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# Rapid chiral characterization of amino acids using non-covalent Cu<sup>II</sup> complexes and TIMS-ToF separation

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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 in the gas phase (with various metal/alkali cations and chiral selectors such as cyclodextrins or carbohydrates [1,2]).
- 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 addition, the resolving power of the TIMS-ToF instrument (Bruker Daltonics) used in this work can be increased ( $R \approx 150-200$ ) using single ion mobility monitoring (SIM<sup>2</sup>) and thus improve the mobility separation of stereoisomers [3].

## Methods

- Direct infusion of proteinogenic chiral L- and D-AA with addition of Cu<sup>II</sup> and <sup>13</sup>C-Phe & <sup>13</sup>C-Pro as chiral references in H<sub>2</sub>O/MeOH (5 μM). Mix solutions consists of each AA at 1 μM, references at 10 μM and Cu<sup>II</sup> at 20 μM.
- TIMS-ToF: ESI in positive mode – SIM<sup>2</sup> with high mobility resolving power (using narrow mobility range detection centered on each ion of interest)
- Study of all combinations between the analyte, references and Cu<sup>II</sup> (dimers AA/Pro, AA/Phe and AA/AA; trimers AA/Pro/Pro, AA/Phe/Pro, AA/Phe/Phe, AA/AA/Pro, AA/AA/Phe and AA/AA/AA, see Table 1).
- Mobility separation ability is evaluated with  $\Delta CCS\% = 2 \frac{(CCS_B - CCS_A)}{(CCS_A + CCS_B)} \times 100$

## Results and Discussion

### Study of individual solutions of (L+D) AA enantiomers

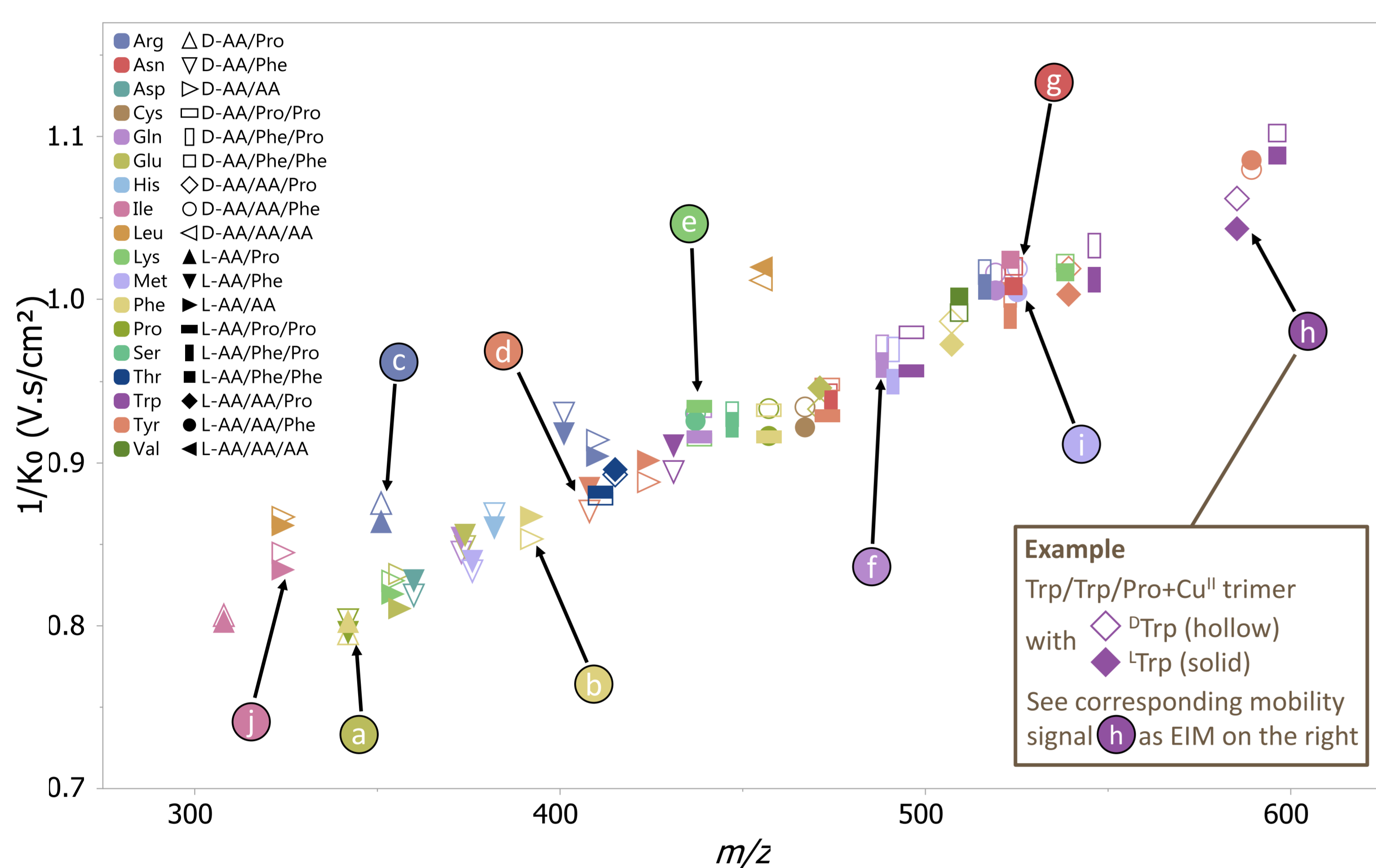
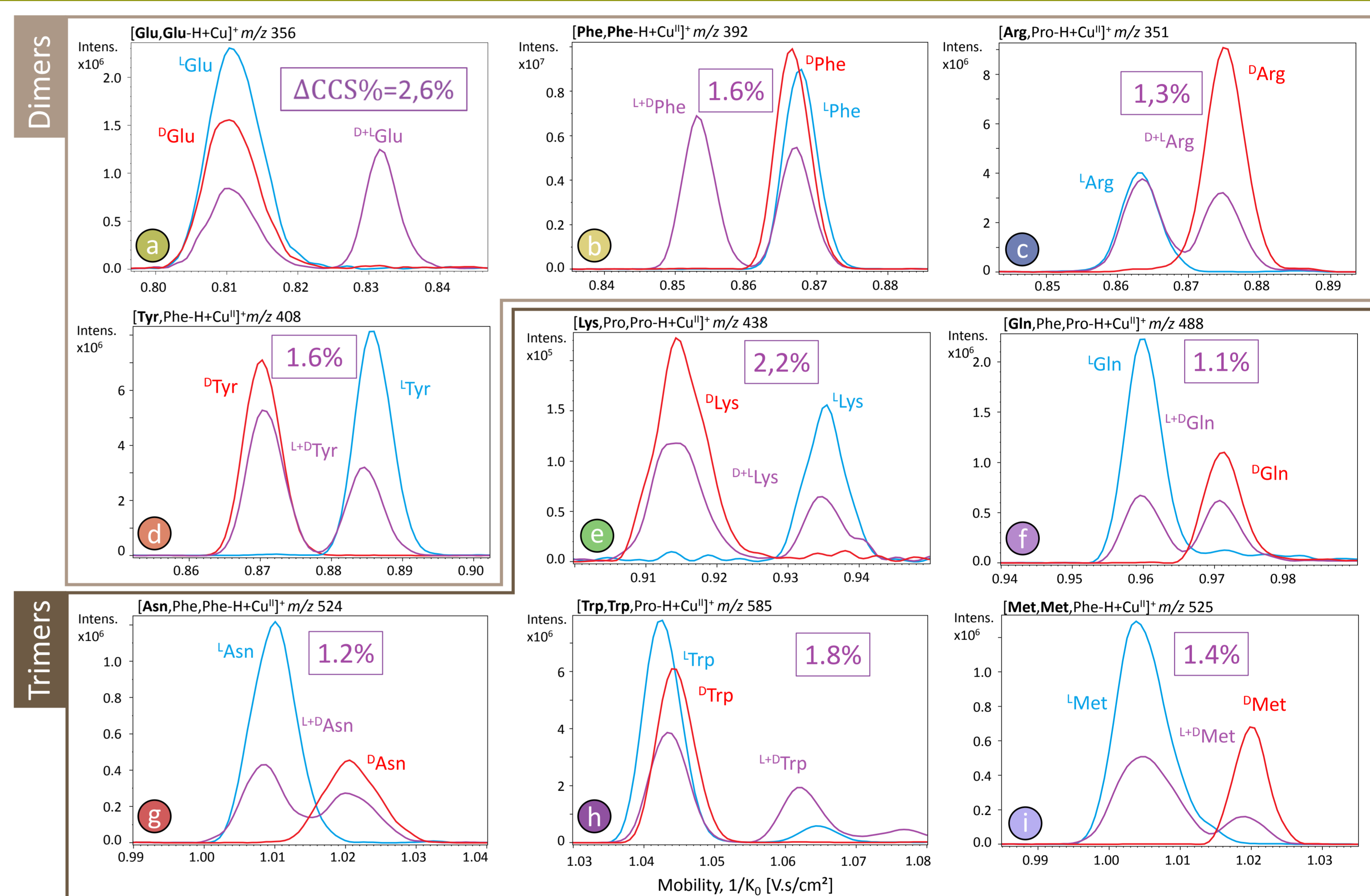


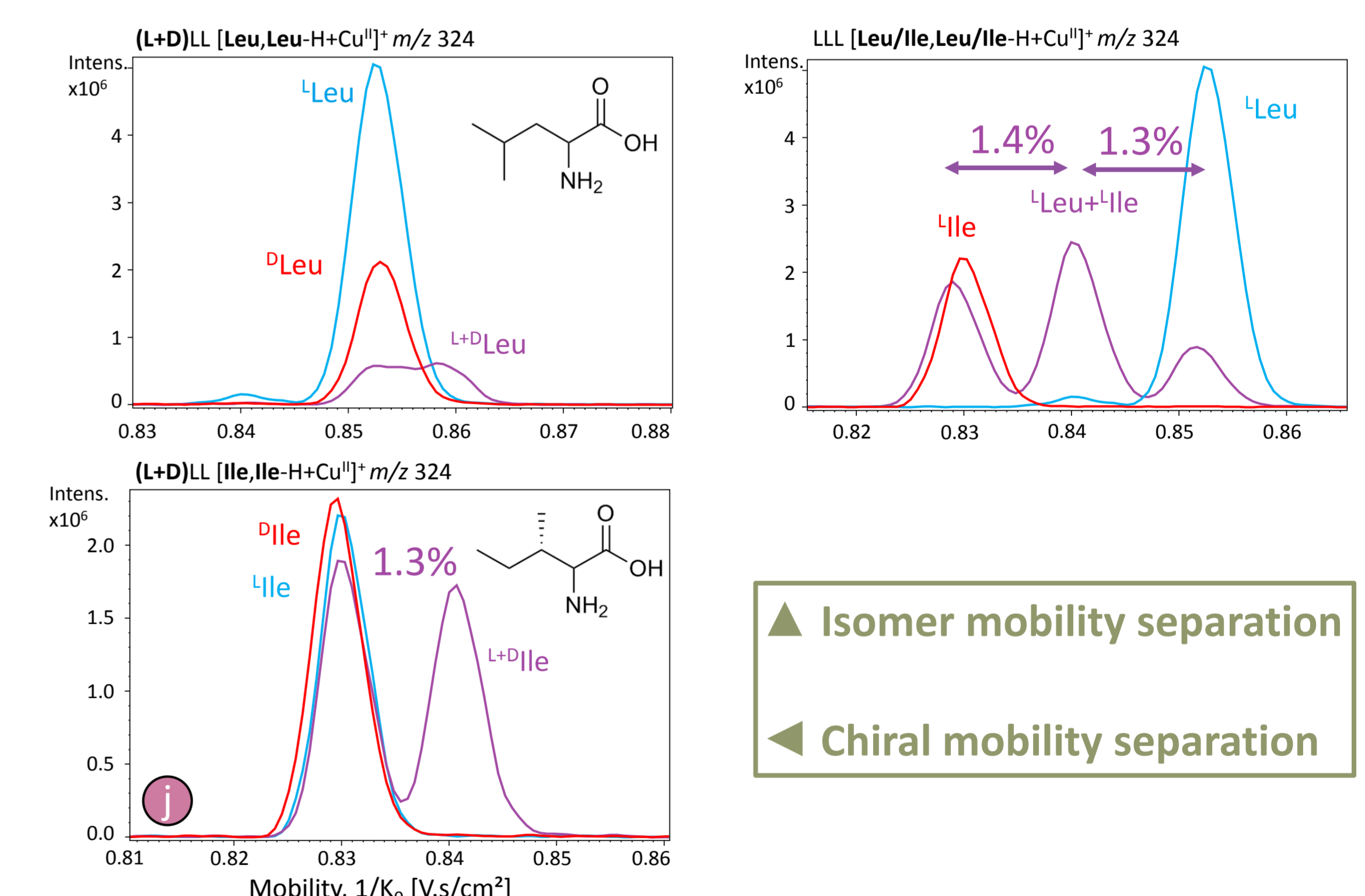
Fig. 1 (Above) Reduced mobility plot of the resolved non-covalent complexes of L- and D-AA with <sup>13</sup>C-Phe, <sup>13</sup>C-Pro and Cu<sup>II</sup> in individual enantiomer solutions using SIM<sup>2</sup> mode. (Right) Extracted ion mobilitygrams (EIMs) of selected complexes of chiral L- or D-AA and Cu<sup>II</sup> (dimers and trimers formed with <sup>13</sup>C-Phe and/or <sup>13</sup>C-Pro).



[...-H+Cu <sup>II</sup> ] <sup>+</sup>	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%	-	-	X	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%	-	2.0%	1.3%	-
AA,Phe,Phe	-	X	0.6%	-	-	1.2%	-	-	-	X	-	-	0.9%	-	-	-	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%	-	-	0.5%	-
AA,AA,AA	X	X	X	X	-	1.3%	-	X	-	X	-	X	-	0.8%	-	-	-	-	X

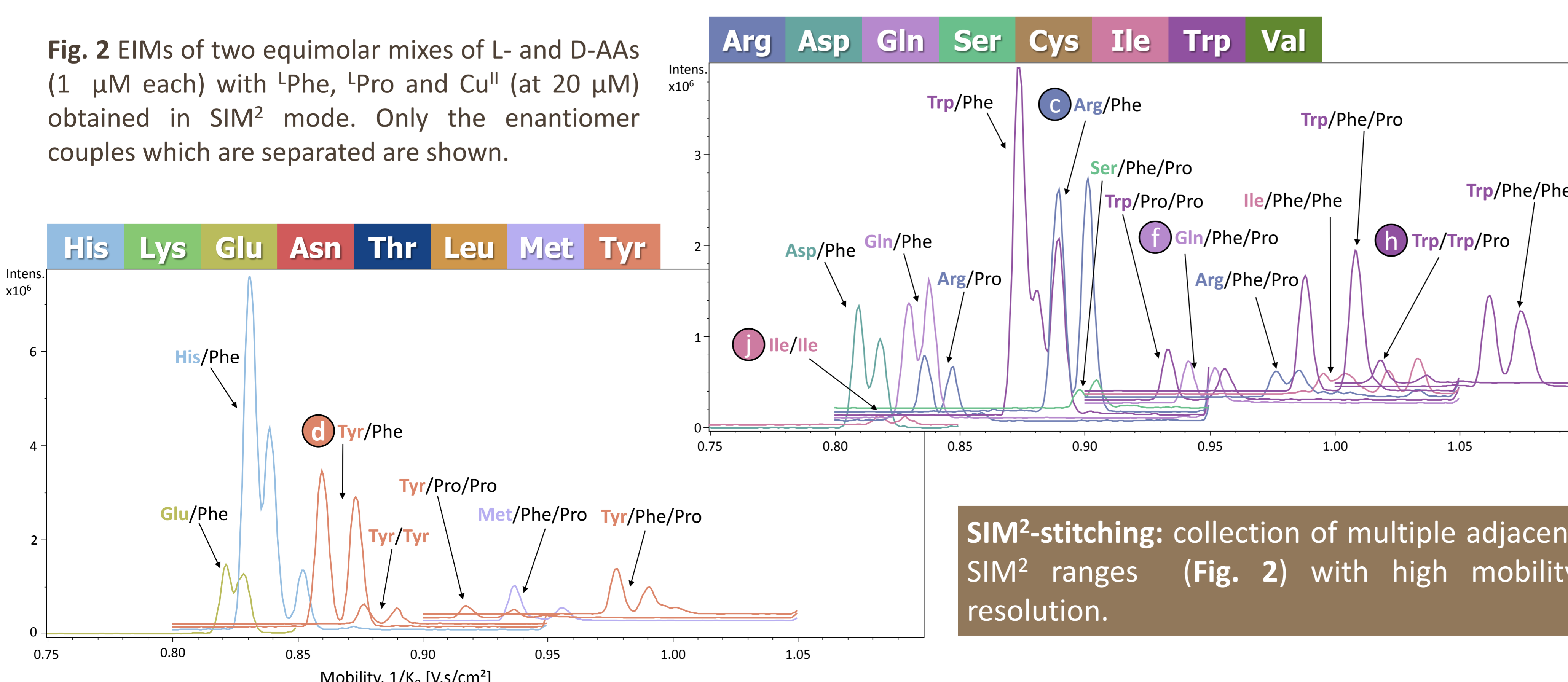
Table 1: Chiral separations obtained for each AA in enantiomer solutions (L+D)LL in SIM<sup>2</sup> mode expressed as ΔCCS% values. (x) No complex found in individual and enantiomer solutions (LLL, DLL and (L+D)LL); (-) No mobility separation or several peaks found in the enantiomer solutions (L+D)LL; Orange Chiral separation in the enantiomer solutions (L+D)LL only.

### Leucine & Isoleucine isomers



### Rapid analysis of chiral AA mixtures with SIM<sup>2</sup>-stitching

Fig. 2 EIMs of two equimolar mixes of L- and D-AA (1 μM each) with <sup>13</sup>C-Phe, <sup>13</sup>C-Pro and Cu<sup>II</sup> (at 20 μM) obtained in SIM<sup>2</sup> mode. Only the enantiomer couples which are separated are shown.



SIM<sup>2</sup>-stitching: collection of multiple adjacent SIM<sup>2</sup> ranges (Fig. 2) with high mobility resolution.

### Conclusions

- Most proteinogenic L- and D-AA can be discriminated using non-covalent diastereoisomer complexes with Phe, Pro and Cu<sup>II</sup> with at least one combination of references and analyte and/or through self-association.
- Chiral separation is achievable in mix solutions, although not all ion complexes are found. Isomeric separation of Leu & Ile is possible through self-association, however chiral separation (L vs D) of each AA is more challenging.

#### ACKNOWLEDGMENTS

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#### REFERENCES

- S. Yang, L. Gu, F. Wu et al.; Talanta, 243 (2022)
- J. Czekner, E.K. Scheider, P. Weis et al.; J Am Mass Spectrom, 33 (2022)
- E. Rathahao-Paris, A. Delvaux, M. Li et al.; J Mass Spectrom, 57 (2022)