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Development of a high-throughput and high ion mobility resolution approach using automatic stitching of multiple ion mobility ranges on a TIMS-ToF instrument: application to chiral analysis

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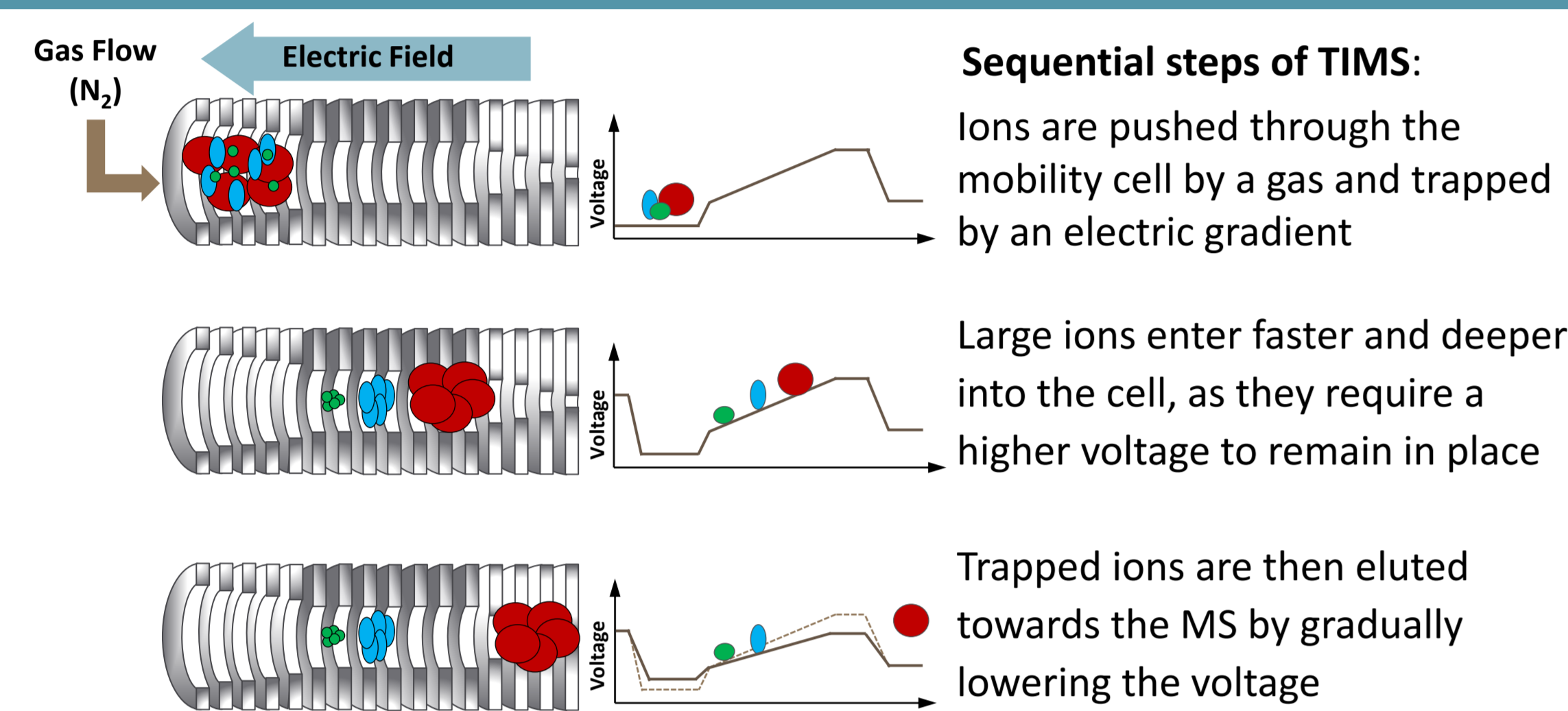
INTRODUCTION

- The characterization and identification of metabolites of interest in biological matrices is often achieved using hyphenated methods such as liquid chromatography coupled with **mass spectrometry** (LC-MS) but it remains an analytical challenge.
- Ion mobility spectrometry** is based on gas-phase mobility and its coupling with MS (IM-MS) offers an additional separation dimension and may allow CCS determination of analytes without lengthening the acquisition time. High resolution conditions are nevertheless essential to distinguish ions with very close ion mobilities such as **chiral compounds**.
- A high-throughput and high IM resolution approach was developed to allow exhaustive IM-MS measurements on a trapped ion mobility spectrometry time-of-flight (**TIMS-ToF**) instrument. **Chiral amino acids** (AAs) were chosen as model chiral compounds, and enantiomer couples were separated as non covalent complexes with Cu^{II}.

MATERIALS

- Direct infusion of proteinogenic chiral **L- and D-AAs** (1 μM each) with the addition of **Cu^{II}** and **¹Phe & ¹Pro** as chiral references (20 μM each) in H₂O/MeOH. Analysis of each individual AA at 5 μM.
- Study of all combinations between the analyte, references and Cu^{II} (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).
- Mobility separation evaluated with $\Delta CCS\% = 2 \frac{(CCS_B - CCS_A)}{(CCS_A + CCS_B)} \times 100$

INSTRUMENTATION



TIMS separation

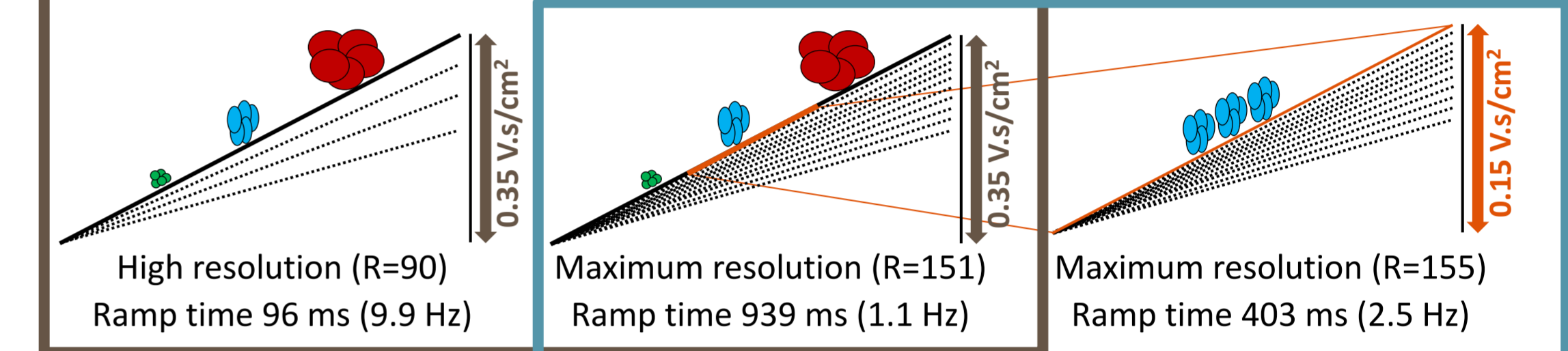
In practice

Three mobility modes are available (normal, high and maximum resolution), each corresponding to fixed **spectra rate** values (Hz).

The **mobility range** 1/K₀ (V.s/cm²) is fixed, and the **ramp time** (ms) is adjusted to fit the spectra rate.

High resolution => low spectra rate & long ramp time

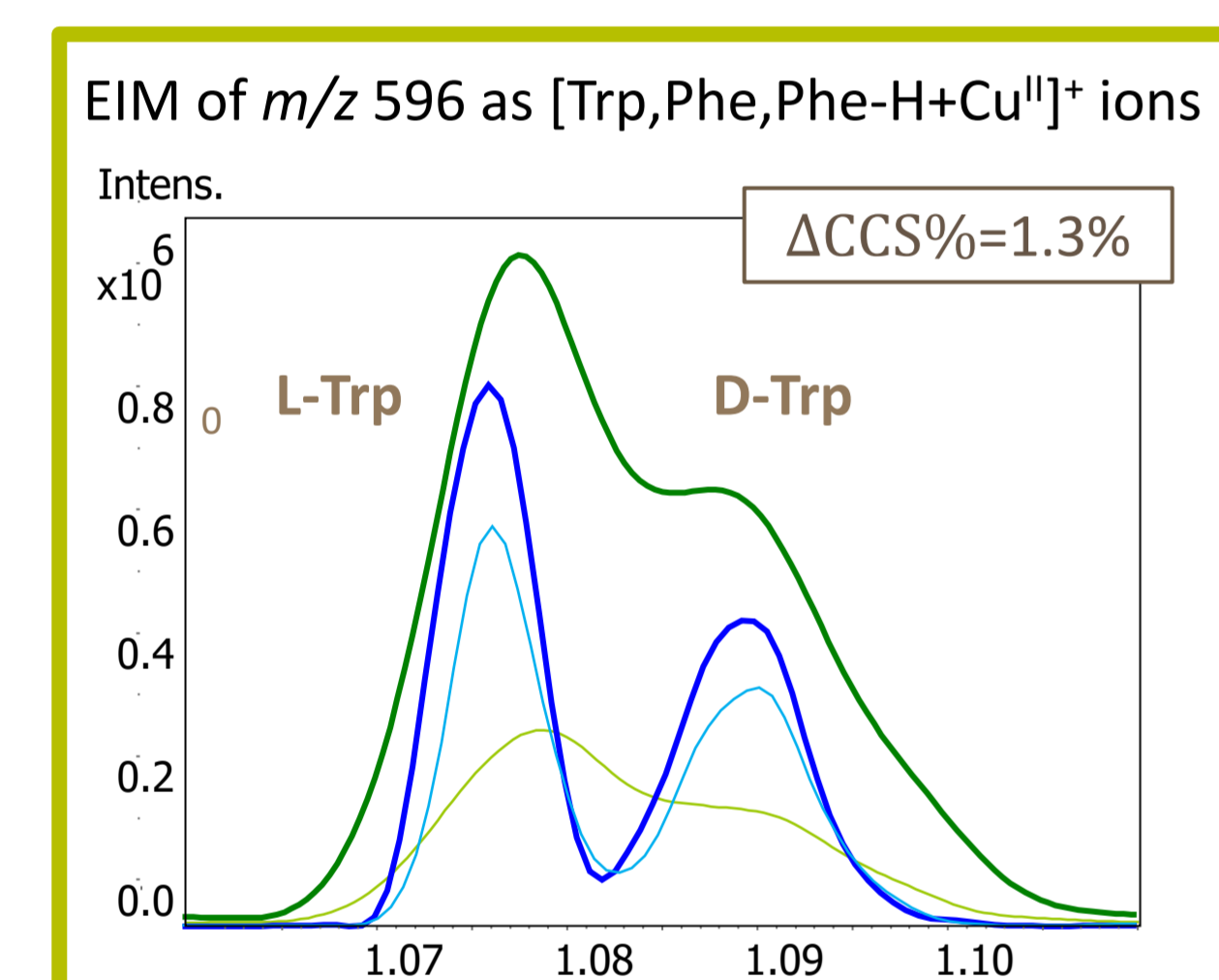
For a given mobility range, a higher resolution mobility mode will require longer ramp times => more MS scans per elution step



For a given mobility mode, a narrow mobility range will require shorter ramp times to fit the spectra rate and keep the resolution constant, while increasing sensitivity => **SIM² mode** (Single ion mobility monitoring) [1]

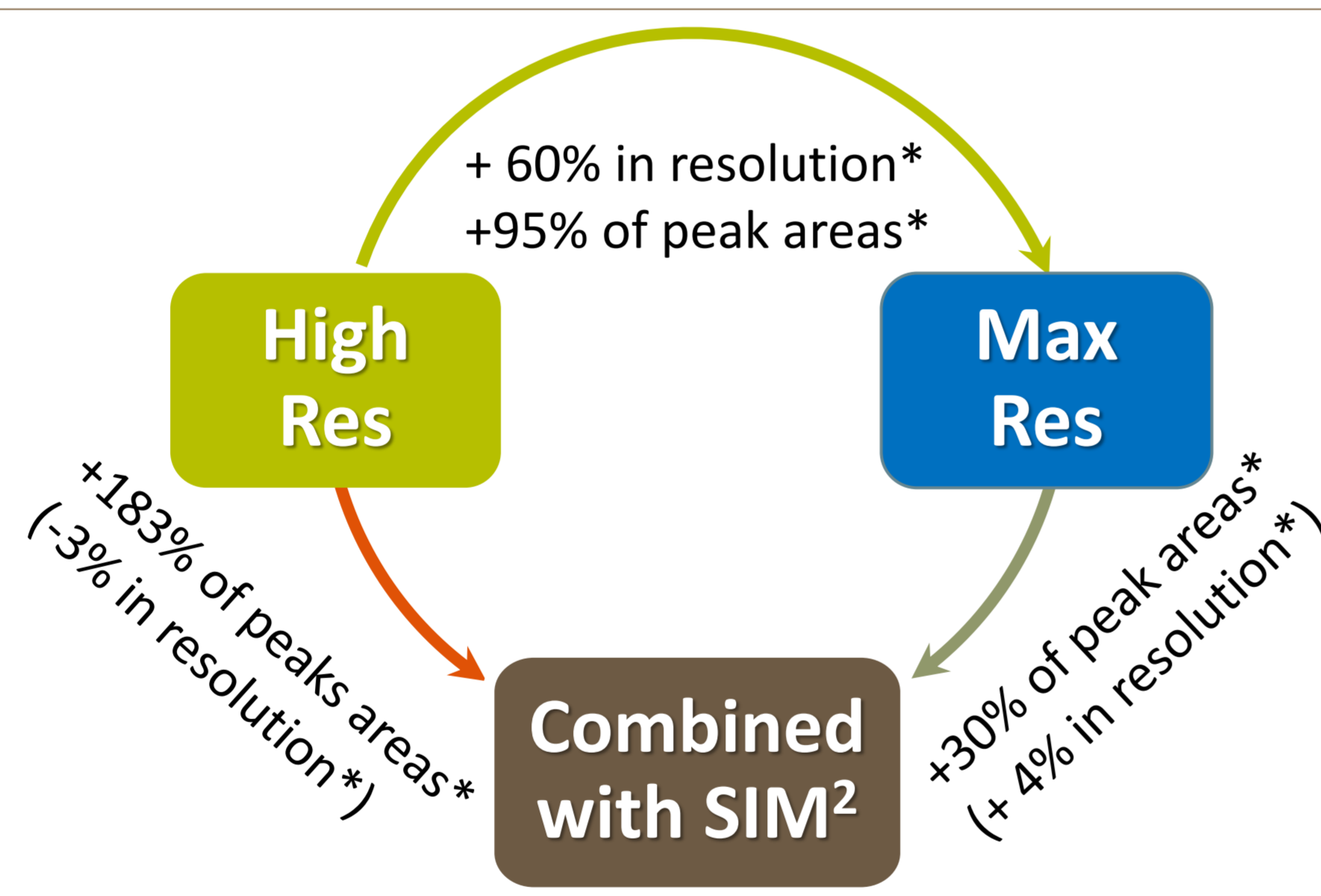
RESULTS

High resolution ion mobility for chiral analysis



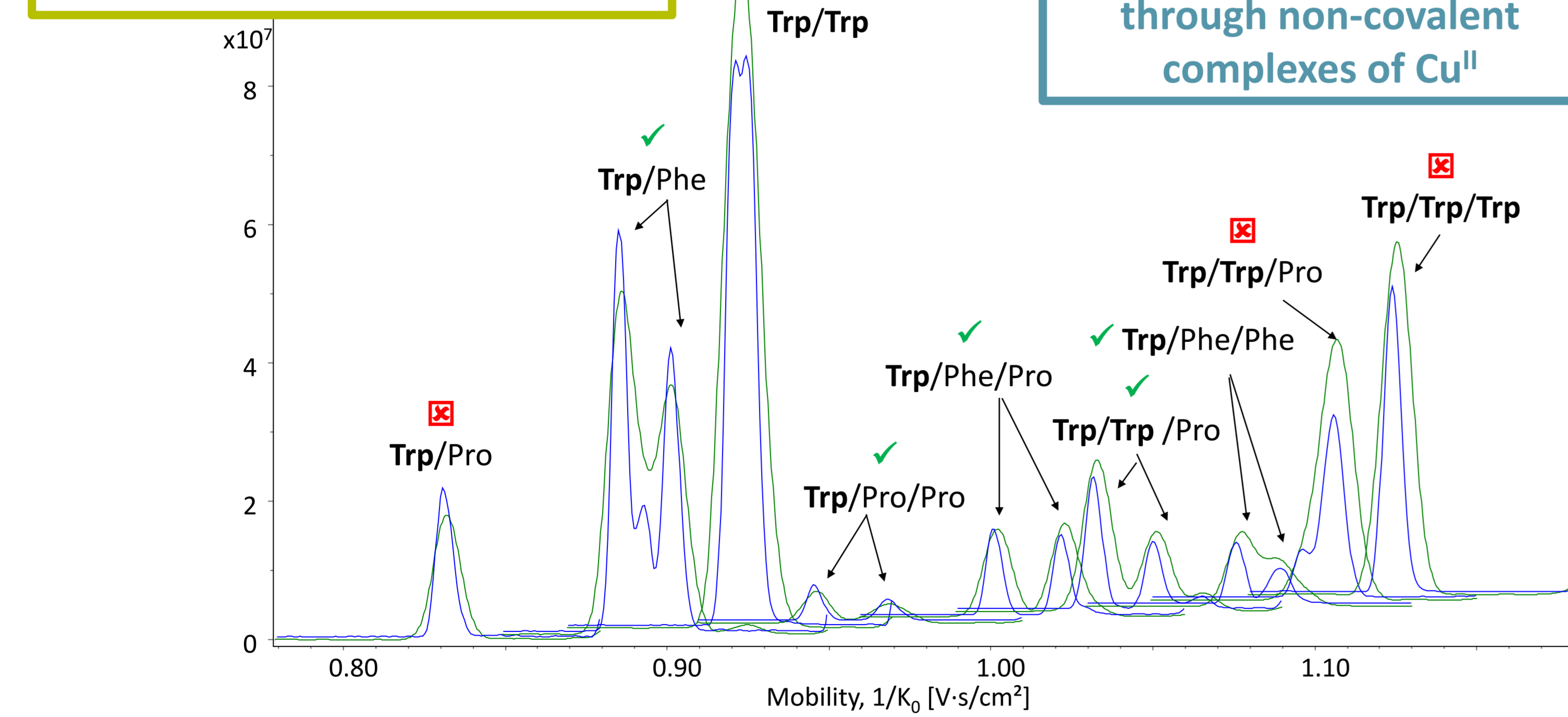
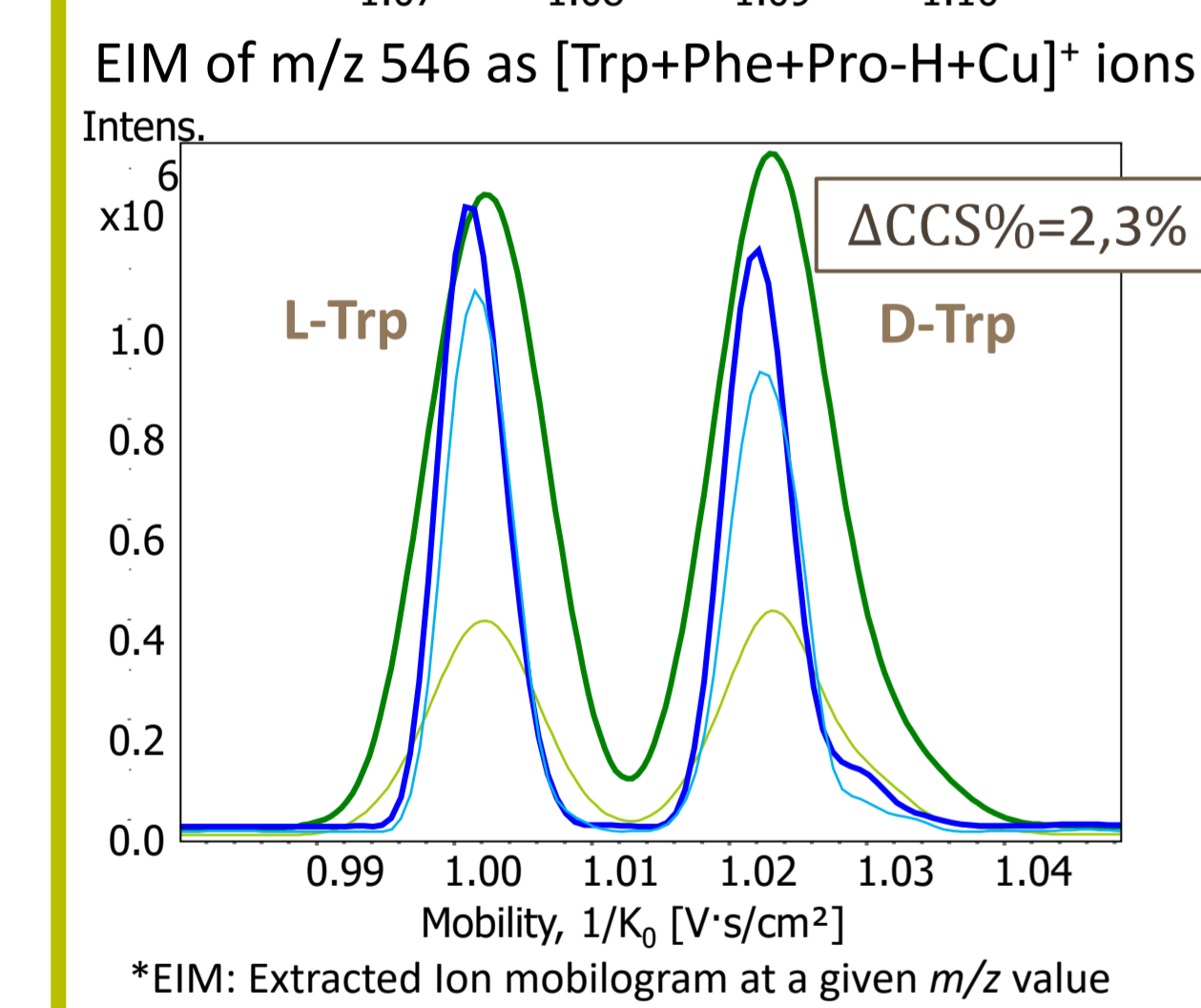
Optimization of TIMS conditions

Traces	Mobility range (1/K ₀ in V.s/cm ²)	Ramp time (ms)	Spectra rate (Hz)	# Scans
High Res full scan	0.80-1.17	101	9.41	281
High Res SIM ²	Window size of 0.10	30	28.4	827
Max Res full scan	0.80-1.17	994	1.00	31
Max Res SIM ²	Window size of 0.10	273	3.60	108



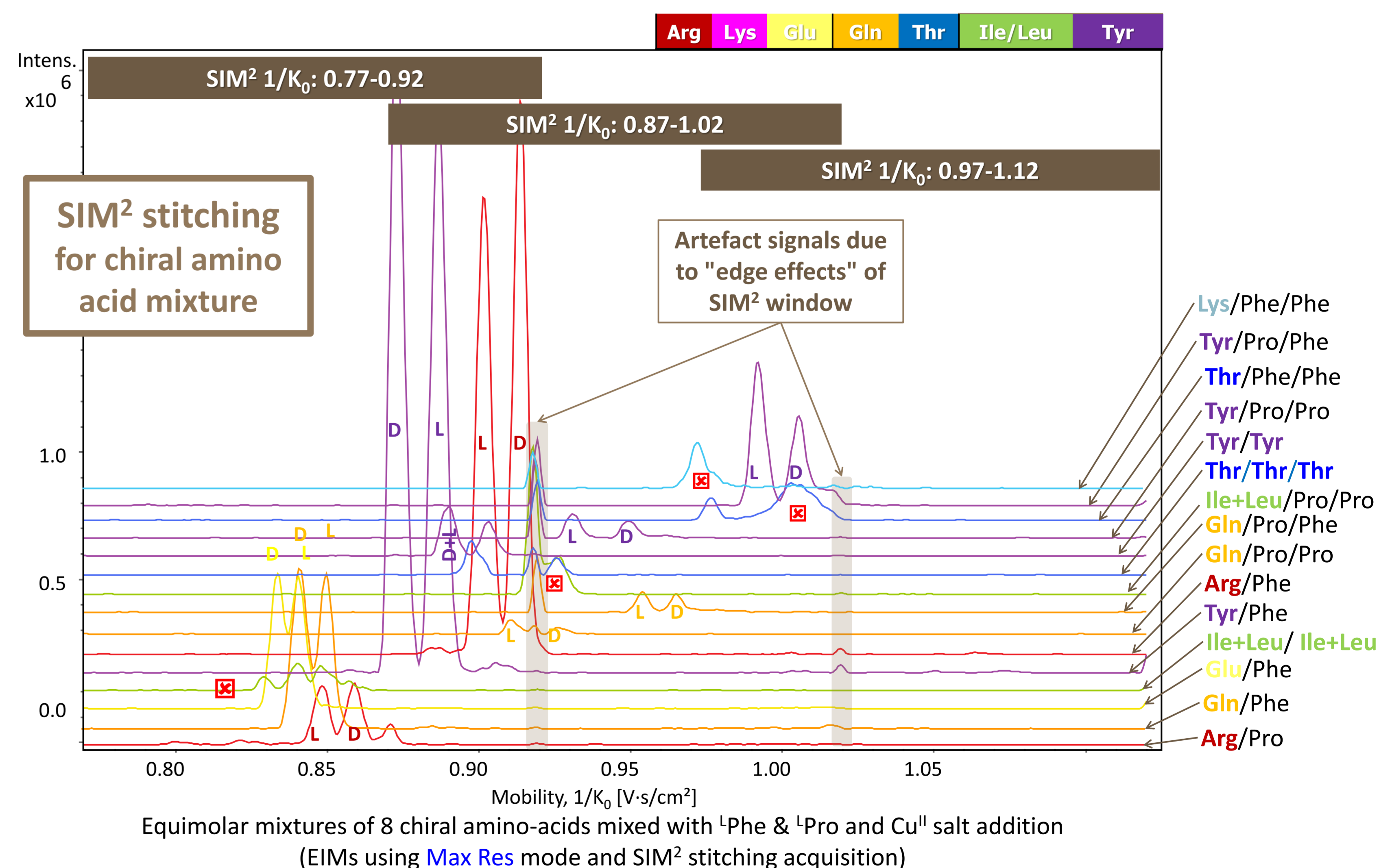
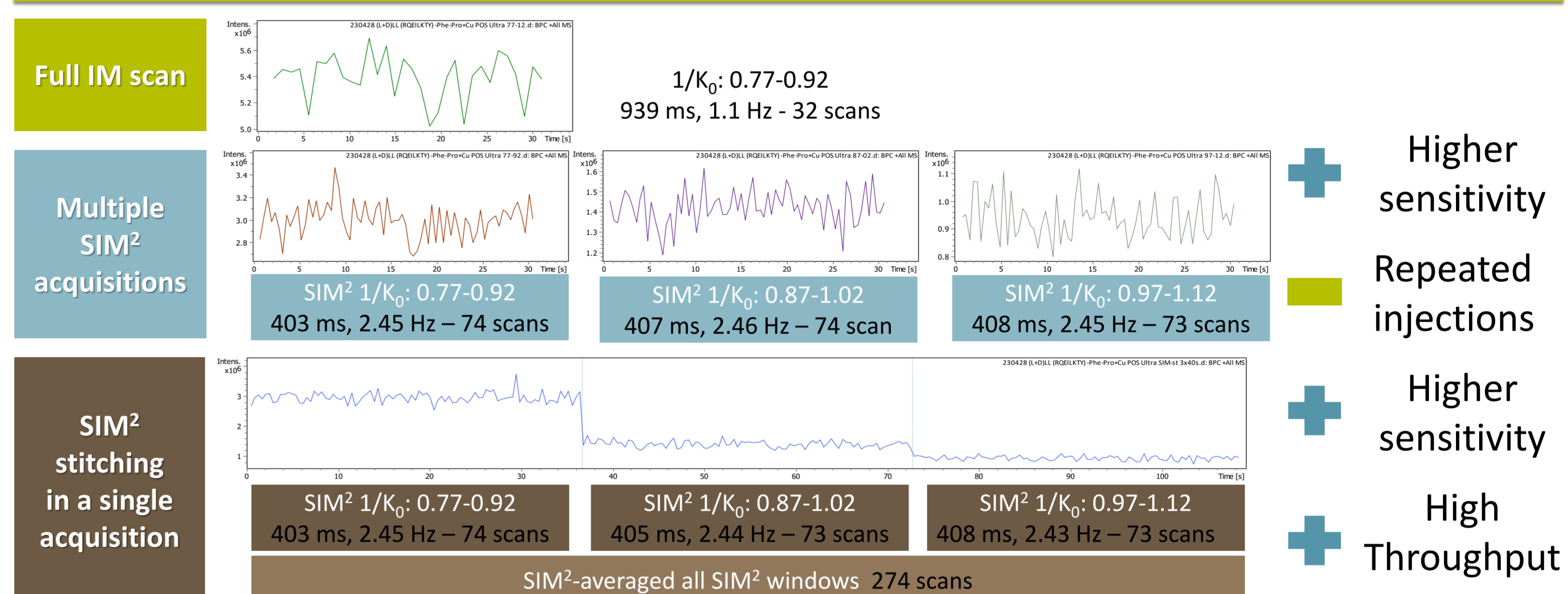
* Calculations from all Trp complex ions, see Figure below

Chiral ion mobility analysis through non-covalent complexes of Cu^{II}



L- and D-Trp mixed with ¹Phe & ¹Pro and Cu^{II} salt addition (EIMs from High Res & Max Res detection)

SIM² stitching for high throughput and high resolution analysis of complex mixture



CONCLUSION

- Fast chiral IM analysis of AAs as non-covalent Cu^{II} complexes with Phe and Pro as chiral references
- 70% of all possible complexes are detected in a mix solution (92% in individual mix), and 19% are separated (vs 28%)
- SIM² stitching allows exhaustive ion mobility measurements while maintaining high resolution and sensitivity on large mobility range

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REFERENCES

[1] E. Rathahao-Paris, A. Delvaux, M. Li et al.; J Mass Spectrom, 57 (2022)

Full scan	<ul style="list-style-type: none"> Large mobility range in a single acquisition No mobility separation below 1.5% (moderate mobility resolution)
Ultra with SIM ²	<ul style="list-style-type: none"> Highest mobility resolution & sensitivity, good for targeted analysis and known compounds Narrow mobility range, may require repeated injections, long ramp times (LC compatibility)
SIM ² stitching	<ul style="list-style-type: none"> Single acquisition by collection of adjacent SIM² windows, high mobility resolution & sensitivity (possible edge effects)