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Quantitative Determination of Isotope Ratios from Experimental Isotopic Distributions

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Abstract

Isotope variability due to natural processes provides important information for studying a variety of complex natural phenomena from the origins of a particular sample to the traces of biochemical reaction mechanisms. These measurements require high-precision determination of isotope ratios of a particular element involved. Isotope Ratio Mass Spectrometers (IRMS) are widely employed tools for such a high-precision analysis, which have some limitations. This work aims at overcoming the limitations inherent to IRMS by estimating the elemental isotopic abundance from the experimental isotopic distribution. In particular, a computational method has been derived which allows the calculation of $^{13}\text{C}/^{12}\text{C}$ ratios from the whole isotopic distributions, given certain caveats, and these calculations are applied to several cases to demonstrate their utility. The limitations of the method in terms of the required number of ions and S/N ratio are discussed. For high-precision estimates of the isotope ratios, this method requires very precise measurement of the experimental isotopic distribution abundances, free from any artifacts introduced by noise, sample heterogeneity, or other experimental sources.

Introduction

Elemental isotopic composition variation in biological products due to natural processes is known and provides important information for a diverse variety of studies. The isotopic signatures of biomolecules depend upon the geographical parameters like latitude, distance from the sea, altitude, and seasonal effects.[1] For example, Oxygen isotope ratio ($^{18}\text{O}/^{16}\text{O}$) indicates the source along with the authenticity control information for products like fruit juices, wine, milk, butter, cheese etc.[2–4] Isotopic analysis of bone and dental remains of past species are used to determine the dietary patterns of ancient humans.[5] Natural isotope ratio variability also provides information on studies like gender-specific physiology,[6] nitrification and nitrate turnover rates in forests,[7] history of earth's climate,[8] determining origins of a given sample,[2] and diets of contemporary animals.[9] In biomedical sciences, isotope ratios based tracer methods are employed for protein turnover studies, fat metabolism, [10] and breath tests for clinical testing purposes[11], not to mention the drug testing of athletes.

Conventionally, high-precision carbon isotope ratio measurements are expressed in terms of the delta notation.[12,13] The Delta notation is defined as the relative difference in parts per thousand between the sample isotope ratio and an isotope ratio of an international standard. For carbon, the accepted international standard is Pee Dee Belemnite (PDB), a belemnite from the Cretaceous Pee Dee formation, South Carolina USA. It is expressed as following:

$$\delta^{13}\text{C}_{\text{PDB}} = \left(\frac{R_{\text{SPL}} - R_{\text{PDB}}}{R_{\text{PDB}}} \right) \times 1000 = \left(\frac{R_{\text{SPL}}}{R_{\text{PDB}}} - 1 \right) \times 1000 \quad (1)$$

$$R_x = \frac{[^{13}\text{C}_x]}{[^{12}\text{C}_x]} \quad (2)$$

Where $[^{13}\text{C}]$ and $[^{12}\text{C}]$ are the abundances of the respective isotopes in the sample or PDB, $R_{\text{PDB}} = 0.0112372 \pm 0.0000090$. [14] $\delta^{13}\text{C}$ values are expressed as “per mil” values or ‰. Using this definition, C3 [15,16] and C4 plants have a $\delta^{13}\text{C}$ value of -26.5 ‰ and -12.5 ‰, with the corresponding abundances for ^{13}C being 1.082 ‰ and 1.097 ‰ of total carbon respectively, indicating the subtle differences in the isotopic signatures. Examples of C3 plants include trees, shrubs, flowering plants and temperate zone grasses. C4 plants include maize, sugar cane, and tropical grasses.

Since the isotopic variations in various categories are extremely subtle, measurements of δ values require very high-precision determination of the isotope ratios of a particular element involved. Isotope Ratio Mass Spectrometers (IRMS) [17] are widely employed tools for such high-precision analysis. These instruments typically require complex compounds to be reduced to simpler molecules before measurement. For example, organic compounds are combusted to CO_2 , H_2O and N_2 which are then separated and detected individually. Current IRMS instruments thus accept the sample analyte in the form of only a limited number of gases, which, in turn, must represent the isotopic characteristics of the original sample. This represents a major limitation for the range of molecules that can take advantage of isotope ratio mass spectrometry. This work aims at overcoming the limitations inherent to IRMS by estimating the elemental isotopic abundance directly from the Experimental Isotopic Distribution (EID). This implies that any mass spectrometer capable of providing isotopic resolution can be used for isotope ratio analysis. Another substantial advantage of this method is that the samples do not need to be reduced to simpler molecules, extending the utility of isotope ratio mass spectrometry to molecules that are not amenable to the reduction process. This will also provide increased sensitivity since the sample preparation process will involve fewer steps, and hence, fewer losses.

Computational approaches have been proposed previously for stable isotope enrichment experiments. [18,19] These allow for measuring the isotope enrichment ratios by comparing the predicted, enriched isotopic distributions with the measured isotopic distributions. [18,19] Progress has also been made for the measurement of the distribution of isotopomers in a labeled compound by means of linear algebra. [20] However, the purpose of the current approach is to determine the natural abundances of elements from their experimental isotopic distributions. This work should not be confused with the approaches intended for monitoring the progress of isotope enrichment ratios used in isotope labeling experiments.

An isotopic distribution (ID) [21,22] is a direct measure of the isotopic abundances of its constituent elements, convolved by the number of atoms of each element present in the molecule under consideration. If the isotopic abundance contributions from all but one element are known, an EID can be analyzed mathematically to determine the isotopic abundance contribution of the unknown element. Known isotopic abundance contributions can be deconvolved from the EID, allowing for solution for the unknown element.

Theory: Let the elemental composition of a given molecule be $\text{C}_{\text{Nc}} \text{H}_{\text{Nh}} \text{N}_{\text{Nn}} \text{O}_{\text{No}} \text{S}_{\text{Ns}}$. Let

$$\left. \begin{aligned} P_c &= \begin{bmatrix} 1 - p_c \\ p_c \end{bmatrix} \\ P_H &= \begin{bmatrix} 1 - p_h \\ p_h \end{bmatrix} \\ P_N &= \begin{bmatrix} 1 - p_n \\ p_n \end{bmatrix} \end{aligned} \right\} \mathbf{A}+1 \text{ Elements} \quad \left. \begin{aligned} P_o &= \begin{bmatrix} 1 - (p_{o_1} + p_{o_2}) \\ p_{o_1} \\ p_{o_2} \end{bmatrix} \\ P_s &= \begin{bmatrix} 1 - (p_{s_1} + p_{s_2}) \\ p_{s_1} \\ p_{s_2} \end{bmatrix} \end{aligned} \right\} \mathbf{A}+2 \text{ Elements}$$

represent the isotopic abundances of C, H, N, O and S respectively, where $P_x(i)$ represents the i th isotope of element X.

Let $T[n]$ represent the Theoretical Isotopic Distribution (TID). Thus,[22]

$$T[n] = P_c[n] *^{N_c} P_c[n] * P_H[n] *^{N_H} P_H[n] * P_O[n] *^{N_O} P_O[n] * P_N[n] *^{N_N} P_N[n] * P_S[n] *^{N_S} P_S[n] \quad (3)$$

where $*$ denotes the convolution[23] operator:

$$z[n] = x[n] * y[n] = \sum_{j=1}^{\infty} x[j] \times y[n+1-j] \quad (4)$$

and $*^N$ denotes multiple convolutions defined as follows:

$$x[n] *^N x[n] = x[n] * x[n] * x[n] \dots \mathbf{N} \text{ times} \quad (5)$$

Assuming that the isotopic abundances of all the elements except any one element, say C, are known, the goal is to find the unknown isotopic abundance of ^{13}C , denoted by p_c . In order to extract the p_c values from the convolved isotopic distributions, it is convenient to reorder $T[n]$. The theoretical isotopic distribution $T[n]$ can be represented as the following:

$$T[n] = T_1[n] * T_2[n] \quad (6)$$

where $T_1[n]$ represents the isotopic abundance contribution from carbon as follows:

$$T_1[n] = P_c[n] *^{N_c} P_c[n] \quad (7)$$

and $T_2[n]$ contains known isotopic abundance information about the other elements defined as the following:

$$T_2[n] = P_H[n] *^{N_H} P_H[n] * P_O[n] *^{N_O} P_O[n] * P_N[n] *^{N_N} P_N[n] * P_S[n] *^{N_S} P_S[n] \quad (8)$$

$T_2[n]$ is assumed to be completely known since its constituents depend upon the known isotopic abundance values of all the elements other than carbon. Since $P_x[n]$ is non-zero only for positive values of n , where x denotes a particular element, the same holds true for $T_1[n]$ and $T_2[n]$. Thus,

$$T[n] = \sum_{j=1}^{N_c+1} T_1[j] \times T_2[n-j+1] \quad (9)$$

since the length of $T_1[n]$ is N_c+1 . Let the coefficients of expansion of $T_1[n]$ be $\alpha_1, \alpha_2, \dots, \alpha_{N_c+1}$. Thus, $T[n]$ can be expanded as follows:

$$T[1] = T_1[1] T_2[1] = \alpha_1 T_2[1] (1 - p_c)^{N_c} \quad (10)$$

$$T[2] = T_1[1] T_2[2] + T_1[2] T_2[1] = \alpha_1 T_2[2] (1 - p_c)^{N_c} + \alpha_2 T_2[1] (1 - p_c)^{N_c-1} p_c \quad (11)$$

$$\begin{aligned} T[k] &= T_1[1] T_2[k] + T_1[2] T_2[k-1] + \dots + T_1[k] T_2[1] \\ &= \alpha_1 T_2[k] (1 - p_c)^{N_c} + \alpha_2 T_2[k-1] (1 - p_c)^{N_c-1} p_c + \dots + \\ &\quad \alpha_k T_2[1] (1 - p_c)^{N_c-k+1} p_c^{k-1} \end{aligned} \quad (12)$$

When the number of ions representing an experimental isotopic distribution is extremely large, the experimental distribution approaches the theoretical isotopic distribution.[24] Hence, the terms on the left of each of the above equations can be substituted by the corresponding values from the EID. Thereafter, each of the above polynomial equations can be solved for p_c , with each equation representing one of the isotopes observed in the experimental isotopic distribution.

Using Chebyshev's inequality, it is possible to determine the number of ions required to generate an isotopic distribution that are sufficient to determine the $\delta^{13}\text{C}$ value within a given accuracy. This is done as follows. Assume that the true value of $\delta^{13}\text{C}$ is δ_{true} , and the estimate is desired within, say, $\pm\Delta$. Since the $\delta^{13}\text{C}$ value depends upon $[^{13}\text{C}]$ (denoted by p_c) and $[^{12}\text{C}]$ ($1 - p_c$) as seen in equations 1 and 2, the corresponding values of p_c , say $p_{true} \pm \Delta_{pc}$, for $\delta_{true} \pm \Delta$ can be determined from equations 1 and 2. Now the isotopic abundances in an EID depend upon the value of p_c . Let the isotopic abundances for ideal EID (which is the same as the TID) be represented by the vector E_{true} when δ_{true} is the true value of $\delta^{13}\text{C}$. Due to the limited number of ions, there is a variation in the EID.[24] Let this variation be represented by $E_{true} \pm e$, and be such that the variation is the same as that is caused by the variation in p_c in the range of $p_{true} \pm \Delta_{pc}$. The goal here is to determine the number of ions so that the vector of observed isotopic abundances, E , lie within $E_{true} \pm e$. Each of the isotopic peaks in the EID is treated individually for the analysis purposes. According to Chebyshev inequality,

$$P(|E(i) - E_{true}(i)| \geq e(i)) \leq \frac{\sigma_i^2}{(e(i))^2} \quad (13)$$

where i denotes the i^{th} isotopic peak, and σ_i^2 indicates the variance of the i^{th} isotopic peak, and $e(i)$ denotes the magnitude of deviation of the observed i^{th} isotopic peak from its true value. It means that the probability that the observed isotopic abundances in EID, E , differs from the true values, E_{true} by more than a certain value e , is bounded by the expression of the right hand side of the above equation, which depends upon the variance in the given isotope and its deviation from the true value. The variance σ_i^2 is given by[24]:

$$\sigma_i^2 = \frac{E_{true}(i) \times (1 - E_{true}(i))}{N} \quad (14)$$

where N denotes the number of ions used to generate the EID. Now, assume that it is desired that the estimated delta value deviates from the true value by no more than Δ with a probability $\hat{P}$. Then number of ions, N , can be solved for using the above equations and $\hat{P}$ as follows:

$$N(i) = \frac{E_{true}(i) \times (1 - E_{true}(i))}{\hat{P} \times (e(i))^2} \quad (15)$$

Note that this number can be calculated for each isotope in the EID. This number will ensure that if the EID is generated using a certain number of ions satisfying the above equation, $\delta^{13}\text{C}$ will be bounded within the limits as described above.

Methods

Experimental spectra of chlorophyll-b (Molecular formula = $\text{C}_{55}\text{H}_{70}\text{MgN}_4\text{O}_6$) purchased from Sigma (St. Louis, MO) were obtained with 1 μM concentration using ethanol and 1% formic acid. The spectra were obtained on a previously described [25,26] custom hybrid electrospray FTICR instrument. Bovine ubiquitin sample was prepared with 1 μM concentration in a 50:50 mixture of water and methanol, and the spectra were obtained on the same instrument. Matlab 7.1 (Natick, MA) was used for the data analysis.

Results and Discussion

In order to test this theory (per equations 10–12), performance analysis was first carried out on modeled EIDs generated using Monte-Carlo simulations in silico. EIDs were generated by varying the number of ions to study the effect of error in determining $\delta^{13}\text{C}$ as a function of the number of ions used to generate the EID. The number of ions was varied from 500 to 22000, in the increments of 500, and the true value of delta used to generate the isotopic distribution was -25.5‰ (typical value for C_3 plants), corresponding to $p_c = 1.0832\text{‰}$. Ions were generated for an “average” protein [27] with a molecular weight of 9000 Da. Each estimate was generated using an average of 10 simulations, with number of ions being the same in each of the 10 simulations. Fig 1 illustrates that with the increase in the number of ions used to generate the EID, the error drops substantially. This is intuitive since the higher the number of ions, the closer the EID approaches to the TID.[24]

The higher the molecular weight of the biomolecule, the greater is the number of carbon atoms and the number of isotopic peaks present in its isotopic distribution, because a greater number of combinations of isotopes becomes possible with increasing molecular weight. So, an investigation of the effect of molecular weight on the delta estimate was carried out. Isotopic distributions were generated using Monte-Carlo simulations, with molecular weight varying from 1000 Da to 20000 Da, with increments of 500 Da. Each distribution was generated using 100000 ions, with the true value of delta being again -25.5‰ . The results are plotted in Fig 2, which indicate that with the increasing molecular weight, the error in delta drops since more information is available from a higher number of isotopes present in the isotopic distribution. An important observation is that as the molecular weight increases, the number of ions required for the EID to approach the TID also increases [24] due to the widening isotopic distribution. Thus, the improvement in the estimate with the increasing number of observed isotopic peaks may be somewhat offset by the higher variance in the EID for higher molecular weight for the

same number of ions. Thus, figure 2 shows that, for 100000 ions, error in the observation of $\delta^{13}\text{C}$ values is minimized in the mass range of ~8–15 kDa, which includes the mass convenient for proteins such as ubiquitin, cytochrome *c*, and lysozyme, among others. All the isotopic peaks with peak heights greater than 5% of the highest peak were used to arrive at the final estimate, because in practice, not all the isotopic peaks are observed in the EID. The less abundant ones are difficult to observe since their abundance often falls below the noise baseline, and their abundance is usually highly distorted by noise making their value in the estimate highly suspect.

Thus, the theory from equations 10–12 works well using simulated EIDs. But what about the performance for real life spectra? Thus, the theory established above was subsequently applied to experimental spectra obtained on a custom hybrid electrospray FTICR instrument.[25, 26] A chlorophyll *b* sample was prepared using ethanol and 1% formic acid. Chlorophyll *b* (C3 plant, $\delta^{13}\text{C} = -26.5$) spectra were acquired and signal averaged over 100 scans. Mg present in the molecule is removed in the acidic medium and is replaced by 2 H atoms, leading to a monoisotopic molecular weight of 885.55 Da. Fig 3 shows one such spectrum of chlorophyll-*b*. The inset in the picture show the expanded m/z region of the EID, along with the TID. The EID, unfortunately, clearly consists of multiple overlapping components, and hence, has an isotopic pattern substantially different from the TID as the insets in the figure illustrate. Since the goal of this study is to differentiate between extremely subtle variations of the isotopic abundances, the interference in the EID of the desired molecule from any other components cannot be permitted, since it will distort the EID and lead to erroneous results. In this case, the overlapping isotopic distributions extend the EID to high mass, which would greatly overestimate $\delta^{13}\text{C}$. This example illustrates one of the challenges faced by using this method. It is critical that any EID used for quantitative determination of isotope ratios consist of one and only one component, and that all of the isotopic variance be due to the variance in the isotope ratios.

Experimental spectra of bovine ubiquitin (8.5 KDa) were obtained in order to further evaluate the theory against the experimental data. Fig 4a shows the mass spectrum of bovine ubiquitin with isolation of 10+ charge state in the quadrupole, Q1, averaged over 550 scans. The insets show the expanded view of the EID and the TID. Fig 4b shows the plot of peak areas of the EID and the TID for the same isotopic distribution in circles and stars respectively. There appears to be a systematic bias in the EID when compared against the TID, the peak areas of the first few isotopes in the EID are smaller than their theoretical counterparts, and the difference becomes smaller for higher isotopes. This bias may be attributed to the ion selection in the front end quadrupole. Some of the isotopes may be preferentially accumulated over the others due to the m/z window on the quadrupole. This phenomenon was observed despite the fact that 10 Da wide window was used for ion selection. Fig 4c shows the histogram of the delta estimates obtained using 19 spectra of bovine ubiquitin. The results were generated from 103 isotopic peaks for the 10+ charge state. Due to the artifacts present in the EID because of isolation, the EID does not represent the true TID, and hence, the delta estimates obtained are also likely to be erroneous since the theory established above requires the EID to represent the TID very closely in order to give good estimates. Fig 4c shows that the delta values don't converge to a median value and represent a very wide range of values. Thus, experimental techniques which distort the isotopic distributions, such as quadrupole isolation shown here, prevent calculation of the true δ values. This is true even for extremely subtle distortions like the one shown.

Due to the problems associated with the EID distortion because of the isolation in the quadrupole, isolation was disabled, and all the charge states were allowed to pass through the ion optics and collected in the cell. The resulting spectrum with the averaging of 500 scans is shown in Fig 5a, with the inset showing the EID of 10+ charge state within the spectrum. The

peak areas of the EID and the corresponding TID are shown in Fig 5b, with the EID and the TID being represented by circles and stars respectively. This figure shows that the EID matches very closely to the expected TID once the EID distortion caused by quadrupole isolation has been eliminated. For a complete evaluation, 24 bovine ubiquitin spectra were generated, and a total of 392 isotopic peaks from the charge states 9+ and 10+ were used for estimating the delta values. The resultant histogram of delta estimates is shown in Fig 5c. Note that the estimates tend to converge to a central median value of -27.55 ± 2.89 , which compares favorably with the expected $\delta^{13}\text{C}$ value (-25.5) for animals whose diet primarily consists of the C3 plants.[28,29] It should be noted that this method may not be suited for the analysis of heterogeneous populations of molecules, since it will increase the possibility of having multiple overlapping components in the isotopic distributions, and hence, distorting their shape, as was demonstrated with the chlorophyll spectrum above.

Figure 6 shows the results of a Monte-Carlo simulation to determine the number of ions required to ensure with a 95% probability that the estimated delta value is within $\pm 1\%$ as a function of molecular weight. The true value of $\delta^{13}\text{C}$, δ_{true} was taken to be -25.5 . The results were obtained as follows. Natural abundance values of ^{13}C (p_c) were calculated for the $\delta_{\text{true}} \pm 1\%$. TIDs were generated for the resulting p_c values. The ion number was estimated using Chebyshev's bound results in equation 15, e(i) was obtained by the difference between the ideal TID and the TID resulting from the p_c values corresponding to $\delta_{\text{true}} \pm 1\%$, and probability $\hat{P}$ was taken to be 0.95. The results demonstrate that with the increasing molecular weight, smaller number of ions are required in order to estimate the δ value with the same accuracy. This is intuitive since the higher molecular weight ions have greater number of carbon atoms and hence, subtle changes in δ , and thus p_c , will result in greater changes in the isotopic distribution. This is because as seen in equation 3, the isotopic distribution involves the term containing convolution of the natural abundances of carbon N_c times, where N_c is the number of carbon atoms present in the molecule. Hence, subtle changes in $\delta^{13}\text{C}$ values will produce a more pronounced effect for higher molecular weights due to greater number of multiplications of p_c involved than with the smaller molecules.

With FTMS, the space charge limit bounds the total number of charges that can be detected without peak distortion. Fig 6 indicates that, the larger the molecule, the smaller is the number of ions required to achieve the same level of accuracy in $\delta^{13}\text{C}$ values. Thus, for FTMS, increasing the mass of the molecule used for $\delta^{13}\text{C}$ measurements and decreasing the charge state increases the accuracy, provided baseline isotopic resolving power is achieved.

This approach can be used for the determination of isotope ratio for any element. The equations above can be modified to treat the isotopic abundance of the desired element to be unknown, and then solve for the unknown value. The discussion here was based primarily on the isotope ratio of carbon because of its utility in a wide variety of applications. This approach may be extended to determine the variations in natural abundances of multiple elements if mass spectra from multiple samples originating from the same source are available. If there are m unknown abundances, and $\geq m$ different spectra from the same source are available, m different sets of equations (one from each spectra) similar to equations 9–12 can be used to solve for m variables using algebraic methods.

As shown, this method is very sensitive to the distortions introduced in the experimental isotopic distributions arising due to many factors such as quadrupole voltages, overlapping isotopic distributions, distortions due to modifications such as deamidation. Hence, this approach is not suitable for use with ion trap or quadrupole instruments. Time of Flight instruments may be better suited for this purpose due to minimal isotopic distribution distortion, provided that the observed isotopic distribution provides baseline resolved isotopes.

It is possible to improve the performance of this method with an FTICR instrument using SWIFT (Stored Waveform Inverse Fourier Transform). [30] SWIFT excitation will allow equal excitation to all the isotopes within the isotopic distribution, and hence, minimize the signal artifacts. Also, using SWIFT, it is possible to eject ions that are not of interest, providing an opportunity to accumulate a greater number of ions of interest within the cell, which will lead to a smoother experimental isotopic distribution, and result in a more accurate final estimate, provided the space charge limit of the cell is not exceeded.

This approach can be useful for many applications that require detection of subtle distortions. For example, this may be a method of choice to check whether the instrument response is linear and stable in a given narrow m/z range. A known molecule, whose elemental isotopic abundances are known, can be used to generate the experimental spectrum. The experimental spectrum may then be compared against the theoretical spectrum in order to test for any isotopic distortions using this method. Any distortions observed may be attributed to the non-linear response of the instrument.

The number of ions sufficient for the experimental isotopic distribution to approach its theoretical counterpart depends upon the molecular weight of the molecule of interest. For higher mass molecules, greater numbers of ions are thus required for the experimental and theoretical distributions to “match” against each other. There is a tradeoff between the number of ions and molecular weight for a “smooth” experimental isotopic distribution. Higher molecular weight molecules are advantageous because of the greater number of carbon atoms and isotopic peaks in the distribution. Lower molecular weight molecules require fewer ions to provide ideal experimental isotopic distribution, but they contain fewer carbon atoms, and hence, less information.

In this study, isotopic peaks were fitted to Lorentzian peak shapes [31], and the areas of the fitted peaks were calculated in order to calculate the peak intensities. Careful consideration must be given to avoid any peak distortions due to space charge effects in the ICR cell to avoid erroneous results. And care must be taken to use the best possible peak shape fitting method in order to calculate the most accurate peak areas which are true representatives of isotopic intensities. Peak height intensities are generally unacceptable due to approximation errors.

Conclusions

A theoretical framework has been developed and tested for estimating the elemental isotopic abundances from the experimental isotopic distributions. The estimate improves with increasing number of ions generating the isotopic distribution. Higher molecular weights are particularly useful for a better estimate because the higher number of carbon atoms and isotopic peaks observed lead to a greater amount of information. However, higher molecular weights also require a higher number of ions in order for the EID to converge to the theoretical isotopic distribution, which is required for reliable results. This method circumvents some of the limitations experienced by the traditional IRMS by providing a greater flexibility about the kind of samples that may be used for the analysis. This approach is applicable for isotopically resolved spectra from any kind of instrument. For optimal results, experimental isotopic distribution is required to have minimal artifacts due to the subtle nature of the measurements being made. It is very important to avoid any perturbations in the experimental isotopic distributions due to noise or other sources like influences from overlapping isotopic distributions, intensity artifacts due to bias in quadrupole voltages etc.

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Figure 1.

The estimate of the $^{13}\text{C}/^{12}\text{C}$ ratio improves with the increase in the number of ions used to generate the simulated isotopic distribution, $\delta_{\text{True}} = -25.5$, $\text{MW} = 9000$, each estimate was generated using 10 monte-carlo simulations of isotopic distributions

Figure 2.

The estimate of the $^{13}\text{C}/^{12}\text{C}$ ratio improves with the increase in molecular weight due to the higher number of atoms represented in the isotopic distribution, $\delta_{true} = -25.5$, $\delta_{Est} = -25.68$

Figure 3.

The mass spectrum of chlorophyll-b ($C_{55}H_{70}MgN_4O_6$, MW=906.83, Mg is replaced by 2 H atoms in acidic medium, leading to MW=885.55) from spinach, the desired EID consists of multiple overlapping components demonstrating one of the difficulties of this approach.

Figure 4.

(a) The mass spectrum of bovine ubiquitin with quadrupole isolation of the 10+ charge state at $m/z=857.5$ (b) The EID (circles) differs from the TID (stars) due to the isolation artifacts, showing that care must be taken to prevent isotopic distribution distortion during the experiment (c) $\delta^{13}\text{C}$ estimate using 19 spectra of bovine ubiquitin, with isolation of 10+ charge state, delta median value=7.62 from the 103 estimated values from different isotopes

Figure 5.

(a) The mass spectrum of bovine ubiquitin without quadrupole isolation (b) The EID (circles) matches well with the TID (stars) (c) Δ estimate values using 26 spectra of bovine ubiquitin, median value = -27.55 from 392 estimated values from different isotopes, indicating that the cow(s) from which this sample is derived fed primarily on C3 plants

Figure 6. Number of ions required vs. molecular weight to measure the $\delta^{13}\text{C}$ value within 1 per mil with a probability of 0.95