



# Setting the STORI Straight: Improved CDMS Results Via misSTORI Analysis

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## Abstract

### Purpose:

Large ions often change in mass while being analyzed via charge detection mass spectrometry (CDMS). The misSTORI approach has been developed to correct for such mass shifts, significantly improving the final absolute mass spectra.

### Methods:

CDMS data was collected using Direct Mass Technology (DMT) mode on a Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. Data was processed using either custom Python scripts or commercially-available STORIBOARD software (Proteinaceous), using either traditional STORI algorithm, which does not correct for frequency shifts, or the more advanced misSTORI algorithm, which attempts to correct for frequency shifts.

### Results:

The misSTORI process can improve CDMS results in various ways. It can improve mass accuracy, especially for long acquisitions. It can also provide interesting insight into the behaviors/properties of ions (e.g. “empty” viral capsids changing mass more than “full” capsids, ions increasing in mass during analysis, etc.).

## Introduction

The data quality in Charge Detection Mass Spectrometry depends on accurate determination of ion charge. The method of Selective Temporal Overview of Resonant Ions (STORI)<sup>1</sup> has proven useful for determining the charge of ions that survive for variable amounts of time. However, if an ion is shifting in  $m/z$  during acquisition collection, traditional STORI processing will underrepresent the ion’s charge, therefore biasing its absolute mass low. The misSTORI method is an extension of STORI which can detect and correct for mass/frequency shifts that can occur during mass analysis, providing more accurate charge and mass assignments for individual ions.<sup>2</sup> In addition to improving mass accuracy, the information obtained from the misSTORI calculations can also provide interesting insights into the fundamental processes occurring inside the mass analyzer.

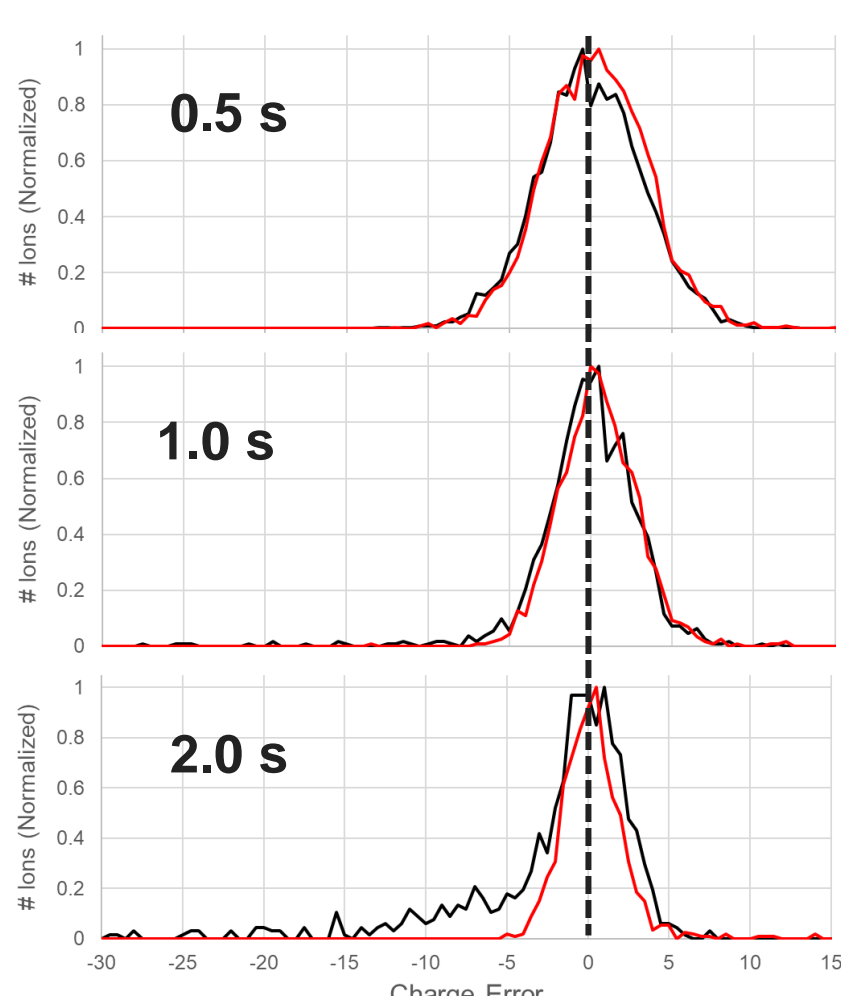
## Materials and methods

CDMS data was collected using Direct Mass Technology mode on a Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. Data was processed using either custom Python scripts or commercially-available STORIBOARD software (Proteinaceous). For any given peak, an initial centroided frequency is calculated, for which a traditional STORI curve is generated. A misSTORI plot is then generated by taking the derivative of the complex STORI values. A plot of misSTORI phase is then fit into multiple linear segments. The segment slopes represent the error between the signal frequency and centroid frequency at any time point. These frequency errors can be used in a second STORI calculation, in which the frequency is adjusted dynamically over time to match the signal’s frequency.

## Enabling longer acquisitions

In CDMS measurements, charge precision should improve with the S/N of the signal, which would theoretically scale with the square root of acquisition length. For maximum charge precision, the longest practical acquisition length should be used. However, longer acquisitions have a higher likelihood of collisions with neutrals, which may result in  $m/z$  changes. These  $m/z$  changes would cause errors in traditional STORI processing, meaning that long acquisitions may not provide improved performance. However, since misSTORI processing can account for the  $m/z$  shifts that occur during analysis, it should allow for improved charge precision to be realized from longer acquisitions.

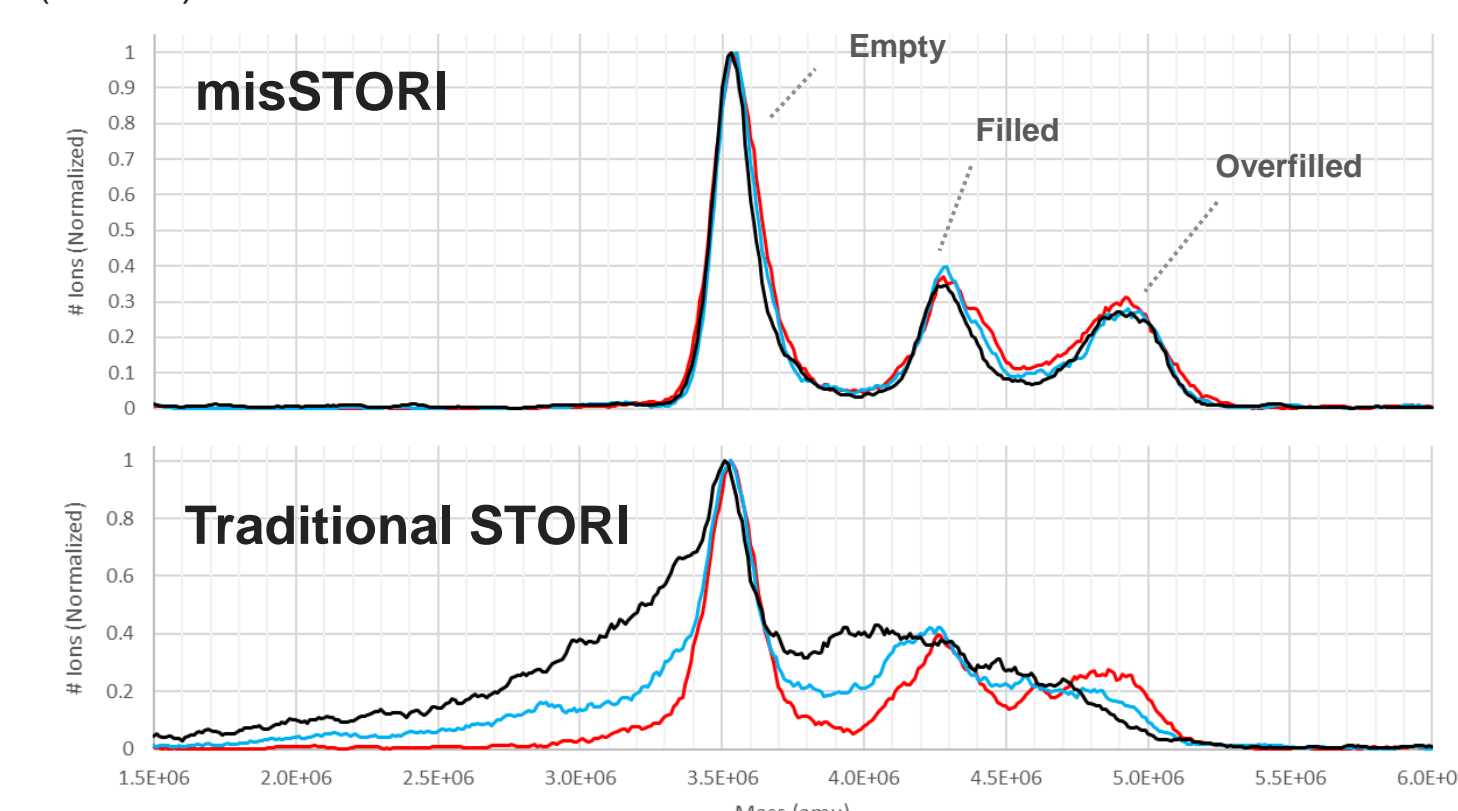
This improvement is demonstrated in Figure 1, which compares charge precision for traditional STORI and misSTORI processing at various acquisition lengths, using VP3-only adeno-associate virus (AAV) capsid ions. When going from 0.5 to 1.0 second acquisitions, charge precision improves for both traditional STORI and misSTORI. However, when extending to 2.0 seconds, the traditional STORI quality degrades, with a bias toward lower charge due to  $m/z$  changes. Meanwhile the misSTORI quality continues to improve. This concept should allow for analyses of ultralong acquisitions<sup>3</sup> without a drop in performance due to  $m/z$  shifts.



**Figure 1. Charge precision of empty VP3-only AAV capsid ions at various acquisition lengths using traditional STORI processing (black) and misSTORI processing (red). With 0.5 and 1.0 second acquisitions (top and center), the two algorithms are indistinguishable. However, with 2.0 second acquisitions (bottom), traditional STORI processing degrades, while the misSTORI processing improves.**

## Effect on AAV full:empty ratios

AAVs are often used in gene therapy, consisting of a capsid shell that encapsulates a DNA payload. The relative concentrations of “empty” and “full” capsids is a critical metric in the analysis such AAV samples, and CDMS is growing in popularity for such measurements. Ideally, the CDMS signal processing algorithm should not affect the full/empty ratio. To determine the degree to which STORI and misSTORI algorithms affect full/empty ratios, acquisitions of various lengths (1, 2 and 4 seconds) were collected for partially filled AAV2 capsids. The data shown in Figure 2 demonstrates that with misSTORI processing, the full:empty ratios would remain consistent across transient lengths (top). However, traditional processing would make it difficult, if not impossible, to determine proper ratios with transient lengths beyond 1 second (bottom).

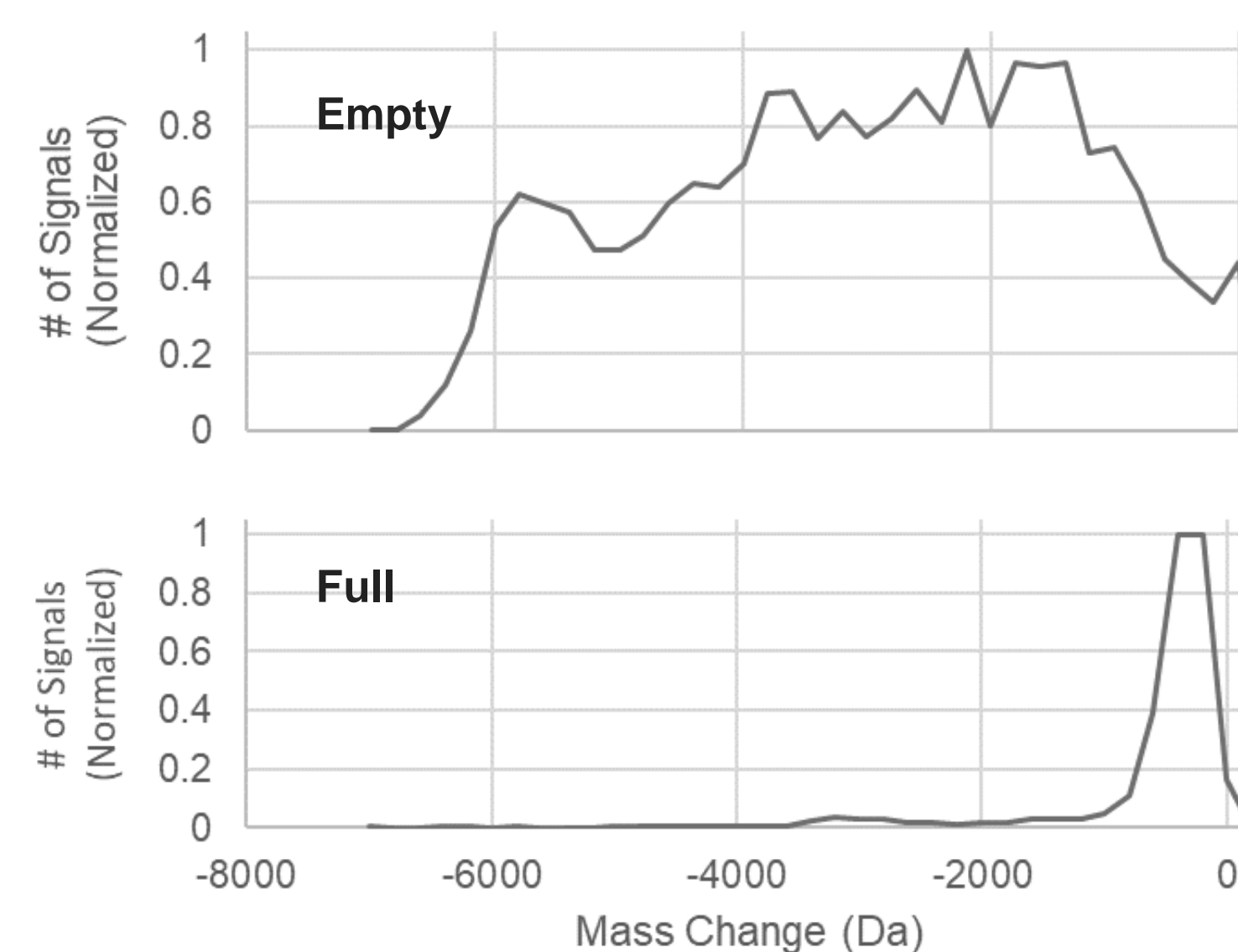


**Figure 2. Absolute mass spectra obtained for a partially filled AAV2 sample, using traditional and misSTORI processing at various acquisition lengths. While the resolution improves with longer acquisitions due to higher charge precision with misSTORI (top), the empty:full ratio does not change appreciably. However, with traditional processing, the ratio is ill-defined beyond 1s acquisition.**

## Varied desolvation of empty/full AAV capsids

Under some experimental conditions (e.g. low ion transfer tube temperatures, high liquid flow rates, etc.), AAV ions can be poorly desolvated, resulting in significant mass shifts during analysis, as the residual solvent molecules are sequentially removed from the ions. When misSTORI analysis is performed on these ions, the absolute mass loss can be estimated from the magnitude of the  $m/z$  shift and the measured charge.

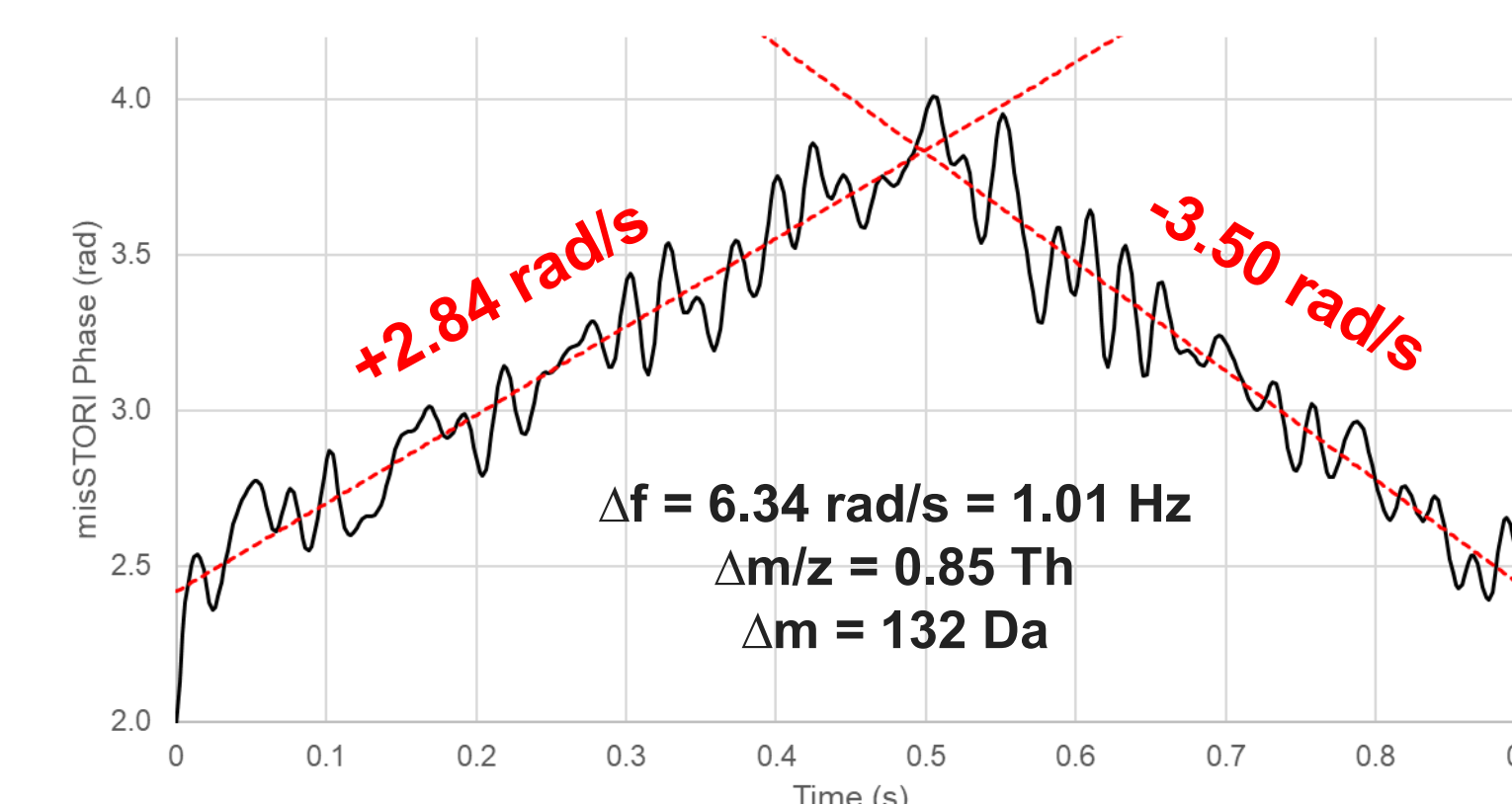
Figure 3 shows shiftograms for full and empty ions under poor desolvation conditions, using 2 second acquisitions from an AAV8 sample. Interestingly, the empty capsid ions undergo much larger mass shifts than the full capsids, despite experiencing the exact same instrumental conditions (e.g. spray composition, in-source trapping, HCD pressure, etc.). The empty capsids lose thousands of Da of mass during analysis, while the full capsids only lose hundreds of Da. While initially surprised by this result, we hypothesize that the empty capsid ions are not truly “empty” at all, but rather “full” of solvent molecules. This large number of solvent molecules continues to slowly bleed out of the “empty” capsids during analysis. Meanwhile much of the interior volume of the full capsids is occupied by the large genome payload, meaning there are less solvent molecules to be lost during analysis. It should be noted that other AAV samples/conditions have shown comparable shifting between empty and full capsids. As such, there may be interesting nuance in what conditions lead to such behavior. With the information provided from misSTORI analyses, such interesting glimpses into underlying chemical/physical phenomena can be had.



**Figure 3. Distributions of mass changes for empty (top) and full (bottom) AAV8 capsids under poorly desolvating conditions. The empty capsids lose much more mass than the full capsids. This suggests that the “empty” capsids may actually be “full” of solvent.**

## Ions gaining mass during analysis

Other interesting phenomena can also be observed via misSTORI analyses. For example, while many AAV ions undergo mass losses during analysis, some examples have been seen in which ions appear to increase in mass. As an example, a misSTORI phase plot of a 1 second acquisition of a VP3-only AAV capsid ion (+155 charge state), using Xenon as the collision gas, is shown in Figure 4. There is a clear change in frequency around 500 ms. The negative change in slope indicates that the frequency went down, which would occur with a rise in  $m/z$ . This could be explained by the capture of a neutral particle. Based on the magnitude of the frequency change and the charge of the ion, it’s estimated that the gained mass is approximately 132 Da, although the absolute precision of this estimate is limited by the S/N of the signal. Nevertheless, this is very close to the mass of Xenon, which was the collision gas in these experiments. This strongly suggests that the AAV ion collided with a Xenon atom inside the Orbitrap, captured said atom, and then carried it along for the remainder of the acquisition. This demonstrates the power of misSTORI analysis in unraveling fundamental behaviors of these large ions inside the mass spectrometer.

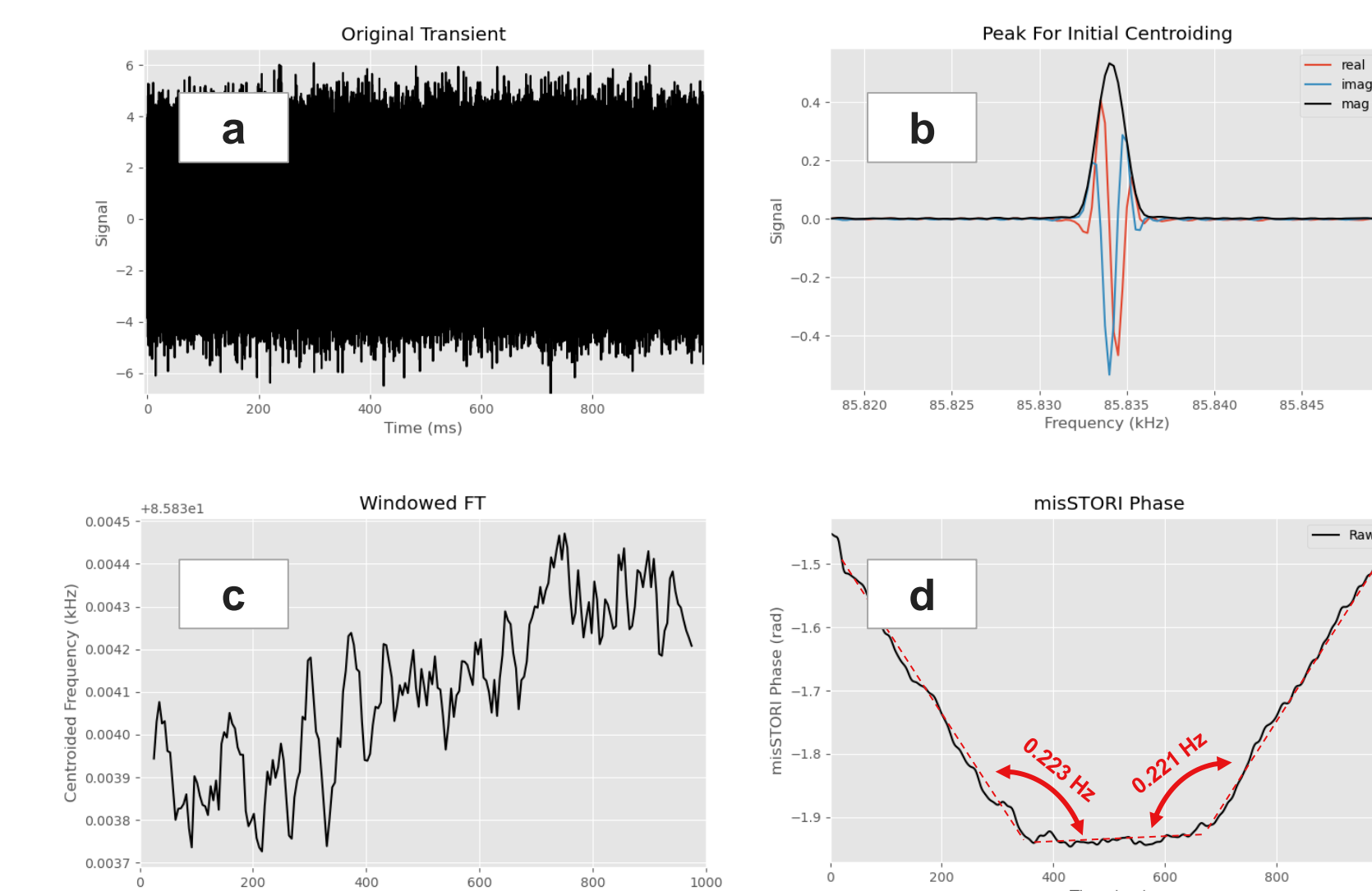


**Figure 4. misSTORI phase plot of an AAV ion that gains ~131.9 amu, closely matching the mass of the gas used in the collision cell, Xenon.**

## Comparison to STFT

Previous attempts to detect and correct for frequency shifts have mostly focused on the “Short Time Fourier Transform” (STFT) concept, in which an acquisition is broken into numerous shorter, overlapping chunks<sup>3-5</sup>. A Fourier transform is then applied to each chunk, and the resulting peaks are fitted/centroided. Shifts in frequency and/or amplitude can be seen via changes in the respective centroid information of each window. However, since each window is generated from a much shorter effective acquisition, the centroided frequencies are less precise. If signal-to-noise is sufficiently low and frequency changes are sufficiently small, it can be hard to pinpoint when frequency changes occur. Since misSTORI processing uses the entire acquisition, it has access to more information and can provide a clearer picture as to when the frequency change occurs.

Figure 5 compares the two approaches’ ability to pinpoint frequency changes. A one second time domain signal was synthesized with a frequency corresponding to a 3.5 MDa virus on the Q Exactive UHMR. It underwent two successive water losses (0.221 Hz frequency shifts), spaced 333 ms apart. A STFT analysis was performed with 50 ms windows, spaced 5 ms apart. Each window was zero filled three times and fitted for a centroid. The resulting evolution of frequency (Figure 5c) shows that there is an overall frequency change of approximately 0.44 Hz, but the noise makes it difficult to determine when the frequency shifts occur. A misSTORI phase plot of the same acquisition (Figure 5d) provides a clearer picture of the underlying processes, with two kinks. The respective changes in slope closely match the true frequency shift.



**Figure 5. (a) A synthetic 1 second long time domain signal of an AAV, undergoing two 0.221 Hz frequency jumps, spaced 333 ms apart. (b) Fourier transformed peak (c) STFT analysis with 50 ms windows in 5 ms increments (d) misSTORI phase analysis**

## Conclusions

The misSTORI algorithm is a significant improvement upon traditional STORI processing. It can detect and correct for shifts in  $m/z$  that are commonplace in the measurement of large ions, such as AAVs. This enables the use of longer acquisitions, which improves overall charge precision. The resulting information can also provide interesting insights into fundamental phenomena occurring inside the analyzer. Lastly, misSTORI processing can more precisely localize when frequency changes occur than traditional STFT approaches.

## References

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