ABSTRACT

Many large, complex species undergo changes in m/z during mass analysis, which can lead to incorrect charge and mass estimations using conventional STORI processing. The STORI algorithm can be modified to track changes in m/z and therefore frequency in the Orbitrap. This information can be fed back into the same generation, significantly improving the measurement accuracy when changes occur.

INTRODUCTION

Orbitrap-based Charge Detection Mass Spectrometry (CDMS) has evolved as a powerful tool for studying large, heterogeneous systems which are unsuitable for conventional mass spectrometry (1). The STORI (Selective Temporal Overview of Fisher of All K) of these species using CDMS by measuring the rate at which an ion accumulates signal over a specified time interval. (2) Conventional STORI processing presumes a fixed frequency signal and monitors the rate at which signal builds over a set time for ions changing mass/frequency during the detection period (e.g. from loss of non-covalently bound solvent inside the Orbitrap), the resultant frequency shift can significantly lower the charge estimate. Since many large species may lose mass during detection, it is beneficial to enhance STORI processing to accommodate variable frequency.

PRELIMINARY STORI ANALYSIS

A real sample example, which was artificially constructed using only minor capsid protein VP3, was electrosprayed into a Thermal Scicraft™ Q Exactive™ UltraH. Mass spectra were collected with a 1 second acquisition for 45 minutes. Injection time was fixed at 20 milliseconds. The flux was such that any single spectrum contained several thousand ions. As a baseline reference, STORI plots were generated for peaks that met the criteria in Table 1. Virtually all ions provided signal for the full length of the transient. Two example peaks of ~150 charge were used throughout to demonstrate the observed behaviors, complications and remedies.

STORI ANALYSIS

In order to understand the relationship of frequency changes in these signals, the time derivative of the complex STORI data was taken. This analysis, termed "STORI", generates a frequency data set and plots the rate at which a signal is building for cosine and sine waves (real and imaginary parts, respectively) of the STORI frequency over time. When the signal frequency and STORI frequency are well matched, signal builds at a constant rate in both components, in flat STORI plots, as seen for Peak #1 (Figure 3, left). However, data is not matched, the signal frequency and the STORI frequency during the acquisition, signal will not build at a constant rate for the cosine and sine waves, but rather transitions between the two over time. This nature in STORI plots are evident for Peak #2 (Figure 3, right).

STORI PHASE AND FREQUENCY ERRORS

The rate at which signal transfers between cosine and sine waves is evident in the phase angle of the STORI data. The slope of the STORI phase angle is a direct measure of the difference between the signal frequency and STORI frequency at any point in time. (Peak #2) Since the STORI plots are flat, the signal frequency is the STORI frequency. This represents a difference of 0.00 Hz. Peak #3's STORI phase plot is in Figure 4, right. Until 0.4 seconds, the slope is -0.04 Hz, meaning the ion frequency is 0.04 Hz (8.6 ppm) higher than the STORI frequency. The inflection point at 0.4 ms indicates a discrete jump in frequency of ±1.48 Hz (26.85 ppm). This translates to a 53.7 ppm drop in m/z (1.2 m/z) charge. For Peak #2 the STORI phase plot is even more pronounced, with a -0.12 Hz (23.0 ppm) drop in m/z (0.5 m/z) for a charge change of +100, this corresponds to a decrease of -192 amu in absolute mass.

FEEDING BACK THE FREQUENCY ERROR

Since the slope of the STORI phase plot is a direct measure of the difference between the STORI frequency and the true signal frequency, that information can be fed back into the algorithm, dynamically adjusting the STORI frequency over time to match the true frequency of the ion as the transient progresses. This results in much straighter STORI plots for ions that undergo mass changes during the transient while maintaining accurate shapes for ions that don't change (Figure 4). A histogram of charge error for both traditional and "corrected" STORI plots shows a dramatic improvement in accuracy when changes occur are encountered (Figure 5), essentially eliminating the fronting to low charge error errors.

ABSOLUTE MASS SPECTRA

The absolute mass of each peak is estimated by rounding the estimated charge to the nearest integer and multiplying with m/z. The absolute mass for these individual ions can then be binned to generate an absolute mass spectrum. Such spectra for the traditional and corrected STORI approaches are shown in Figure 7. The mass spectrum from traditional STORI processing has the tell-tale fronting that stems from reduced charge estimations when mass shifting occurs. Conversely, the corrected STORI processing has a much more symmetric distribution. This improved precision should prove especially useful when more complex systems, with many overlapping distributions, are analyzed.

CONCLUSIONS

Mass shifts during transient collection are quite common for large, complex species (e.g. from continued desolvation inside the Orbitrap). Modifications to the STORI algorithm allow for accurate tracking and correction for any such mass shifts. This can potentially provide significant improvement in charge accuracy (and therefore mass accuracy) for many analyses. It should also allow for longer transient acquisitions to be performed without compromising charge precision, since the higher likelihood of shifting with longer transients can now be properly accounted for.

REFERENCES


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