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STEREOSPECIFIC RECOGNITION OF AMINO ACIDS IN QUATERNARY COPPERED COMPLEXES VIA ENANTIOSELECTIVE REDUCTION

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INTRODUCTION

- Chirality poses a unique challenge in life sciences since individual enantiomers may exhibit different biological activity and toxicity. Efficient chiral analytical methods are thus required.
- Enantiomeric distinction of amino acids (^{D/L}AA) based on the relative rate constants of competitive fragmentations of quaternary coppered complexes (QCCs) is an efficient method for chiral differentiation [1,2].
- Phenylalanine (Phe) was used as a model compound with proline (Pro) and non-natural AA phenylglycine (PhG) as chiral selectors [3] for a detailed study of the enantioselective reduction of homochiral or heterochiral QCCs [Cu^{II},(^{D/L}Phe,^DPhG,^DPro-H)]⁺ in gas phase (see adjacent panel). Additional *in silico* simulations were carried out for further kinetic insights.

METHODS

- Mixtures of ^DAA or ^LAA and Cu^{II} salt were prepared in H₂O/MeOH (1:1) at 20 μM and directly infused in an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific) in positive ESI mode.
- QCCs were subjected to resonant activation using collision-induced dissociation (CID) while varying collision energy (CE) and activation time (ActT). The resulting energy-resolved mass spectrometry (ERMS) data was represented via the survival yield (SY) method.
- CID experiments were simulated within the frame of RRKM theory using MassKinetics Scientific Demo software [4].

RESULTS

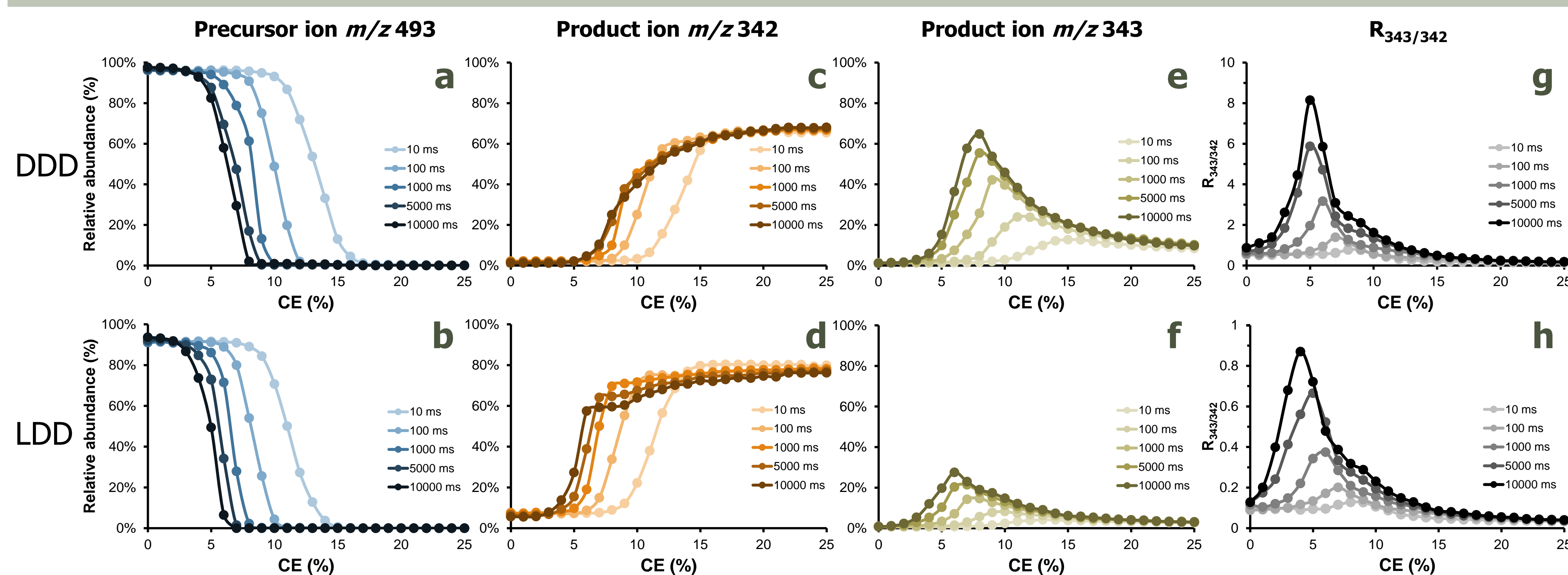


Fig. 1: ERMS of DDD and LDD [Cu^{II},(Phe,PhG,Pro-H)]⁺ obtained at ActT of 10, 100, 1000, 5000 and 10000 ms; precursor ion [Cu^{II},(Phe,PhG,Pro-H)]⁺ *m/z* 493 (a and b), product ion [Cu^{II},(Phe,Pro-H)]⁺ *m/z* 342 (c and d), product ion [Cu^I,(Phe,Pro)]⁺ *m/z* 343 (e and f), ratio of product ions *m/z* 343 over *m/z* 342, *R*_{343/342} (g and h).

- The extent of the reduction, *i.e.* the abundance of the product ion *m/z* 343, is enhanced when increasing ActT (Fig. 1e-1f).
- LDD complexes are less stable than DDD complexes (Fig. 1a-1b).
- Surprisingly, the abundance of the reduced ion *m/z* 343 was found to decrease when increasing CE. The ratio of the product ions *m/z* 343 over *m/z* 342 *R*_{343/342} follows a similar pattern.

These results can be described using the RRKM theory [5]:

- Rearrangements (such as the formation of the product ion *m/z* 343) have low entropy of activation and low critical energy *E*₀, and are favoured at low CE and long ActT.
- Conversely, direct cleavage (*i.e.* formation of the product ion *m/z* 342), characterized with higher entropy and critical energy, is favoured at high CE and short ActT.

Preliminary simulations using MassKinetics were performed to explore these kinetic findings.

Second form of QCC

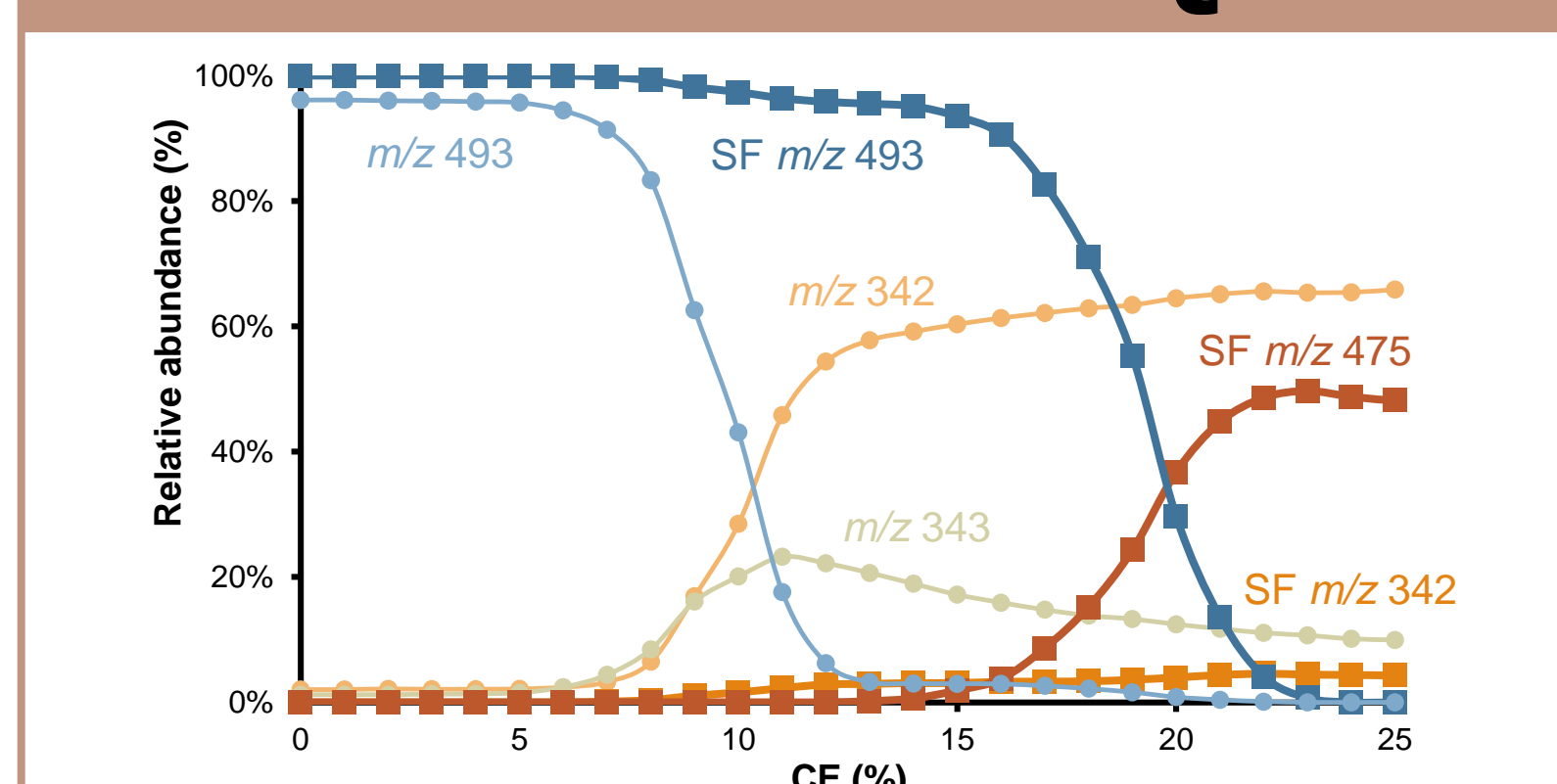


Fig. 4: ERMS of DDD [Cu^{II},(Phe,PhG,Pro-H)]⁺ obtained at an ActT of 100 ms with (SF, square) and without in-source fragmentation (circle).

Under certain circumstances, the abundance of the QCC precursor ion was found to plateau before its complete disappearance (Fig. 4). This phenomenon hints that at least two forms of QCC co-exist, the most abundant of which leads to the chiral effects described above.

The second form was isolated using in-source fragmentation (SF) to remove the most abundant form (Fig. 4). Sequential MS experiments on this second form show different fragmentation patterns, most notably the fragment [M-H₂O]⁺ *m/z* 475, which suggest two distinct structures.

The second form could not be observed using ion-mobility (TIMS-ToF, Bruker), either because the CCS of both forms are similar or because the source of the instrument does not allow the appearance of the second form.

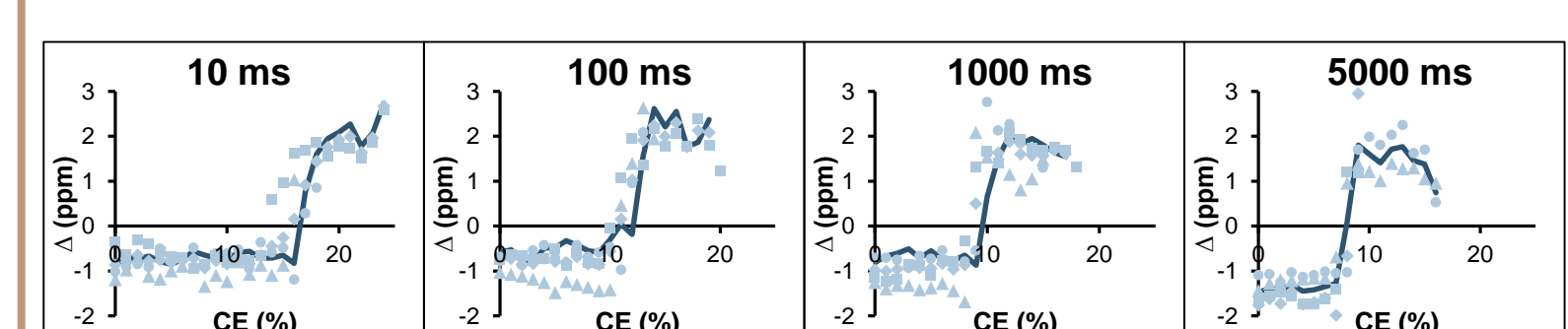


Fig. 5: Difference between the accurate and exact mass of the precursor QCC [Cu^{II},(Phe,PhG,Pro-H)]⁺ *m/z* 493 for DDD (circle and square) and LDD (diamond and triangle) complexes at ActT of 10, 100, 1000 and 5000 ms (solid line, mean).

The switch between the two forms was accompanied by an unexpected *m/z* shift of about 3 ppm (Fig. 5). Additional experiments using an FT-ICR at very high resolution (1-2.10⁶ at *m/z* 500) could neither evidence a second form nor the *m/z* shift.

- ERMS curves simulated with MassKinetics are able to show the influence of ActT on the dissociation of the precursor ion [Cu^{II},(Phe,PhG,Pro-H)]⁺ (Fig. 2). In particular, the unexpected behavior of the reduced ion *m/z* 343 can be reproduced within the simulation.

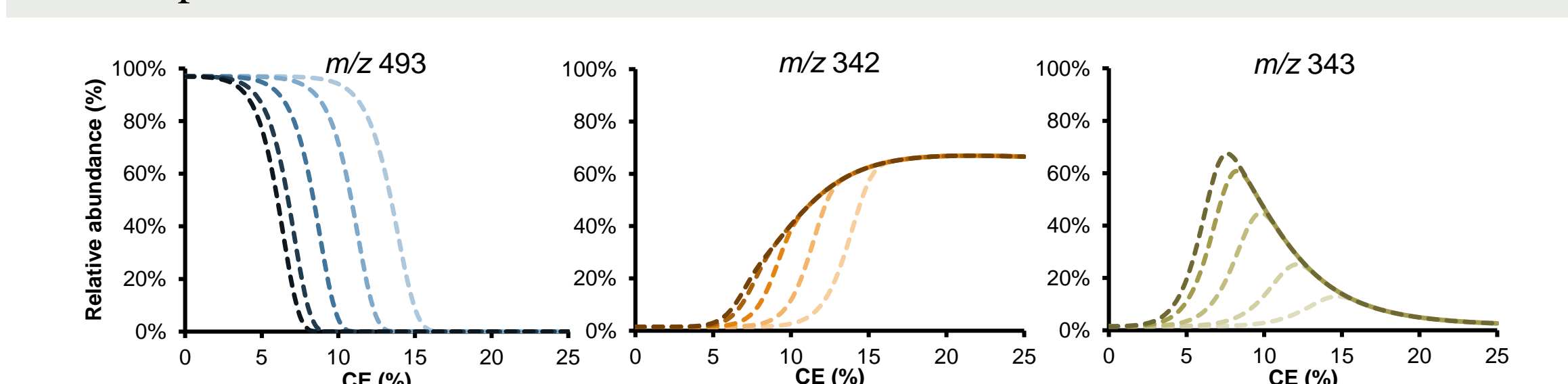


Fig. 2: Simulated ERMS of DDD [Cu^{II},(Phe,PhG,Pro-H)]⁺ for ActT of 10, 100, 1000, 5000 and 10000 ms (precursor ion *m/z* 493 (blue), product ions *m/z* 342 (orange) and *m/z* 343 (green)).

- The critical energy *E*₀ values used in the simulation for the formation of the reduced ion *m/z* 343 are comparable in DDD and LDD complexes (0.85 eV). However, the formation of the product ion *m/z* 342 requires a lower *E*₀ in LDD (1.12 eV) compared to DDD complexes (1.20 eV) (Fig. 3a and 3b). The product ion *m/z* 342 is thus preferentially formed in heterochiral QCCs, rather than the reduced ion *m/z* 343 being favored in homochiral QCCs.

- The increase of *R*_{343/342} followed by its decrease would be an artefact caused by the presence of prompt product ions at 0% CE (Fig. 3c), likely formed by the isolation waveform in the ion trap. Without these preliminary dissociations, the abundance of the reduced ion *m/z* 343 would always be higher than the product ion *m/z* 342 at low CE and *R*_{343/342} would only decrease (Fig. 3d).

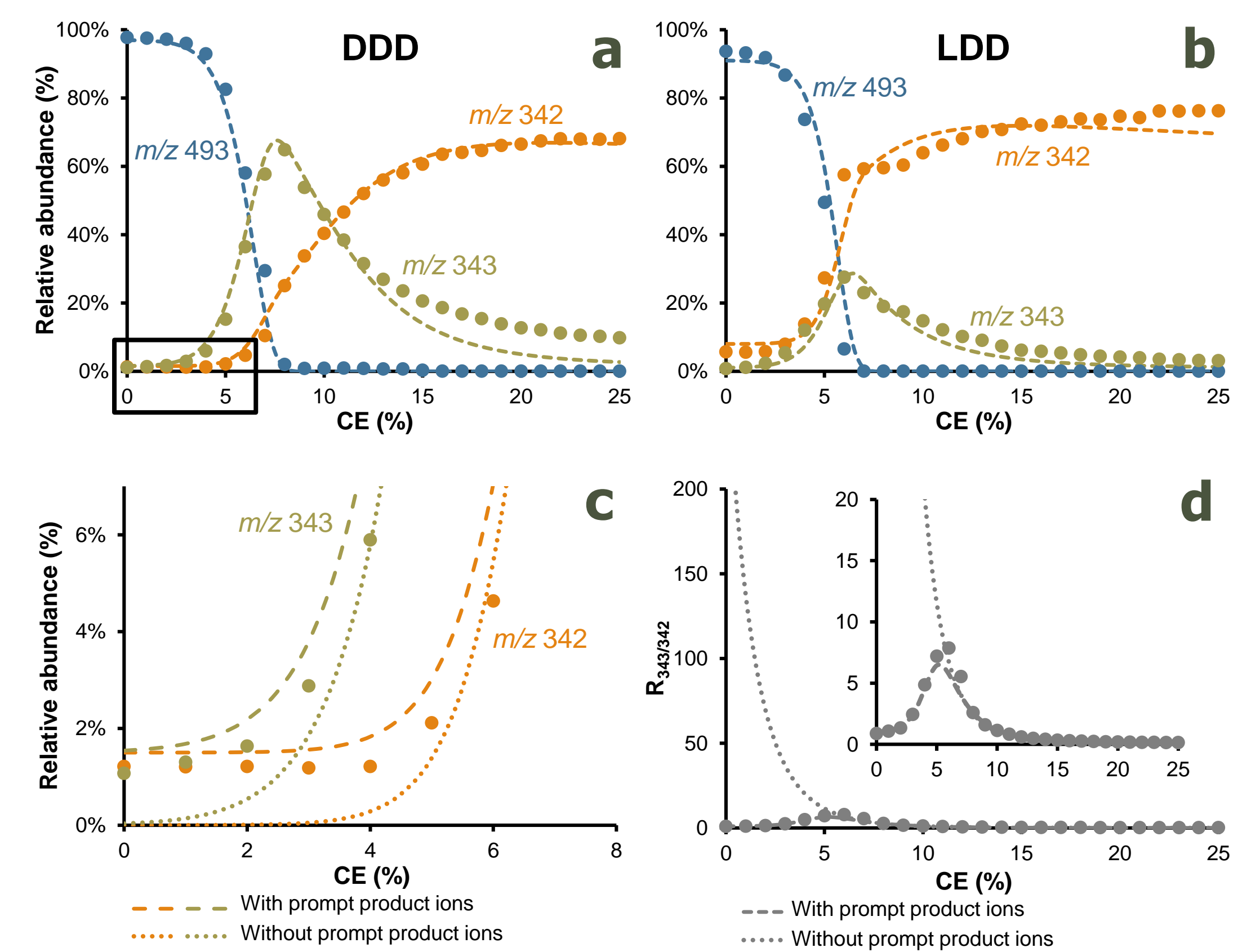


Fig. 3: ERMS of (a) DDD and (b) LDD [Cu^{II},(Phe,PhG,Pro-H)]⁺ obtained at an ActT of 10000 ms (circles) and simulated curves generated with MassKinetics (dashed line). (c) Zoom of Fig. 3a and MassKinetics simulated curves of DDD precursor QCC with (dashed line) and without prompt product ions (dotted line). (d) Ratio of product ions *m/z* 343 over *m/z* 342 and MassKinetics simulated curves of DDD precursor QCC with (dashed line) and without prompt product ions (dotted line).

CONCLUSION

- Enantioselective reduction in [Cu^{II},(Phe,PhG,Pro-H)]⁺ QCCs would be a consequence of the instability of heterochiral complexes, which would favour direct cleavage and the product ion [Cu^{II},(Phe,Pro-H)]⁺ *m/z* 342, rather than the result of an increase in the formation of the reduced product ion [Cu^I,(Phe,Pro)]⁺ *m/z* 343 in homochiral QCCs.
- A putative second form of the precursor QCC was found, which hints at a possibly distinct conformation but was not identified.

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