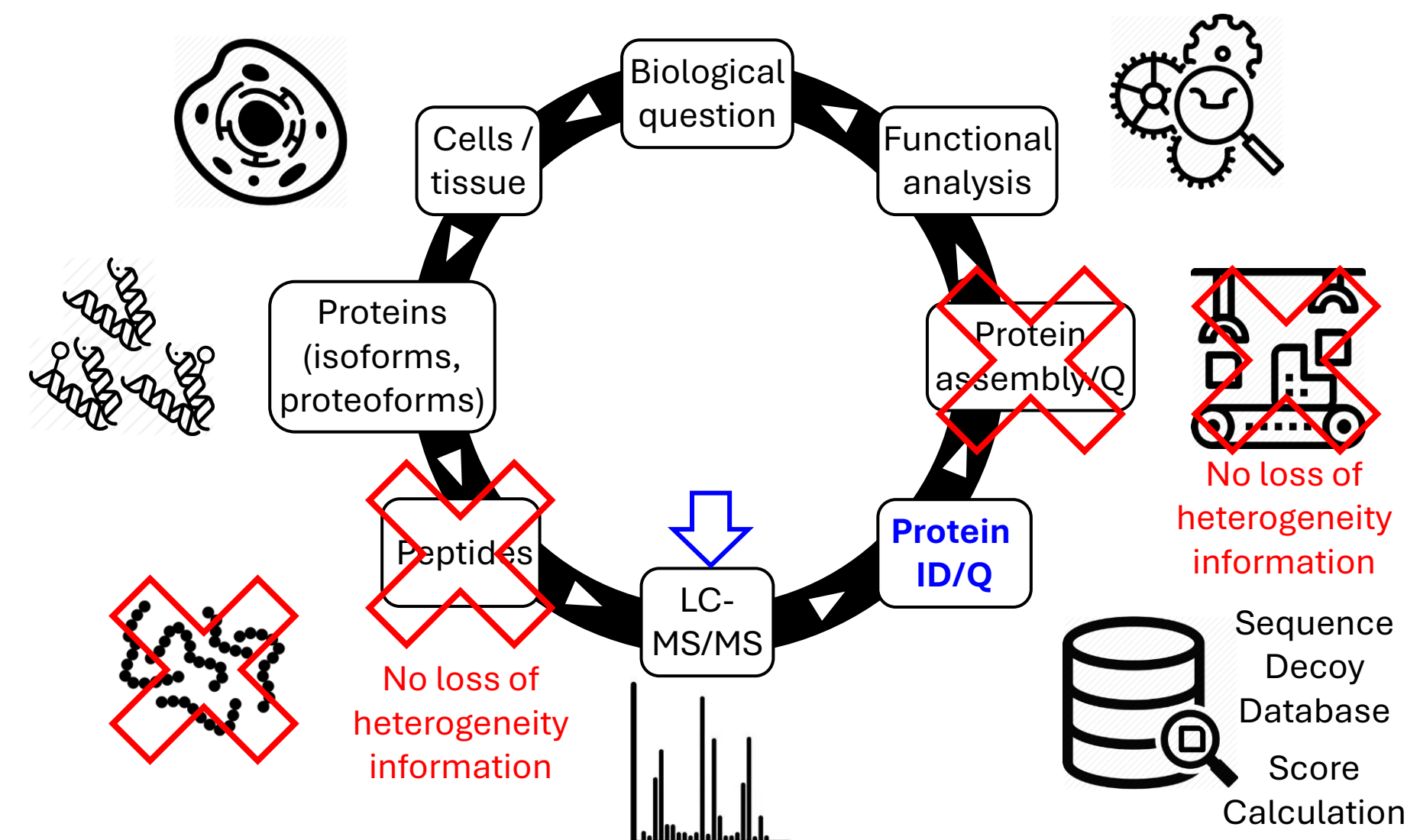


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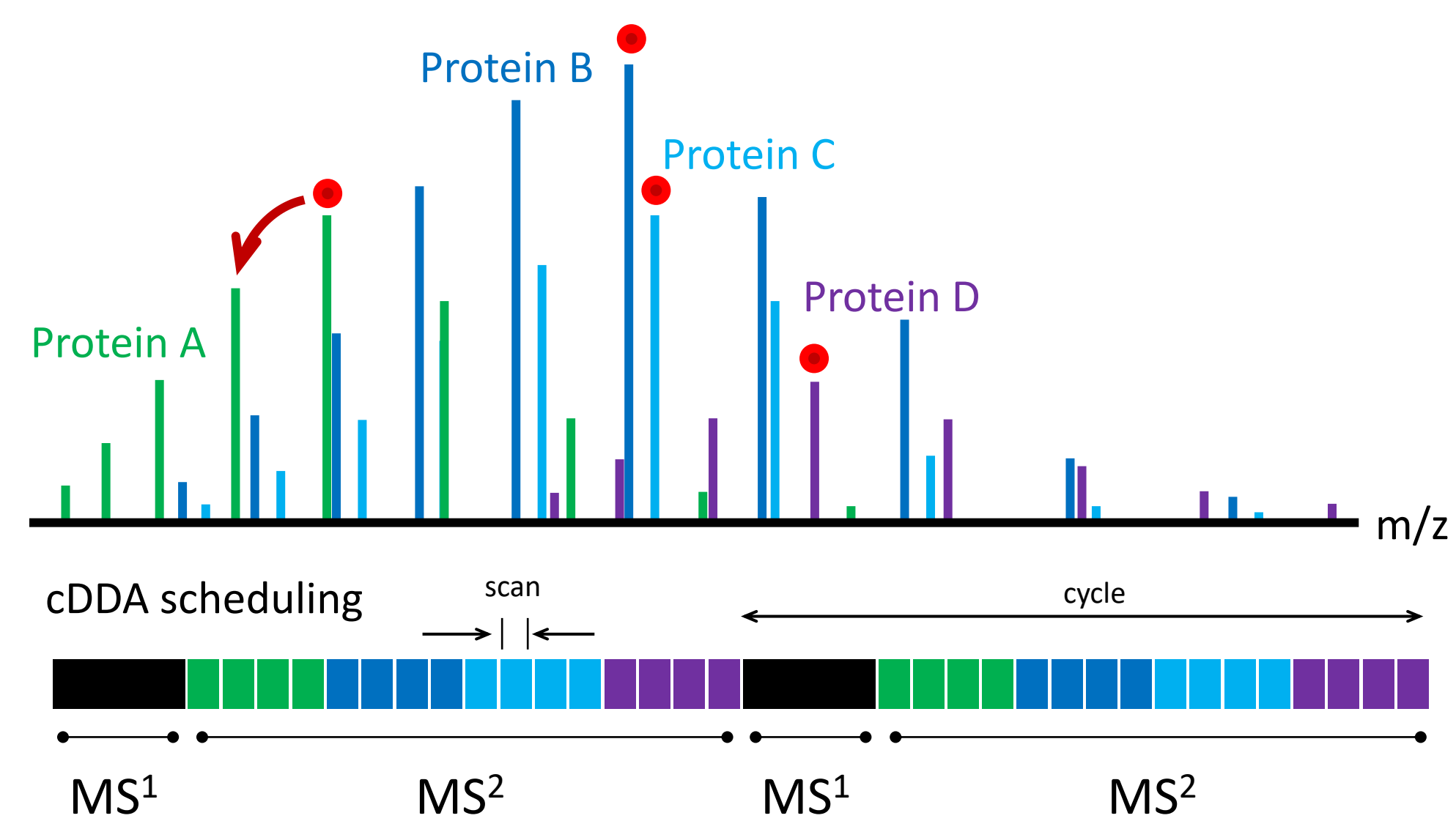
Introduction

- Protein-centric proteomics is an emerging MS field where to answer a biological question related to cells or tissues, one analyzes proteins directly, without relying on their digestion into smaller peptides.
- Isoforms and proteoforms are separated using liquid chromatography before being analyzed using MSⁿ mass spectrometry, using techniques ranging from collision induced activation to electron capture dissociation.
- In protein-centric proteomics, proteoforms are identified and quantified directly without the need to rely on peptide assembly into proteins.



Methods

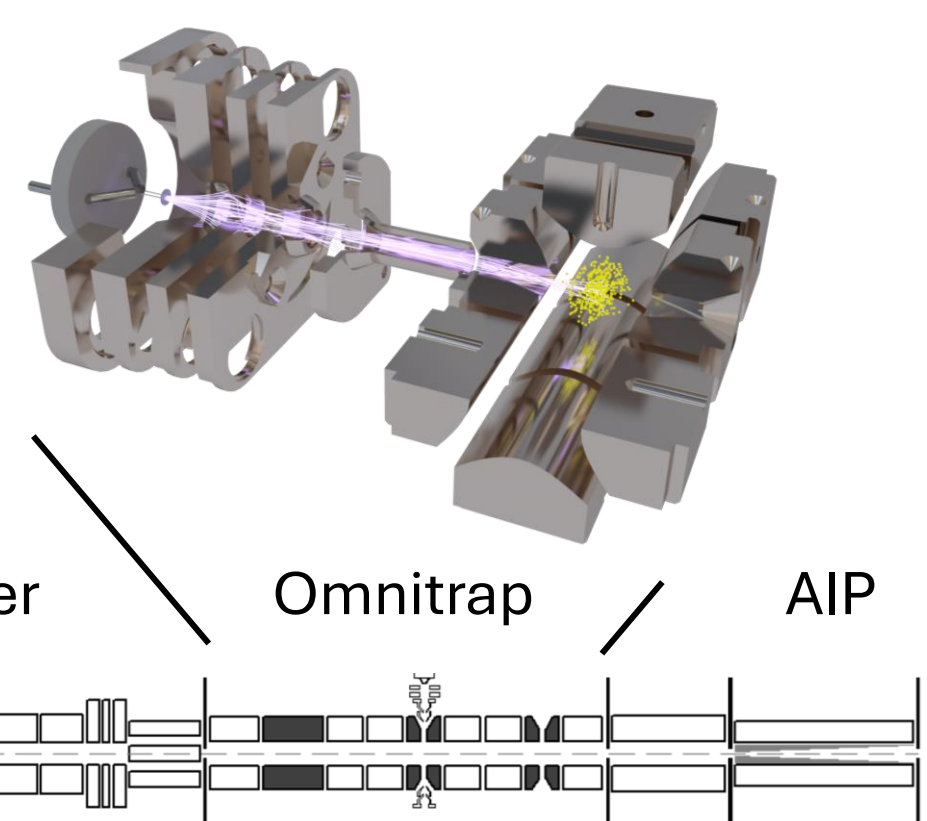
- Charge DDA (cDDA) enables on-the-fly charge-state deconvolution during LC-MS analysis, allowing precise targeting of coeluting proteoforms across a wide dynamic range, and eliminates redundant fragmentation of highly abundant species. By dynamically shifting the isolation window to non-overlapping charge state regions of the m/z spectrum, cDDA significantly reduces chimeric spectra.



Instrumentation

- Schematic of a Bruker timsOmni[™] IMS Q-Omnitrap[™]-ToF mass spectrometer. The Omnitrap cell with its electron gun is highlighted.

- CaptiveSray
- isCID 40 eV
- Dynamic accumulation
- Dynamic ECD at ~1 eV KE

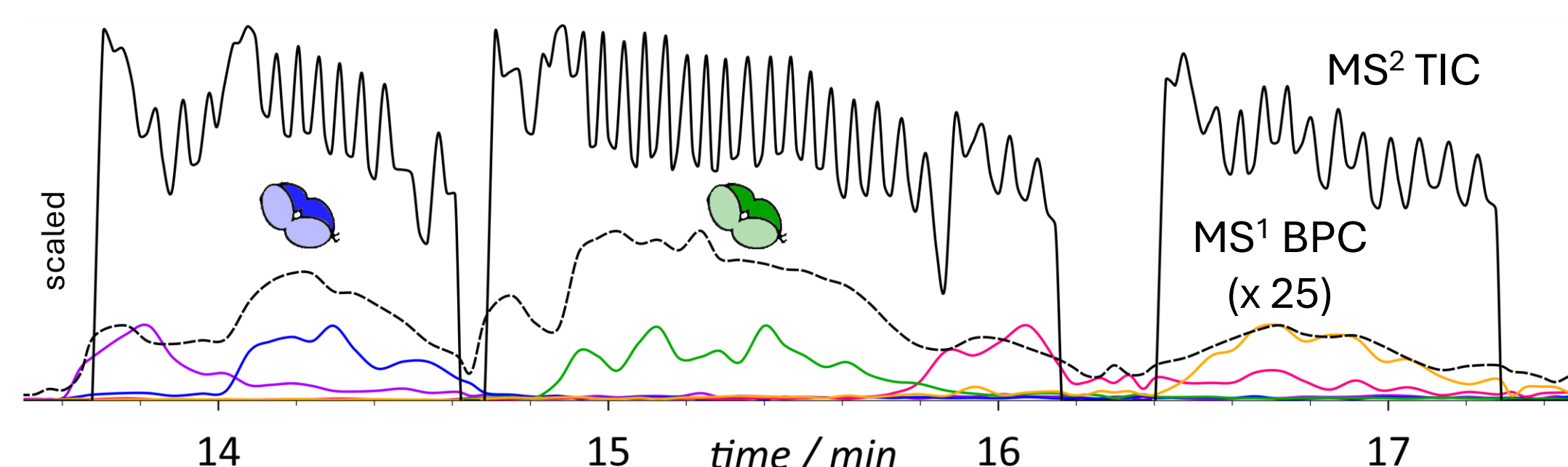


Top-down analysis on a LC-MS time scale of 6 Fabs mixture

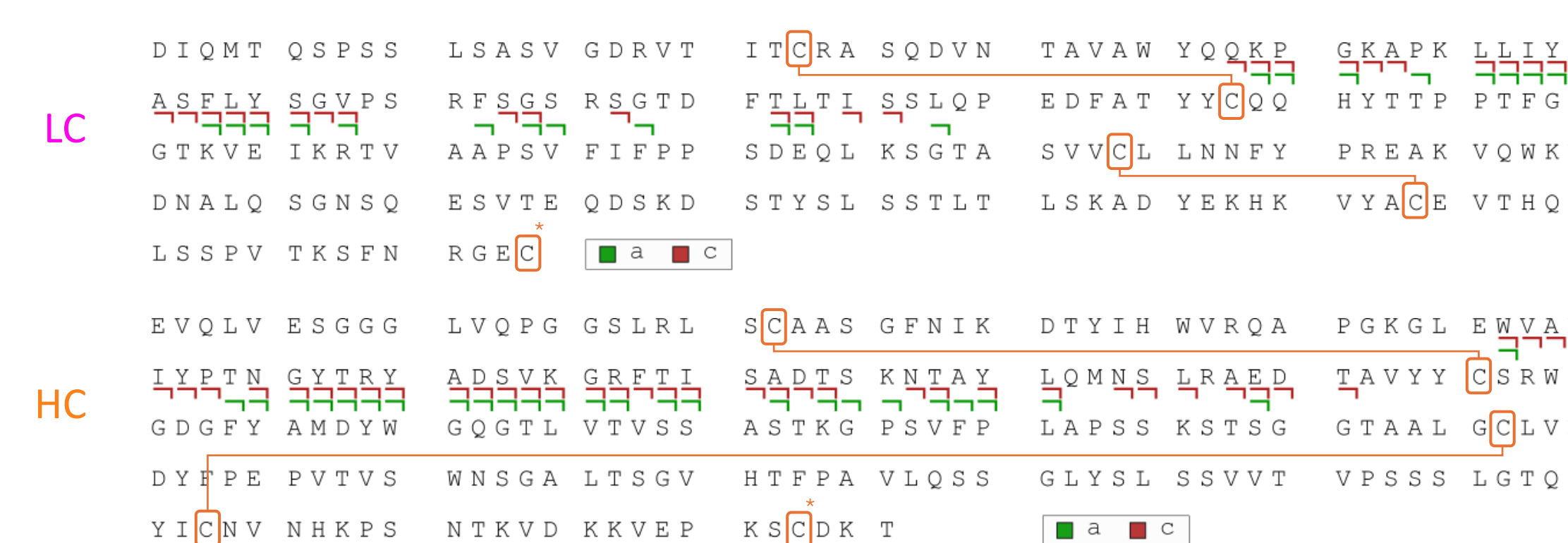
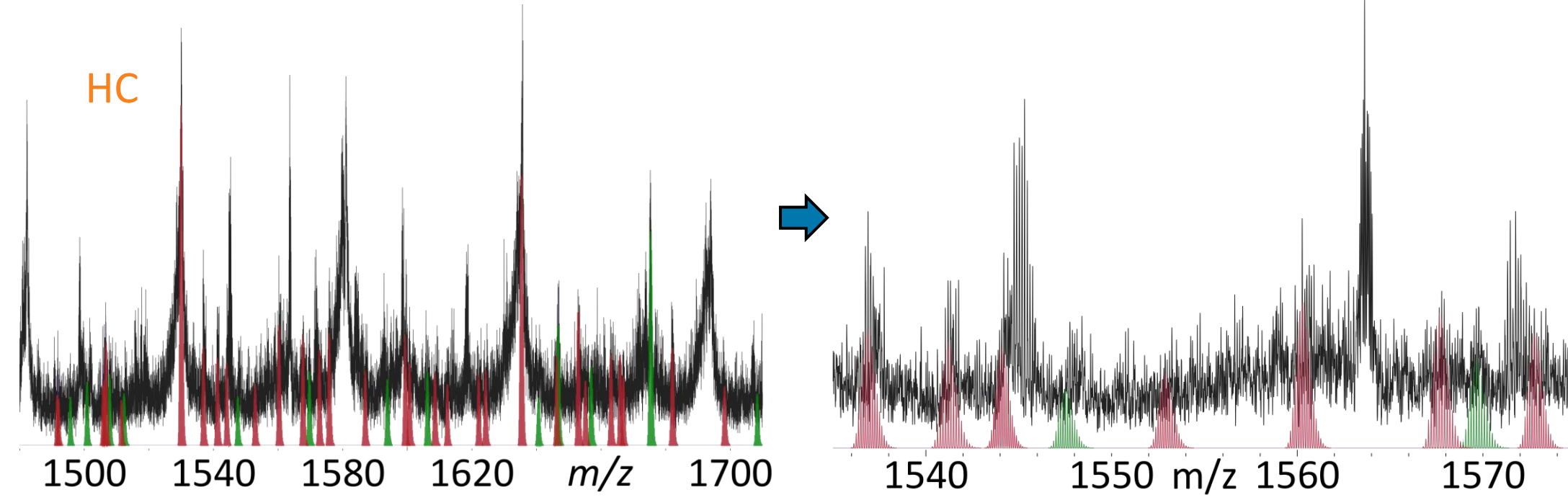
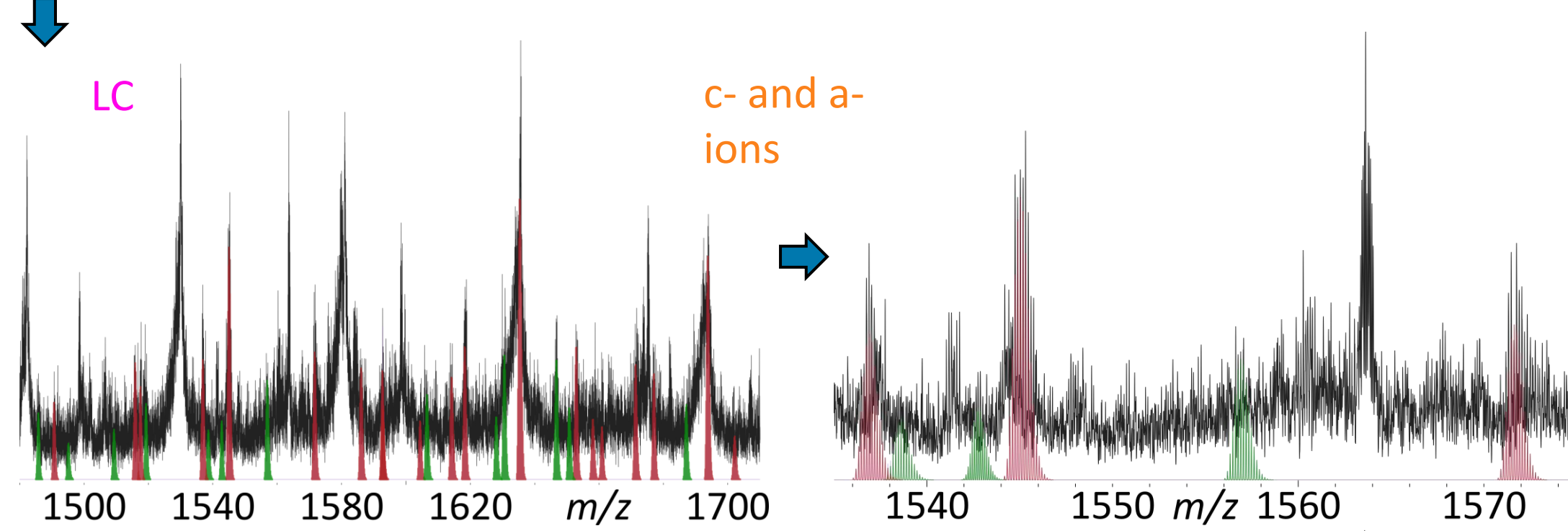
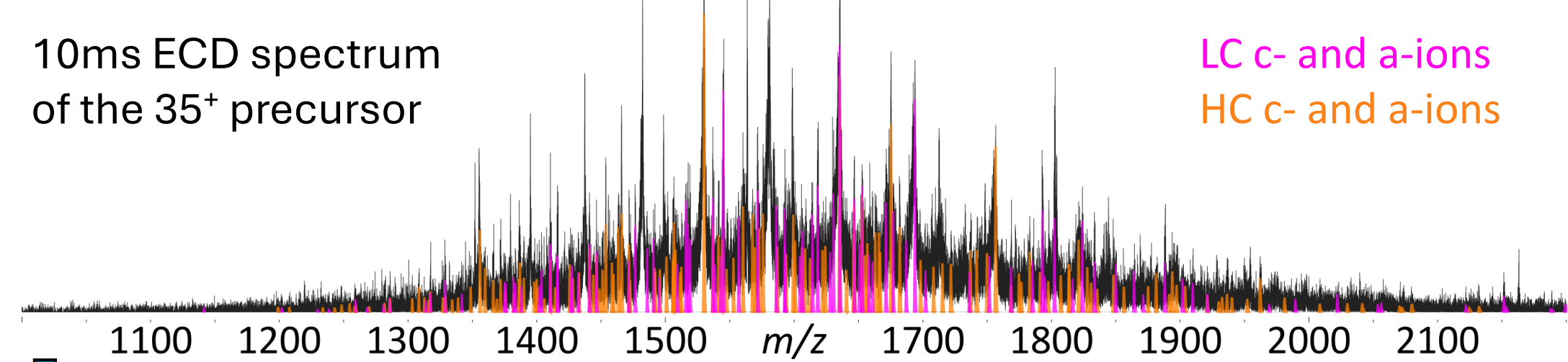
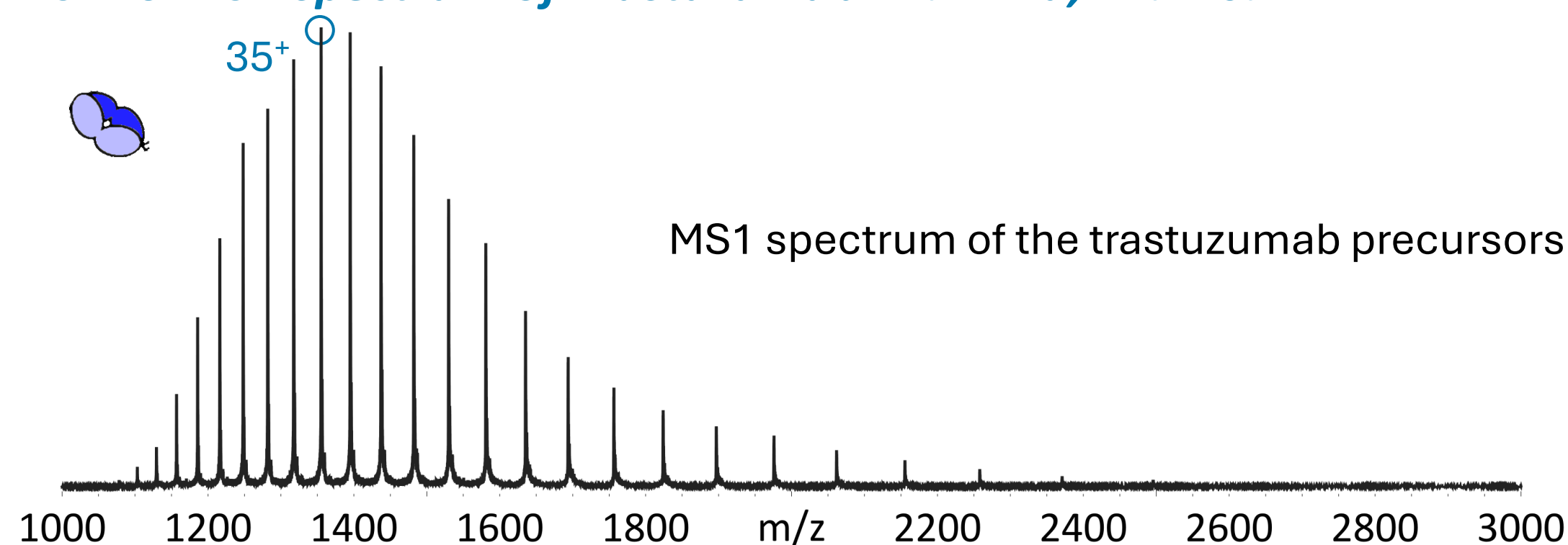
- Fabs were produced by enzymatic digestion from 300 µg of a mixture of 6 IgG1 monoclonal antibodies (eq.:eq.) using the hinge directed proteases Igde. The Fabs recovered in 80 µL of H₂O + 0.1% FA were aliquoted and snap frozen.
- Following a x10 times dilution in H₂O + 0.1% FA, 1 µL of Fabs was separated using reverse phase chromatography (5_{0-1min}, 23-33_{1.5-20min}, 95_{21min}% of 100% ACN + 0.1% FA) on a MAbPac[™] Capillary Reversed Phase HPLC Column.
- Electrosprayed Fabs, spanning the 33⁺ to 37⁺ range, were identified from MS1 scans, mass selected and accumulated thanks to the cDDA algorithm, fragmented by ECD in the Omnitrap, before being detected.
- The results were processed using DataAnalysis[™] and Omniscope[™].

Liquid chromatographic separation

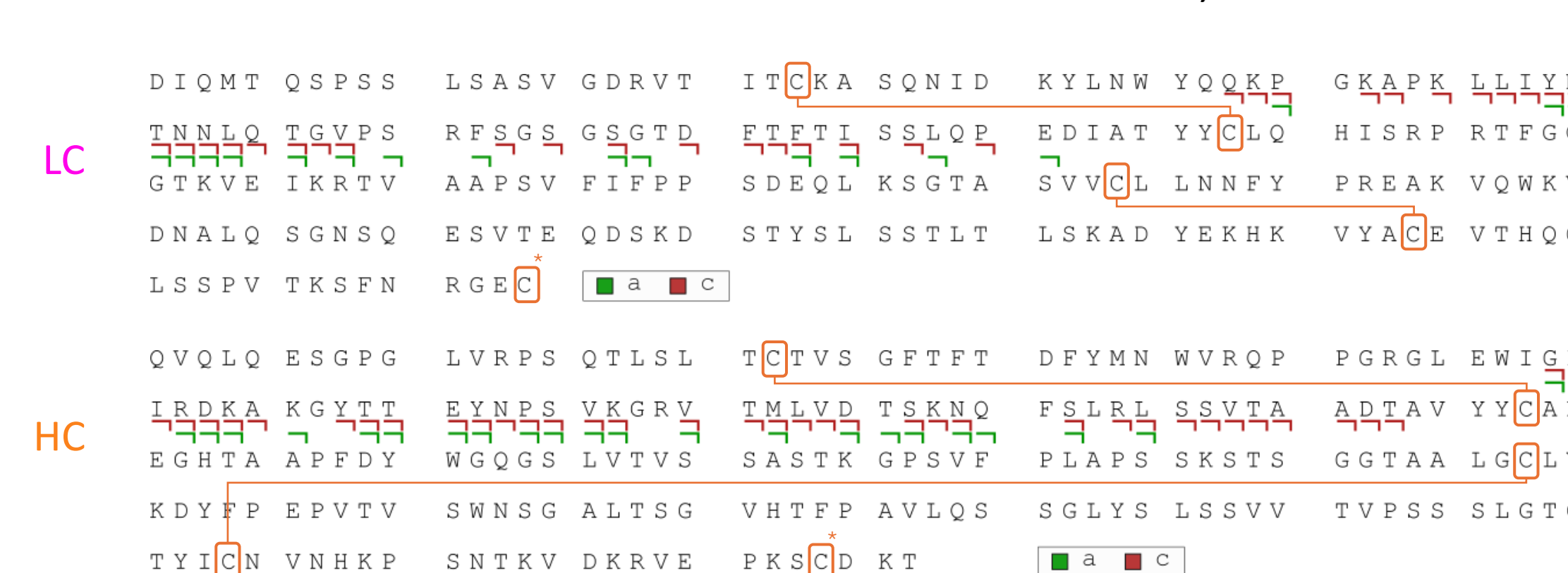
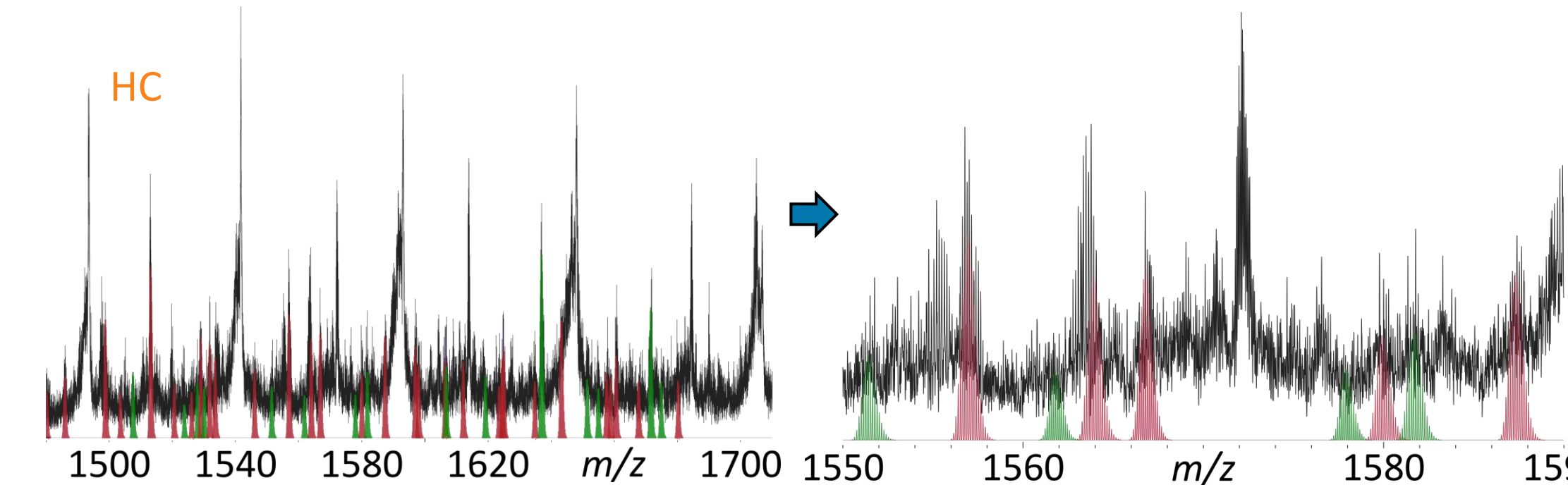
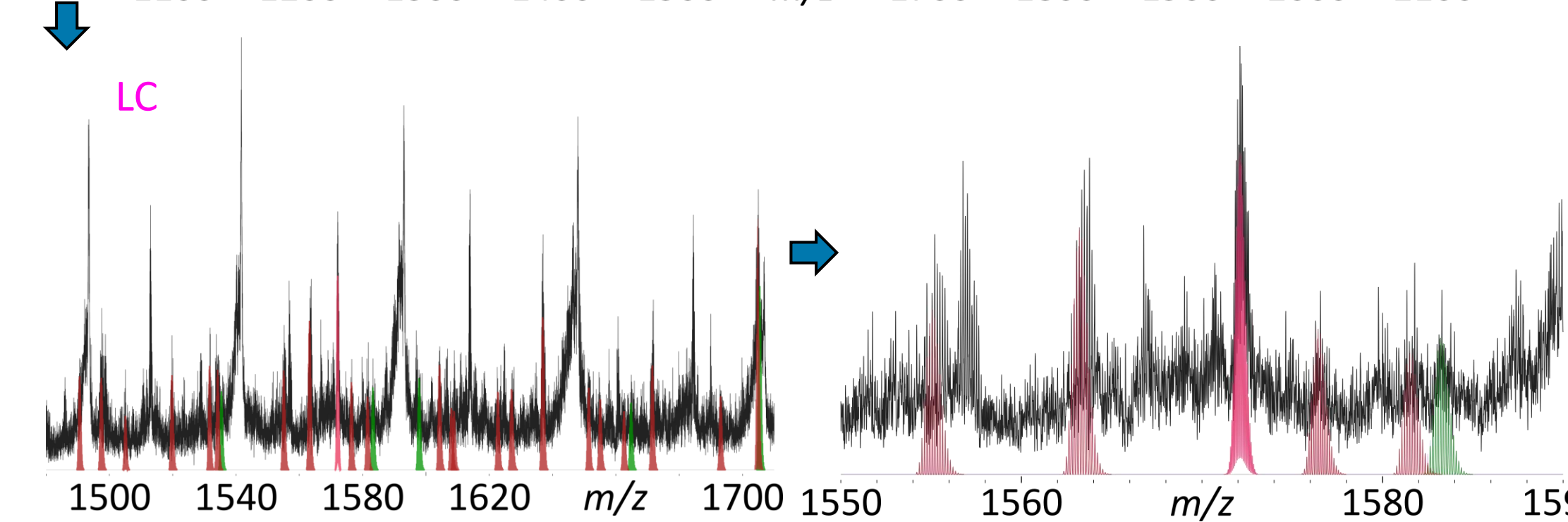
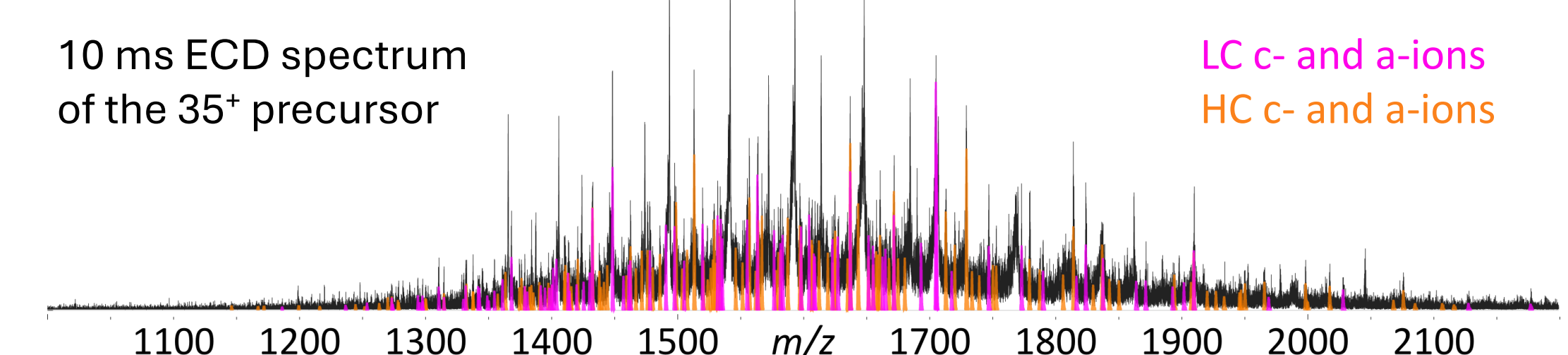
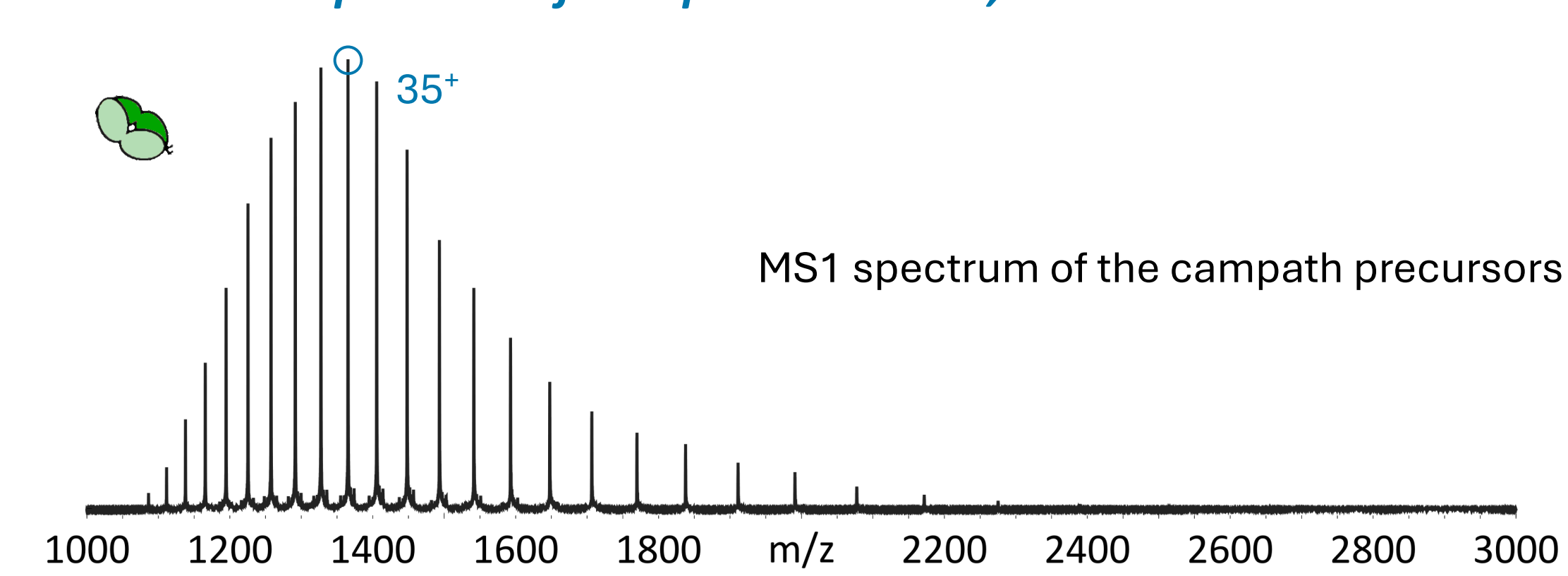
- Throughout the chromatographic run (black dashed line), dynamic precursor accumulation (black solid line) actively regulates ion flow in MS² scans enabling optimal trapping and fragmentation of precursor ions. The approach ensures the high fidelity recording of isotopic distributions for high mass fragments thereby enhancing sequence coverage and analytical confidence.



LC-MS² ECD spectrum of Trastuzumab 47.4 kDa, 14.2±0.4 min RT

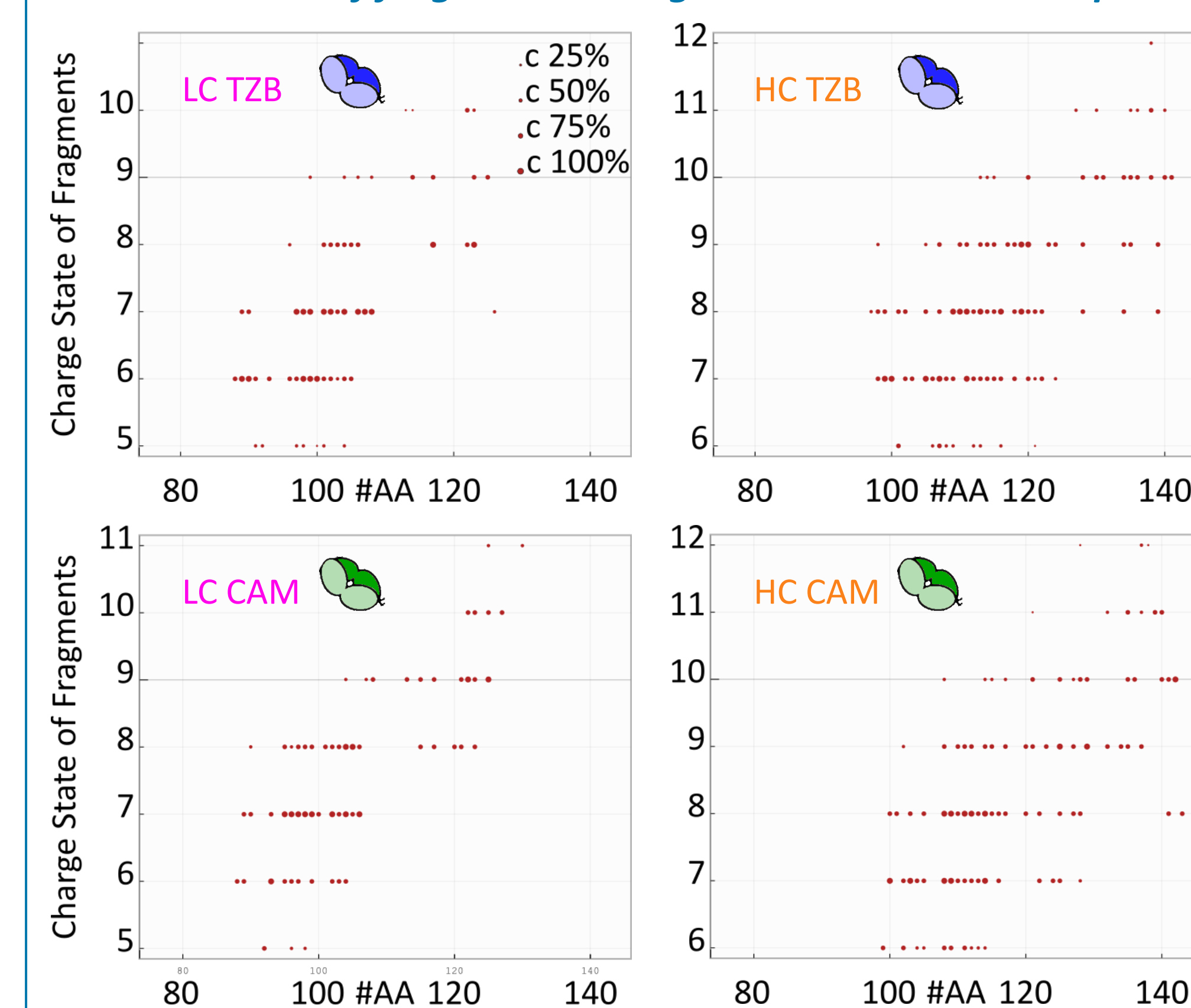


LC-MS² ECD spectrum of Campath 47.8 kDa, 15.4±0.5 min RT

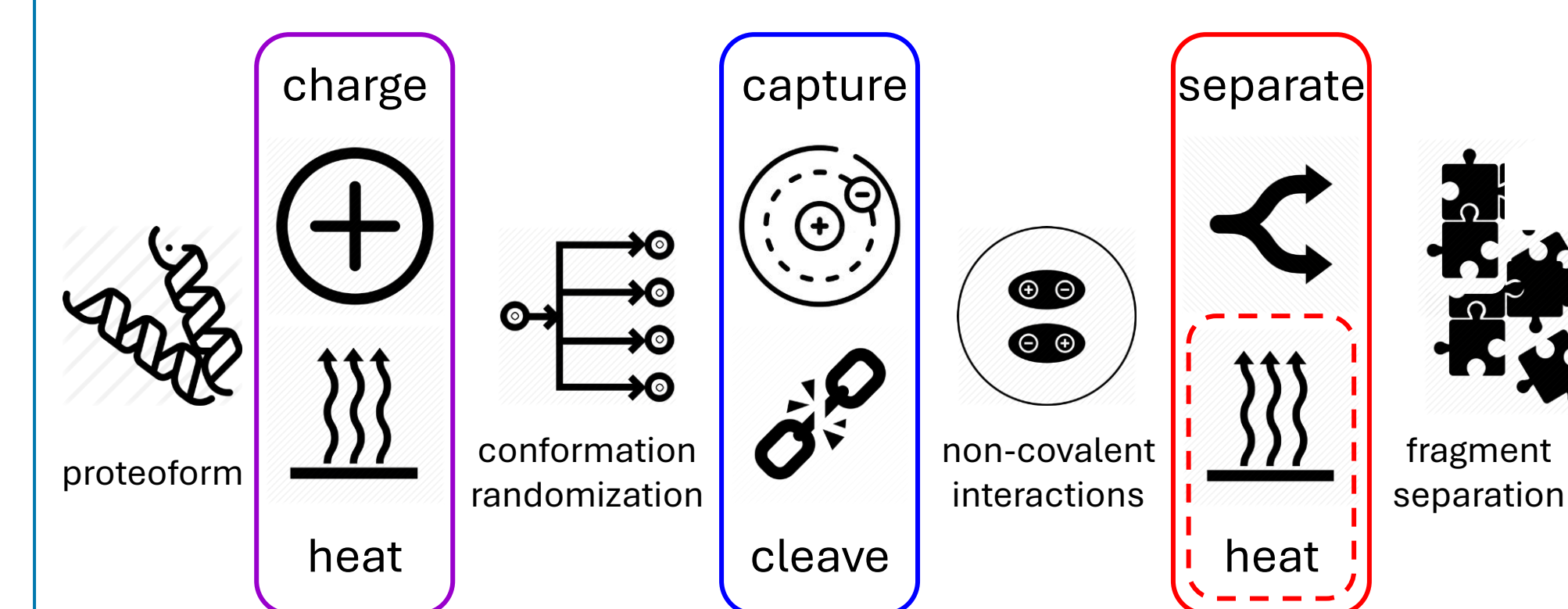


- Operating the timsOmni on a LC-MS timescale, the contributions of different precursor Fab ions can be disentangled via dynamic precursor selection, accumulation, and fragmentation.
- The resulting ECD fragmentation yields spectra with signal to noise enabling unambiguous fragment assignment thanks to high fidelity isotopic distributions.
- Sequence coverage and analytical confidence is further enhanced by the correlation of Fab fragments with different charge states.

Contributions of fragments' charge states to Fabs' ECD spectra



Tunability of the ECD process in the timsOmni's Omnitrap



Conclusions

- The present work demonstrates the potential of top-down cDDA-ECD on the timsOmni for the untargeted characterization of complex samples, such as serum Fabs, or purified samples, such as biopharmaceuticals.
- Averaging multiple TOF spectra sequentially at kHz rep rate, the timsOmni produces high signal-to-noise ratio mass spectra enabling unambiguous assignments on a LC-MS timescale.
- Multiple sequential MS² scans yield fragmentation spectra with high-fidelity isotopic distributions addressing top-down sequencing needs.
- In short, sample processing with the timsOmni enables characterizing proteoform heterogeneity as well as monitoring modifications and mutations occurring during the production and storage of proteins, on a LC-MS timescale using electron capture dissociation.

Future work

- Use electron impact dissociation (EID) on the timsOmni to increase fragment yield and sequence coverage in Fabs regions inaccessible to electron capture dissociation (ECD).
- Optimize liquid chromatographic separation for Fabs originating from human serum, eventually complemented by ion mobility separation.

Acknowledgments & contact

- Ref. 1. Selectivity over coverage in de novo sequencing of IgGs. den Boer MA, Greisch JF, Tamara S, Bondt A, Heck AJR. Chem Sci. 2020, doi: 10.1039/d0sc03438j
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