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N-terminal charge-driven *de novo* sequencing by using ASDF-incorporated Curved Field Reflectron

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Overview

- *De novo* sequencing consisted of N-terminal derivatization and informative MALDI-MS/MS bolstered by high resolution MS/MS and HE-CID is reported.
- Differentiations of Ile/Leu are performed with HE-CID successfully.
- A precise assignment of Gln/Lys is conducted.
- Switching PSD to HE-CID rapidly is valuable to realize weak d-ions.

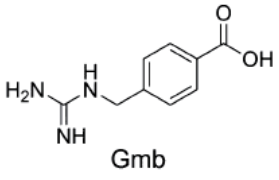
Introduction

Fixing strong charge at N-terminus of peptide has been reported to be effective derivatization for *de novo* sequencing. While giving high proton affinity at N-terminus of peptide facilitates the generation of a- and b-ions, it is impossible to differentiate isobaric Ile/Leu residues. Side chain fragmentation generated by high energy CID on MALDI-TOF/TOF has a potential to overcome this issue. On the other hand, a precise assignment of Gln/Lys by the previous MALDI-TOF/TOF is quite difficult due to insufficient MS/MS resolution and accuracy. We will report discrimination of Ile/Leu and a capability of the assignment of Gln/Lys. We will apply newly developed MALDI-TOF/TOF, which has high resolution in MS/MS and a high energy CID at 20keV, to analysis N-terminal derivatized peptides.

Methods

Chemistry of N-terminal derivatization [3,4)]

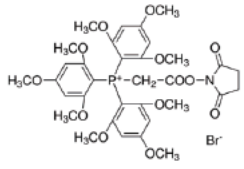
Gmb; 4-Guanidinomethylbenzoic acid



Gmb

Gmb was synthesized as previously reported. Gmb (1 mmol) dissolved in DMSO at a concentration of 2.5 mM was mixed with peptides (100 nmol, 2.5 mM) and DMT-MM (1 mmol, 2.5 mM). The resultant mixture was stirred at room temperature overnight. The derivatized peptides were purified by RP-HPLC.

TMPP-Ac; N-Succinimidyloxycarbonylmethyl)tris(2,4,6-trimethoxyphenyl)phosphonium bromide



TMPP-Ac-NHS was synthesized as previously reported. TMPP-Ac-NHS (1 mmol) dissolved in 0.5 M phosphate buffer (pH8.5) at a concentration of 2.5 mM was mixed with peptides (100 nmol, 0.25 mM). The resultant mixture was stirred at room temperature overnight. The derivatized peptides were purified by RP-HPLC.

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MALDI-tandem TOFMS

Instrument	: MALDI-7090 (Shimadzu/Kratos)
Measurement	: PSD and high-energy CID-MS/MS in positive ion mode.
Collision gas	: helium
Collision energy	: 20 keV (laboratory frame of reference).

Principle of Axial Spatial Distribution Focusing (ASDF)

MS/MS resolution is improved with correcting the spatial distribution of ions in ASDF cell. This is achieved by applying a pulsed electrostatic field at the point, at which the precursor and fragments are velocity focussed but are spatially resolved.

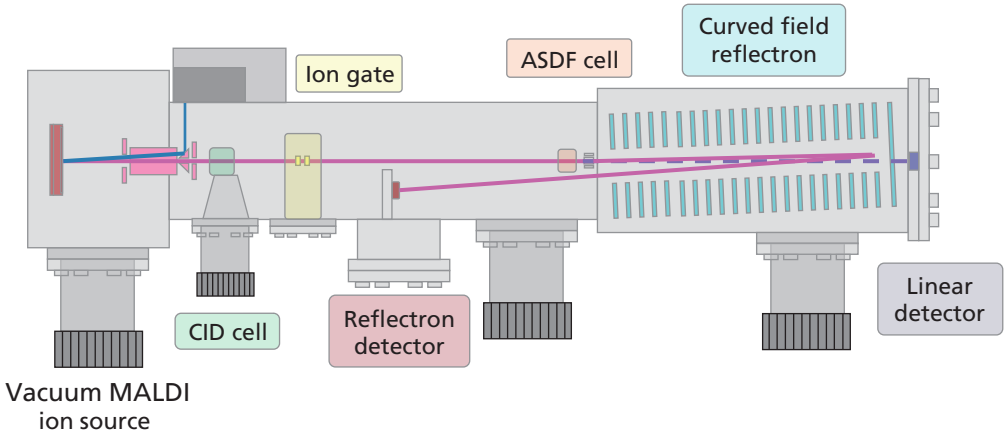

Fig.1 Inside view of MALDI-7090

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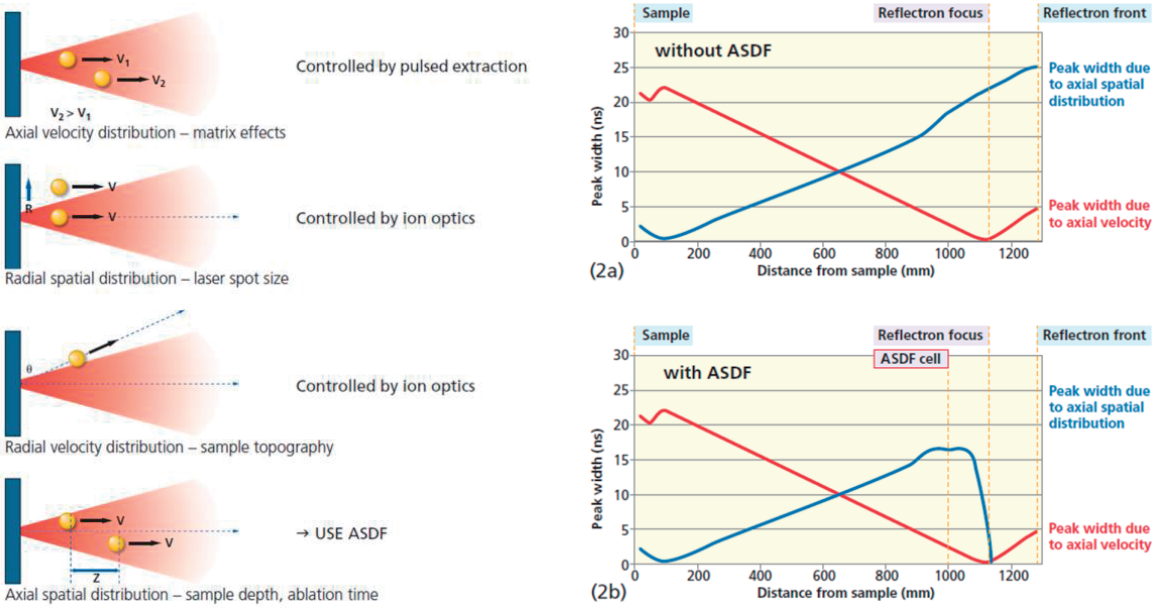

Fig.2 Axial Spatial Distribution Focusing

Results

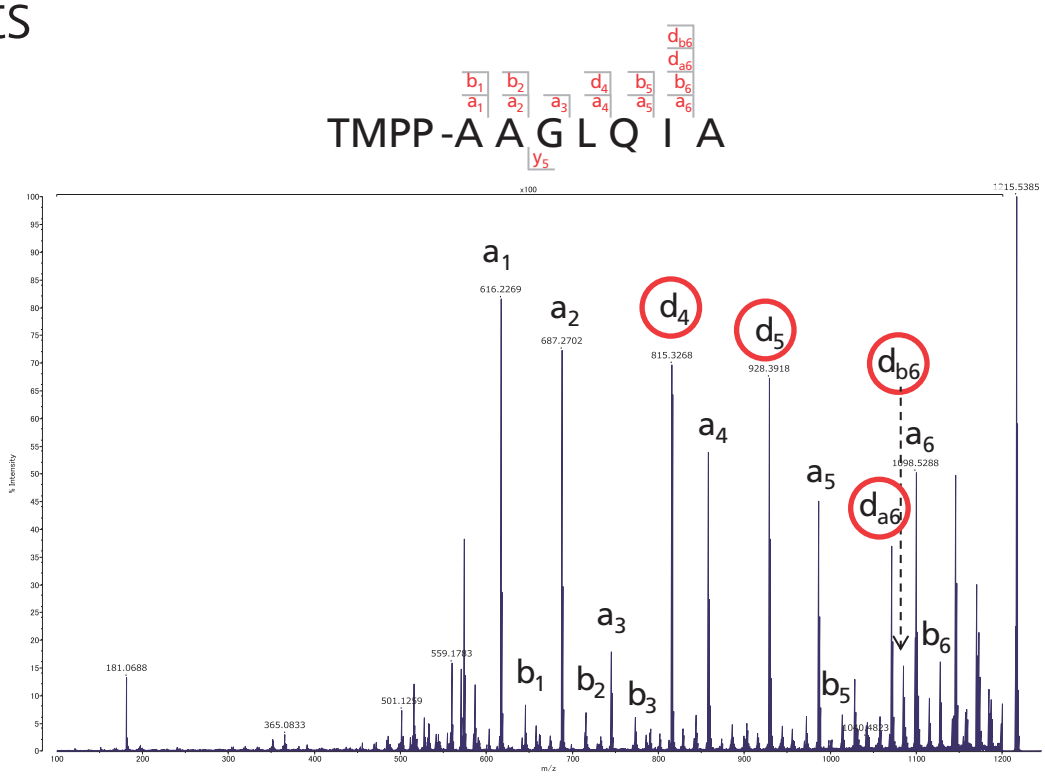

Fig.3 CID-MSMS of TMPP-Ac derivatized peptide

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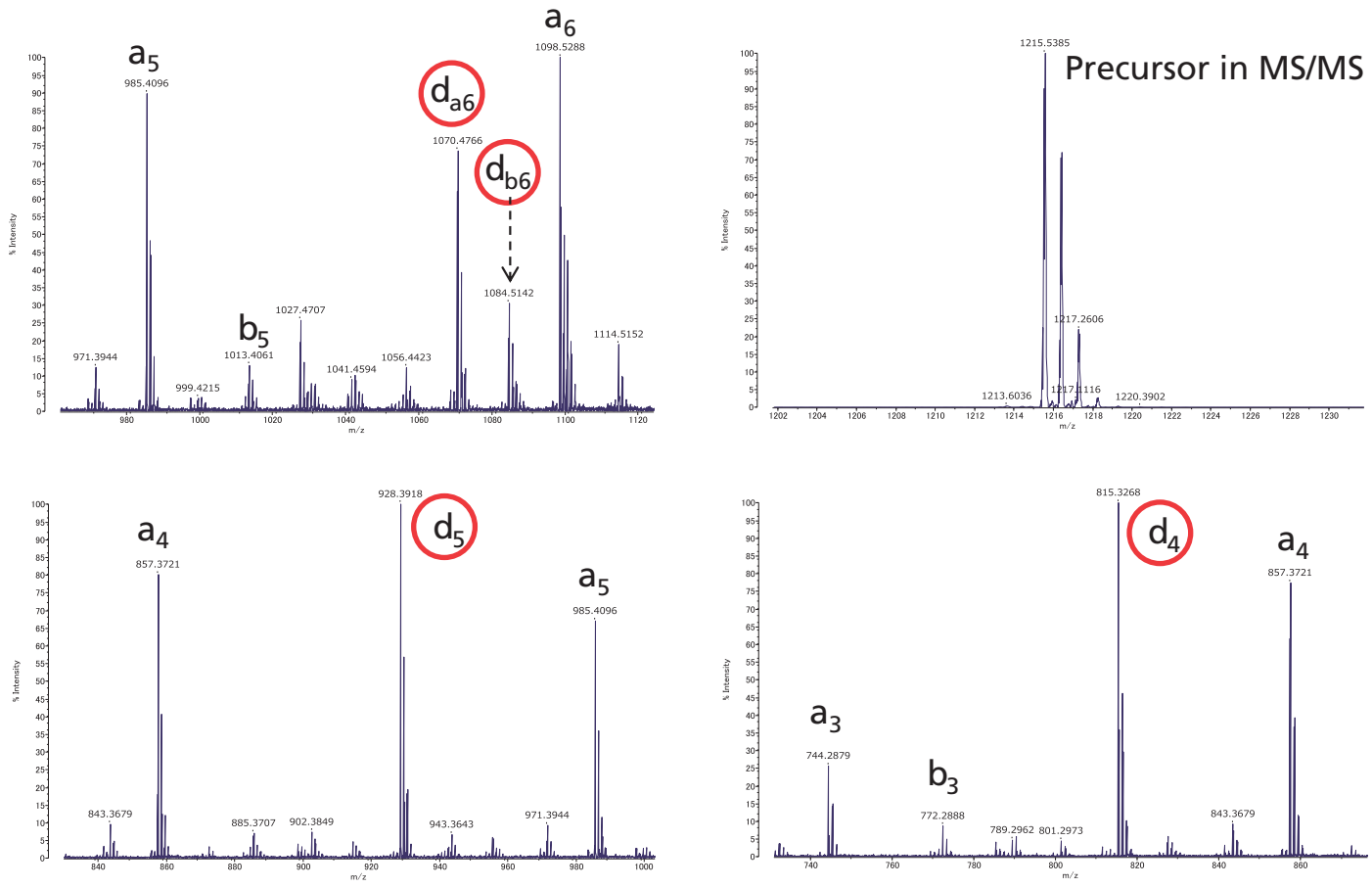

Fig.4 Enlarged spectrum in "Fig.3"

	Theoretical	Observed	Diff.
a1	219.1240	219.1577	0.0337
b1	247.1190	247.2139	0.0949
a3	290.1612	290.1436	0.0176
b2	318.1561	318.1411	0.0150
y2	331.1976	331.1648	0.0328
a2	347.1826	347.1615	0.0211
b3	375.1775	375.1761	0.0014
n2	418.2667	418.1995	0.0672
a4	460.2667	460.2628	0.0039
b4	488.2616	488.2570	0.0046
y4	572.3402	572.3271	0.0131
n3	588.3253	588.3220	0.0033
b5	616.3202	616.3046	0.0156
[M-H2O+H]+b6	673.4093	673.3571	0.0522
[M-H2O]+b6	687.4093	687.3678	0.0415
a6	701.4093	701.3843	0.0250
b6	729.4042	729.3939	0.0103

128.0582

Fig.5 List of fragment ions of TMPP-Ac derivatized peptide

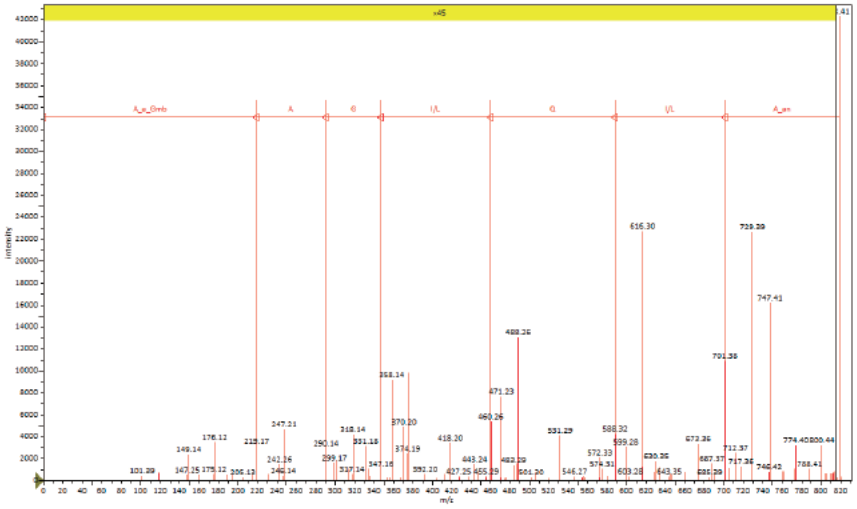

Fig.6 Assignment of TMPP-Ac derivatized peptide by SIMSE in Mass++ [5)]

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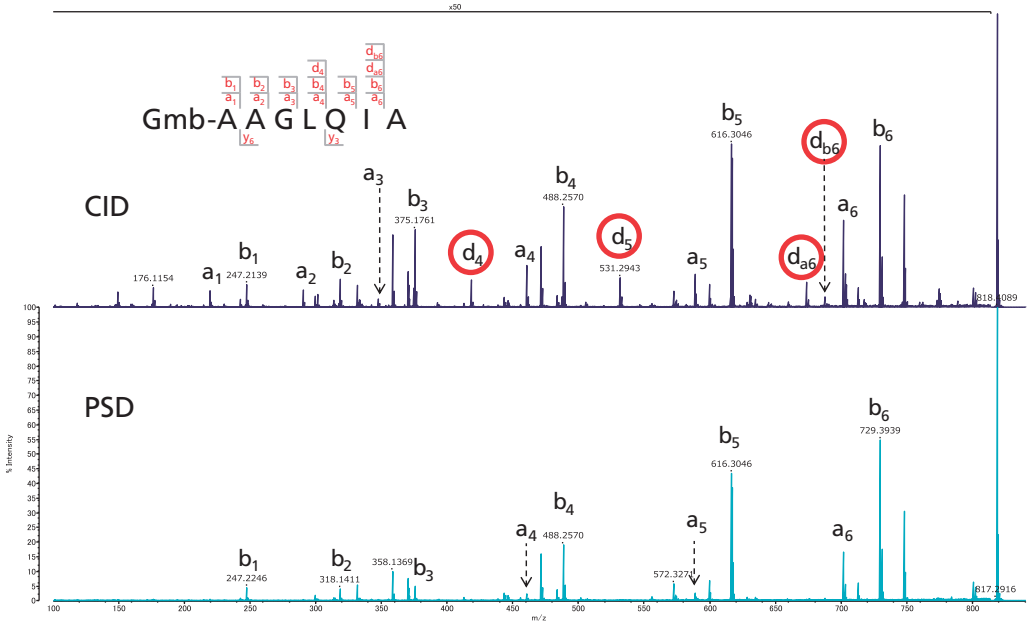

Fig.7 CID (upper) and PSD (lower) of Gmb derivatized peptide

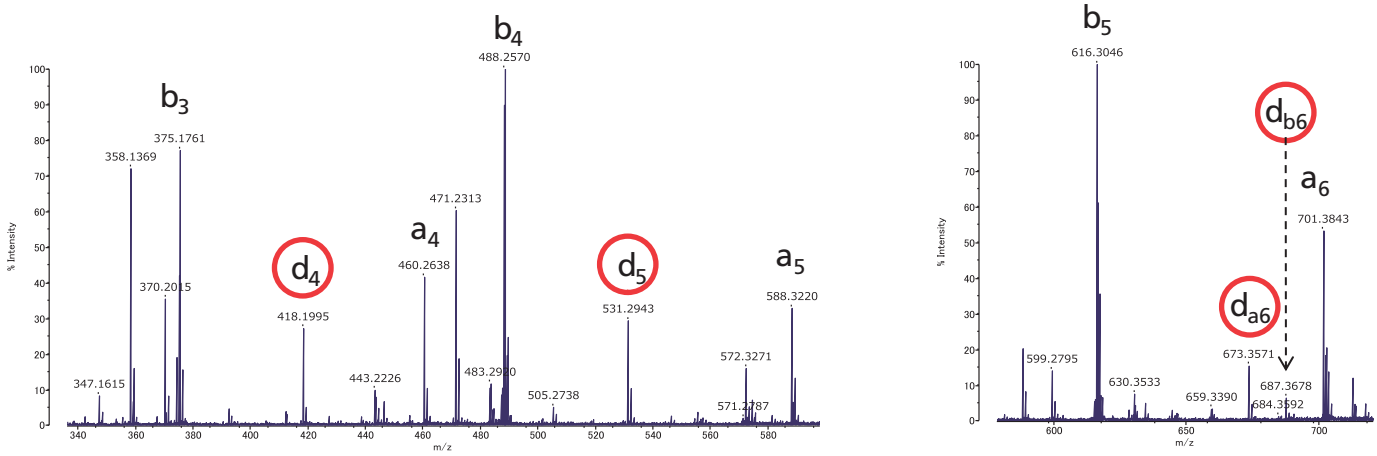

Fig.8 Enlarged CID spectrum in "Fig.7"

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	Theoretical	Observed	Diff.
y_5	501.3031	501.1259	0.1772
a_{11}	616.3306	616.3269	0.0037
b_1	644.3255	644.3331	0.0076
a_3	687.2677	687.2702	0.0025
b_4	715.2626	715.2753	0.0127
a_{12}	744.3892	744.3879	0.0013
d_{11}	815.3733	815.3268	0.0465
a_{11}	857.3733	857.3721	0.0012
a_8	985.4318	985.4096	0.0222
b_5	1013.4270	1013.4061	0.0209
d_{a5}	1070.5160	1070.4766	0.0394
d_{a7}	1084.5160	1084.5142	0.0018
a_8	1098.5160	1098.5288	0.0128
b_6	1126.5110	1126.5200	0.0090

128.0375

Fig.9 List of fragment ions of Gmb derivatized peptide

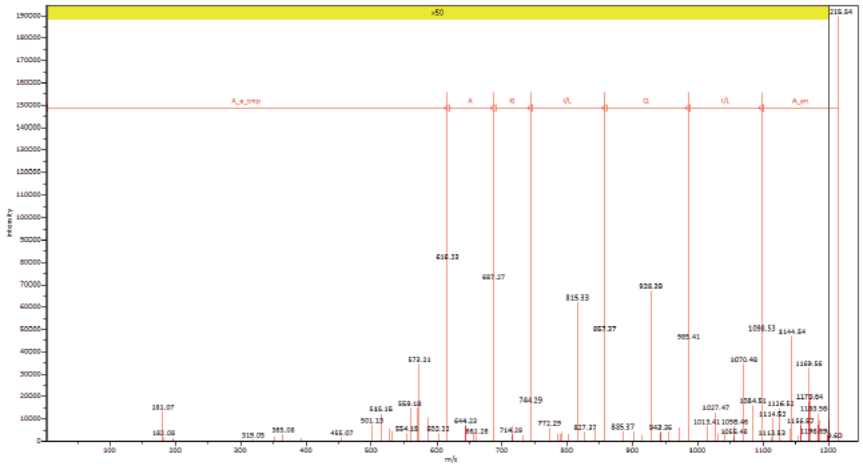
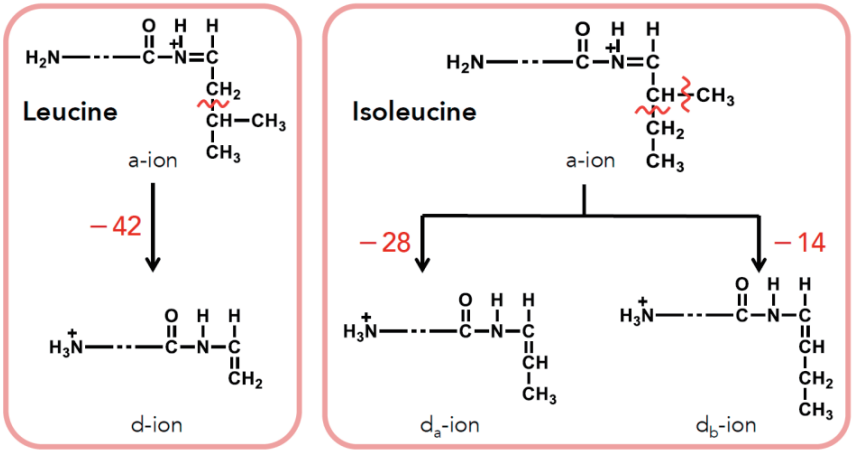


Fig.10 Assignment of Gmb derivatized peptide by SIMSE in Mass++ [5)]



Amino Acid	Residual mass (mono)
Ile	113.0841
Leu	113.0841
Gln	128.0586
Lys	128.0950

Fig.11 Side chain cleavage of Ile/Leu, and exact mass of amino acid residues.

Conclusions

- Ile and Leu in N-terminal derivatized peptides is differentiated successfully by high energy CID-MS/MS, where specific side chain fragmentations of both residues were observed.
- Particular MS/MS accuracy achieved by ASDF enable to apply a precise assignment of Gln/Lys, the mass difference between which is only 0.036 Th.
- High MS/MS accuracy facilitates an interpretation of the data by a dialogical *de novo* software, for instance Mass++.
- Comparing PSD and CID could be useful to recognize fragment ions generated by high energy CID, even if intensities of them will be weak.

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References

(1) Cordero MM, et al; Rapid Commun Mass Spectrom., 1995, 9, pp1356-61.
(2) Cornish TJ, et al; Rapid Commun Mass Spectrom., 1993, 7, pp1037-40.
(3) Miyashita, M., et al; Rapid Commun. Mass Spectrom., 2011, 25, pp1130-1140.
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(5) download site: http://www.first-ms3d.jp/english/achievement/software/mass2

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