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High throughput separation of chiral molecules using non-covalent complexes and SIM² analysis on a TIMS-ToF

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INTRODUCTION

- Chiral molecules pose a unique challenge in life sciences, since enantiomers may exhibit different biological activity/toxicity while having similar physical and chemical properties.
- Separation of enantiomers using mass spectrometry (MS) mostly relies on derivatization or formation of non-covalent complexes (NCCs) in the gas phase (with various metal/alkali cations and chiral selectors such as cyclodextrins or carbohydrates [1,2]). Alternatively, ion-mobility spectrometry (IMS) separates ions based on their m/z ratio and their collision cross section (CCS), which makes it a promising tool for the rapid analysis of isomeric compounds.
- In this work, chiral analyses were conducted on a trapped ion mobility spectrometry time-of-flight (TIMS-ToF) instrument, using serial acquisitions by applying single ion mobility monitoring (SIM²) at high mobility resolving power and at different ion mobility ranges [3]. The method was applied on various natural products such as amino acids (AAs) and biologically relevant molecules.

MATERIALS

- Direct infusion of chiral compounds with addition of Cu^{II} and ¹Phe & ¹Pro as chiral references in H₂O/MeOH (5 μM). Chiral mix solutions consists of each AA at 1 μM, references at 10 μM and Cu^{II} at 20 μM.
- TIMS-ToF: ESI in positive and negative mode – SIM² with maximum mobility resolving power (narrow mobility range detection centered on each ion of interest)
- Mobility separation evaluated with:

$$\Delta CCS\% = 2 \frac{(CCS_B - CCS_A)}{(CCS_A + CCS_B)} \times 100$$

RESULTS

Fast IM analysis of chiral AAs

Table 1: Chiral separations expressed as ΔCCS% values obtained in positive ESI for each AA in enantiomer solutions (L+D)LL, in SIM² mode.

(x) No complex found in individual and enantiomer solutions (LL, DLL and (L+D)LL); (-) No mobility separation or several peaks found in the enantiomer solutions (L+D)LL; Orange Chiral separation in the enantiomer solutions (L+D)LL only.

[...H+Cu ^{II}] ⁺ adduct	Positively charged			Negatively charged		Polar				Special		Hydrophobic							
	Arg	His	Lys	Asp	Glu	Asn	Gln	Ser	Thr	Cys	Pro	Ala	Ile	Leu	Met	Phe	Trp	Tyr	Val
AA,Pro	1.3%	-	-	x	-	x	x	x	x	x	x	-	0.4%	-	x	0.9%	-	-	-
AA,Phe	1.4%	1.0%	-	1.1%	0.8%	-	1.0%	-	-	-	1.0%	-	-	-	0.7%	-	1.8%	1.6%	-
AA,AA	1.1%	-	1.0%	-	2.6%	x	-	x	x	-	-	-	1.3%	0.6%	-	1.6%	-	1.5%	x
AA,Pro,Pro	-	x	2.2%	-	-	x	1.7%	-	0.5%	x	x	-	-	-	-	1.8%	2.4%	2.0%	-
AA,Phe,Pro	0.9%	x	-	-	-	0.4%	1.1%	0.7%	-	x	x	-	-	-	2.1%	x	2.0%	1.3%	-
AA,Phe,Phe	-	x	0.6%	-	-	1.2%	-	-	-	x	-	-	0.9%	-	-	x	1.3%	-	1.0%
AA,AA,Pro	x	x	x	x	1.4%	-	-	x	0.9%	-	-	x	-	-	-	1.4%	1.6%	1.6%	-
AA,AA,Phe	x	x	x	x	-	x	1.1%	0.5%	-	1.3%	1.8%	-	x	x	1.4%	x	-	0.5%	-
AA,AA,AA	x	x	x	x	-	1.3%	-	x	-	x	-	x	-	0.8%	-	-	-	-	x

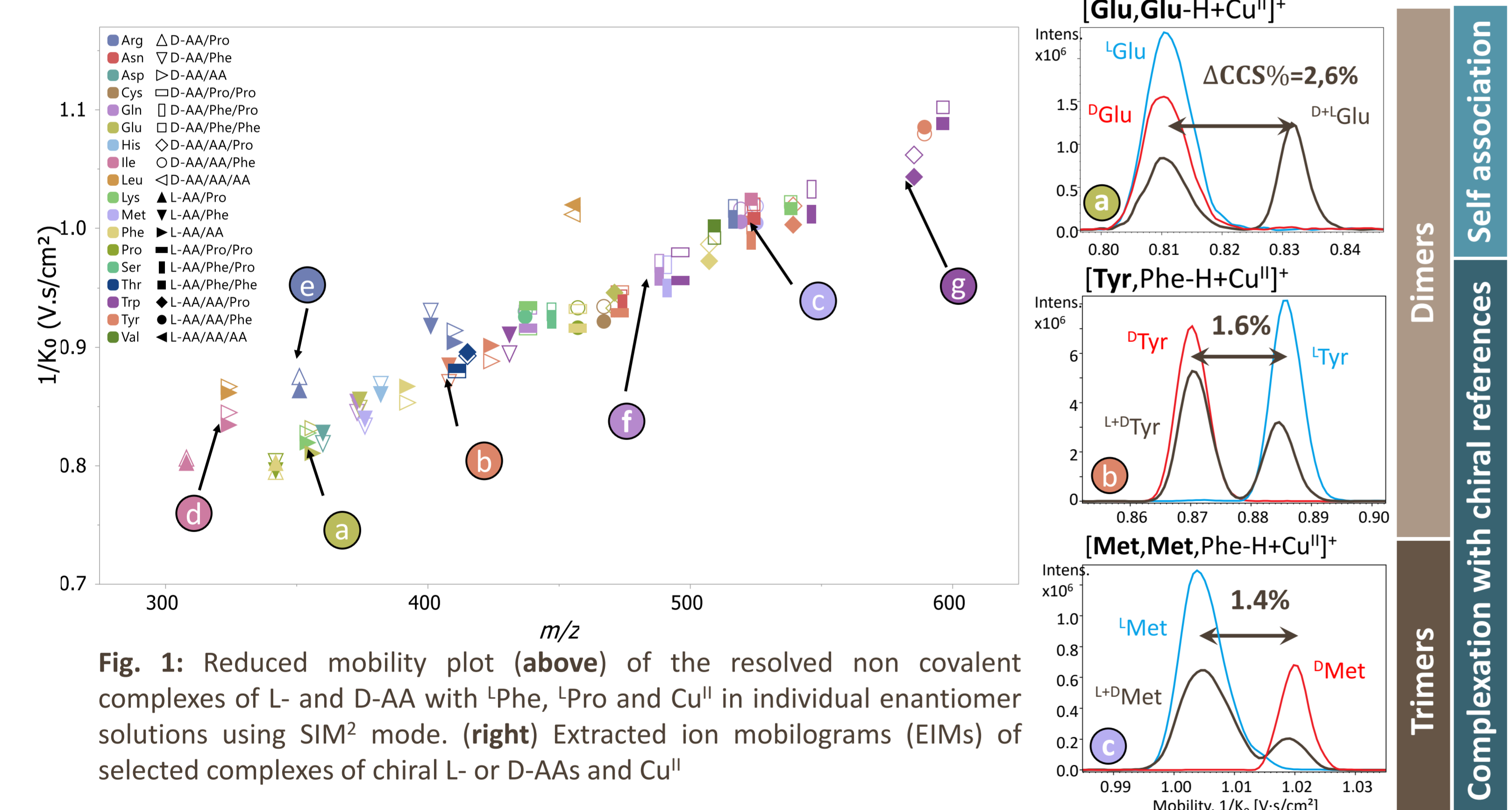
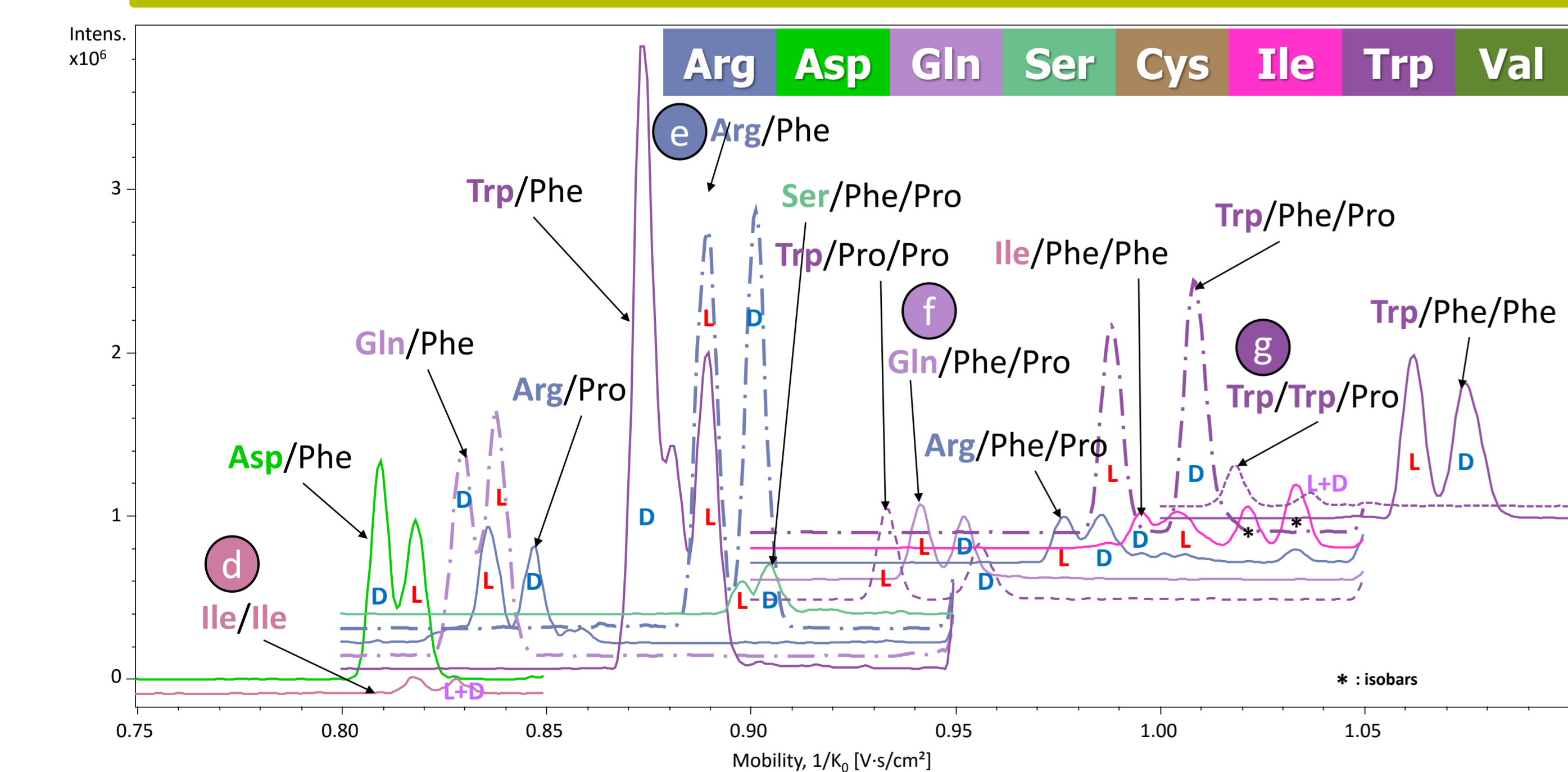


Fig. 1: Reduced mobility plot (above) of the resolved non-covalent complexes of L- and D-AA with ¹Phe, ¹Pro and Cu^{II} in individual enantiomer solutions using SIM² mode. (right) Extracted ion mobilograms (EIMs) of selected complexes of chiral L- or D-AAAs and Cu^{II}

High resolution and high throughput IM analysis of chiral AA mixtures using SIM² stitching



SIM² provides the highest mobility resolution and high sensitivity on a limited range, while SIM² stitching* maintains these performances on a broad mobility range

* SIM²-stitching: collection of multiple adjacent SIM² ranges

More details about TIMS and SIM² stitching in poster by Chalet et al.

Chiral analysis of natural products

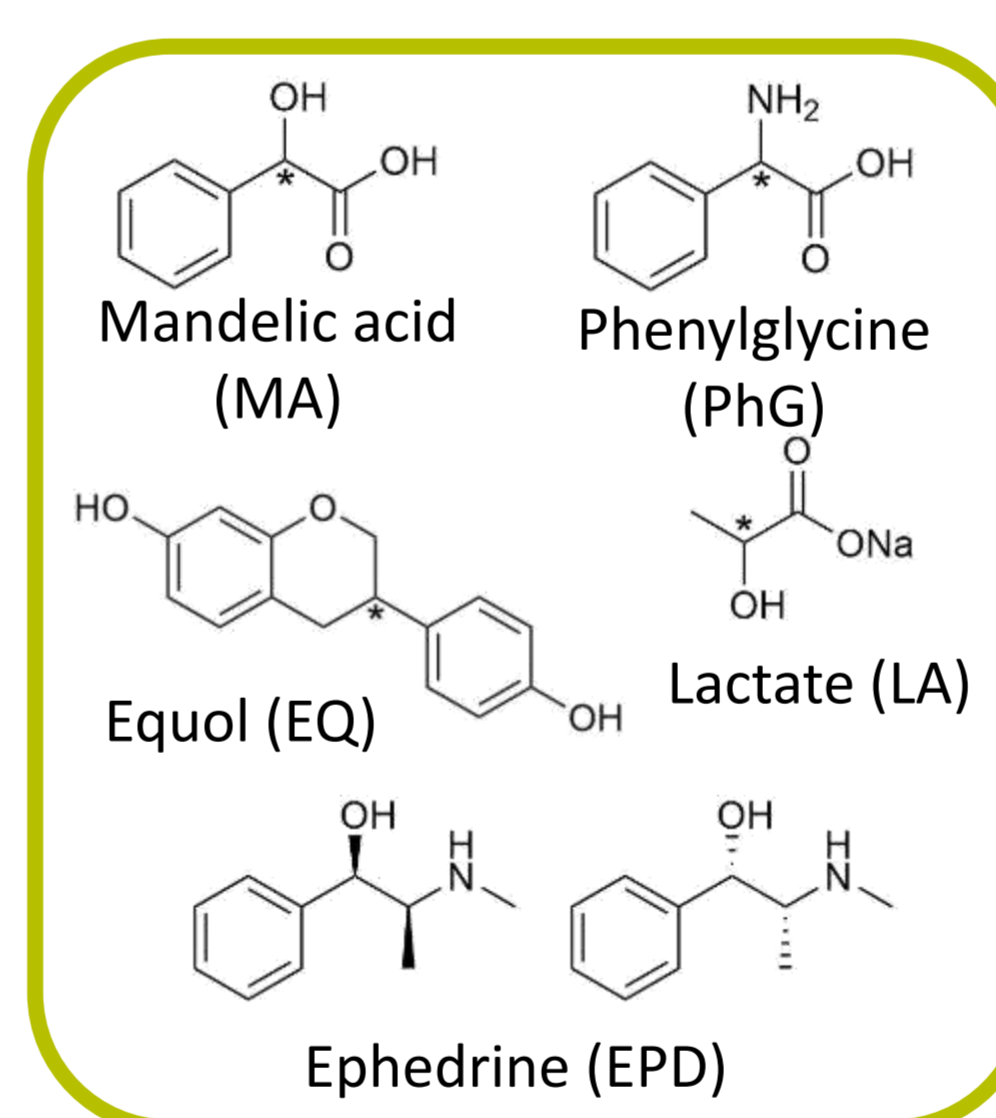
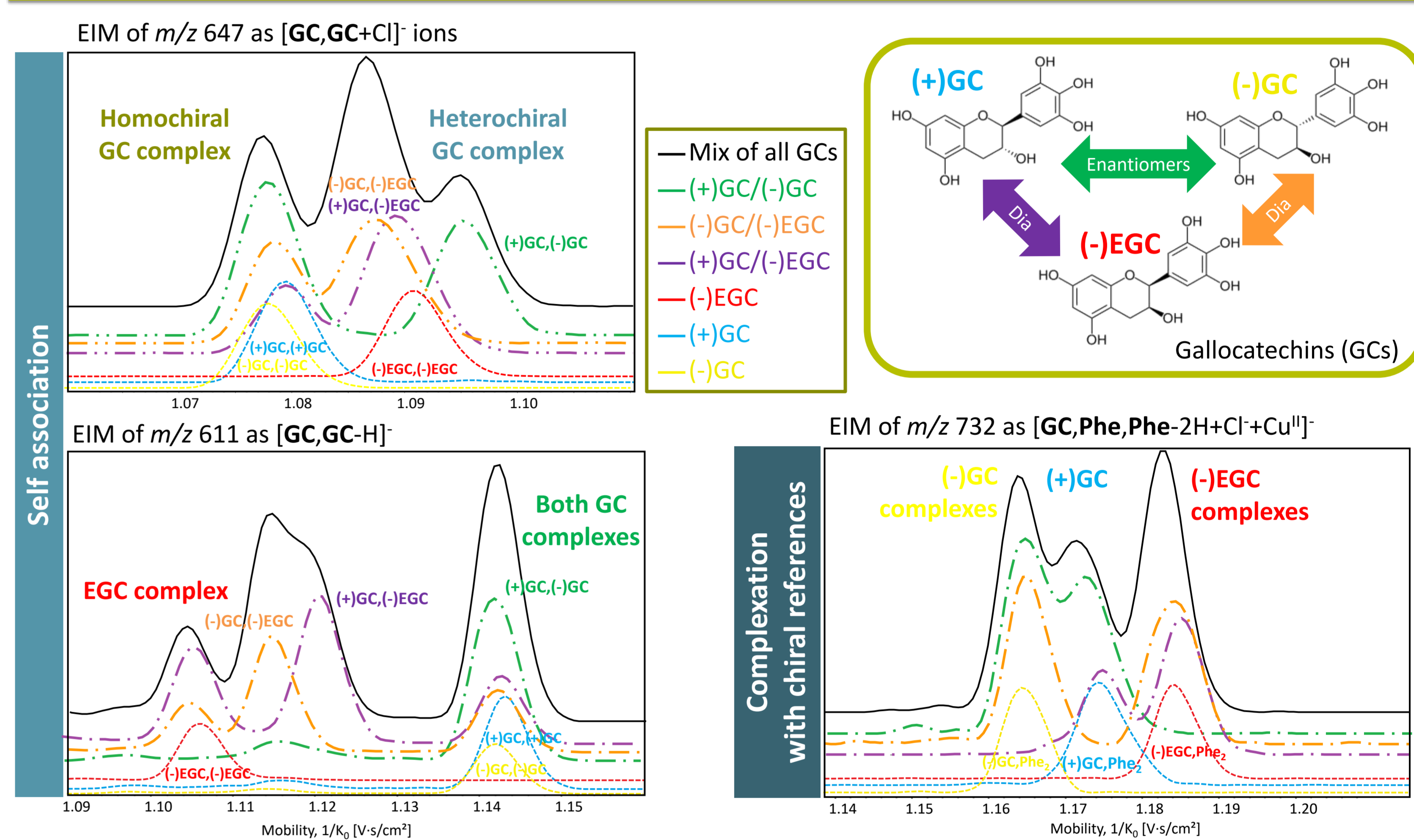


Table 2: Chiral separations expressed as ΔCCS% values obtained for biologically relevant chiral molecules (structures above and right) in individual or mix solutions; SIM² mode, positive and negative ionization.

(x) No complex detected; (-) Complex detected but no mobility separation; () not tested

Complex	ESI	Adducts	(-)GC (-)EGC	(+)GC (-)EGC	(+)GC (-)EGC	All GCs	MA	PhG	EQ	LA	EPD
X,Pro	POS	+Cu ^{II}	-	-	-	-	1.2%	1.0%	()	()	-
	NEG	+Cu ^I	()	()	()	()	()	()	-	-	-
X,Phe	POS	+Cu ^I	2.8%	1.8%	2.4%	1.6%	1.4%	1.3%	()	()	-
	POS	+Na ⁺	4.5%	4.7%	2.9%	4.6%	3.1%	-	-	-	-
X,X	POS	+Cu ^{II}	3.9%	4.6%	2.9%	3.9%	0.7%	2.8%	1.8%	2.3%	()
	POS	+Cu ^I	2.8%	4.9%	1.1%	2.7%	2.1%	1.1%	-	-	-
X,X	NEG	-H ⁻	0.9%	1.4%	-	0.9%	-	-	-	-	-
	NEG	+Cl ⁻	2.4%	2.0%	-	2.4%	-	-	-	-	-
X,Pro,Pro	POS	+Cu ^{II}	0.8%	0.9%	1.6%	0.9%	0.7%	-	-	-	-
	POS	+Cu ^{II}	-	-	-	-	-	x	0.9%	()	()
X,Phe,Pro	POS	+Cu ^{II} Cl	-	-	-	-	-	-	0.9%	()	()
	NEG	+Cu ^{II}	1.0%	1.0%	-	1.0%	()	()	-	-	-
X,Phe,Phe	POS	+Cu ^{II}	-	-	-	-	-	-	-	-	-
	POS	+Cu ^{II} Cl	1.6%	0.9%	0.7%	0.6%	1.0%	()	()	-	-
	NEG	+Cu ^{II}	-	-	-	-	-	()	()	-	-
X,X,Pro	POS	+Cu ^{II}	-	-	-	-	-	-	-	-	-
	POS	+Cu ^{II}	-	-	-	-	-	x	1.5%	()	()
X,X,Phe	POS	+Cu ^{II}	-	-	-	-	-	x	-	()	()
	NEG	+Cu ^{II}	-	-	-	-	-	()	()	-	-
X,X,X	POS	+Cu ^{II}	-	-	-	-	-	-	-	-	-
	NEG	-H ⁻	2.7%	2.2%	-	2.4%	()	()	-	-	-

CONCLUSION

- Most proteinogenic L- and D-AAAs can be discriminated using non-covalent complexes with Phe, Pro and Cu^{II} in positive mode with at least one combination of references and analyte and/or through self-association.
- Chiral separations are successful for chiral AA mix solutions, although not all complexes of interest may be detected. SIM² stitching acquisition ensures high resolution and high throughput onto large reduced mobility ranges.
- Chiral analyses of other biologically relevant molecules using complexation were less efficient. The choice of references, adducts and analytical conditions is thus important to ensure proper chiral separations with NCCs.

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