

Data dependent acquisition with peptide identification probability estimation during comprehensive analysis

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1. Overview

Quantitative estimation of identification probability is proposed for the purpose of data dependent acquisition for MALDI-MS (matrix-assisted laser desorption/ionization mass spectrometry).

2. Introduction

Characteristics of MALDI-MS

- Widely used for structural analysis of peptides.
- Allows analysis of the same samples iteratively for detailed analysis.

Issues with MALDI-MS analysis

- Comprehensive manual analysis is time-consuming
- Sample exhaustion
 - Difficult to analyze all MSⁿ precursors

Data dependent acquisition strategy for MALDI-MS (iterative MS/MS acquisition)

- Identification probability modeling**
 - Derivation of estimation model from spectra of modeling samples
- MSⁿ analysis for real samples**
 - Select appropriate MSⁿ precursors and determine acquisition parameters (See Fig. 1)

Automatic & effective data dependent acquisition

- Automatic : Precursor selection and determination of other acquisition parameters
- Effective : Maximize the expected number of peptides identified

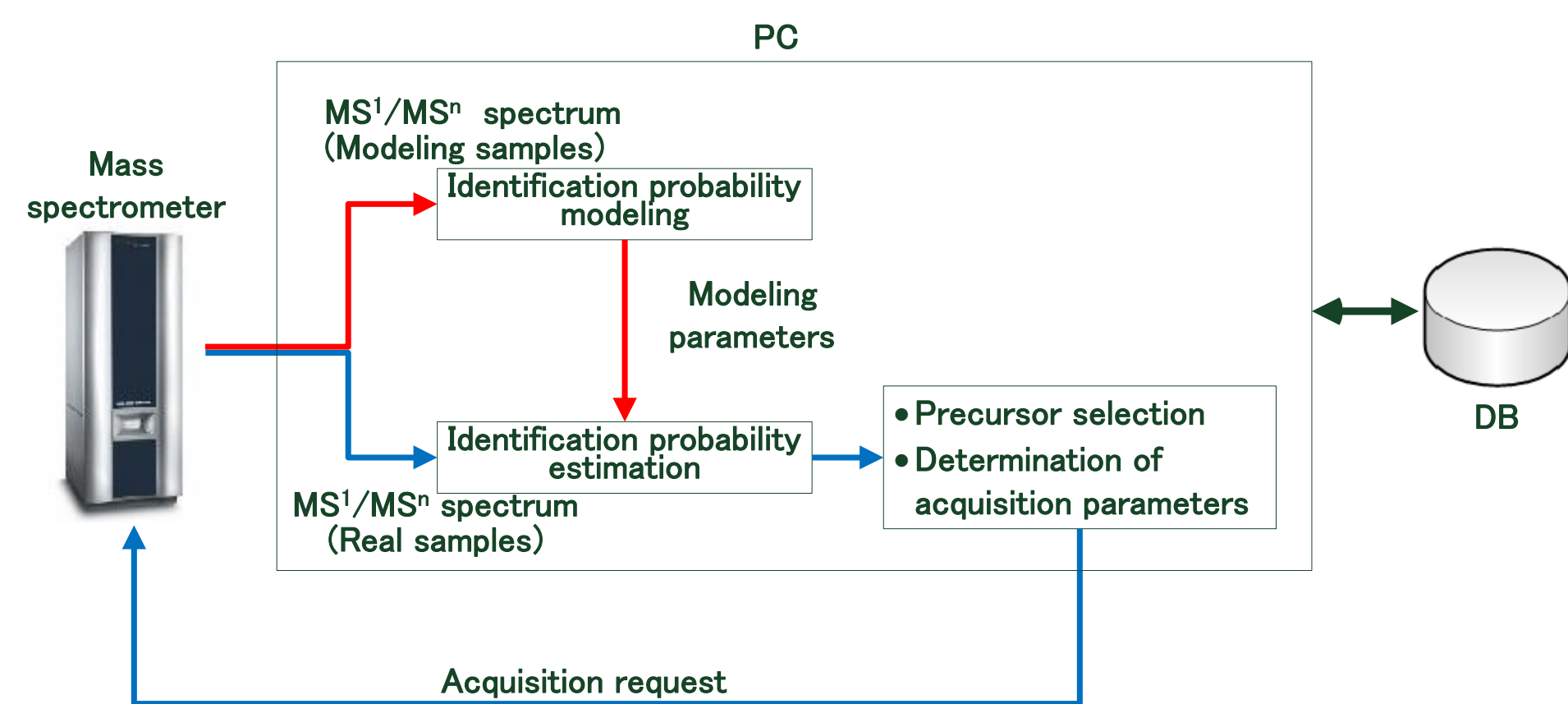


Fig. 1 Data flow of data dependent acquisition using identification probability model

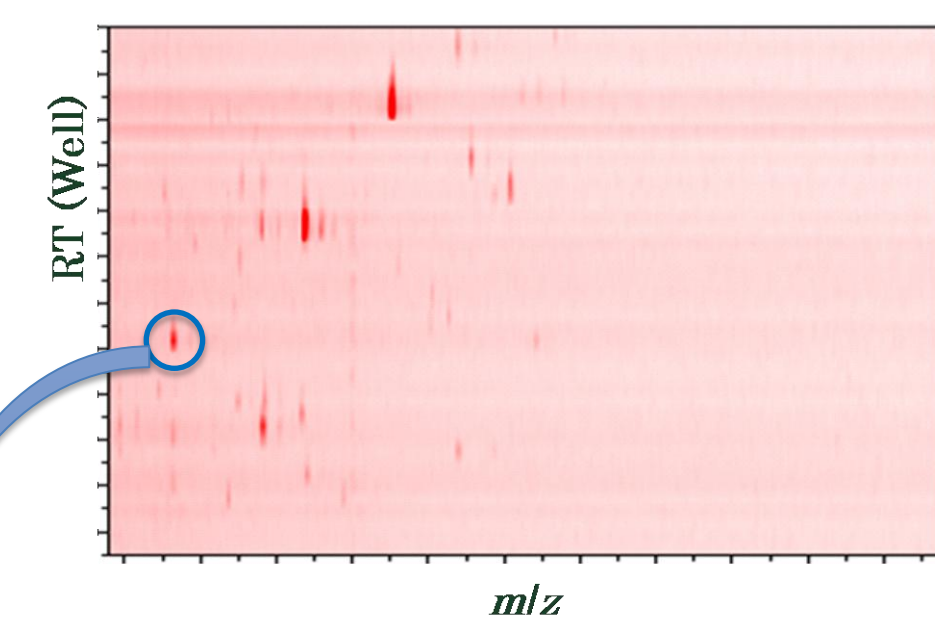
2. Methods

As a preliminary investigation, we tried to derive an identification probability model from S/N ratios of MS¹ peaks from which *m/z* values were selected as MS² precursors.

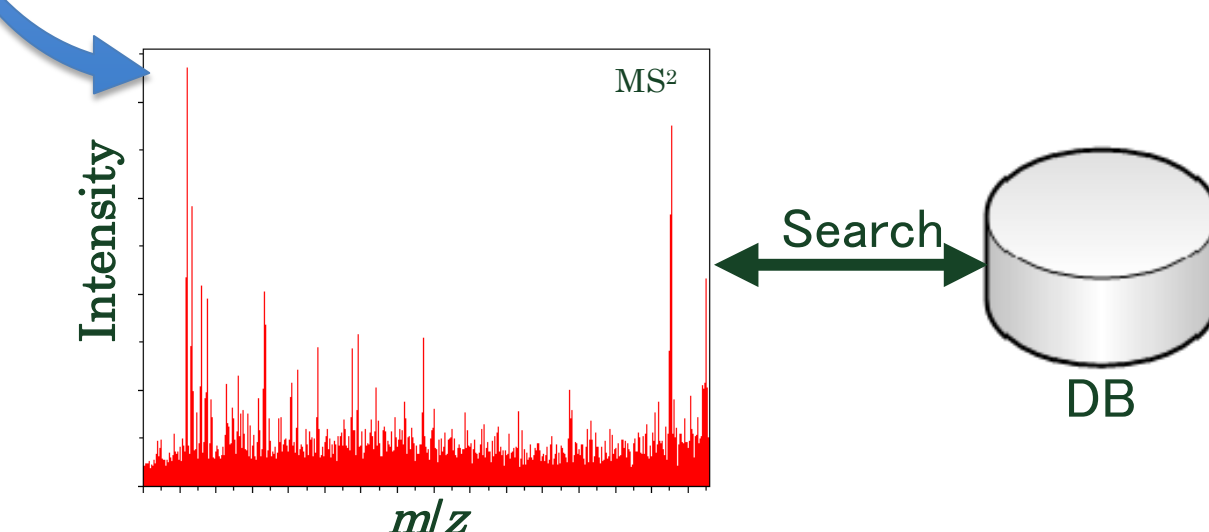
Derivation of identification probability model

Acquiring data for modeling

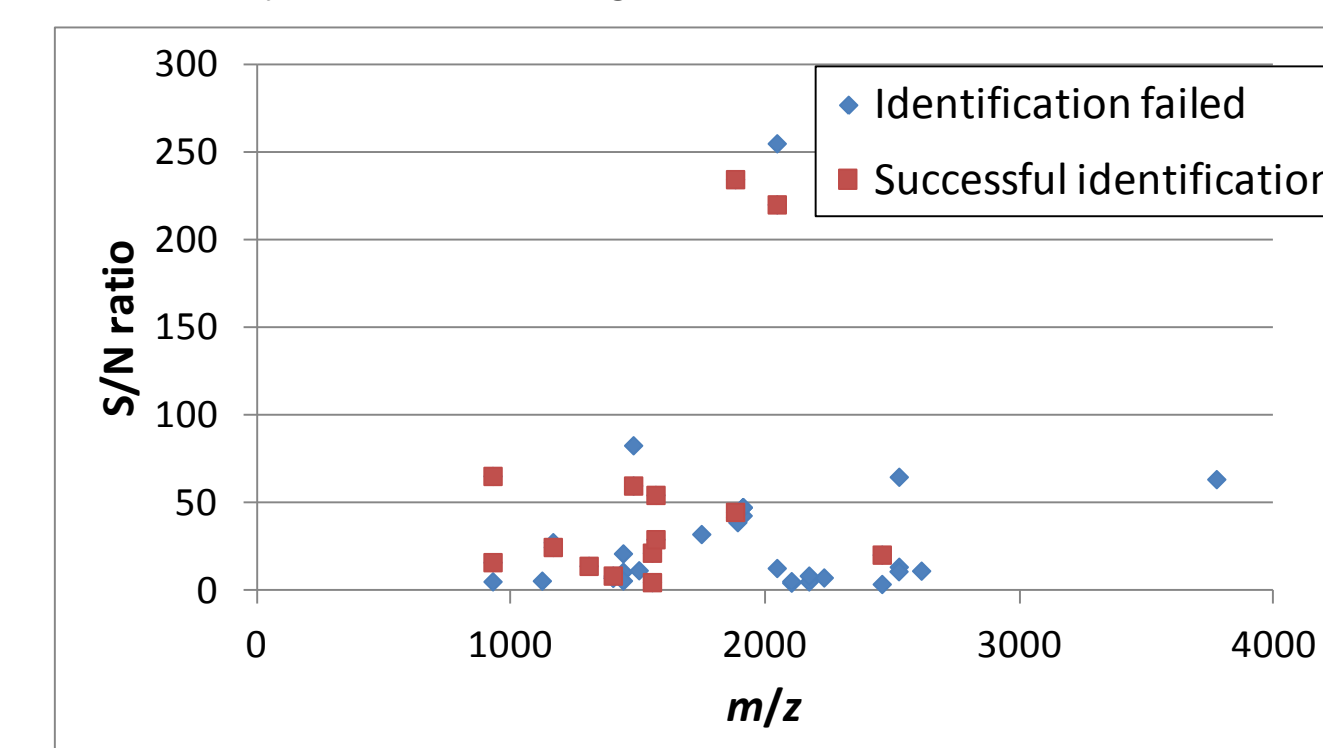
- MS¹ acquisition of identification probability modeling samples



- MS² acquisition at major 2D peaks & DB search

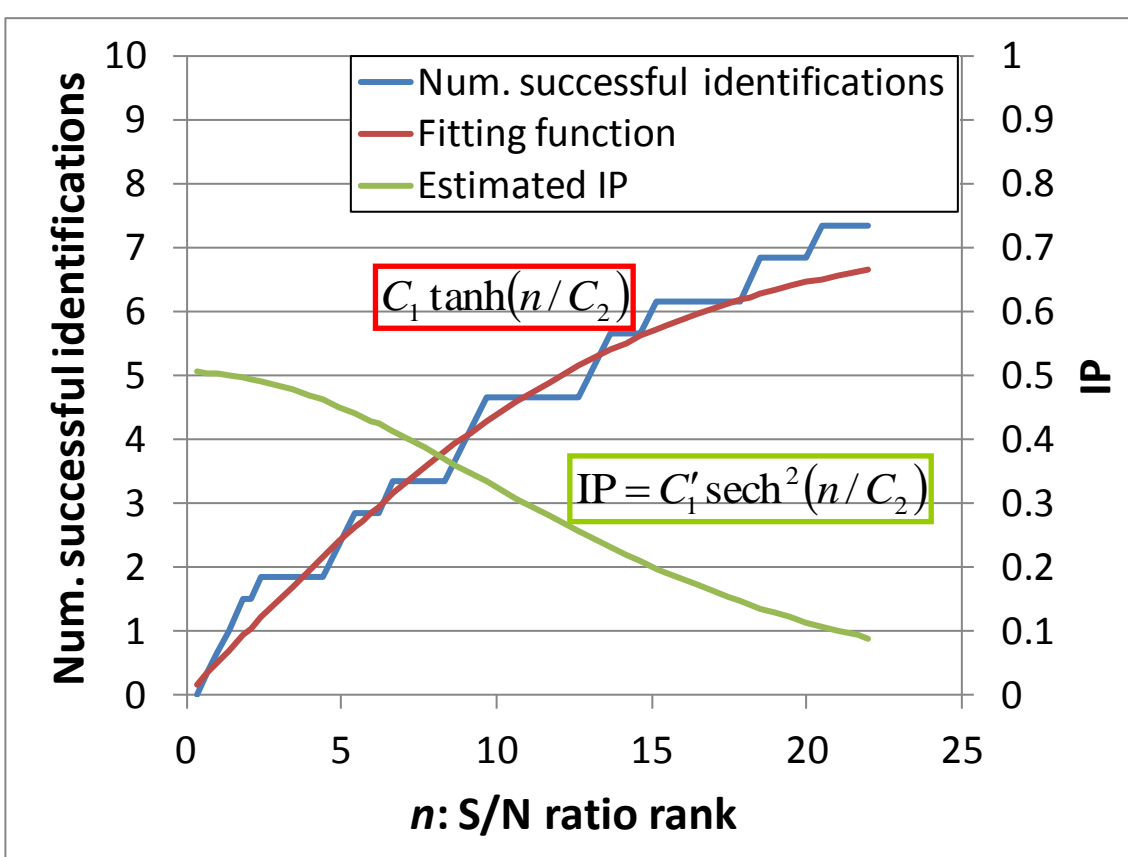


- Classify 2D peaks using the result of DB search



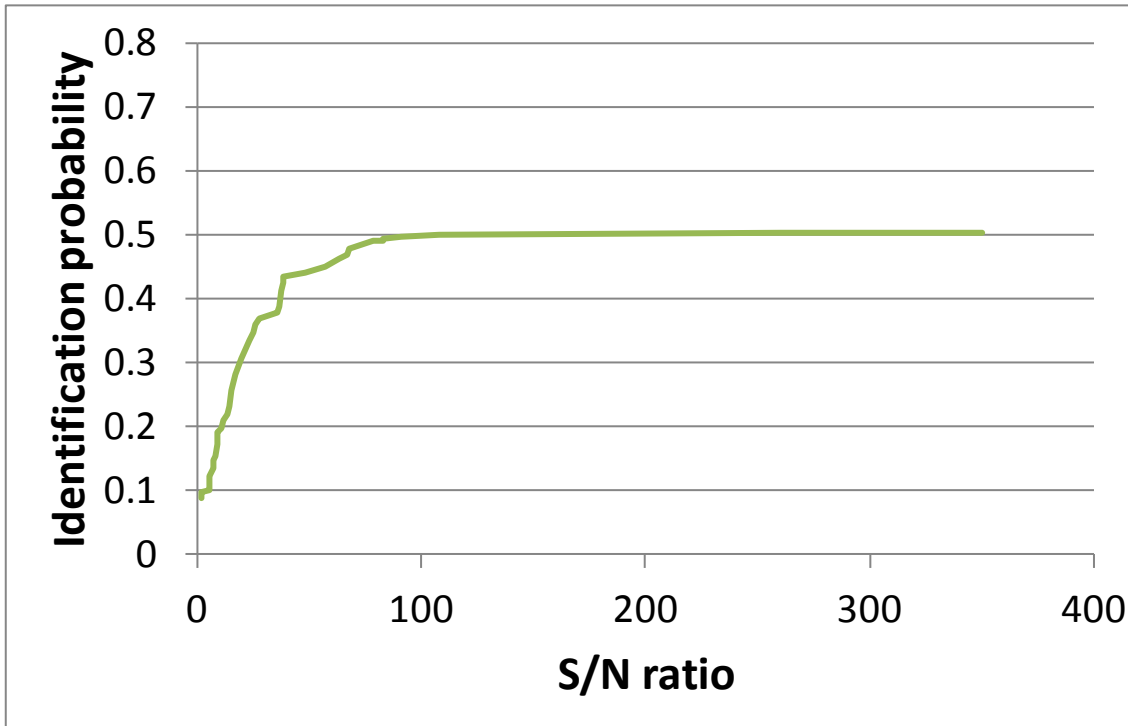
Calculation of Modeling Parameters

- Identification probability estimation using S/N ratio rank



C_1, C_1', C_2 : Fitting parameters

- Identification probability estimation for given S/N ratio



Data for derivation of identification probability model

- Samples & preparations**
 - Commercially available tryptic digest mixtures (Waters MassPREP BSA, Yeast Enolase, Rabbit Phosphorylase B)
 - Mixing above mixtures (without LC separation) into Matrix CHCA at three content levels (100 fmol/ μ L, 1 pmol/ μ L, and 10 pmol/ μ L)
- Instrument** : Axima Performance™ (MALDI-TOF-MS, Shimadzu/Kratos, UK)
- S/N ratios at MS² precursors** : Averaged from at least four MS¹ acquisitions
- Search Engine** : Mascot™

3. Results

Derived identification probability model (See Fig. 2)

- Derived from the result of 131 MS² acquisitions (See Table 1)
- Improvement of Identification probability saturates at about S/N=100.
- Estimated identification probabilities increase with increased number of accumulations of MS² acquisitions.

Table 1 Number of Successful Identifications for Different Number of Accumulations of MS² Acquisitions

Sample	Num. MS ² Acquisitions	Num. Successful Identifications		
		500 acc.	1000 acc.	2000 acc.
BSA 100 fmol	13	1	1	1
BSA 1 pmol	20	5	8	9
BSA 10 pmol	11	4	4	4
Yeast Enolase 100 fmol	10	0	0	0
Yeast Enolase 1 pmol	19	0	1	2
Yeast Enolase 10 pmol	22	2	3	3
Rabbit Phosphorylase B 100 fmol	16	1	2	5
Rabbit Phosphorylase B 1 pmol	10	3	5	5
Rabbit Phosphorylase B 10 pmol	10	4	3	3
Total	131	20	27	32

acc. : accumulations

Precursor selection and determination of number of accumulations of MS² acquisitions (See Fig. 3)

- ① A 500 acc.
 - ①~② A 500 acc. , B 500 acc.
 - ①~③ A 1000 acc. , B 500 acc.
 - ①~④ A 1000 acc. , B 500 acc. , C 500 acc.
 - ...
- Possible accumulations increase.

4. Conclusion

Achievements

- Identification probability model can be derived from S/N ratios of modeling samples
- Identification probability model gives proper precursor selection and number of accumulations

Future tasks

- Application of identification probability model for data dependent acquisition
- Evaluation of effect on data dependent acquisition when identification probability depends on the type of peptides (See Fig. 4)

Acknowledgment

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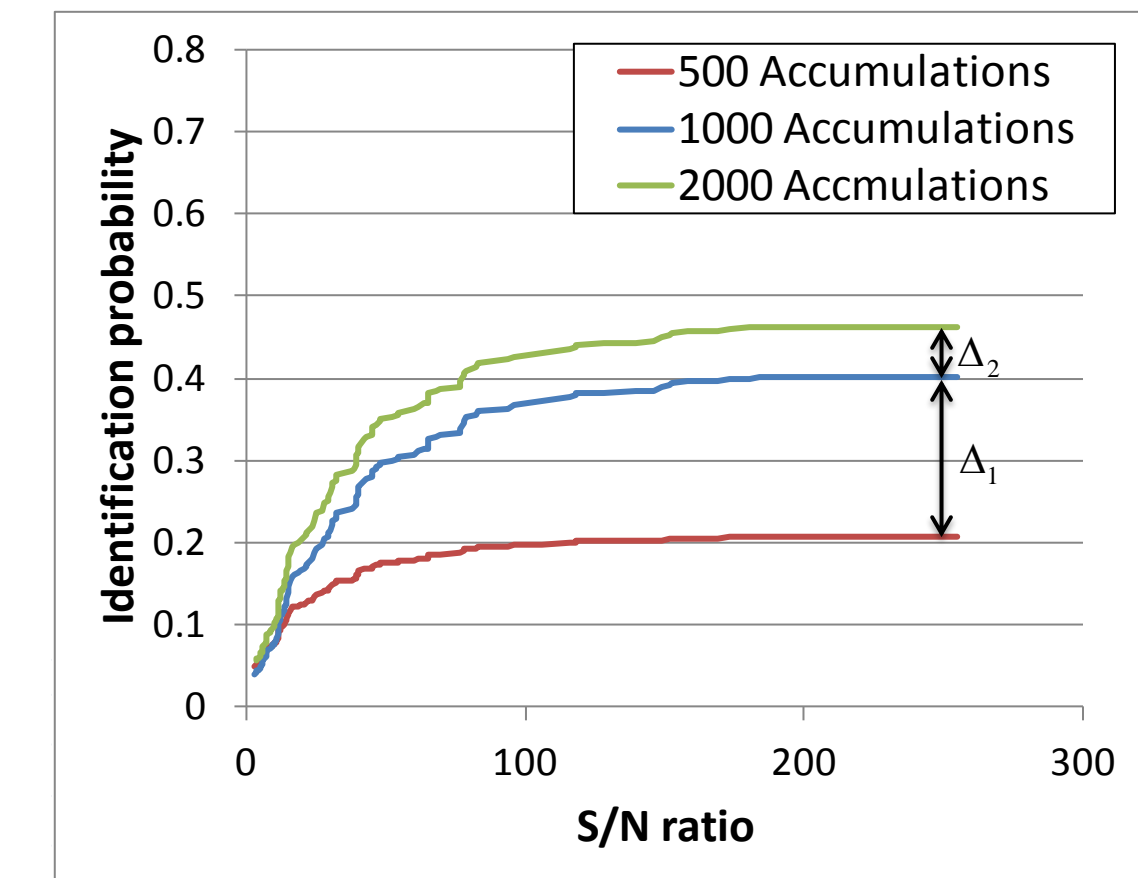


Fig. 2 Identification probability for Different Number of Accumulations

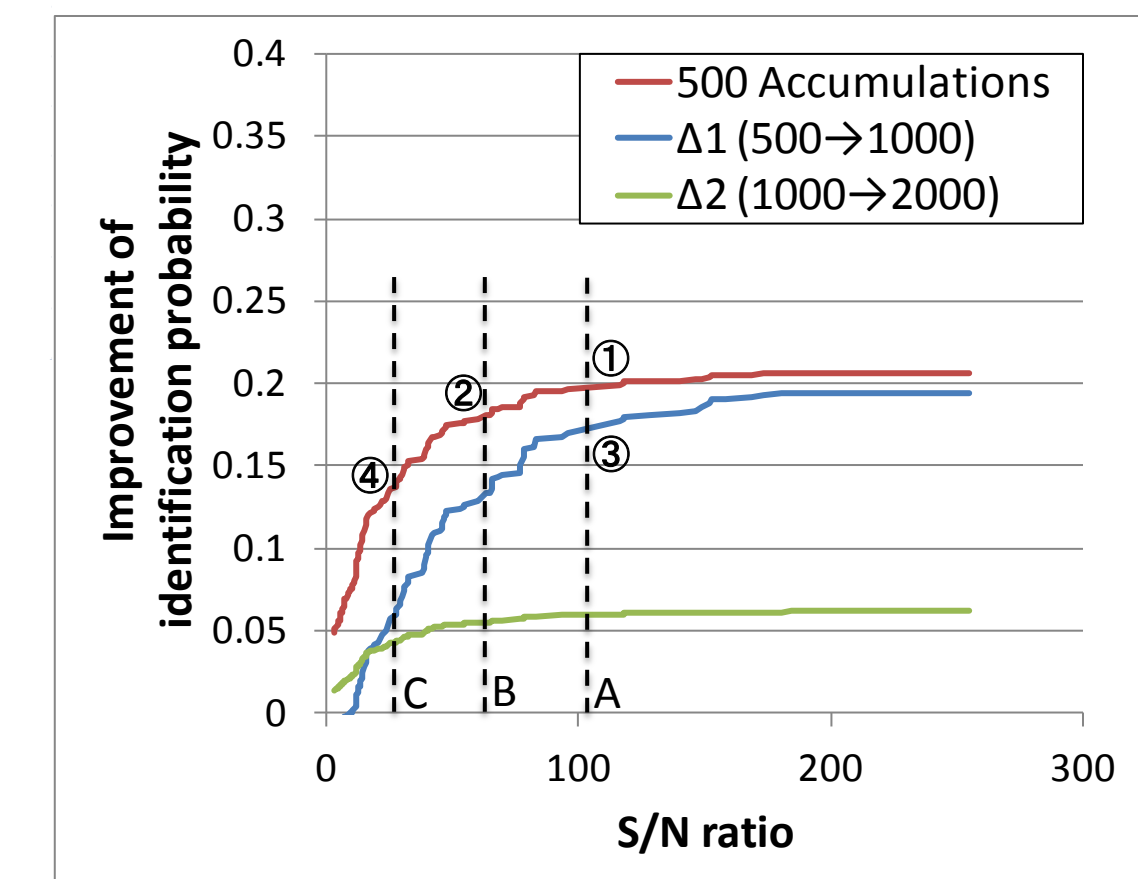


Fig. 3 Example of Precursor Selection and Determination of Number of Accumulations

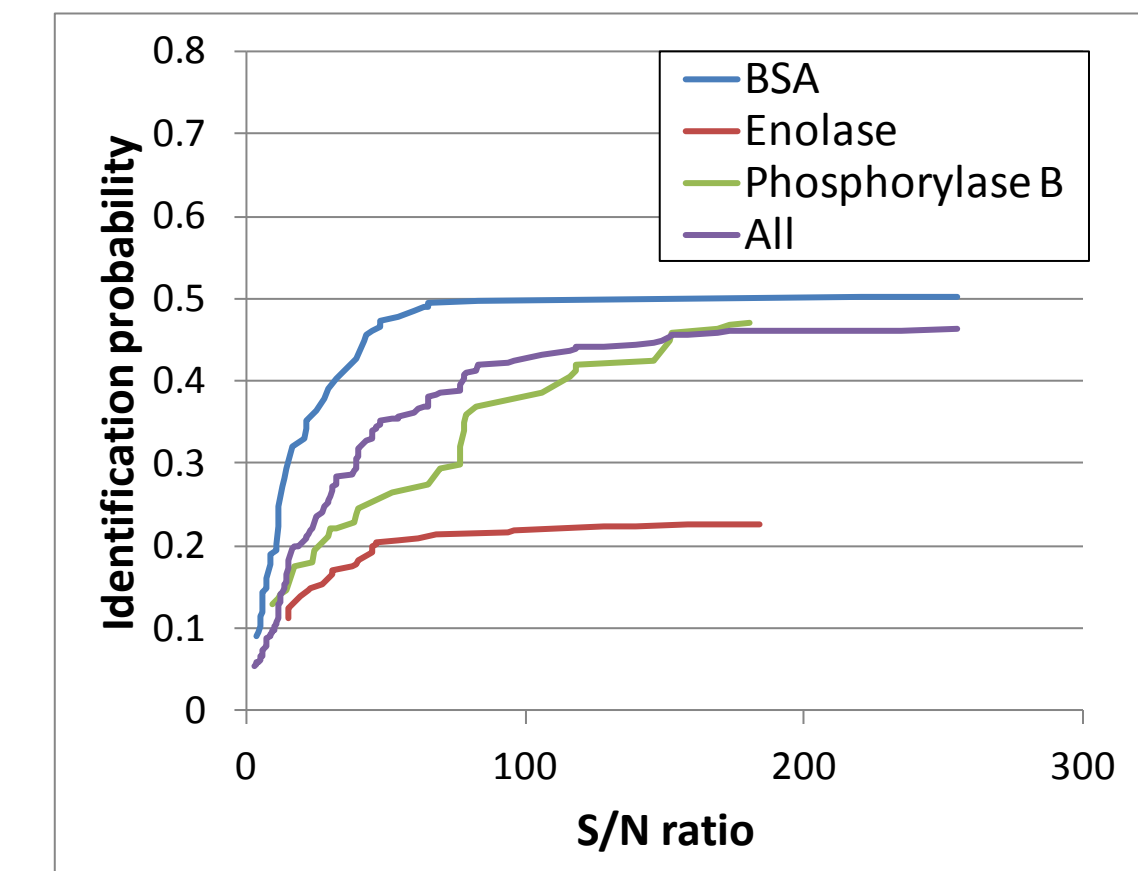


Fig. 4 Dependency of Identification Probability on Peptide Type