Enhanced Detection of non-covalently bound Enzyme Complexes using a Dedicated Large Molecule Autotune on a Q-TOF Mass Spectrometer

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Introduction

The analysis of intact proteins is of great importance to the biopharmaceutical industry. Many released therapeutically relevant drugs are based on monoclonal antibodies.

Nevertheless, for a comprehensive understanding of enzyme complexes different approaches are practiced. The most commonly used method is the infusion of the complexes suspended in high salt containing aqueous buffers at biological pH 7, such as ammonium acetate to maintain their native-like structure in solution. Once in the gas-phase, declustering and desolvation are critical parameters, as described extensively in the literature.

The difference in size, based on collision cross section [1], between a small molecule like acetaminophen and a non-covalent complex as GroEL is about 200.

Due to this large difference, adjustments of the optical rail of the mass spectrometer is a recommended step to accommodate the anticipated different behavior of large molecules.

The previously developed SWARM tune for small molecules has already shown a substantial benefit in small molecule analyte abundance [2], so utilizing the same methodic for large molecules is an extension to the applications based tuning approach in the acquisition software.

Figure 1 shows how multiple elements of the optical rail are tuned simultaneously to find unambiguously the global maximum.

Experimental

Experimental conditions: LC-MS

A fully automated System Tune is performed by introducing calibrant solution via the built-in calibrant delivery system. Using a prototype software, the newly developed large molecule Autotune was performed, and the signal abundance of the calibrant ion with the highest m/z value compared to the standard System tune.

In the next step, the signal abundance of a chromatographically separated monoclonal antibody was compared under the different tunes. Separation was done at standard flowrates, using a 2.1x50mm PLRPS- column (Agilent Technologies, CA, USA) with formic acid as modifier.

Experimental conditions: native-MS

For native MS analysis, alcohol dehydrogenase was resuspended in 100 mM Ammonium acetate to a final concentration of 1μM. GroEL was prepared according to Campuzano [3] to a final concentration of 1μM GroEL in 200 mM ammonium acetate. All enzymes and chemicals were obtained from (Sigma Aldrich, St. Louis, MO, USA).

Infusion was performed using a syringe pump at 200 nl/min, using emitter tips with an 8 micron tip size (SIS, Ringoes, NJ, USA). MS analysis was done on a 6545XT AdvanceBio LC/Q-TOF (Agilent Technologies, Santa Clara, CA, USA).

Figure 2: Schematic view of the 6545XT AdvanceBio LC/MS-QTOF.

For fragmentation of GroEL, nitrogen as collision gas was replaced with pure SF₆. Due to SF₆ having different properties, fragmentation can be achieved with much lower energy compared to nitrogen or argon. In addition, a newly designed differential pumping step on the 6545XT AdvanceBio LC/Q-TOF (Figure 2) allowed the use of sulfur hexafluoride.

A prototype software was used to smooth the GroEL fragment spectrum, performing a Savitzky-Golay smoothing of the raw spectra.

Figure 1: Screenshot from the MassHunter Acquisition UI, showing simultaneously tuning of multiple optical elements. Caption lists various SWARM particles.
Results and Discussion

Most Autotunes of Q-TOF instruments are tunes which are designed to transmit ions in both low and high mass range. We recently demonstrated the benefits of dedicated small molecule tunes, and with this work continue to further develop this tailored strategy. When comparing the standard Autotune to the newly developed large molecule Autotune, we could observe a factor of >3 increase in abundance of 2722 calibrant ion (Figure 3).

![Figure 3: Comparison of high mass calibrant signals using Large Molecule SWARM Autotune (black) and Standard Mass Range SWARM Autotune (red), executed on the same instrument.](image)

The same increase in abundance were observed when using a monoclonal antibody, demonstrating the proof of principle that the changes in the optical rail of the Q-TOF mass spectrometer behave similar for calibrant ions as for intact proteins in a similar m/z range. With formic acid as modifier, the most abundant observed charge state was at about m/z 3090.

![Figure 4: Intact mAb Mass Standard, average mass spectrum using Large Molecule SWARM Autotune and Standard Mass Range SWARM Autotune on the same instrument.](image)

To further explore the utilization of the tune, we analyzed alcohol dehydrogenase (ADH), a 151 kDa tetramer, via nano-infusion under native MS conditions. Using the new tune with an acquisition rate of 1 sec, we had ~4000 counts per spectrum absolute abundance of ADH in the high m/z range above 5000. This high abundance makes it possible to fine tune method parameters by solely looking at the real-time acquisition window, compared to the previous need of acquiring and averaging data before decision making (Figure 5). No rolling average or other data manipulation were performed.

![Figure 5: Screenshot from the Acquisition UI acquired at 1 spectra/sec for ADH (A) and GroEL (B).](image)

Using the ion current obtained by the tune, a very fast fine tuning for collision cell pressure and collision energy is possible to obtain best spectra quality.

Figure 6 shows the benefit of the large molecule SWARM Autotune compared to the Standard SWARM Autotune for the Alcohol Dehydrogenase tetramer.

![Figure 6: Alcohol Dehydrogenase, average mass spectrum using Large Molecule SWARM Autotune and Standard Mass Range SWARM Autotune executed on the same instrument.](image)
Results and Discussion

In a next step to further explore the functionality and suitability of the newly developed Large Molecule SWARM Autotune we infused GroEL. The tetradecameric complex of in total 802kDa was readily visible in the Acquisition UI. Optimization of the native complex did not show a substantial difference between moderately applied collision energies and pressure. Notably, we used sulfur hexafluoride as collision/cooling gas, and presumably due to the need of ion-cooling lowering the pressure lead to an immediate loss of abundance.

The averaged (non-smoothed) spectrum over a 2 min acquisition is shown in Figure 7, showing well resolved charge states between 10500 m/z and 11500 m/z, as well as more higher m/z species up to 14000 m/z.

Figure 7: GroEL-14mer, unsmoothed spectrum of 2 min infusion at 200 nl/min of 5pmol GroEL in 200mM Ammonium acetate.

The purpose of using the large sulfur hexafluoride was to induce the ejection of a monomer from the tetradecameric complex, resulting in a tridecameric complex with much higher m/z as previously reported on a modified instrument [4]. Here we used the commercially available 6545XT AdvanceBio LC/Q-TOF to demonstrate that with the newly developed Large Molecule Autotune the detection of large complexes with high abundances is possible.

Figure 8 shows the smoothed spectrum of the tridecameric species after successful dissociation from a monomer at the 20,000 m/z region.

Figure 8: GroEL 13-mer, generated using SF₆ as collision gas at 200eV collision energy.

Conclusions

- Large Molecule SWARM Autotune shows 3-4x increase in large molecule abundance.
- Increased abundance allows to tune instrument via Acquisition UI interaction
- Differential pumping allows maintaining ultra-low pressure in the TOF region despite using SF₆ as collision gas
- Extended mass range on 6545XT AdvanceBio LC/Q-TOF allows for detection of non-covalent complexes at very high m/z ratios.

References

1 Bush Lab, University of Washington http://depts.washington.edu/bushlab/ccsdatabase/

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