

pMod – An Advanced Protein Deconvolution Algorithm with Automated Peak Modeling for Charge Deconvolution of Mass Spectrometry Data

Application Note

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Introduction

The benefits of the widely-used maximum entropy deconvolution algorithm include transforming a multiple-charged spectrum into a zero charged mass spectrum and significantly reducing the complexity of the results. However, this method can introduce many artifacts. To improve the deconvolution algorithm and deliver the most probable results that are virtually free of artifacts, Agilent Technologies has developed the peak modeling deconvolution (pMod) algorithm, an advanced data processing methodology. This application note describes the pMod method, compares it to the maximum entropy deconvolution methodology, discusses precision of zero-charge mass measurement, and shows enhanced resolution of protein peaks for precise analysis.



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The pMod algorithm

Maximum entropy charge deconvolution is a well-known and powerful data analysis algorithm for determining the neutral mass for intact proteins using electrospray mass spectra¹. This method transforms an m/z raw spectrum of one or more intact proteins into the most probable zero-charge mass spectrum (in Dalton units). Maximum entropy deconvolution works reliably for low complexity (low number of modifications) protein data or a relatively simple protein mixture.

The pMod deconvolution algorithm starts with maximum entropy deconvolution. Based on the maximum entropy result, pMod automatically generates mass spectra peak models without manual intervention and applies these models through fitting and validating procedures. The peak modeling method also applies physical rules so that only masses consistent with the experiment rules are reported. Spectral data that does not fit the model is rejected as noise. Therefore, the pMod result is much cleaner than the maximum entropy deconvolution result and produces fewer artifacts. Using this process pMod produces a highly resolved zero-charge spectrum and a set of mass precision assessments for each peak.

Results obtained using maximum entropy deconvolution and pMod deconvolution

Maximum entropy deconvolution results show (Figure 1A) improved mass resolution compared to the raw data, especially for the less resolved small features. Limitations in this method, however, arise in two instances: 1) when harmonics and artifacts are generated in deconvoluted results, and 2) when protein peaks are not well defined due to the limited resolution or low signal-to-noise (S/N) ratio. Figure 1B shows the peak modeling deconvolution result demonstrating these characteristics of deconvolution. The pMod result has enhanced resolution and overlapped peaks are well-resolved providing higher confidence for protein confirmation.

The precision of zero charge mass measurement

pMod deconvolution also assesses the mass measurement precision from each charge state in the raw data and calculates the standard deviation of the zero-charge mass. This value is called uncertainty in pMod. The uncertainty value can be used as the peak width for the pMod deconvoluted peaks. As a result, the peak width of the deconvoluted peak presented no longer reflects the isotope width of the protein molecule. A narrow peak indicates higher confidence of the protein at this mass. A broad peak indicates lower confidence of the protein at this mass (Figure 1).

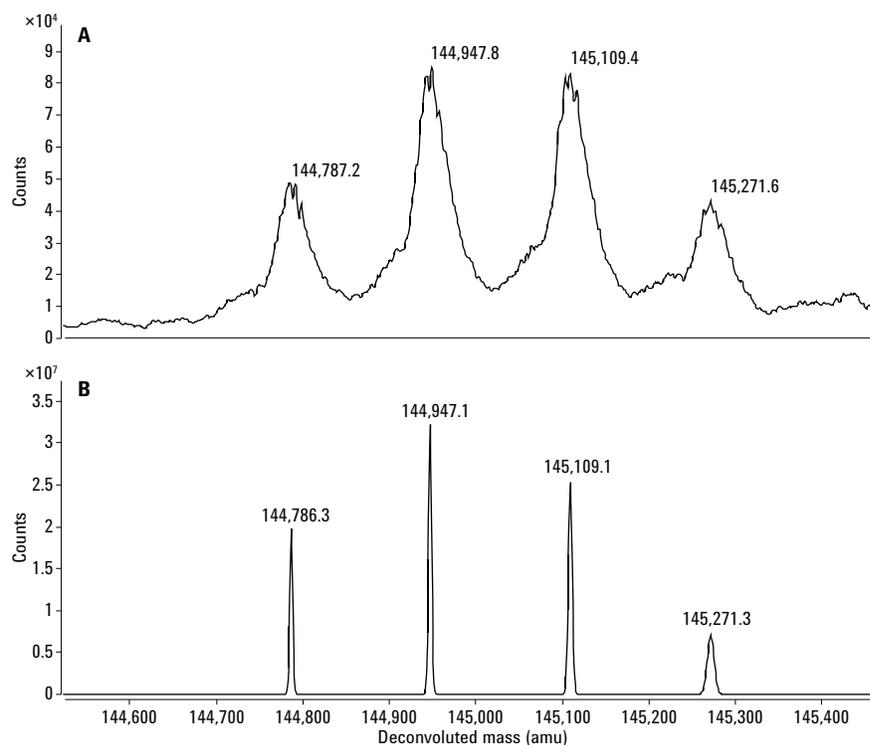


Figure 1. Deconvoluted spectra of 100 ng IgG1 acquired on an ESI-Q-TOF instrument. A) Maximum entropy deconvoluted spectrum. B) Peak modeling deconvoluted spectrum.

Table 1 shows the mass measurement standard deviation (uncertainty) of a mAb zero charge mass at different concentrations using an Agilent 6550 Q-TOF LC/MS. Less than 2.3 Da mass precision was achieved from 10 ng to 5,000 ng mAb. Mass precision reflects the distribution of the mass measurement in the analysis.

The ability of pMod to resolve overlapped spectral peaks

In the sample IgG1 and IgG2 mixture, peaks with masses at 144,784 and 144,823 are 39 Da apart and not well resolved by maximum entropy deconvolution. The mass peaks are much narrower and exhibit better resolution

after pMod has been applied. pMod results have improved S/N ratios over maximum entropy. In addition, the width of the peaks provides the precision of the mass measurement for that protein. Overlapped peaks from IgG1 and IgG2 are well resolved from each other and can be identified as separate proteins.

Table 1. Average mass measurement precision (Da) of mAb at different concentrations. Five replicates at each concentration.

	G0F+G0F	G0F+G1F	G1F+G1F	G1F+G2F	G2F+G2F
5,000 ng	0.7	0.5	0.4	0.8	2.3
1,000 ng	0.7	0.5	0.4	0.7	0.9
500 ng	0.8	0.5	0.5	0.4	0.8
100 ng	0.6	0.5	0.5	0.6	1.3
10 ng	1.0	0.8	0.8	N/A	N/A

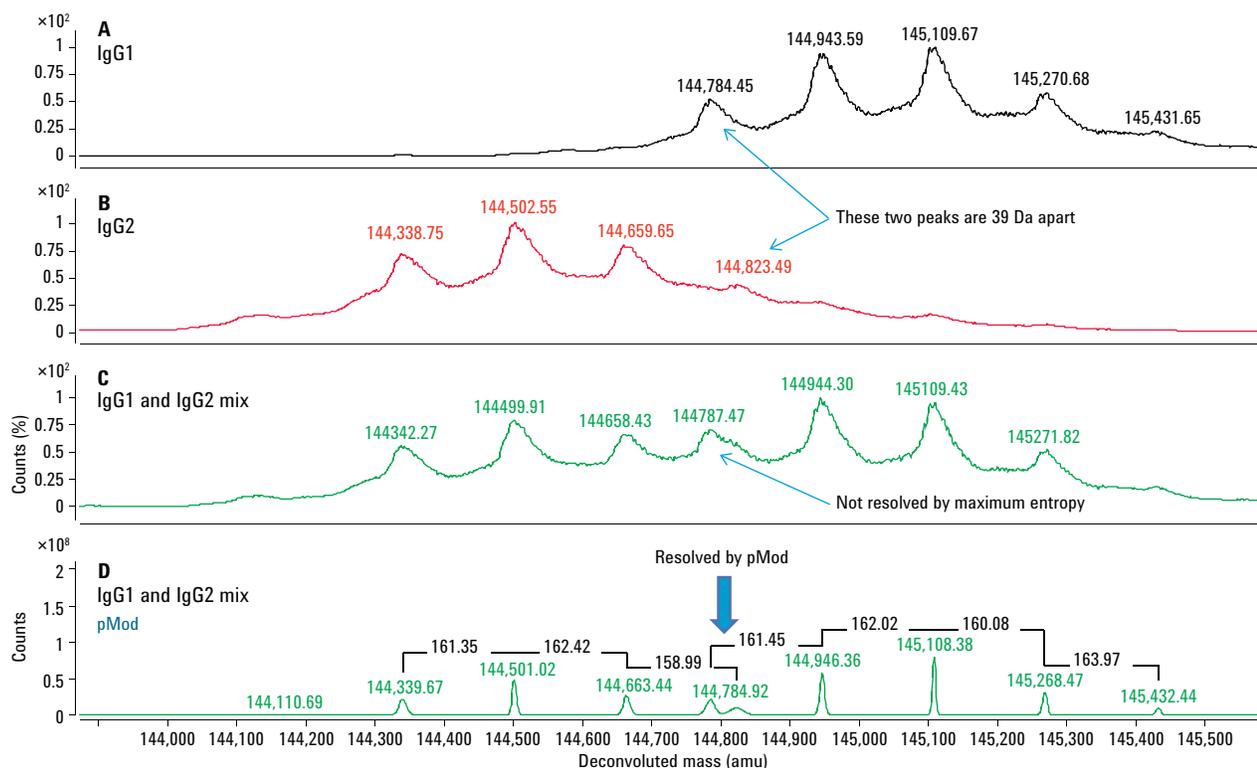


Figure 2. A) Maximum entropy deconvoluted spectrum of IgG1. B) Maximum entropy deconvoluted spectrum of IgG2. C) Maximum entropy deconvoluted spectrum of mixture of IgG1 and IgG2. D) Peak modeling deconvoluted spectrum of IgG1 and IgG2 mixture.

Relative quantitation using pMod

We collected IgG1 data over a wide range of concentrations, ranging from 5,000 ng/ μ L to 5 ng/ μ L, with five replicates for each concentration. The raw data was deconvoluted using the peak modeling deconvolution method.

Figure 3 shows the deconvoluted spectra at different concentrations. Figure 4 shows the calibration curves for the top four deconvoluted peaks at 144,786, 144,947, 145,109, and 145,270 Da. Linear dynamic range with $R^2 > 0.99$ was achieved between 10 ng to 1,000 ng.

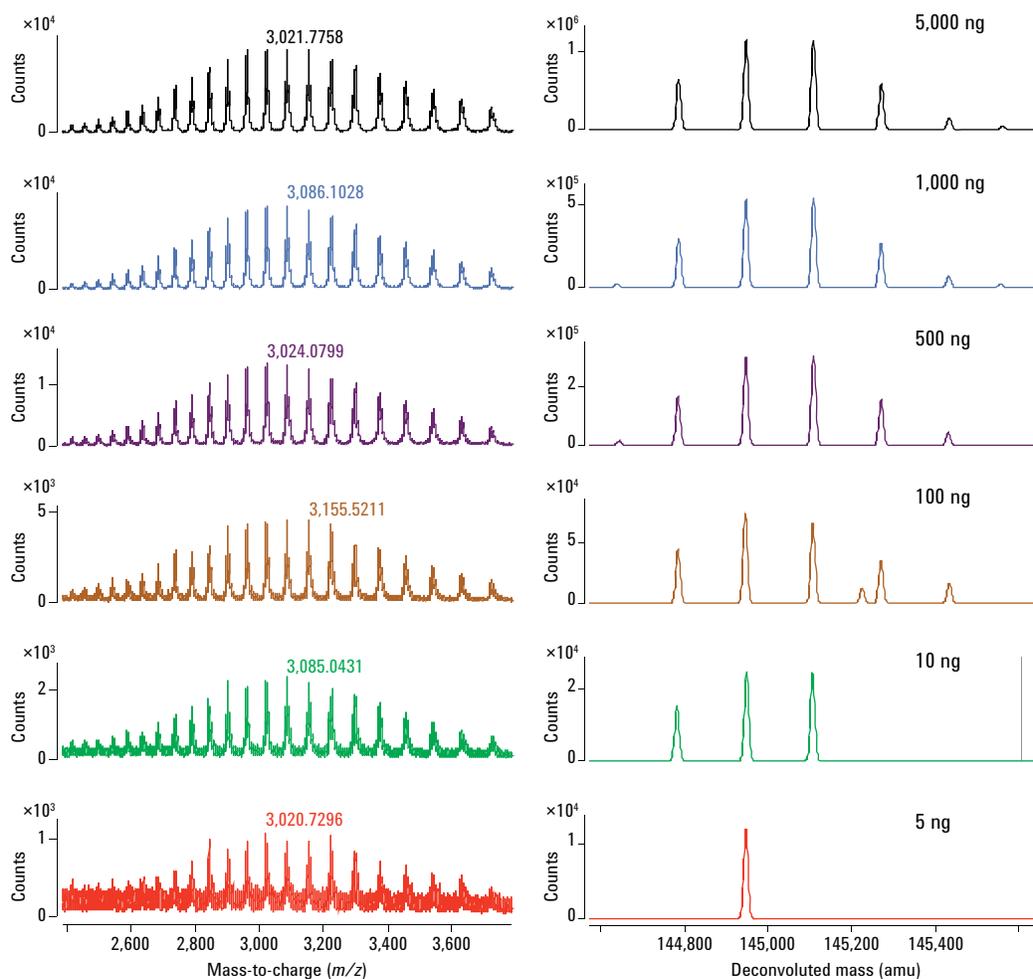


Figure 3. Raw data and peak modeling deconvoluted spectra of IgG1 at different concentrations. From top to bottom are spectra for concentrations 5,000, 1,000, 500, 100, 10, and 5 ng/ μ L.

Conclusions

- Peak modeling deconvolution provides deconvolution results that present few artifacts. The obtained results have improved S/N ratios and report the standard deviation of the mass measurement.
- The peak modeling deconvolution result has an enhanced resolution result in overlapped peaks frequently being well resolved. This has enabled the differentiation of small modifications from the main heterogeneous glycoprotein profile with much greater clarity.
- Peak modeling deconvolution results have been shown to be useful for relative quantitative analysis in determining the relative concentration of protein isoforms.

Reference

1. A. G. Ferrige, M. J. Seddon, B. N. Green, S. A. Jarvis, J. Skilling, and J. Staunton, Disentangling electrospray spectra with maximum entropy, *Rapid Communications in Mass Spectrometry*, Volume 6, Issue 11, Pages 707-711 (1992).

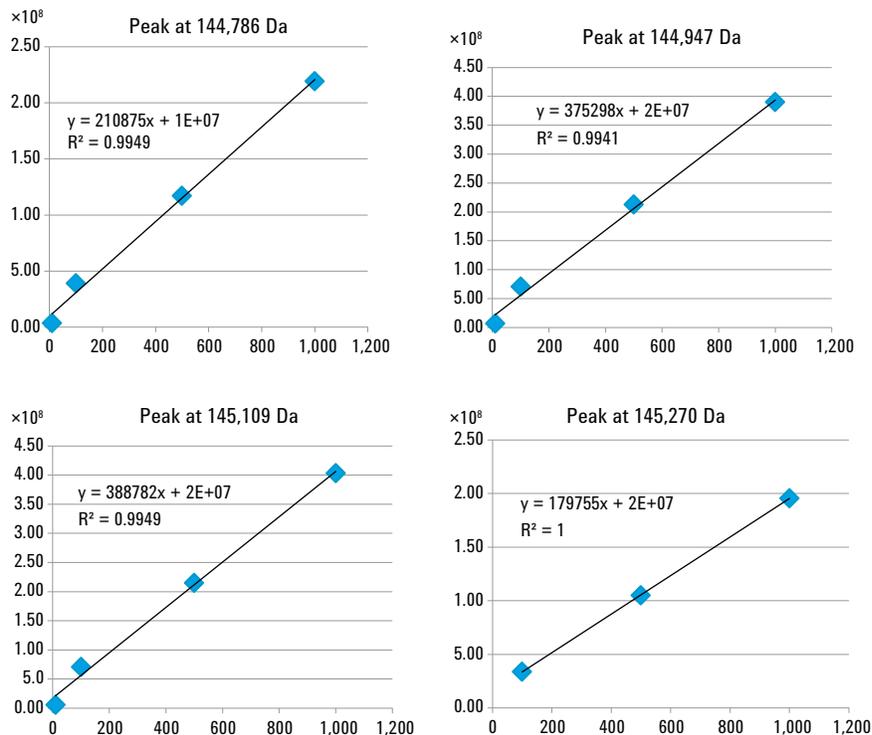


Figure 4. Calibration curves for the top four deconvoluted peaks. From top left, clockwise, are calibration curves for peaks at 144,786, 144,947, 145,109 and 145,270 Da.

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