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BE PATIENT AND RELEASE THE PRESSURE: RECIPE FOR HIGH-RESOLUTION CHARGE DETECTION MASS SPECTROMETRY

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Within the last decades, native mass spectrometry (MS) has become a key tool in the study of proteins and their higher-order assemblies. With the introduction of single-molecule charge-detection MS (CDMS) even large heterogenous biological systems, with relevance to virology and biopharma, such as adeno-associated viruses (AAVs) and plasmid DNA can now be interrogated by native MS.
However, mass analyzing such high mass heterogenous macromolecular assemblies comes still with several challenges, notably the need for optimal transmission of these giant particles through the mass analyzer, and better spectral resolution to resolve co-occurring species and attain high mass accuracy.
To improve spectral resolution, we coupled a FTMS Booster (Spectroswiss) to a UHMR Orbitrap (Thermo Fisher Scientific), which allowed us to record long transients of up to ~20 sec. By recording ultra-long transients of AAVs ions, we observed that these particles can survive long periods of trapping in the Orbitrap, despite travelling over 100s of kilometres, experiencing ~1000s of collisions with background gas. Sampling the AAV mass distributions over many scans after 1 second- or 24-seconds transient recording time displayed an enhanced mass resolving power with a ~40% reduction of their peak widths. With this enhanced resolution, partially filled capsids could be baseline resolved, revealing details of their heterogeneity and composition that were previously inaccessible.
Recording the long transients in CDMS mode also allowed us to monitor in more depth the behavior of all individual ions, revealing that the stability of ion trajectories is very much dependent on the mass and charge of the ions, but also on the number of collisions the ions experience while being trapped and recorded in the Orbitrap.
To improve transmission and desolvation the use of external gas and collisional cooling seems to be essential, but this can negatively affect MS measurements, due to leakage of residual gas into the Orbitrap mass analyzer. Although usually not an issue for standard proteomics, this is problematic for native or intact MS where ions can collide with the background gas during transient recording, impairing particle detection and quantification.
To tackle these issues, we implemented an externally driven pulsing valve to regulate the gas flow in an Orbitrap mass analyzer. In this modified set-up external gas can still be applied for transmission and desolvation of particles but is restrained when measurement in the Orbitrap analyzer start. Here we demonstrate that this modification provides profound benefits for measuring challenging samples that otherwise suffer from collisions with background gas in the Orbitrap. Instead of milliseconds, native plasmid DNA, IgM and AAV particles can now be measured for 10s of seconds in the mass analyzer with extraordinary stability of their ion trajectories. This has broad impact as it dramatically improves the number of ions being detected, enhances the mass resolution and charge uncertainty, and thus the mass accuracy and sensitivity.

Keywords
single-molecule MS, Orbitrap ion trajectories, charge-detection MS, mass resolution, signal-to-noise ratio.