SHIMADZU ASMS2012 ThP30-No. 286

Data dependent acquisition with peptide identification probability estimation during comprehensive analysis

Yoshihiro Yamada, Yuko Fukuyama, Natsumi Funakoshi, Hidenori Takahashi, Masaki Murase, Shigeki Kajihara, Koichi Tanaka
Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Corporation, Kyoto, Japan

ms3d FIRST Program

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 MS^n precursors

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

- Identification probability modeling
  - ✓ Derivation of estimation model from spectra of modeling samples
- MS^n analysis for real samples
  - ✓ Select appropriate MS^n 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

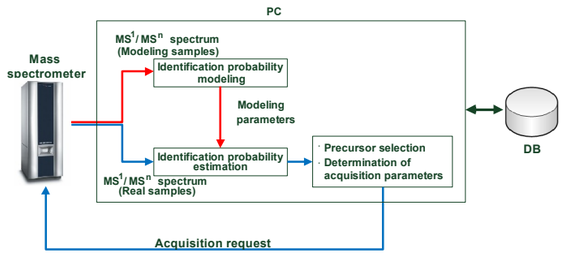


Fig. 1 Data flow of data dependent acquisition using identification probability model

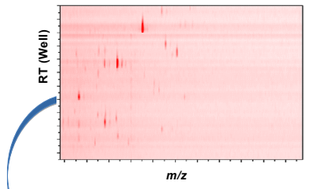
2. Methods

As a preliminary investigation, we tried to derive an identification probability model from S/N ratios of MS^1 peaks from which *m/z* values were selected as MS^2 precursors.

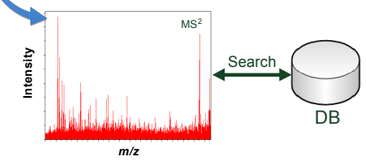
Derivation of identification probability model

Acquiring data for modeling

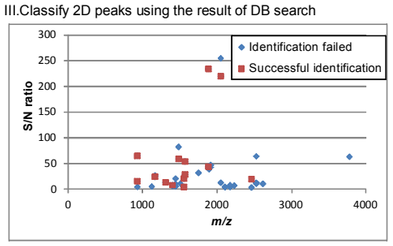
I.MS^1 acquisition of identification probability modeling samples



II.MS^2 acquisition at major 2D peaks & DB search

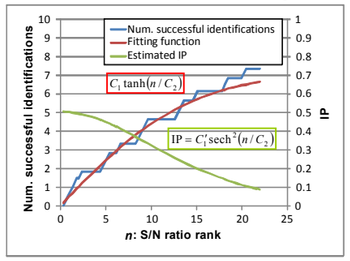


III.Classify 2D peaks using the result of DB search



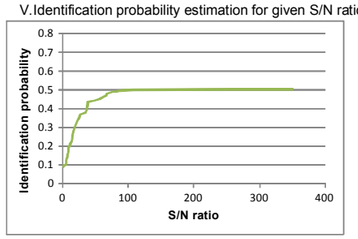
Calculation of Modeling Parameters

IV.Identification probability estimation using S/N ratio rank



C_1, C_1', C_2 : Fitting parameters

V.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/μL, 1 pmol/μL, and 10 pmol/μL)
- Instrument : Axima Performance™ (MALDI-TOF-MS, Shimadzu/Kratos, UK)
- S/N ratios at MS^2 precursors: Averaged from at least four MS^1 acquisitions
- Search Engine : Mascot™

3. Results

Derived identification probability model (See Fig. 2)

- Derived from the result of 131 MS^2 acquisitions (See Table 1)
- Improvement of Identification probability saturates at about S/N =100.
- Estimated identification probabilities increase with increased number of accumulations of MS^2 acquisitions.

Table 1 Number of Successful Identifications for Different Number of Accumulations of MS^2 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 MS^2 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.

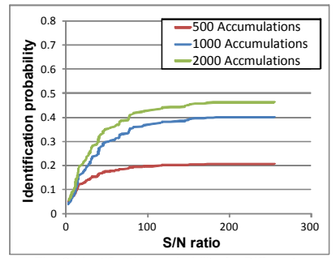


Fig. 2 Identification probability for Different Number of Accumulations

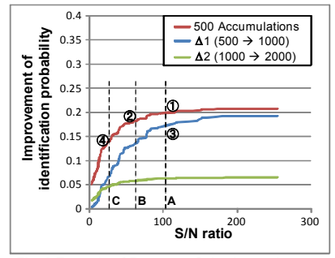


Fig. 3 Example of Precursor Selection and Determination of Number of Accumulations

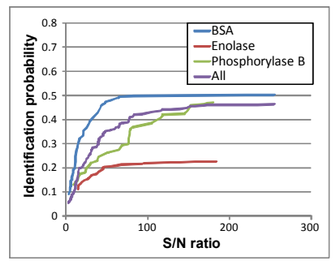


Fig. 4 Dependency of Identification Probability on Peptide Type

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

This research is supported by the Japan Society for the Promotion of Science (JSPS) through its "Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program).