Sensitivity improvement on megadalton protein measurement

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ABSTRACT

More than 10x improvement of ion transmission in the QJet ion guide region for megadalton proteins, *m/z* > 10,000.

INTRODUCTION

Native mass spectrometry is gaining popularity due to its ability to obtain information on tertiary and quaternary structure of proteins and protein complexes. Because native proteins and protein complexes have higher *m/z* values due to fewer charges carried in native conformations compared to denatured states, native MS requires mass spectrometer with high *m/z* ranges. In theory, time-of-flight (TOF) instruments have unlimited *m/z* range, and thus are a natural fit for native MS. However, conventional QTOF instruments designed for general use tend to have low ion transmission efficiency for high *m/z* ions in the free-jet expansion region (QJet ion guide region). Improvement of ion transmission in the QJet ion guide region is critical to improve sensitivity for megadalton proteins.F

MATERIALS AND METHODS

Sample preparation:
Pyruvate kinase (Sigma P9136-5KU) and β-galactosidase (Sigma 10745731001) were dissolved directly in 50 mM ammonium acetate at 1 mg/mL and used without any further purification. NISTmAb (NIST monoclonal antibody reference material #8671) was diluted to 10 mg/mL with 50 mM ammonium acetate.

GroEL (Sigma C7688) was dissolved at 20 µM (monomer concentration) in 20 mM tris-HCl, 50 mM potassium acetate (pH 7) with 2 mM adenosine-5′-triphosphate (ATP). An acetone precipitation step was used to precipitate the protein, separating it from the impurities in the sample that do not precipitate. The sample was shaken slowly on a vortex for 1 h. Then, methanol was added to 20% of the final volume and the sample was shaken for another hour. After that, the protein was precipitated with 50% acetone by volume. The precipitate was resolubilized in 20 mM tris-HCl, 50 mM potassium acetate with 2 mM ATP, at a final protein concentration of 20 µM for the monomer, as recommended for refolding. The solution was shaken slowly on a vortexer for 1 h at room temperature and then concentrated using a microcentrifugation device (Amicon Ultra 0.5 mL, 30 kDa cutoff, Millipore, Billerica, MA). The concentrate was diluted to 20 µM of GroEL monomer with in 20 mM Tris-HCl, 50 mM potassium acetate with 2 mM ATP and shaken again for 1 h followed by the filtration step. The shaking and filtration were repeated four times to maximize refolding efficiency of the tetradecamer complex. The solution was eventually concentrated to give 6 µM of GroEL tetradecamer, which was then buffer exchanged into 50 mM ammonium acetate.

HPLC conditions:
A Shimadzu liquid chromatography system was used for on-line SEC separation with a Waters BEH200 SEC column (2.1 or 4.6 mm × 150 mm, 1.7 µm). The SEC LC method was fast and works reproducibly with 10 min run time. All proteins eluted before 3 min by 25 mM ammonium acetate.

MS conditions:
A TripleTOF 6600 system (SCIEX) was modified to increase ion transmission efficiency of high *m/z* ions. Ions were captured and collisionally focused in the high pressure (Torr) QJet ion guide before entering the lower pressure Q0 ion guide (mTorr) and further being transmitted into the high vacuum Q1 quadrupole region (10^{-5} Torr). RF frequencies of QJet ion guide, 1350 kHz (original), 600 kHz and 300 kHz, were evaluated for the transmission efficiency of high *m/z* ions.

RESULTS

Sensitivity improvement
The sensitivity gain was evaluated with 4 high mass proteins, NISTmAb (146 kDa), pyruvate kinase (232 kDa), beta-galactosidase (464 kDa) and GroEL (801 kDa). Besides monomers, in-source induced dimers, trimers and tetramers from above proteins were also used to extend *m/z* coverage up to 16,000. The maximum intensity of each charge state along with reconstructed protein peak at 300 kHz and 600 kHz are compared with the original QJet ion guide RF (1.35 MHz).

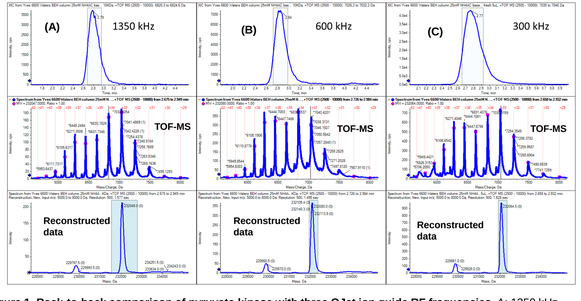

Figure 1. Back-to-back comparison of pyruvate kinase with three QJet ion guide RF frequencies. A: 1350 kHz (original); B: 600 kHz and C: 300 kHz, respectively. Top panes show XIC, middle panes the average TOF-MS spectra and bottom shows the reconstructed data. Average intensity gain was ~ 2x for 600 KHz and 4.6x for 300 kHz.

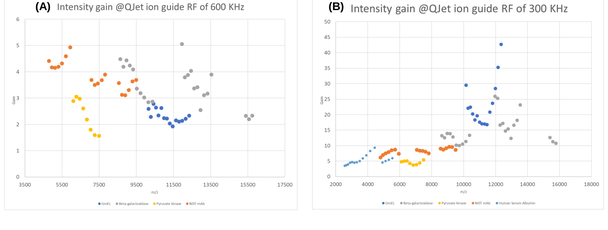

Figure 2. Sensitivity gain with different QJet ion guide frequencies. Gain of 600 kHz (A) and 300 kHz (B) compared to original frequency at 1.35 MHz. The intensity gain 1.5 – 5 x for 600 kHz and 4 – 40 x for 300 kHz. A mass-dependent gain was observed for 300 kHz, but not much for 600 kHz. > 20x gain was observed on GroEL ions.

Improved detection of megadalton proteins

> 20x sensitivity improvement with 300 kHz on ions with *m/z* >11,500 enables the detection of low abundant in-source induced GroEL-GroEL at 1.6 MDa, which was not detectable with original QJet ion guide RF. Further examination of high m/z region, > 16,500, in-source fragment of GroEL, 13-mer (higher *m/z* with less charges) than intact GroEL was identified at extremely low abundance only with 300 kHz QJet ion guide RF. Similar observation was made on β-galactosidase, where in-source induced trimer and tetramers were only observed with 300 kHz.

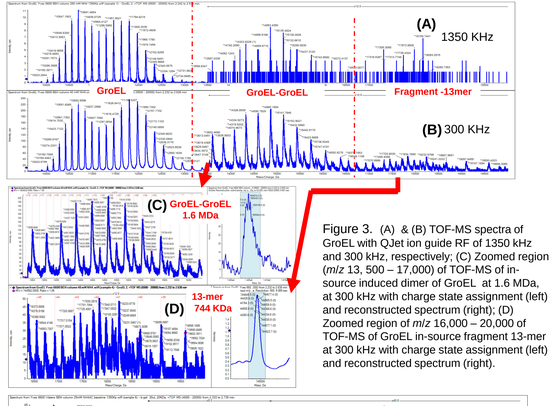

Figure 3. (A) & (B) TOF-MS spectra of GroEL with QJet ion guide RF of 1350 kHz and 300 kHz, respectively; (C) Zoomed region (*m/z* 13, 500 – 17.000) of TOF-MS of in-source induced dimer of GroEL at 1.6 MDa, at 300 kHz with charge state assignment (left) and reconstructed spectrum (right); (D) Zoomed region of *m/z* 16,000 – 20,000 of TOF-MS of GroEL in-source fragment 13-mer at 300 kHz with charge state assignment (left) and reconstructed spectrum (right).

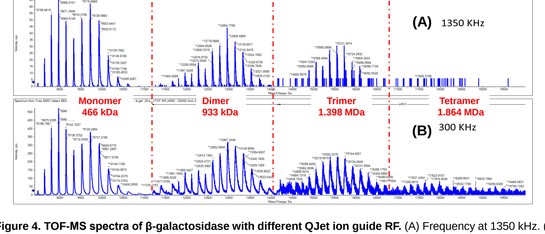

Figure 4. TOF-MS spectra of β-galactosidase with different QJet ion guide RF. (A) Frequency at 1350 kHz. (B) Frequency at 300 kHz. In-source induced dimer β-galactosidase trimer (1.398 MDa) and tetramer (1.864 MDa) could only be detected with 300 kHz.

Impurity analysis – Aggregation of pyruvate kinase

Aggregates are common product-related impurities. Characterization and quantification of low-level impurities are always a challenge. >10x improvement of ion transmission of high-mass proteins enables better detection and quantification of aggregates.

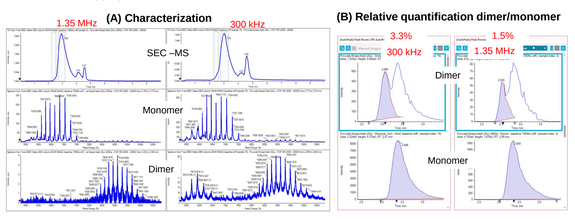

Figure 5. (A) Characterization of pyruvate kinase aggregate with original QJet ion guide RF (1.35 MHz, left), and 300 kHz (right); (B) Quantification of pyruvate kinase dimer with original QJet ion guide RF (1.35 MHz, left), and 300 kHz (right). The relative quantification result is lower with 1.35 MHz QJet ion guide RF due to lower intensity of dimer. Peak deconvolution was employed to differentiate between aggregate dimer and in-source-induced dimer.

CONCLUSIONS

- Optimization of the operating parameters of the ion optics enabled the detection of low abundant in-source induced GroEL-GroEL and fragment 13-mer as well as of aggregates of pyruvate kinase.

REFERENCES

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