II</sup>, Co<sup>II</sup>. In solid line: ion mobility spectra for <sup>D</sup>AA; in dotted line: ion mobility spectra for <sup>L</sup>AA compounds.