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Identification of Two Post-Translational Modifications via Tandem Mass Spectrometry

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Abstract

Post translational modifications (PTMs) play most important roles in the accomplishment of biological processes and molecular functions. Tandem mass spectrometry (MS/MS) has become one of the most promising methods for the detection and characterization of PTMs. Most MS/MS based researches for the detection of PTMs have achieved good performance by searching protein databases with short peptide sequence tags or by exhaustive searching on one PTM. It is challenging to identify two PTMs for a tandem mass spectrum. In this paper, we proposed a new algorithm to detect two PTMs with unknown types. First, we constructed a Pair of Peak Set (PPS) which is composed of pairs of peaks that have the highest sum of intensities. We used PPS instead of the whole experimental spectra to compare with the theoretical spectra from a peptide sequence database. Second, we revealed the relationship between PPS and the whole experimental spectrum. Third, a series of logic conditions was proposed to detect PTMs from a MS/MS spectrum. The conditions were used to filter a peptide sequence database and get candidate hits. Finally, we used a scoring function to rank the candidate hits. We applied the method to a large MS/MS dataset and the experimental results demonstrated that the proposed method achieved better performance of identifying any types of PTMs in a blind mode than current existing methods. In our future work, we will use this method to identify more than two PTMs from a MS/MS spectrum.

Keywords

tandem mass spectrum; post-translational modification (PTM); peptide sequence tags

1 Introduction

Protein post-translational modifications (PTMs) have been increasing the functional diversity of proteome. The modifications encompass phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, lipidation and proteolysis, and they

modulate the activities of most proteins in almost all aspects of normal cell biology and pathogenesis. Tandem mass spectrometry (MS/MS) is a feasible technique for detecting and characterizing unknown protein structural changes especially PTMs. However, with the incomplete human knowledge about the PTMs and biological measurement techniques, the identification of PTMs is a still challenging task especially for the mass spectra that contain many noisy peaks.

Currently, there are two major methods for PTM identification. One uses the alignment approach to find the best match between the candidate and the identified sites. The methods include Sequest (Jimmy K. Eng, 1994), and Mascot (Perkins et al., 1999). The other is to obtain the peptide sequence without retrieving the peptide sequence database, and the major advantage of this method is that it can find some unknown sequences. But this method is sensitive to the quality of the experimental data. If a mass spectrum contains much noise, the recognition rate will drop down sharply. The major disadvantage of this method is that in most cases, it can't find the whole sequence. Han et al (Han et al., 2005) propose a dynamic programming algorithm for an experimental spectrum. They use a heuristic method to get the peptide sequence which depends on the quality of the mass spectrum like Searle et al., (Searle et al., 2004). Due to the missing peaks and noises which are included in a MS/MS spectrum, some approaches select peptide sequence tags as a query filter to search a peptide database. Liu, et al. (Liu et al., 2006) construct a spectrum graph to find the longest path in a MS/MS spectrum. Some of previous approaches like Yates et al (Yates et al., 1995) use an exhaustive search that analyzes every possible PTM position for each peptide from the database. The major drawback of this search method is that it can only take into account a few modifications and is very time consuming.

Recently, a novel method for blind identification of Post Translational Modifications (PTMs) was proposed; which can detect all types of PTMs without knowing which PTMs exist in a sample. The first blind approach to PTM identification (spectral alignment) was proposed by Pevzner et al. (Pevzner et al., 2000; Pevzner et al., 2001). Searle et al. 2004 (OpenSea) (Searle et al., 2004) and Han et al. (Han et al., 2005) (SPIDER) proposed another approach of blind PTM identification. The identification of all types of PTMs is a challenging task, especially the identification for a MS/MS spectrum with multiple PTMs.

It is not applicable to use an exhaustive searching method that analyzes each possible PTM site in the protein due to huge time consuming. InsPecT (Tanner et al., 2005) generates limited candidates to speed up the search and uses a scoring function to rank the hits. In order to find the best way to identify the PTMs, we focus on candidate generation and the scoring of all found hits.

In this paper we present a novel algorithm to gain the PTMs candidate set and scoring the hits by analyzing the feature of multiple modifications. We can identify PTMs and get better accuracy and efficiency than other tools. Firstly, we construct a Pair Peak Set (PPS) out of a MS/MS spectrum that reflects the real peak values corresponding to the MS/MS spectrum. Secondly, we demonstrate a relationship between the PPS and the experimental data in our dataset. Lastly, we introduce a new approach to identify PTMs using the PPS.

2 The method

2.1 Quartet weights and the global quartet weight matrix

Assuming a modification Δ of the peptide P corresponds to position i , we can define a theoretical spectrum of any modified peptide. Preliminary definitions are as follows:

1. Let $A = \{a_1, \dots, a_{20}\}$ be the set of amino acids.

2. A peptide $P = p_1 \dots p_n$ is a sequence of amino acids, with peak value $p_{vi} = \text{mass}(p_i)$. For an experimental spectrum S , $\text{PM}(S)$ is the set of peak values of the spectrum, where n is the length of the amino acid sequence.
3. The parent value for the experimental spectrum is $\text{Exp}P$, and the parent value for the theoretical peptide is $\text{The}P$.
4. The $b\text{Set}$ contains all the b ions in the peptide and the $y\text{Set}$ contains all the y ions in the peptide.
5. Δk is the value of the k th modification of the peptide; the position of the modification for the $b\text{Set}$ is set as $b\text{Start}k$ and the position of the modification for the $y\text{Set}$ is set as $y\text{start}k$.

For example, for the peptide sequence AsDFGKSTmD, the lower character means there is a modification, where $n=11$; the number of modifications $k=2$. The formula is as follows:

$$b\text{start}k = n - y\text{start}k \quad (1)$$

$$\text{Exp}_p - \text{The}_p = \Delta 1 + \Delta 2 \quad (2)$$

We assume that if two PTMs occur in a peptide, the first modification is $\Delta 1$ and the position is $b\text{start}1$. The second modification is $\Delta 2$ and the position is $b\text{start}2$.

There are four kinds of peak values in a spectrum with two PTMs, which are (peak) , $(\text{peak} + \Delta 1)$, $(\text{peak} + \Delta 2)$, and $(\text{peak} + \Delta 1 + \Delta 2)$ respectively. The positions of two modifications will determine the ratio of these four types of peak values. Since the positions of the two PTMs are random, the distribution for the four types of peak values is random. The number of peaks with type $(\text{peak} + \Delta 1)$ and the number of peaks with type $(\text{peak} + \Delta 2)$ are equal due to PTMs only occur on b or y ion. The distribution for them is shown in Figure 1.

2.2 Method description

We propose a new method for detecting $\Delta 1$ and $\Delta 2$. The key idea of our algorithm is to use the most possible PPS to represent the corresponding MS/MS spectra. With this model, the comparison of a theoretical mass spectrum and an experimental mass spectrum is turned into the comparison of the PPS and the experimental spectrum. Our method has the following steps:

1. Create a database: Note that a typical peptide sequence database very large. We create a smaller database that contains the peptides whose corresponding parent mass values are in the range of $[-160, 0.5]$ from the parent mass value of the experimental spectrum.
2. Construct the PPS: Let $\text{PPS} = \{(a_{11}, a_{12}), (a_{21}, a_{22}), \dots, (a_{m1}, a_{m2})\}$ be the set of pairs of peaks which include peak values that have the highest possibilities, where m is the number of PPS, and $\{a_{i1}, a_{i2}\}$ are complementary peak values. These pairs are selected from the corresponding experiment with constraining conditions given in this section.
3. State machine: We use a state machine to detect the exact PTM positions.
4. Candidate generation: We use a number of 'principles' between PPS and the experiment data to produce a list of hits against the dataset gained from step 1.

5. Scoring: We use Dynamic Time Warping (DTW) (Teddy Siu Fung Wong, 2003) alignment algorithm as the scoring function to rank and output the hits.

The key idea of this algorithm is to build a PPS for every experimental MS/MS spectrum. Let m be the size of the PPS. We observe that pairs with high mass intensities are more possible to be real peaks than the pairs that have low mass intensities. A PPS is represented as the following:

$$PPS = \{(a_{11}, a_{12}), (a_{21}, a_{22}) \dots (a_{m1}, a_{m2})\} \quad (3)$$

In a MS/MS spectrum P , let In_i be as the intensity of a peak and p_i is the peak value. Note that the size of the PPS is m , which is computed dynamically according to the length of the corresponding theoretical spectrum (Len). The shortest length of the experimental spectrum is $Ep/278$, where Ep is the parent mass value of the experimental spectrum and 278 is the maximum mass of an amino acid. The longest length of the experimental spectrum is $Ep/57$, where 57 is the minimum mass of an amino acid. The pairs should have maximum possibilities to be real peaks. We introduce $Len = (Maxlen + MinLen)/2$ as the number of peaks that are shifted by the PTMs. The pairs can be easily computed according to the following cases:

1. $m = Len/3$, m is the number of pairs in the PPS.
2. $Peakvalue[i] + Peakvalue[j] = Ep$, i and j are two complementary peaks;
3. $Pairvalue = Intensity[i] * Intensity[j]$. We use Pairvalue to rank the pairs and add top m pairs of peak values into the PPS.

We use its PPS instead of the whole experimental spectrum to align with the theoretical spectrum. The relationship between a PPS and the experimental spectrum is described as the following:

1. Each of the pairs is composed of a pair of complementary peak values.
2. The ratio between signal and noise in PPS is about 96%, while it is only 40% in the entire experimental spectrum.
3. The PPS we constructed reflects the feature distribution of the experimental spectrum.
4. The PPS can be viewed as a sampling from the signals of the experimental spectrum, which is shown as Figure 2.

The advantage of the PPS is as the following:

1. For tandem mass spectrometry, PPS $\{(s_1, s_2), \dots\}$ are likely to contain real peak values extracted from the experimental spectrum P . It effectively removes the noise out of P .
2. The PPS is equivalent to the compressing data of P ; it reduces the computing space and speeds up the search of the peptide database.

As shown in Figure 3, we use the PPS to compare with the theoretical spectrum. The difference for each pair is described by shifts $\Delta 1$, $\Delta 2$, and $\Delta 1 + \Delta 2$. The third column is the bset of the theoretical spectrum, while the fourth column is the yset of the theoretical spectrum. According to the above discussion, the distribution of the four types ($peak$, $(peak + \Delta 1)$, $(peak + \Delta 2)$, $(peak + \Delta 1 + \Delta 2)$) in an experimental spectrum can be classified into three categories without taking account into noise as follows:

1. If the distance of $bstart1$ and $bstart2$ is far away, most of peaks in the experimental spectrum fall into the categories $\{peak + \Delta 1, peak + \Delta 2\}$.
2. If the $bstart1$ and $bstart2$ are very close or equal, most of peaks in the experimental spectrum belong to $\{peak + \Delta 1 + \Delta 2, peak\}$.
3. If the distance of $bstart1$ and $bstart2$ are not very close and neither $bstart1$ nor $ystart1$ is in the start or the end of the experimental spectrum, the peaks have equal chance to fall into either one of the four kinds of peaks.

Because the PPS is a map of the whole experimental spectrum, the PPS reflect the exact distribution of the whole experimental data. We use three logic relations to map three distributions of the mass spectral data. Here, m is the number of pairs. $Np1plus2$ is the number of $\{\Delta 1 + \Delta 2, peak\}$ pairs, while $Np1or2$ is the number of $\{\Delta 1 + 0, \Delta 2 + 0\}$ pairs.

When we use the PPS to match the dataset, we will get $\Delta 1$ and $\Delta 2$. We take note that $\Delta 1$ and $\Delta 2$ will belong to b set or y set. Another rule is $\Delta 1 + \Delta 2$ should be equal to the difference between the parent mass of the experimental mass spectrum and the parent mass of the theoretical mass spectrum. According to the two rules above, we introduce the judge conditions as follows:

$$\left\{ \begin{array}{l} P_{i1} + \Delta 11, P_{i2} + \Delta 12, \text{ where } P_{i1} \text{ and } P_{i2} \text{ are a pair in the PPS, } \Delta 1 + \Delta 2 = EXPP - THEP, \\ \text{where } EXPP \text{ is the parent mass value of the experimental spectrum and } THEP \text{ is the} \\ \text{parent mass value of the theoretical spectrum.} \\ P_{j1} + \Delta 21, P_{j2} + \Delta 22 \\ P_{jk} + \Delta 21, P_{jk} + \Delta 22 \\ P_{jm} + \Delta 21, P_{jm} + \Delta 22 \\ \text{if } ((\Delta 11 = \Delta 22) \& \& (\Delta 12 = \Delta 21)) \\ \{ \\ \text{If } ((P_{i1} \in bset \& \& P_{j1} \in bset) || (P_{i1} \in yset \text{ and } P_{j1} \in yset)) \\ \{ \Delta 1 = \Delta 11; \Delta 2 = \Delta 12; \\ Np1or2 \text{ is increased by one} \end{array} \right.$$

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    }
  }
  if ( $\Delta 11 == \Delta 21$ ) and ( $\Delta 12 == \Delta 22$ )
  {
    If ( $(P_{i1} \in bset \ \& \ P_{j1} \in bset) || (P_{i1} \in yset \text{ and } P_{j1} \in yset)$ )
    {
       $\Delta 1 = \Delta 11$ ;  $\Delta 2 = \Delta 12$ ;
       $Np1or2$  is increased by one
    }
  }
}

The logic conditions for ( $\Delta 1 + \Delta 2$ ) are described as follows:
1.  $P_{i1} + 0, P_{i2} + \Delta 11 + \Delta 12$ ,
2.  $P_{j1} + 0, P_{j2} + \Delta 12 + \Delta 22$ ,
if ( $(\Delta 11 + \Delta 12) == (\Delta 21 + \Delta 22)$ )
{
  if ( $(\Delta 11 + \Delta 12) == EXPP\text{-}THEP$ )
  {
    If ( $(P_{i1} \in bset \ \& \ P_{j1} \in bset) || (P_{i1} \in yset \text{ and } P_{j1} \in yset)$ )
    {
       $\Delta 1 + \Delta 2 = \Delta 11 + \Delta 12$ ;
       $Np1plus2$  is increased by one
    }
  }
}

 $Np1plus2/m > 0.75$ , where  $m$  is the number of pairs in the PPS, means that most of the
signal pair is  $\{\Delta 1 + \Delta 2, 0\}$ .  $Np1or2/m > 0.75$  means most of the signal pairs are  $\{\Delta 1,$ 
 $\Delta 2\}$ . if  $Np1plus2/m < 0.25$ , this means the experimental spectrum doesn't contain two
PTMs.

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2.3 State machine for the positions of two PTMs

A correct result needs to recover the original peptide sequence, so the exact positions of modifications in experimental spectra need to be detected. We use a state machine to detect the positions of the modifications. We define *start1* as the first position of the modifications. If the second position *start2* occurs either on *bset* or *yset*, the shift will be $\Delta 1 + \Delta 2$ after *start2* as shown in Table 1.

In *bset*, the first modification $\Delta 1$ happens in the first start position, which is represented as *bstart1*. The second modification corresponds to a mass shift of $\Delta 1 + \Delta 2$, whose position is represented as *bstart2*. Similarly, for *yset*, the first modification is *ystart1* while the second position is *ystart2*.

2.4 Scoring function

Our approach consists of four steps, where the scoring candidates are as the follows. Here, we introduce a Dynamic Time Warping (DTW) (Teddy Siu Fung Wong, 2003) algorithm as our scoring function. The DTW distance between two mass spectra indicates the similarity of two mass spectra.

$$n/2 + 3/2 \leq m \leq 2n - 2 \quad (4)$$

$$3/2 - n/2 \leq (m - n) \leq n - 2 \quad (5)$$

The conditions restrict the active area of the alignment. For each i, j is chosen in the constrained area. A DTW distance [10] is computed by Equation (6):

$$DTW(i, j) = D(E_i, T_j) + \min \left\{ \begin{array}{l} DTW(i-1, j) \\ DTW(i-1, j-1) \\ DTW(i, j-1) \end{array} \right\} \quad (6)$$

$DTW(i, j)$ denotes the cumulative distance from $(1, 1)$ to (i, j) , thus the DTW distance of E and T is $DTW(m, n)$.

After choosing the PPS, we will get $\Delta 1$ and $\Delta 2$ for modifications and then recover the mass spectral data. The peak values which match the theoretical data will be put into the experimental fragment data set (*ExpDataSet*), and the theoretical data will be put into the theoretical fragment set (*TheoryDataSet*).

$Dis = DTW(ExpDataSet, TheoryDataSet)$;

We rank all identified PTMs based on the DTW values which are obtained by the fragment of peptides. The ranking result is shown in the result section.

3 Results

3.1 Datasets

We modify annotated spectra (by randomly shifting some peaks in two positions) in the datasets of 2657 yeast mass spectral data Yan et al's (Yan B, 2006) to simulate modifications. In addition, we apply our candidate generation method to construct a database for each MS/MS spectrum.

3.2 Results on two modification peptide

Here, the process of candidate generation is as follows:

1. **Input:** The input is a list of mass spectrum datasets which were artificially added two PTMs. And we generate a sub-dataset for each query mass spectrum whose parent value has offset 160 from the parent value of the theoretical dataset, then we put amino acids and mass peak values into a hash table which helps us convert the peptide sequence to peak values.
2. We calculate the shifts $\Delta 1$, $\Delta 2$, and $\Delta 1 + \Delta 2$ for each query mass spectrum and theoretical mass spectrum and then classify the query mass spectrum into three categories by the PPS logic conditions. We use the state machine discussed above to detect the start positions for each query experimental spectrum.
3. **Output:** We calculate the overlapped mass peaks between the query experimental mass spectrum and its theoretical mass spectrum and put them into candidate hit sets. We then employ a scoring function of the DTW algorithm to rank the candidates.

The results of the two PTMs are shown in Table 2. The rate of correct identifications seems high (39.9%).

3.3 Sensitivity and specificity analysis on PTMs

We use sensitivity and specificity to evaluate the performance of the method. Sensitivity is used to measure the probability that our method can match successfully peptides that contain two PTMs with the experimentally validated peptides containing two PTMs. Specificity is

used to evaluate the non PTMs recognized by our method while the peptides actually contain two PTMs.

If the value of a mass spectrum satisfies the condition of $Np1plus2/m < 0.25$ mentioned above, this mass spectrum contains two PTMs by our method. Based on the analysis above, the sensitivity of the test is 0.84 and the specificity is 0.79. The sensitivity and specificity show that our method has a good performance.

4 Conclusions

Recently, more and more approaches have been proposed for identifying PTMs using MS/MS. We propose a new approach to identify two PTMs from a large set of spectra.

We use the PPS instead of the entire experimental data to compare to the theoretical data. The PPS we introduce earlier has very high possibilities to be real peak values, so using it reduces the search space and it contains a very small number of noises. We use the logic relationship based on PPS to get candidate hits. We then use a state machine to detect the exact modification positions of PTMs. At last, we use our scoring function to rank the candidates hits.

In future work, we will provide further approach on more than two PTMS. Also, we will make our algorithm as mature tools for the identification of PTMs.

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Figure 1.
The distribution of four types of mass shifts.

Figure 2.
An example of a Pairs Peak Set (PPS).

Figure 3.
A PPS for an experimental spectrum against a theoretical spectrum.

Table 1

The canonical b-ion set and y-ion set of an example spectrum

b-ion set						
peaks	242.98	372.02	501.07	614.15	786.19	
ions	B2	B3	B4	B5	B7	
value	0	0	1	1	2	
position			bstart1		bstart2	
y-ion set						
peaks	146.94	261.97	318.9	432.1	819.2	
ions	Y1	Y2	Y3	Y4	Y7	
value	0	1	1	2	2	
position		ystart1		ystart2		

*The first row indicates the peak values; the second row marks the ion types. The third row indicates the marks for modification, where 1 refers to the shift value is Δ 1 and 2 refers to the shift value is Δ 2. The fourth row is for the start positions of PTMs.

Table 2

Search results obtained for the dataset that contain two PTMs. The table cells R_i are the percentages of the correct peptides that are reported as top i candidates. CPU is the average amount of time in seconds the programs take to analyze a spectrum (our computing was done on a Dell Power Edge 1950 server).

Data	Model	R1	R2	R3	R4	R5	Total	CPU s
2PTMs	PPS	0.39	0.41	0.43	0.44	0.46	0.46	3.17