
Electrospray Ionization Mass Spectrometry: Deconvolution by an Entropy-Based Algorithm

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The ability of electrospray ionization (ESI) to generate high molecular weight gas-phase ions directly from water-soluble molecules provides an effective route to biopolymer mass measurement by using conventional ion optical methods. Electrospray ion sources have been coupled to quadrupoles, magnetic sector, and ion trap instruments, and, from recent reports [1–4], it may be appropriate to conclude that this method of ionization offers advantages uniquely suited for biopolymer molecular weight characterization. In particular, it may bring improved understanding to the difficult problems of constituent microheterogeneity (i.e., phosphoconjugates, sulfoconjugates, and glycoconjugates).

The growing interest in ESI can be related to two important features: a highly efficient ionization process and the generation of multiply charged ions. Investigators have reported femtomole detection for proteins even though present electrospray ion source designs lose much of their potential sensitivity in the transfer of ions from the ambient pressures of the ionization region to the low pressures required for mass analysis. The second important aspect of ESI is the generation of multiply charged ions, a property that has made it possible to measure protein molecular weights in excess of 200 μ with less expensive instrumentation.

ESI produces not only multiply charged ions, but ions with a range of charged states in a unique spectral pattern. Single molecular components become an envelope of distinct peaks with a distribution that complicates the direct interpretation of molecular weights. For situations in which there are only a few ion species in the spectrum, techniques have been published that are effective in extracting molecular weight information. In particular, Mann et al. [5] has introduced an "averaging" and a "deconvolution" algorithm. However, as the complexity of the spectrum increases, the multiply charged components be-

come superposed on a background of other peaks making these methods less effective.

In this communication a novel computational algorithm is presented for extracting molecular weight information from the peak spacings exhibited by multiply charged ions. The algorithm is intended to detect the pattern of a multiply charged ion embedded in a complex spectrum and avoid the complications of contaminating components, overlapping peaks and poor baseline. An entropy measure is used to signal the presence of a specified pattern, the envelope of charge states corresponding to a trial parent mass, in the observed set of spectra. Treatment of the data in this manner suppresses artifacts associated with the earlier deconvolution approach [5], and improves spectral dynamic range and confidence in peak detection. These adjunct techniques, combined with the ease of high performance liquid chromatography interfacing, enhanced ionization efficiency, and high molecular weight analysis, could prove most useful in resolving the demanding problems in glycoprotein and other posttranslational modifications.

Theory

The use of entropy in an algorithm for interpreting the mass spectra of multiply charged ions can be understood by contrasting it with the deconvolution algorithm of Mann et al. [5]. Both approaches are analogous in that the output or deconvoluted spectrum is a plot of a "distance" function serving as an ion detector. What distinguishes the algorithms are different formulations of the "distance" function. In particular, the Mann et al. [5] deconvolution can be represented as measuring a conventional Euclidean metric distance as a function of a single parameter, the mass of the parent, while the entropy-based deconvolution uses a probabilistic or information-theoretic distance in the same way.

Consideration of an entropy distance requires a mathematical formulation of mass spectra as stochastic or probabilistic events and thus one needs to state

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formal relationships among entropies, fluctuation probabilities, and stochastic convergence. In an appendix we have summarized results from this well-developed field [6] to document the use of entropy as a "distance" function. Accepting the entropy measure, how it functions as an ion detector is a topic that is independent of the mathematical details. Our objective in this report is to focus on ion detection only, and this will become a contrast of the distance functions as they apply to electrospray data. This contrast primarily relates to the problem of detection artifacts.

The deconvolution process is equivalent to calculating distances in a vector space and a probability space. This requires translating mass spectra into vectors and into probabilities. If one defines the set $\Gamma = \{m_1, \dots, m_N\}$ as the set of possible mass values established by the instrumental scan parameters and reported by the data acquisition system, then the mass spectrum $\{l(m_1), \dots, l(m_N)\}$, where $l(m_i)$ is the intensity or number of counts for the mass value m_i , can be thought of as a N -dimensional vector. A probability distribution can be constructed from the mass spectrum by normalization, i.e., by dividing each intensity $l(m_i)$ by the sum of the intensities over the N distinct mass values. Any N -dimensional vector $\nu = (\nu_1, \dots, \nu_N)$, where $\{\nu_i\}$ is a set of nonnegative real numbers satisfying eq 1 represents a possible probability distribution on Γ .

$$\sum_{i=1}^N \nu_i = 1 \quad (1)$$

Define all vectors ν satisfying these constraints as $P(\Gamma)$, the set of probability distributions on Γ .

With these definitions it is now possible to contrast the entropy and Mann et al. [5] deconvolution algorithms. Both methods correspond in that they construct a parameter-dependent distance between elements of $P(\Gamma)$. This parameter is the mass of the parent ion M , and for every value M one calculates the difference between a model distribution $\nu(M)$ [representing the expected pattern of a multiply charged ion of that mass] and the data ρ as the normalized spectrum. The plot of this difference as a function of the parent mass is the deconvoluted spectrum. The entropy deconvolution uses the relative or level-2 entropy, given by eq 2, as the measure of the difference between two distributions or points, ρ and ν , in $P(\Gamma)$. (The level-2 entropy is discussed in the Appendix.)

$$I_{\rho}^2(\nu) = - \sum_{i=1}^N \nu_i \log \left(\frac{\nu_i}{\rho_i} \right) \quad (2)$$

This expression is in contrast to the usual formula for the difference between two points in a N -dimensional vector space which is given by the Euclidean metric or

least square difference, i.e.,

$$|\rho - \nu| = \sqrt{\sum_{i=1}^N (\nu_i - \rho_i)^2}$$

The entropy distance defined by eq 2 is not a formal metric or distance function in that it is neither symmetric nor does it satisfy the triangle inequality. As was mentioned above, for the method presented by Mann et al. [5], the deconvolution is essentially plotting the Euclidean distance or least-square difference, i.e., if $\nu[M]$ is the model distribution and ρ is the normalized data, one plots:

$$LS(M) = \sum_{i=1}^N (\nu_i[M] - \rho_i)^2 \quad (3)$$

The relationship between this and the Mann et al. [5] expression will be further detailed below. In the entropy deconvolution method we do not plot the entropy function $I_{\rho}^2(\nu[M])$ itself, but plot instead the M dependence of the function:

$$\exp(I_{\rho}^2(\nu[M])). \quad (2a)$$

As discussed in the Appendix this quantity is more directly related to the probability of a fluctuation in the data producing the model distribution.

It should be stated for emphasis that both the entropy and the Mann et al. [5] deconvolution methods are based on finding values of the parent mass that more "accurately" represent the data. A fundamental distinction between them is that the entropy approach involves a concept of distance as a probability measure instead of a Euclidean measure and this defines the difference between the model and the data in information-theoretic terms instead of metric terms.

Both deconvolution methods require that each parent mass be associated with a pattern or model distribution and that one calculates a difference between this distribution and the observed spectrum. To illustrate how model distributions are generated, we have adopted the notation of Mann et al. [5]. For each ion complex one defines M as the mass of the parent ion (plus adducted neutrals), m_a as the mass of the charge-carrying adducts, and i as the number of charged adducts. This gives an effective mass (m_e) as:

$$m_e = M/i + m_a \quad (4)$$

Generally we have a multiply charged parent ion of mass M with a specified range of charge states denoted by the integer set $A = \{i_{\min}, \dots, i_{\max}\}$. Since the basic theme of both the entropy and sum deconvolution is to produce a model spectrum that is "near" the actual spectrum for the correct value of the parent mass M , one generally selects features in constructing the model distribution ν that are representative of physical features in the data, e.g., a finite width to the ion peaks. This will increase the apparent signal to noise in the deconvoluted spectra. To construct the

model distribution ν , first assume there is a definite mass profile associated with the parent mass M and apply the relation defined in eq 4 to each of the mass values in this profile for each integer in the set A . Actual peak widths in the mass spectrum are a combination of instrumental parameters and isotopic abundance. One could consider the scaled and translated parent ion profile to be then convoluted by a Gaussian envelope to better represent the ion focusing, but this level of description is generally unwarranted since band overlaps and noise generally obscure the details of the bandshape. The programs discussed in this report have approximated the final bandshape as Gaussian with an input parameter describing the halfwidth. An additional set of parameters is employed to describe the varying amplitude of different charge states. This issue has been simplified by describing the distribution of charge states in A as Gaussian. Input parameters describe the width and centering of the multiple-charge states. The result of this is to produce, as the model distribution ν , copies of the (Gaussian) parent ion profile at M that are scaled and mass translated in the pattern that one would expect of multiply charged ions.

From the translation (eq 4), the model distribution ν is explicitly M dependent and this M dependence is inherited by both $LS(M)$ and $I_{\rho}^2(\nu; M)$ from formulas 2 and 3. Thus, to summarize, for each parent mass M one constructs a model distribution ν and obtains the "difference" between this distribution and the actual data. This difference, plotted as a function of M , is the deconvoluted spectrum. The characterization applies to both the entropy and Mann et al. [5] deconvolution methods, but each with their respective notions of difference.

The deconvolution approach developed by Mann et al. [5] can be shown to be a specific case of the least square function corresponding to the choice of a parent ion profile that is concentrated at a single mass in the generation of the model measure. One also selects the amplitudes of the different charge states in the model to be the same. From eq 3 we have the following:

$$LS(M) = \sum_{i=1}^N \nu_i^2 + \rho_i^2 - 2 \sum_{i=1}^N \nu_i \rho_i \quad (5)$$

The first two terms in eq 5 can be ignored because they are constant and independent of M ; the cross term $\nu_i \rho_i$ simply selects the data point in ρ at the mass value $m_e = M/j + m_a$ for $j \in A$. If we now write this as a sum-over-charge states instead of writing it as a sum over the mass range, one recovers what is essentially the deconvolution expression of Mann et al. [5]:

$$LS(M) = C_0 + C_1 \sum_{i \in A} \rho[M/i + m_a] \quad (6)$$

Of course, Mann et al. [5] did not present this expression as a least square approximation. Since the ion current corresponding to a parent ion is distributed over the different charge states, it is clear that one could invert this spreading by summing the observed intensity at the points determined by the relation 4 to obtain the total ion current corresponding to a given parent mass M . This is directly the sum in 6 and the physical motivation for the expression is obvious.

To illustrate how artifacts arise in the least-square deconvolution, and their suppression in the entropy deconvolution, consider the following hypothetical application. Here we construct an electrospray spectrum of two multiply charged components each carrying seven charge states, +8, +9, ..., +14, and four singly charged components. Assume the parent masses of the multiply charged components at 15,000 and 14,700 μ and the masses of the singly charged impurities are 1510, 1480, 1460, and 1300 μ . A representative spectrum of this sample is given in Figure 1.

This mass spectrum, when normalized, is represented by ρ in our equations. We now construct the model distribution ν , corresponding to a single parent ion of mass M with a charge set $A = \{+7, \dots, +14\}$, according to the description given previously. However, to calculate the relative entropy by eq 2, it is necessary to set a large but finite value to the divergent expression

$$\nu_i \log \left(\frac{\nu_i}{\rho_i} \right)$$

when ν_i is nonzero and ρ_i is zero. This situation arises when the model distribution for a given parent mass M predicts a peak which the data do not support. (Technically, for the relative entropy to be defined, ν_i must be absolutely continuous with respect to ρ_i , i.e., ν_i must be zero whenever ρ_i is zero.) In setting this to a large but finite value one decreases the overall probability of the model distribution, for this value of M , being a fluctuation of ρ , i.e., one decreases the likelihood of a multiply charged ion of mass M being present in the data.

Having set a parameter describing a "zero cost," one can construct the M -dependent least-square and entropy plots given by eqs 2a and 3 (Figure 2). Now focus on two values of the parent mass, 14,700 and 14,600, which are peak centers in the least-square deconvolution. One, 14,600, is an artifact value, which appears because a single peak in the model distribution for the parent mass 14,600, corresponding to the charge state +10, overlaps the 1460 peak in ρ . The other least-square peak, 14,700, is a true parent mass as all the peaks in ν overlap peaks in the data. Because of the large entropy cost of a miss, the expression 2 (for the parent mass 14,600 associated with a single overlapping peak), is large relative to the parent mass with all of the charge states overlapping

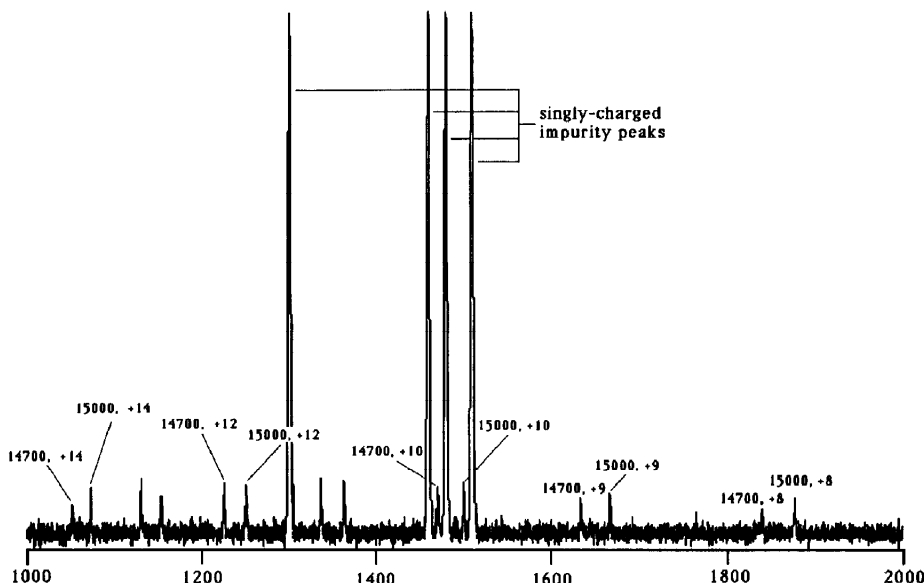


Figure 1. Sum of 10 computer-generated scans: four singly charged components at 1300, 1460, 1480, and 1510 and two multiply charged components at 14,700 and 15,000 with charge states +8, +9, ..., +14.

nonzero values in the data. The application of eq 2a then results in very small values for parent masses with single or no hits compared to parents with multiple hits. Thus, the entropy function effectively serves as a pattern filter for multiple charging. It is the discrimination between single and multiple overlaps that are not handled effectively in the least-square deconvolution algorithm. The relative magnitude of such artifact peaks depends on the intensity of the single peak overlapped compared to the sum of the intensities of the peaks overlapped by the true parent mass. In many analytical settings one has multiple components and the inability to discriminate between artifacts and multiply charged ions over a wide range of intensities has complicated the use of the sum deconvolution technique. These points are illustrated by the model spectra and deconvolution plots in Figure 2.

A couple of important observations should be made. The first concerns the nonlinearity of the entropy processing. This arises because of the logarithmic function in the relative entropy expression (eq 2). The point is of some importance because it introduces the possibility that n independent scans should not always be summed to obtain an average spectrum which is then processed. Letting the superscript indicate the scan number associated with the normalized spectrum, we have the data set as $\{p^j\}$, $j = 1, \dots, n$. The average of this data set is the distribution $\bar{\rho}$ given

by

$$\bar{\rho} = \frac{1}{n} \sum_{j=1}^n \rho^j. \quad (7)$$

If we consider the relative entropy for the set $\{\rho^j\}$, we have

$$-I_{\rho}^n(\nu) = \sum_{j=1}^n I_{\rho^j}(\nu) = \sum_{i,j} \nu_i \log \left(\frac{\nu_i}{\rho_i^j} \right) \quad (8)$$

which is different from the relative entropy (eq 2) for the distribution $\bar{\rho}$ since the sum over scans is outside the log in eq 8, while using eq 7 for $\bar{\rho}$ would place the sum over scans inside the log in eq 2. For the sum deconvolution there is no substantial difference between calculating the least-square difference with the averaged spectrum or averaging the least-square differences for each scan. This is an immediate consequence of eq 5 since the only important M dependence is in the cross terms $\rho_i \nu_i$ and this is linear in the ρ_i .

The formal difference between entropy processing with a set of scans or processing with a single averaged scan is easy to recognize; however, it can be a difficult problem to decide which method to use in practice. The question is complicated by various ancillary processing options related to background suppression, the noise level, and the zero-cost parameter. In the computer simulations (where the total ion count

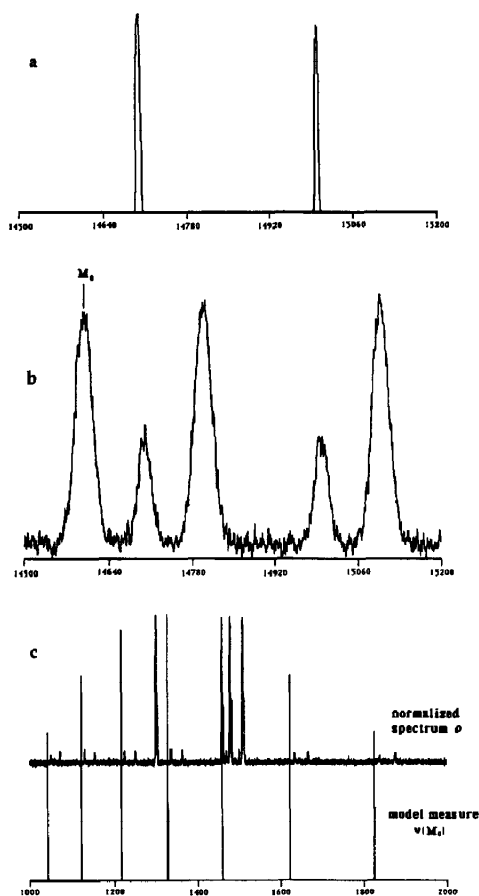


Figure 2. (a) Entropy deconvolution of 10 scans of computer-generated spectrum shown in Figure 1. (b) Mann's sum or least-square deconvolution of same spectrum. (c) Plot of model distribution and mass spectrum showing accidental overlap producing artifact peak in sum deconvolution.

is not too low), the entropy fit to a set of scans, i.e., formula 8, gives improved results. This is due to the accumulation of nonzero values from the noise in the averaged or summed scan. One may think of the application of eq 8 as almost a Boolean "and" operation over all the charge states and over all the independent scans: a peak is detected if it is present with charge state j_1 in scan n_1 and in scan n_2 and so on, and if it is present with charge state j_m in scan n_1 and in scan n_2 and so on. In practical situations the degree to which independent scans are summed into sets of averaged scans will depend on the nature of the data and further processing options.

The relative peak amplitudes in the entropy deconvolution method do not correspond to ion abundance,

although in a qualitative sense more intense ion peaks generally produce stronger entropy peaks. Hence, to obtain a measure of parent ion abundance the sum-deconvoluted spectrum must be used. Thus, in practice, the entropy method is best suited to identify the multiply charged ions and the least-square method to gauge their abundance.

As was discussed above, there are a number of parameters in addition to the parent mass that enter into the entropy deconvolution through the model distribution v . The main point to emphasize is that the best set of parameter values do not necessarily lead to the largest absolute entropy value. What is most significant is the relative difference in entropies between "real" and "artifact" peaks. For example, suppose we have a spectrum with both a range of multiply charged peaks due to a single parent species and a number of singly charged background peaks. A model distribution composed of just two charge states will have a good entropy fit at the correct parent mass since the data supports this pattern quite well. However, there is also a good probability of spurious hits, or artifacts, since any two locations in the mass spectrum could accidentally overlap nearby peaks. Increasing the number of charge states in the model distribution will generally decrease the absolute entropy value for any correct parent mass but by a much greater amount at artifactual parent masses. At the correct parent mass the decrease is due to the increasing detail in the pattern we are seeking (i.e., it is more unlikely that the Gaussian peak shapes and amplitudes of all three peaks would be as closely matched in the data as it was for two peaks). For artifact masses (i.e., masses where the two-charge-state model overlapped peaks in the data), it becomes increasingly likely that one of the correlated peak positions demanded by the three-charge-state model would be absent hence the entropy of the pattern in the data for the artifact mass would decrease by a much greater margin.

These comments also apply when one generates a model distribution with a greater number of charge states than the data supports. This would result in a "miss" due to the unsupported charge state (i.e., a peak in the model and not in the data), and the suggestion has been made that the heavy entropy cost of a miss would prevent detection. This reasoning, however, misses the problem. As detection is based on plotting the entropy as a function of the parameter M (with the other parameters defining the model being fixed), the entropy cost relative to a model with the correct number of charge states is not the relevant quantity. The situation is better represented by the following question: if we have N peaks in the data and we are looking for $N + 1$ peaks from the model are there other (artifact) masses that will have as many (accidental) hits?

In general the question of suboptimal parameters hinges on the relative magnitudes of the entropy over

the parent mass range being investigated and this relates to specific and variable features of the data. The computation time required to generate a single entropy plot is insignificant; in practice we have found the optimization of the model parameters is not a difficult part of the deconvolution analysis. However, this assessment reflects features of the software environment that allow effective interaction with and updating of the parameters.

Application

The entropy and the least-square algorithms have been applied to synthetic spectra (computer generated) and ESI spectra obtained on quadrupole instruments. Synthetic spectra can be of considerable utility in evaluating the various deconvolution routines due to the precise quantitation of spectral features they allow. By application to numerical spectra purely statistical properties of the algorithms can be illustrated. This requires that the statistical properties of the computer-generated spectra reflect the stochastic properties of the data.

The computer spectra were generated by a numerical simulation of a Poisson process in which a parameter representing the expected number of counts for each mass value m_i is defined. Independent scans are obtained by sampling the Poisson distribution corresponding to this parameter [7]. In these simulations noise can arise in two ways. The first is the relative fraction of counts that are real ions to counts that are representing background. From the standpoint of the simulation program they are distinguished only because the background has a mass-independent arrival rate while the signal corresponds to mass-dependent rates which are modeled as multiply charged Gaussian ion profiles. The other noise is shot noise corresponding to the finite ion counts. Of course, background peaks or chemical "noise" are, from the standpoint of the simulation, just additional real peaks.

These numerical simulations have exposed several important observations. As an example, Figure 2 illustrates a major point of this report by processing computer-generated scans from both deconvolution methods and contrasting the results. For low "white" noise levels, taking independent scans is best for reasons mentioned above. However, as the noise level increases, every scan acquires nonzero counts off the ion peaks and it is better to average scans, define a smoother baseline and subtract. Such large white noise levels (i.e., mass independent) would be unexpected in actual data.

Background peaks or chemical noise are a major complication if the molecular species of interest exhibits only a few charge states. To offset this complication it is possible to increase the number of charge states by chemical modification of the analyte. Alternatively, one may increase resolution mathematically

(Fourier techniques) which, in effect, narrows the ion peaks and decreases the possibility of accidental overlap. Application of these Fourier techniques (Fourier deconvolution and Fourier differentiation) are quite effective, and because they are well established and independent of the main point of this article, we will leave further discussion for Figure 3.

The more demanding complication of the entropy processing of ESI spectra is the presence of large, unresolved backgrounds on which many individual peaks are superimposed. In working with these spectra, we generally employ a combination of Fourier deconvolution and baseline subtraction. Such steps can be managed as a prefilter to the entropy deconvolution since the optimization (Fourier cutoff, exponential factor) does not require knowledge of the entropy fit parameters. Figure 4 illustrates the application of these strategies to an ESI mass spectrum of a glycopeptide with two glycosylation sites.

Summary

This report has discussed a novel algorithm for extracting parent masses from spectra containing multiply charged ions, a common feature of ESI. The algorithm works with raw data and does not require the generation of a peak table, and is thus less sensitive to errors introduced by overlapping peaks and other problems associated with peak assignment. As we have discussed in this report, it is less prone to artifacts than the deconvolution method of Mann et al. [5]. Preliminary results suggest this approach to be most effective in analyzing samples of increasing complexity.

Appendix

The first objective is to consider a mass spectrum as a probability distribution on a finite outcome set. In a stochastic interpretation of the ion arrival process, the set $\Gamma = \{m_1, \dots, m_N\}$ is the set of possible outcomes of a random event which, at this level, is simply the detection of an ion within the mass range of the experiment. Counting the number of events of each type m_1, \dots, m_N for a fixed integration time generates the mass spectrum $\{l(m_1), \dots, l(m_N)\}$, where $l(m_i)$ is the intensity or number of counts for the mass value m_i . A probability distribution can be constructed from the mass spectrum by normalization, i.e., by dividing each intensity $l(m_i)$ by the sum of the intensities over the mass range. In this formulation a single mass spectrum represents a large number of distinct random events.

One can also formulate a probability model on Γ in which the mass spectrum corresponds to a single random event. This is a "level-2" formulation of the same process where one selects a probability distribution on Γ and not just elements in Γ . For the set Γ to

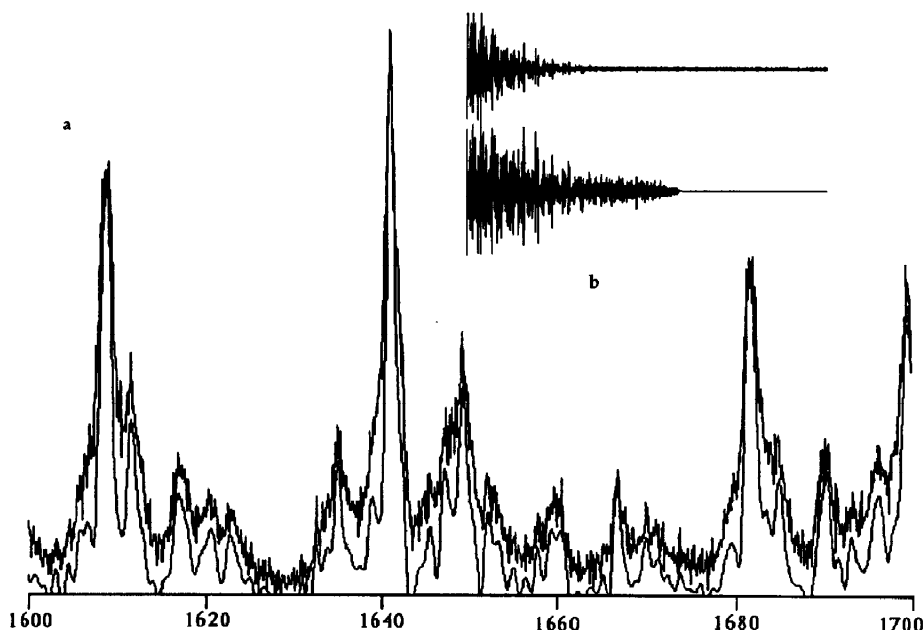


Figure 3. (a) Fourier processing a mass spectrum from 1600 to 1700. The top trace is the raw mass spectrum while the lower trace is the Fourier deconvoluted spectrum illustrating the mathematically enhanced resolution. The smoothing is due to the Fourier cutoff defined in the deconvolution procedure. (b) The Fourier spectrum of the original mass spectrum and the Fourier deconvoluted spectrum. The deconvoluted spectrum has both a frequency cutoff and an amplification of the middle frequencies relative to the lower frequencies.

be distributed by the vector ν means that the probability of the event m_i in a random trial is ν_i . Recall that we defined all vectors ν satisfying these constraints as $P(\Gamma)$, the set of probability distributions on Γ . In a level-2 formulation of the ion arrival process the outcome set is $P(\Gamma)$ and the probabilistic event is selecting a random vector ν in $P(\Gamma)$. Entropy, or more precisely, the relative or level-2 entropy is related to the convergence of random distributions in $P(\Gamma)$. This relationship can be illustrated by the following problem. Assume the set Γ is distributed by a vector ρ in $P(\Gamma)$ and suppose one wishes to determine the probability that an empirical counting of events will produce a distribution ν that is different from ρ . The random process would be to select n elements from the set Γ where the probability of selecting the element m_i is ρ_i . A vector ν is then constructed by setting each component equal to the relative frequency by which m_i was selected, i.e.,

$$\nu_i = \frac{\text{number of times } m_i \text{ was selected}}{n}$$

Now focus on the resulting distribution of ν s in $P(\Gamma)$. As the underlying distribution on Γ is ρ , we know

that ν will converge to ρ as n goes to infinity. The probability of obtaining a value of ν different from ρ is a statement about the rate at which the distribution ν (now a random variable) converges to ρ in $P(\Gamma)$. An entropy function, explicitly the relative entropy, determines this rate. Different entropies arise in characterizing the rates at which different random quantities converge to their asymptotic values [6]. In this problem the asymptotic rate of convergence of the empirical distribution ν to the underlying distribution ρ is exponential with an argument given by:

$$-nI_{\rho}^2(\nu) \quad (n = \text{number of samples})$$

In this expression $I_{\rho}^2(\nu)$ is a level-2 entropy function or relative entropy given by formula 2 for the case of the finite state space Γ [6].

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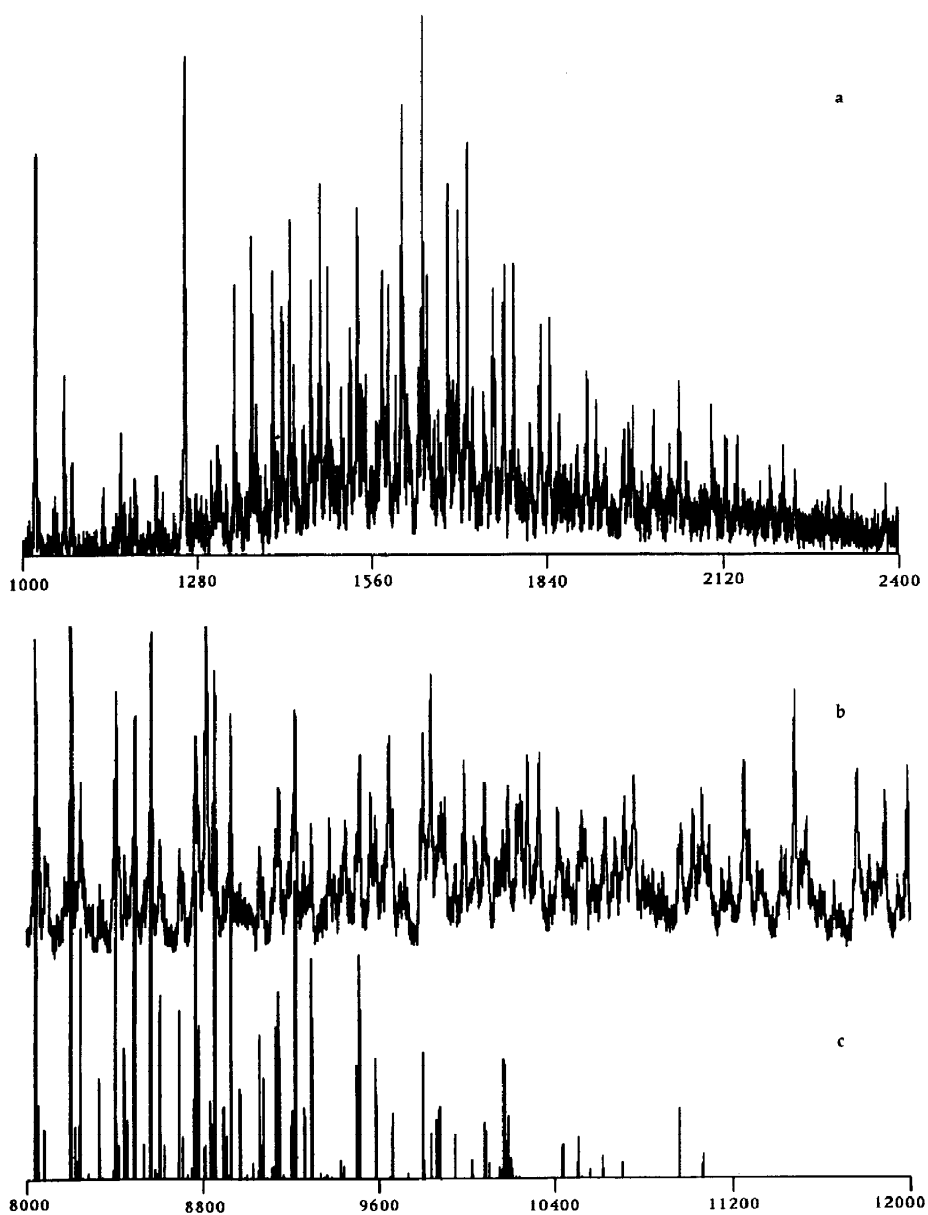


Figure 4. (a) Electro spray mass spectrum of a glycopeptide with two glycosylation sites. (b) Sum or least-square deconvolution of Mann et al. [5]. The artifact peaks above m/z 10,000 dominate the spectrum. (c) The entropy-based deconvolution of the same spectrum. (d) An expanded view of both deconvolutions in the region 8650-9000. The top trace is the sum deconvolution and the bottom trace is the entropy deconvolution. The large peak at 8816 in the least-square deconvolution is an artifact due to an intense peak in the original mass spectrum.

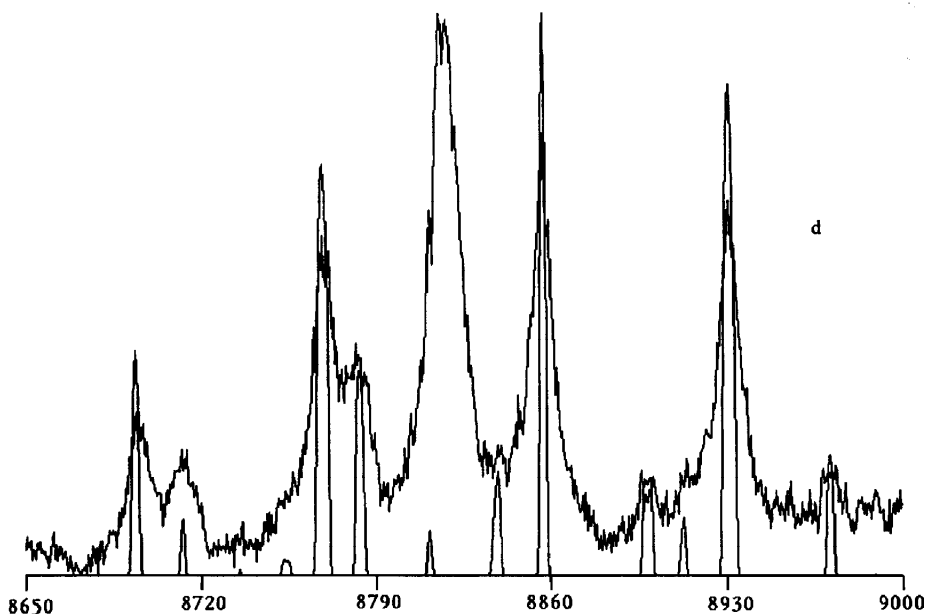


Figure 4. (Continued)

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