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Ultralong transients enhance sensitivity and resolution in Orbitrap-based single-ion mass spectrometry

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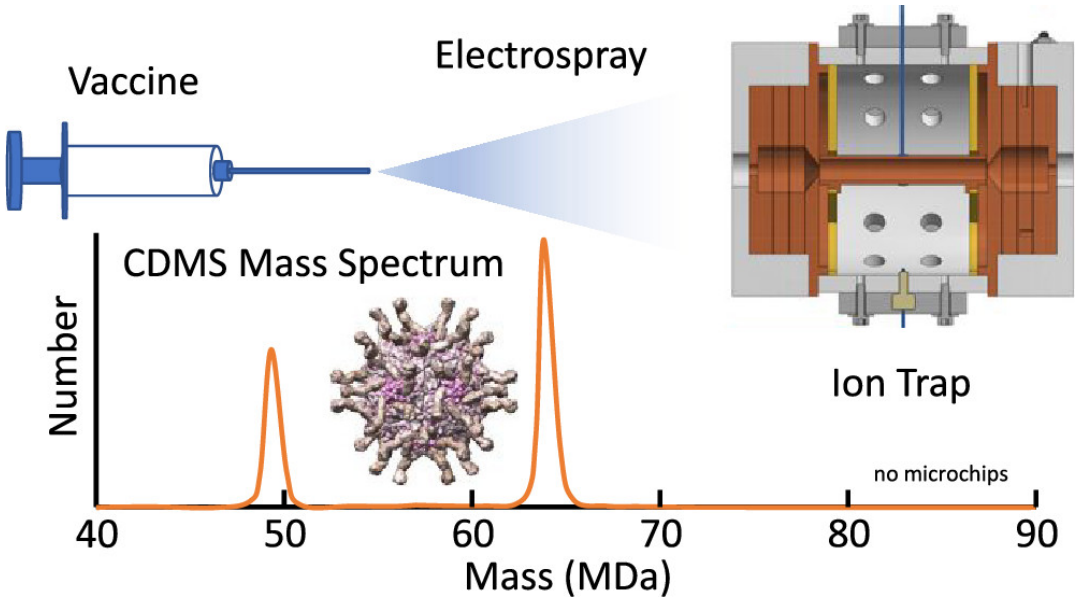
21-12-2024

Key words

CDMS :

- Charge detection mass spectrometry is a single particle technique where the masses of individual ions are determined from simultaneous measurements of each ion's m/z ratio and charge.
- The ions pass through a conducting cylinder, and the charge induced on the cylinder is detected.
- The cylinder is usually placed inside an electrostatic linear ion trap so that the ions oscillate back and forth through the cylinder.
- The resulting time domain signal is analyzed by fast Fourier transformation; the oscillation frequency yields the m/z, and the charge is determined from the magnitudes



MASS SPECTROMETRY

Seeing flying molecular elephants more clearly

Extending mass spectrometry measurements of biomolecules into the megadalton regime is challenging due to the limited resolving power of currently used mass analysers. Now, using single ion-charge detection Orbitrap mass spectrometry, a mass accuracy of 0.001% has been demonstrated for protein particles larger than 9 MDa.

Muhammad A. Zenaidee and Joseph A. Loo

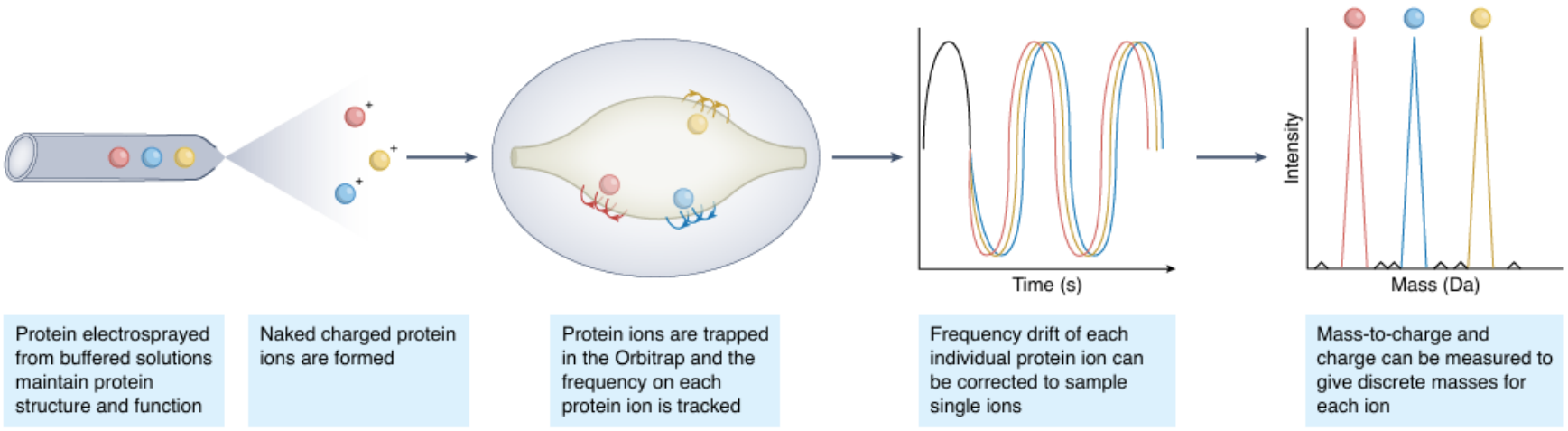


Fig. 1 | Orbitrap-based CDMS. Schematic demonstrating how charge detection mass spectrometry in an Orbitrap analyser is achieved.

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ARTICLES

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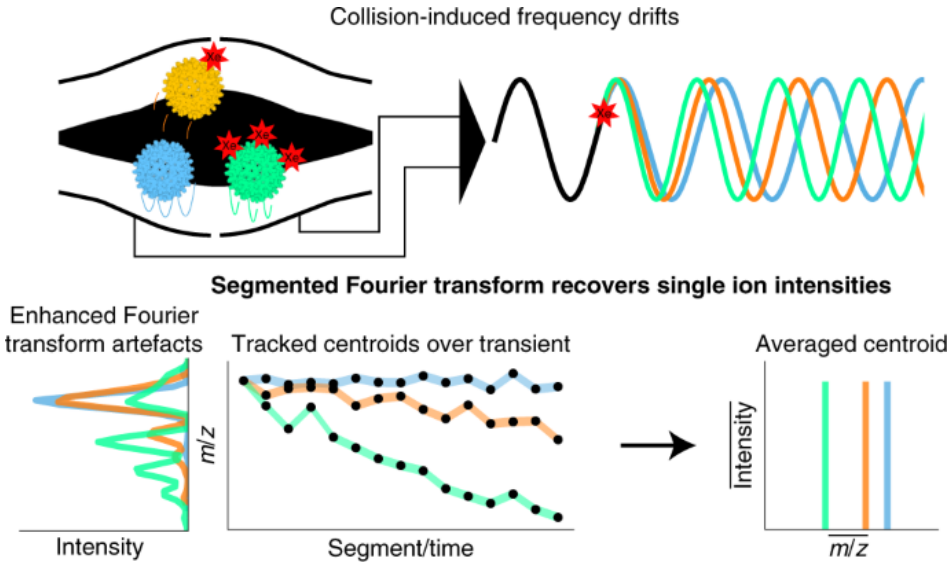
Frequency chasing of individual megadalton ions in an Orbitrap analyser improves precision of analysis in single-molecule mass spectrometry

Tobias P. Wörner[1,2], Konstantin Aizikov[3], Joost Snijder[1,2], Kyle L. Fort[3], Alexander A. Makarov[1,3] and Albert J. R. Heck[1,2] ✉

- CDMS using the Orbitrap analyser offers the possibility to investigate in depth the behaviour of single macromolecular ions within the mass analyser, which is exciting as most of our current knowledge about ion behaviour in the orbital trap is derived from ensemble measurements of smaller and/or denatured particles.

- Here they demonstrate that such knowledge is beneficial for further progress in native MS and CDMS applications on samples of ultra-high mass.



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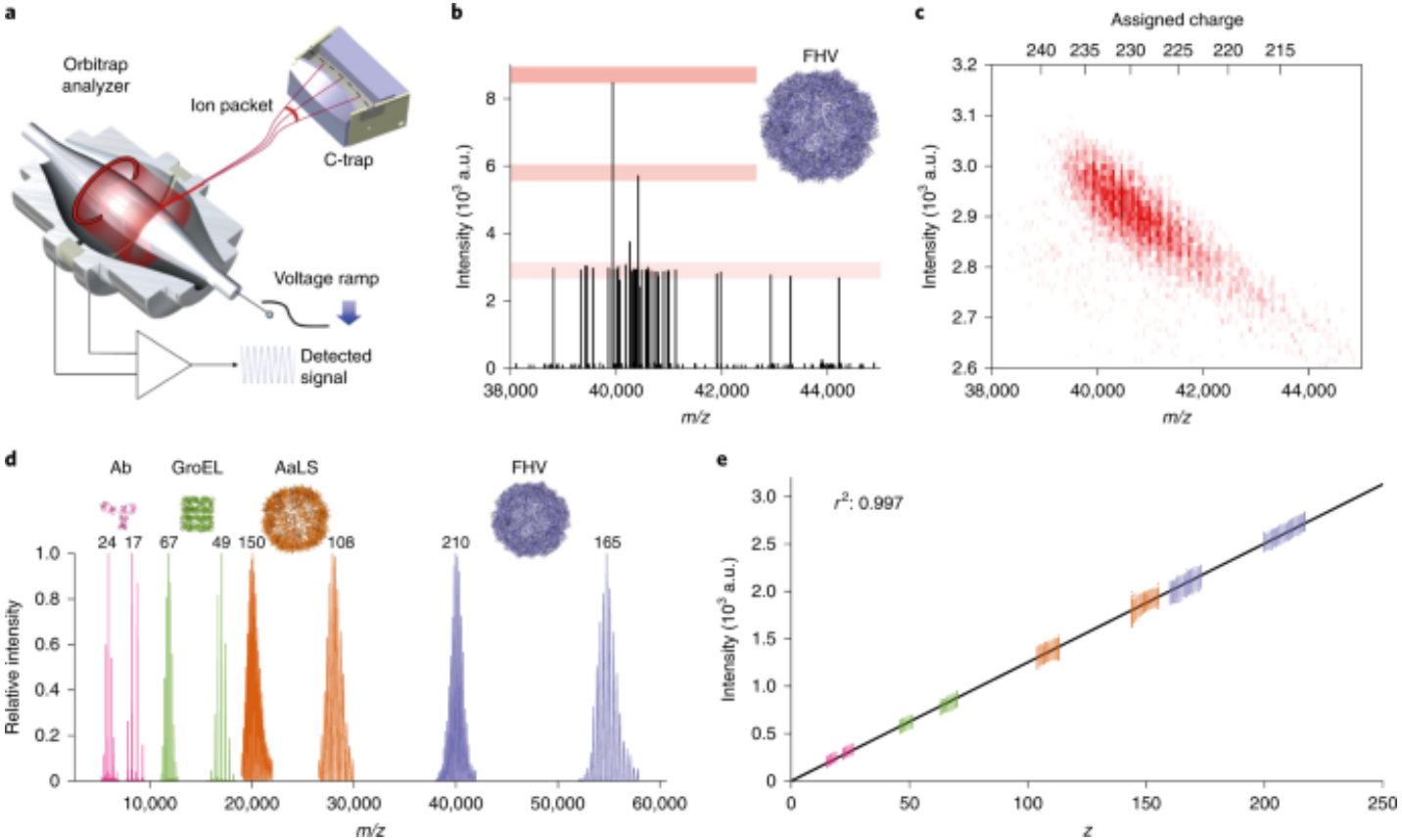
BRIEF COMMUNICATION

https://doi.org/10.1038/s41592-020-0770-7

Resolving heterogeneous macromolecular assemblies by Orbitrap-based single-particle charge detection mass spectrometry

Tobias P. Wörner[1,2], Joost Snijder[1,2], Antonette Bennett[3], Mavis Agbandje-McKenna[3], Alexander A. Makarov[1,4] and Albert J. R. Heck[1,2]



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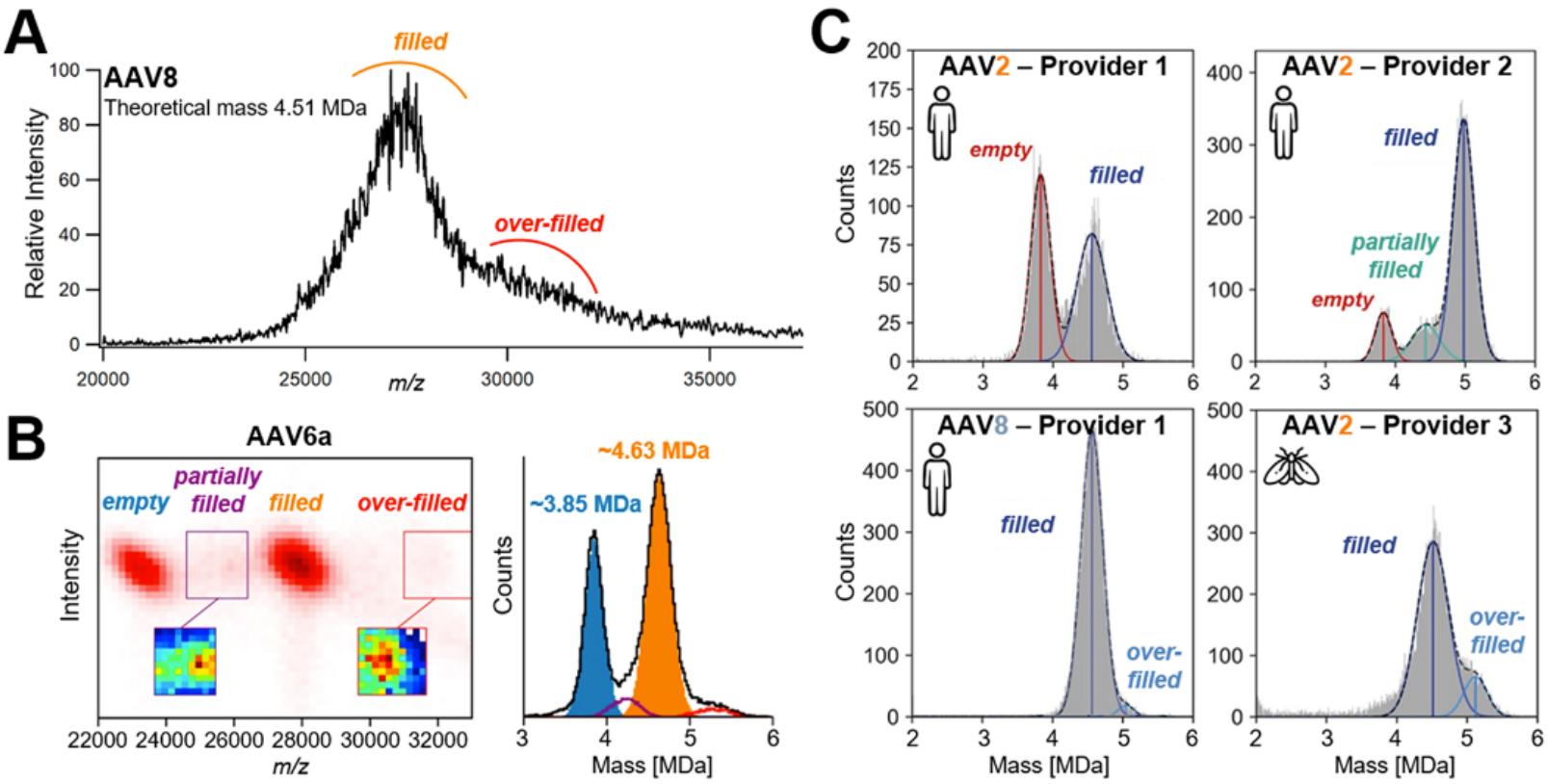
Article

Orbitrap-Based Mass and Charge Analysis of Single Molecules

Evolène Deslignière,[†] Amber Rolland,[†] Eduard H.T.M. Ebberink,[†] Victor Yin,[*] and Albert J.R. Heck[*]



Why this paper

- Orbitrap-based charge detection mass spectrometry utilizes single-molecule sensitivity to enable mass analysis of even highly heterogeneous, high-mass macromolecular assemblies.
- For contemporary Orbitrap instruments, the accessible ion detection (recording) times are maximally ~1–2 s.
- Here by modifying a data acquisition method on an Orbitrap ultrahigh mass range mass spectrometer, we trapped and monitored individual (single) ions for up to 25 s, resulting in a corresponding and huge improvement in signal-to-noise ratio (×5 compared with 1 s), mass resolution (×25) and accuracy in charge and mass determination of Orbitrap-based charge detection mass spectrometry.

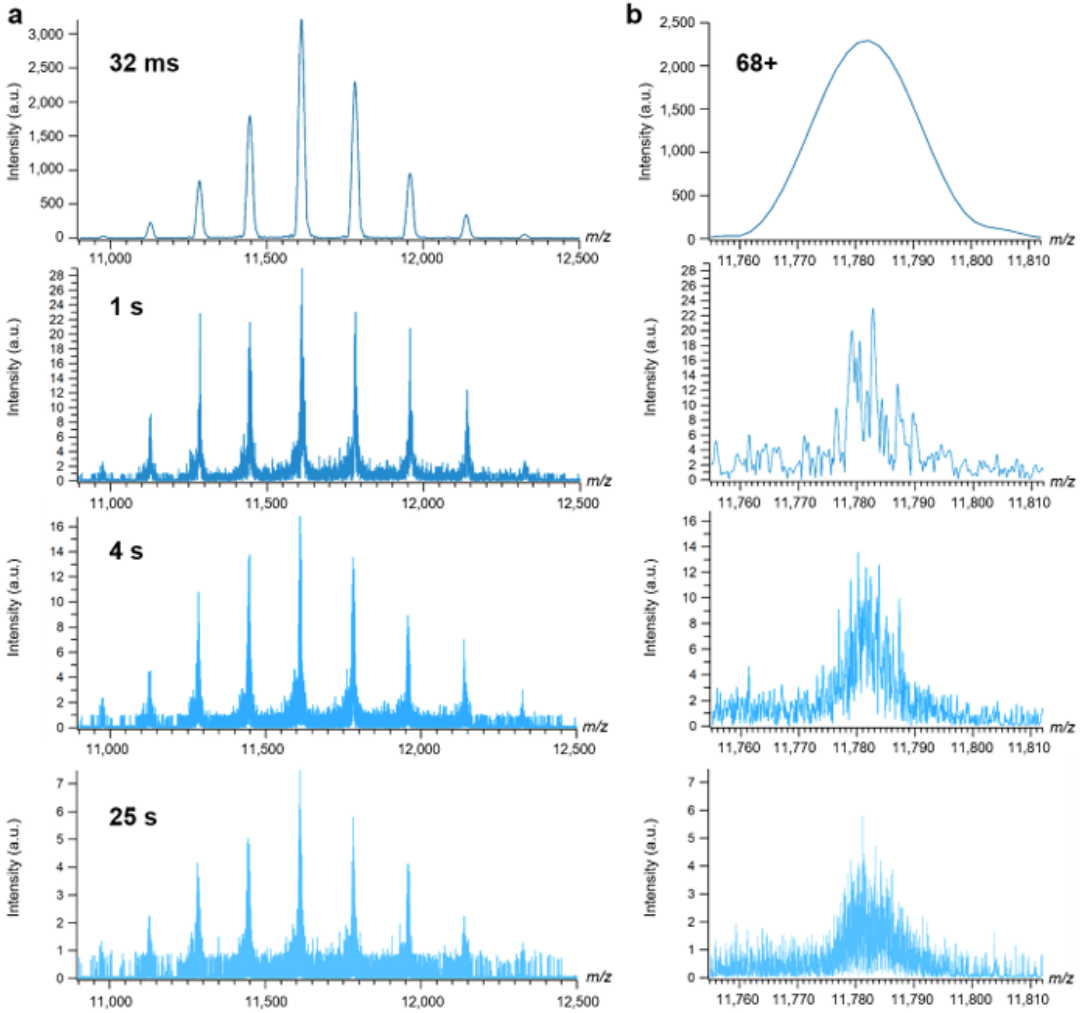
Introduction

- A remaining challenge in Orbitrap-based CDMS, when compared with conventional ion-trap-based CDMS, is the uncertainty in charge determination. Standard deviations in the charge (σz) typically are around 2–3 elementary charges (for 1-s transients), introducing a degree of peak broadening in the mass domain.
- However, as in any Fourier transform (FT)- based MS technology, the charge and mass resolutions, alongside signal-to-noise ratio (S/N), can be enhanced by recording longer induced ion currents (transients) upon ion detection.
- Transient extension is primarily limited by (1) the quality of electrodes (individual for each trap), (2) the hold time of high-voltage transistors in the pulsing electronics of the central electrode power supply and (3) the large amount of spectral information that needs to be read into instrument memory.

- In this Article, we modified the data acquisition (DAQ) of a Thermo Scientific Q-Exactive ultrahigh mass range (UHMR) mass spectrometer to extend the achievable trapping and acquisition timescale by more than an order of magnitude , allowing unparalleled levels of mass resolution (R), sensitivity and accuracy of charge determination in the analysis of large biomacromolecular assemblies.

- Benefits of ultralong transients for Orbitrap-based single-ion MS
- Ultralong transients enhance performance of Orbitrap-based CDMS
- Prolonged ion trapping provides unique high-resolution insight into gas-phase behaviors of single ions

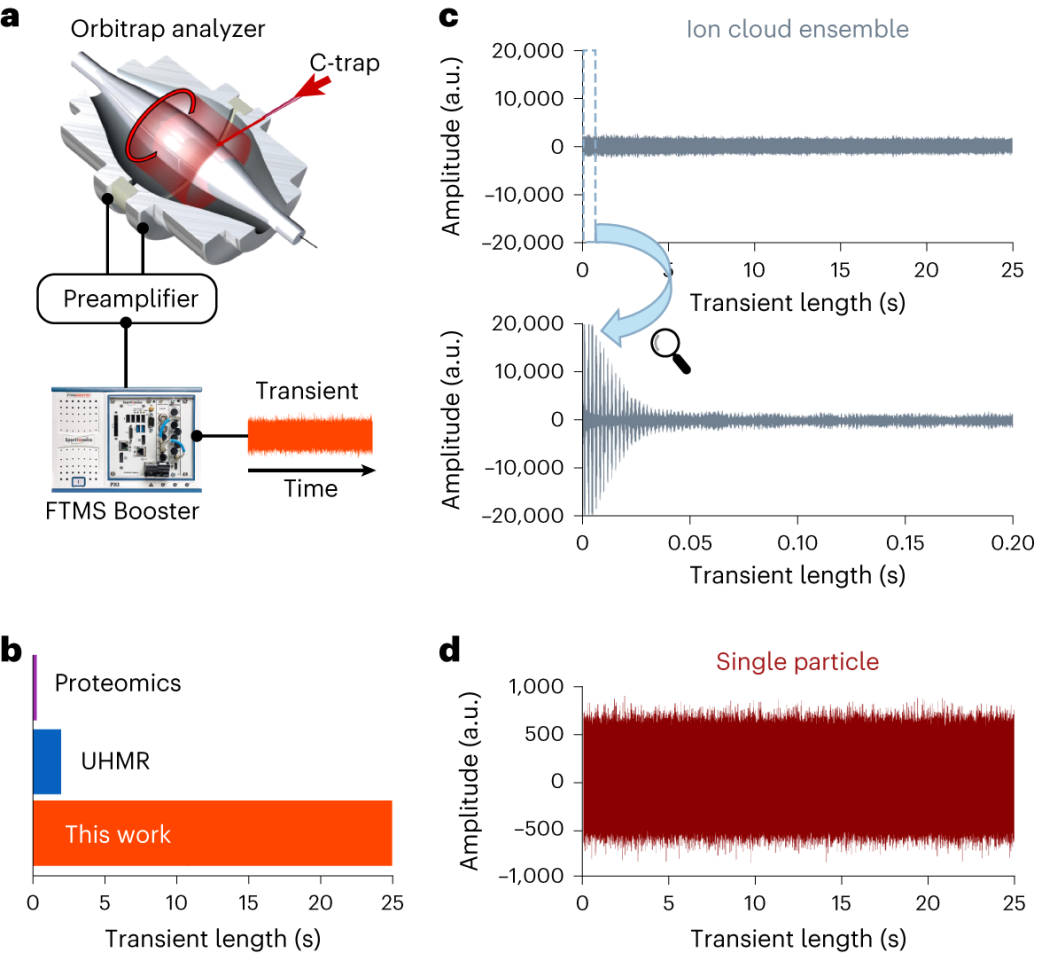
Results and Discussion

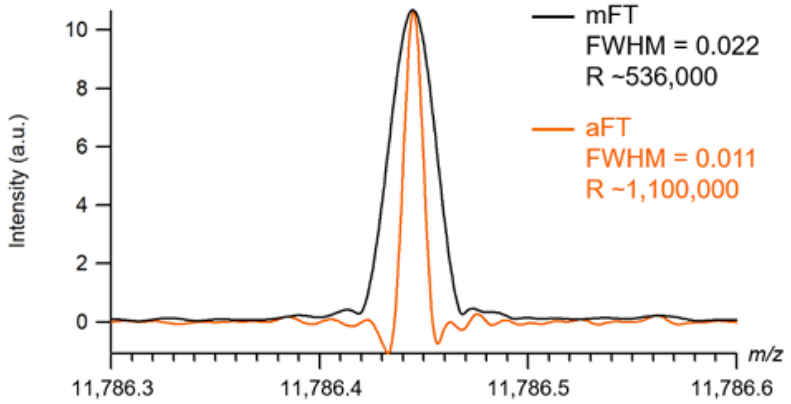


Supplementary Figure 1. Native MS experiments performed on ion ensembles of GroEL using a 25 s-long transient. a, Mass spectra extracted at increasing transient lengths (0.032, 1, 4, and 25 s), showing a substantial decrease of intensities and overall signal quality with extended transient times. **b**, Zoom in on the 68+ charge state of GroEL. At longer transient times, the signal deteriorates, primarily due to the dephasing of ion packets.

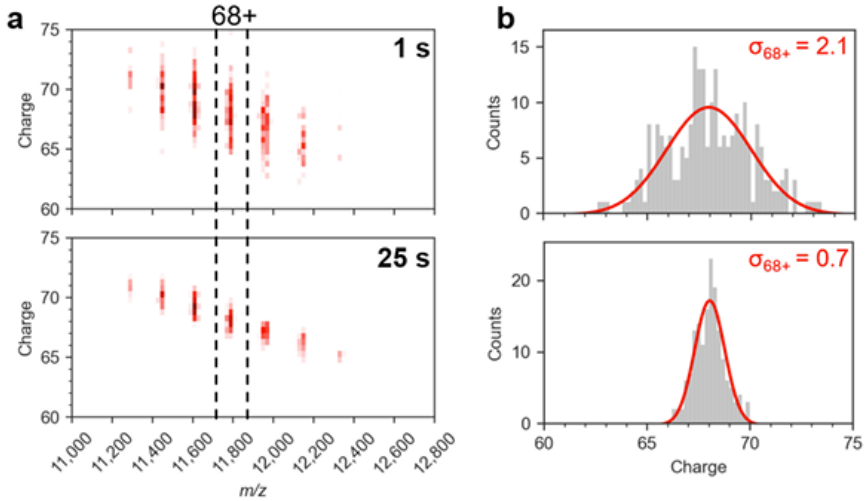
Results and Discussion

Fig. 1: Recording ultralong transients on an Orbitrap analyzer.



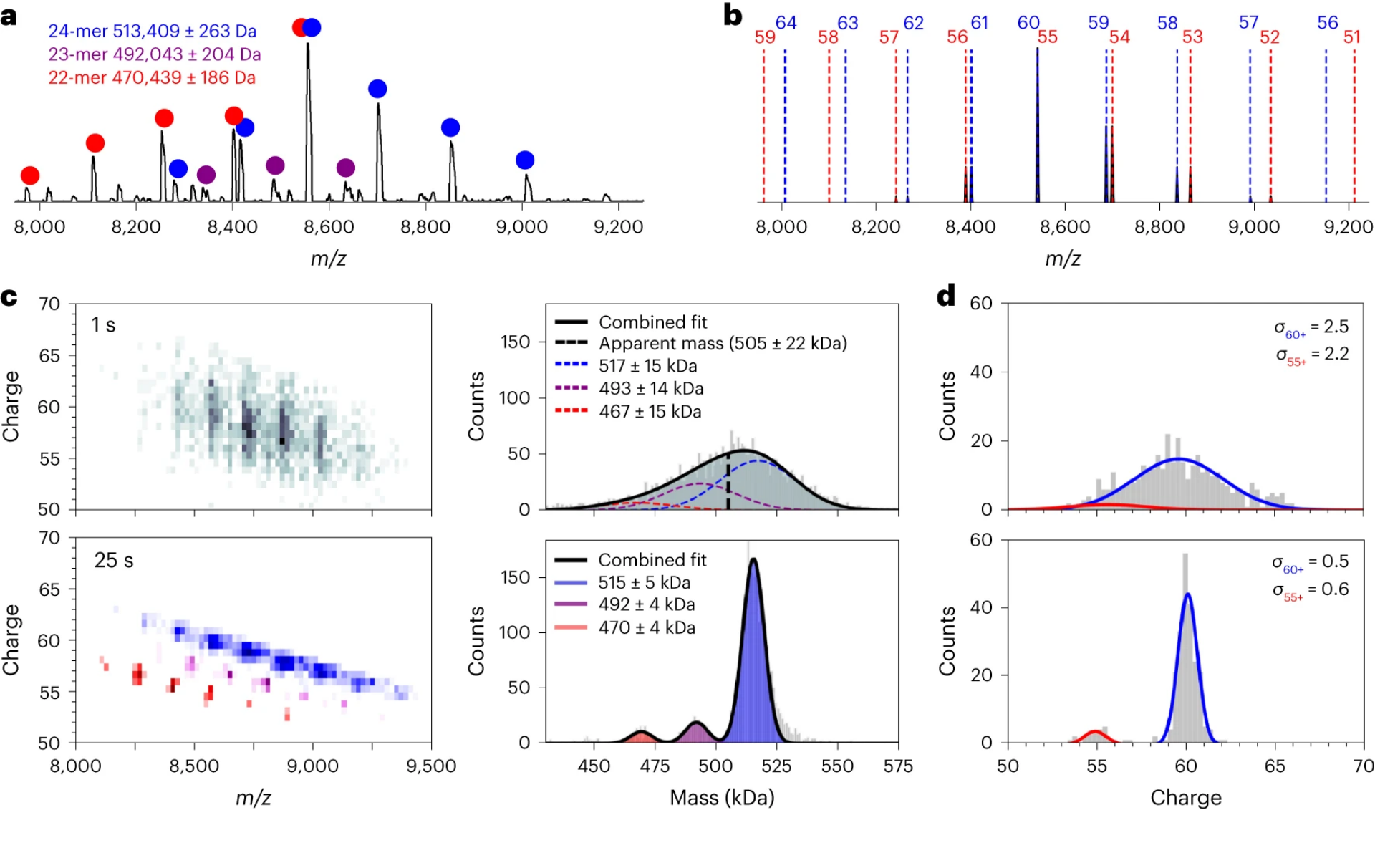


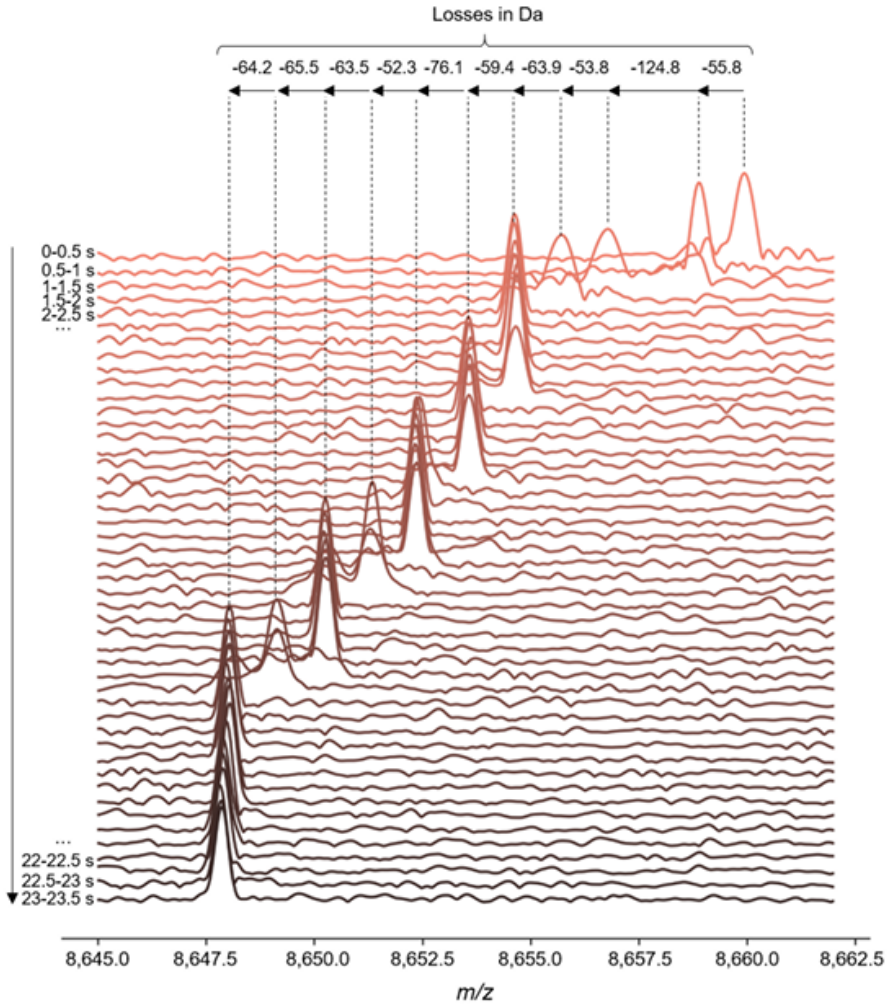
Supplementary Figure 2. Single-ion (GroEL, 68+) signal recorded from a 25 s transient, processed with mFT *vs.* aFT. aFT (orange) yields, as expected, a ~2-fold improvement of the mass resolution over mFT (black) and results in a resolution over 1,000,000 at *m/z* 11,786.



Supplementary Figure 3. Single-particle CDMS of GroEL. a, Two-dimensional histograms of filtered single-ion signals collected over several minutes of acquisition time. Data were extracted at increasing transient lengths (1 and 25 s). **b**, Standard deviation observed for the charge state 68+ of GroEL, showing how ultra-long transients increase the experimental charge accuracy. A three-fold improvement on charge uncertainty is achieved after 25 s (from σ_{68+} = 2.1 to 0.7).

Fig. 2: Ensemble versus single-ion native MS of apoferritin.





Supplementary Figure 8. Neutral losses over the transient course for an apoferritin single ion (57+ charge state of the 23-mer). Each trace represents a 0.5 s-long segment extracted along the transient. The initial mass is 493,555.7 Da. After 25 s, the mass has decreased to 492,876.3 Da, as a result from multiple successive neutral losses, putatively corresponding to the loss of ammonium acetate, acetic acid, ammonia and/or water molecules (77, 60, 17 and 18 Da, respectively). Cases where consecutive small mass losses occur within a single segment can result in an apparent deterioration of resolution (e.g. at 1 – 1.5 s in this Figure). Note that the centroid uncertainty of each peak depends on the segment length chosen for ion tracing.

Conclusions

- In summary, we report the successful implementation of ultralong transients on an Orbitrap mass spectrometer, reporting records and new capabilities for high-resolution mass analysis of large macromo lecular ions.
- A notable future benefit of Orbitrap-based CDMS is its facile access to the suite of ion manipulation techniques afforded by the platform, that is, ion activation and/or tandem MS.
- They anticipate that their realization of prolonged transient acquisitions will benefit the high-resolution analysis of high-mass heterogeneous systems, for example, gene therapy products and/or glycosylated biotherapeutics, while simultaneously setting the stage to leverage the capability ofhigh-resolution ion tracking.
- The resultant increase in S/N offered by extended transients greatly enhances the sensitivity of Orbitrap-based CDMS, whereby we can possibly soon approach the detection of even singly charged ions by Orbitrap-based CDMS.