生物质谱及其方法学

(Bio-mass spectrometry and its methodology)

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主要内容：

一、微生物所现有仪器及检测项目简介；

二、定量蛋白质组学；

三、基于Bottom-up质谱的PTM研究；

四、基于Top-down质谱的蛋白相互作用及PTM研究；

五、离子迁移质谱（IM-MS）及其应用。

微生物所现有质谱仪：

离子阱质谱仪

MALDI-TOF-TOF质谱仪

Q-TOF质谱仪

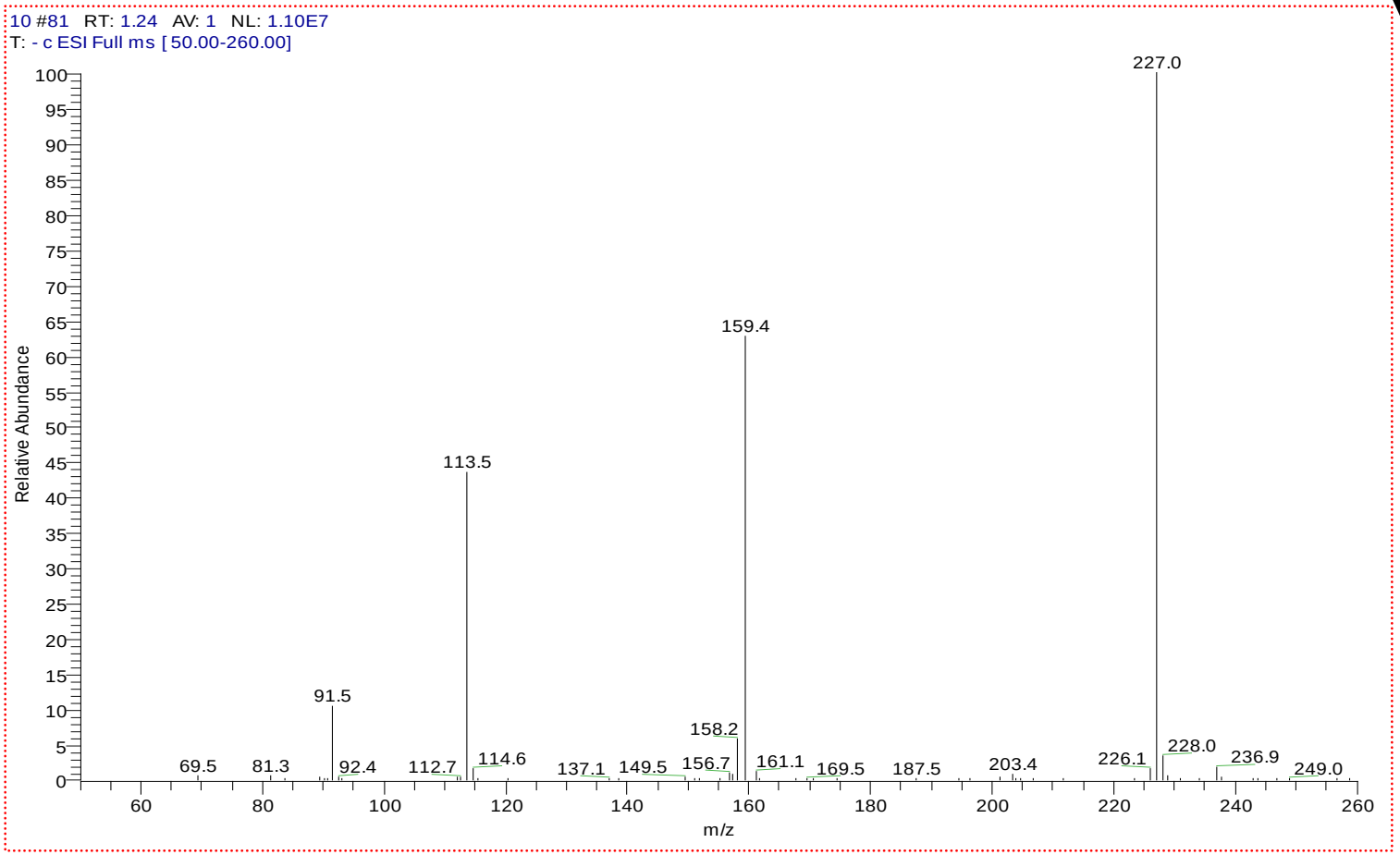
气质联用质谱仪

同位素比质谱仪

串联四极杆质谱仪

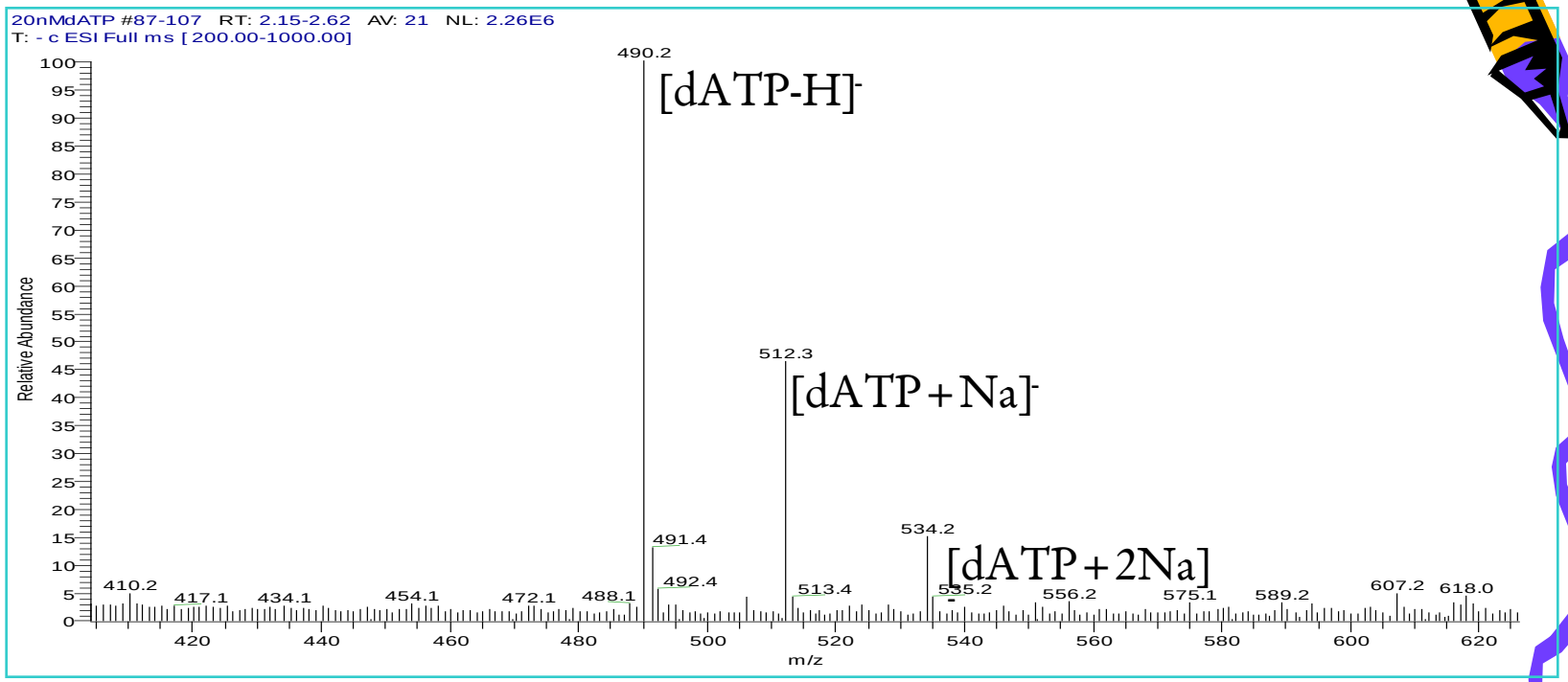
十二五期间已申报质谱仪：GC-Q-TOF、LC-MALDI-TOF/TOF、Q-FT-ICR。

1. 微生物代谢产物小分结构鉴定



ESI-MS spectrum of reaction mixture including substrates, intermediates and products.

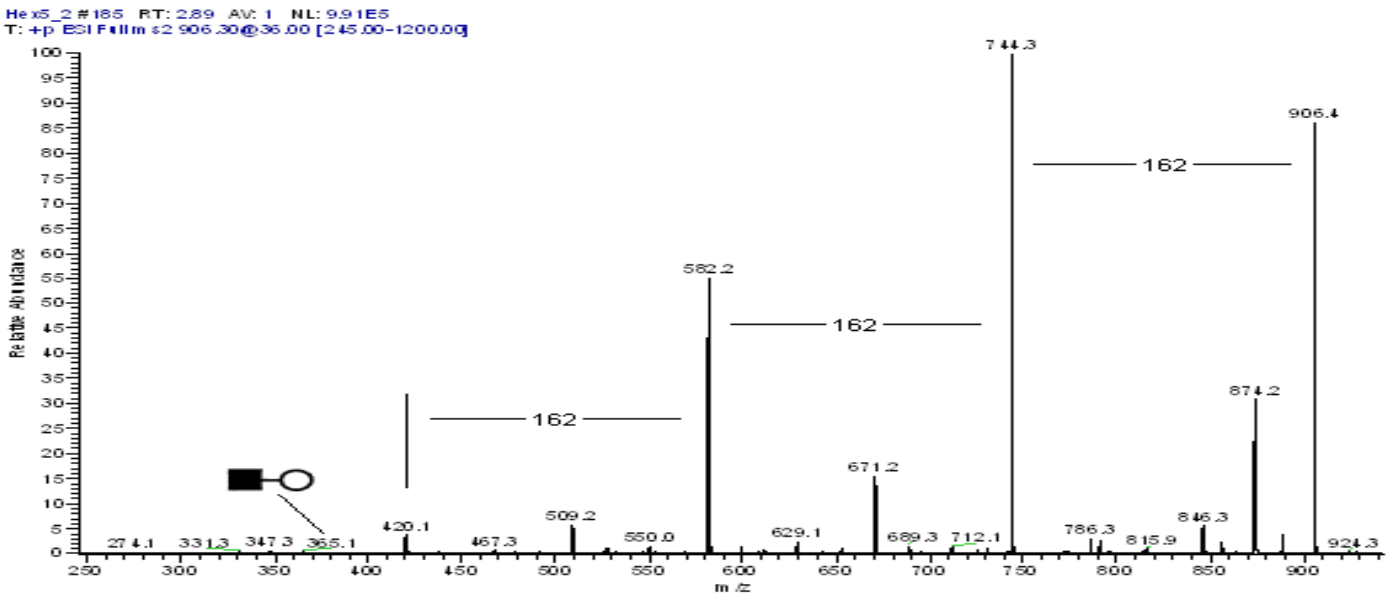
2. 寡核苷酸的鉴定及定量



The MS spectra of dATP from nucleic acid hydrolysis.

Buffer: (A) 50 mM TEAB, pH 8.40, (B) 50 mM TEAB, pH 8.40 20% acetonitrile; linear gradient, 10-60% B in 30 min; flow rate 180μl/min.

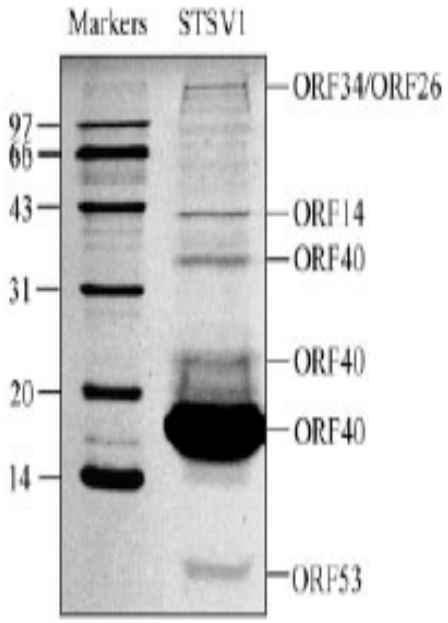
3.寡糖及糖肽鉴定



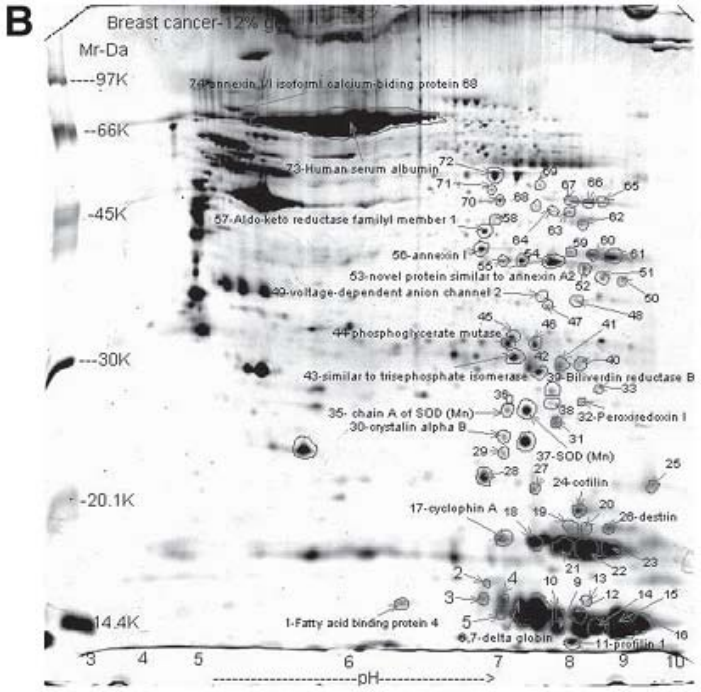
LC-MS/MS质谱鉴定GlcNAc(Man)$_4$ 

■ N-Acetylglucosamine ○ Mannose

4. 蛋白质鉴定(protein identification)



1DE



2DE

5. 表达蛋白质组学研究（Expression Proteomics）

- 细胞质蛋白质组(cytoplasma proteome)
- 膜蛋白质组(Membrane proteome)
- 细胞壁蛋白质组(Cell wall proteome)
- 其他功能性亚细胞蛋白组或细胞器蛋白组(Subcellular proteome and organelle proteome): e.g. 纤维小体蛋白组(Cellulosome proteome)

6.蛋白复合物研究

(Hayano T, Yamauchi Y, et al., J Proteome Res. 2008, 7(9):4183-90;

Xu Y, Piston DW, et al., PNAS, 1999, 96(1):151-156;

Zhou M, Veenstra TD. 2007, 7(16):2688-97.)

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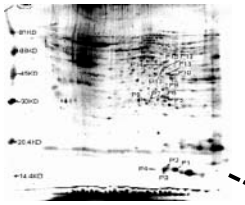
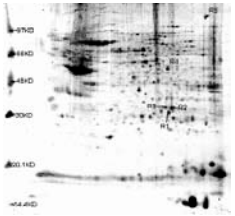
比较蛋白质组学技术路线

2DE, DIGE)

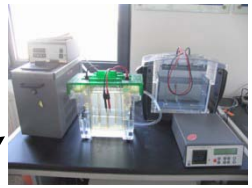


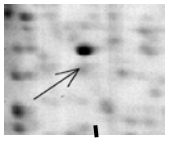


Comparative analysis

State 1

1

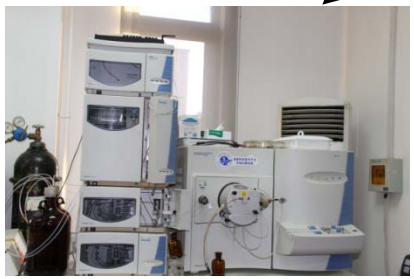




LC-MS/MS

State 2

2 SIL Quantitative Proteomics



PMF

MS/MS

Label-free 3 Quantification

Further functional analysis

Database search

2. 稳定同位素标记的比较蛋白质组学研究 SIL-based comparative proteomics)

(1)代谢标记(Metabolic labeling)(^{13}C, ^{15}N)

Blaqoev B, et al., Nat Biotechnol, 2003, 21: 315-318;
Krijgsveld J, et al., Nat Biotechnol, 2003, 21: 927-931.

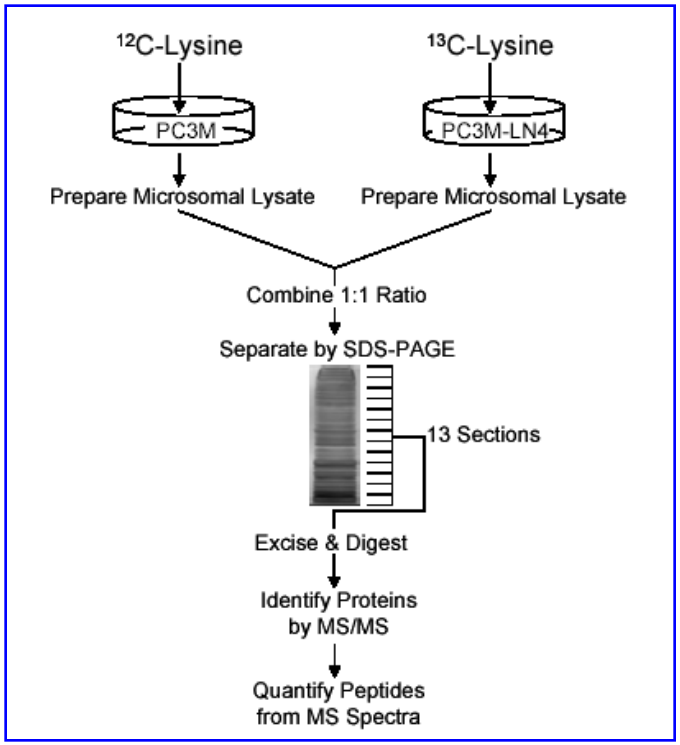
(2) Commercially available ICAT reagent

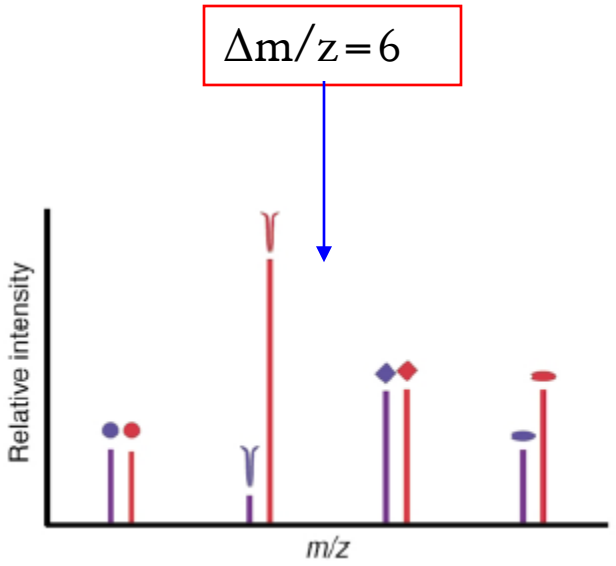
C^{13}ICAT reagent: Oda Y, et al., Anal Chem, 2003, 75(9): 2159-65;
ICAT reagent: Gygi SP. Nat Biotechnol, 1999, 17: 994-999
iTRAQ reagent: Choe LH, et al., Electrophoresis, 2005, 26(12): 2437-49

(3) 酶促同位素标记 (Post-digest isotope Labeling of peptides)(^{18}O)

Schnaubelt M, et al., Rapid Commun Mass Spectrom, 2001, 15: 2456-2465)

SILAC (Stable isotope labeling with amino acids in cell culture)

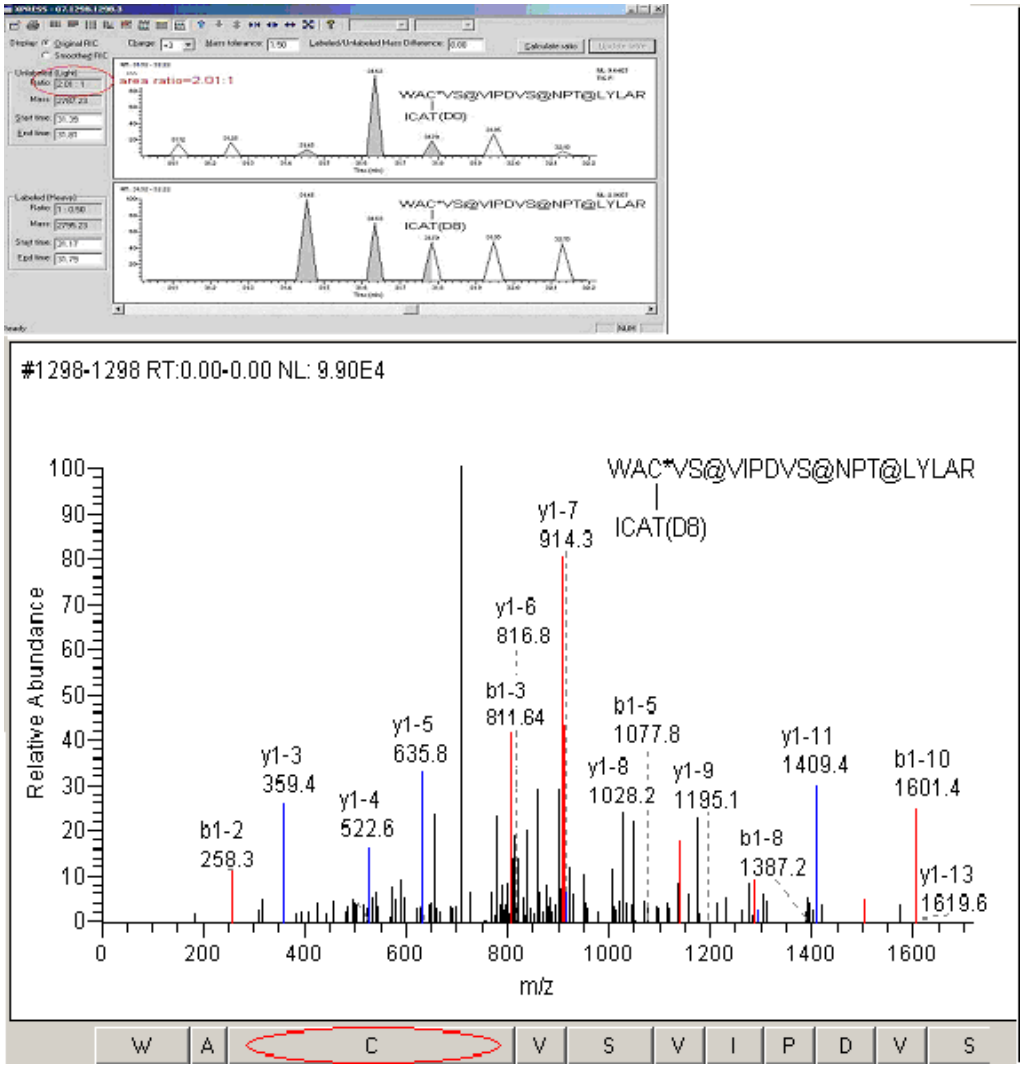




(Blaqoev B, et al., Nat Biotechnol, 2003, 21: 315-318)

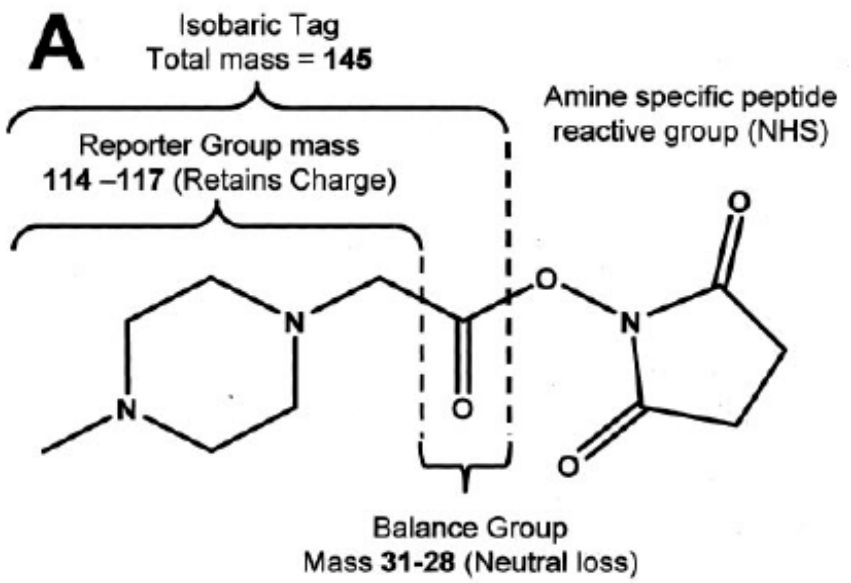
优点：①省去了体外标记化学反应和分离纯化步骤，有利于低丰度蛋白质的检测定量。②完全兼容任何传统的蛋白分离手段。

缺点：①不能对组织样品进行分析定量。②培养细胞在富含稳定同位素介质中生长，可能会影响细胞的繁殖，由此改变蛋白质的表达水平。③昂贵，不适用于人体样品。



Identification and quantification of ICAT-labeled peptide

Isobaric tags for relative and absolute quantitation(iTRAQ)



(*Ross PL,et al., Molecular Cell Proteomics,2004,3(12): 1154-1169*)

(3) 酶促同位素标记
(Post-digest isotope Labeling of peptides)(^{18}O)

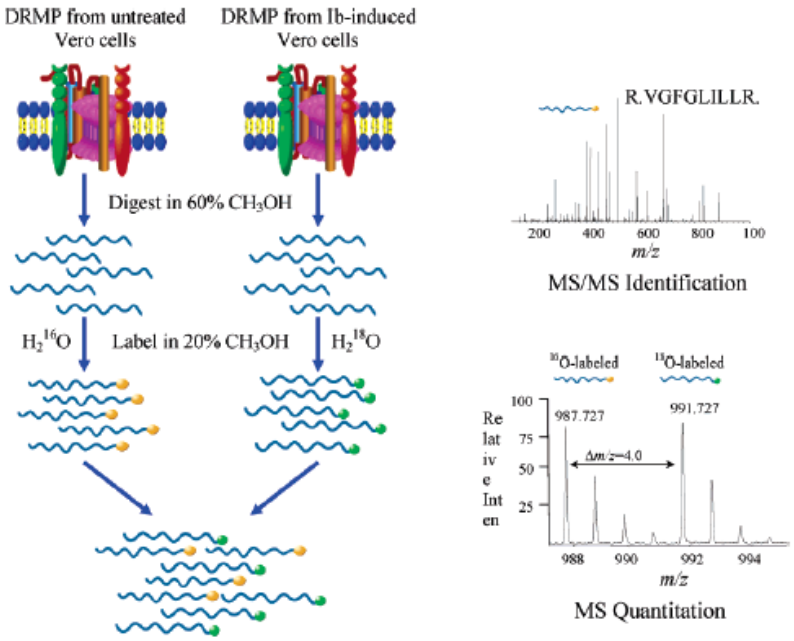


Figure 3. Outline of the $^{18}O/^{16}O$ proteolytic labeling procedure used for multidimensional comparative membrane proteomics of Ib-induced Vero cells.

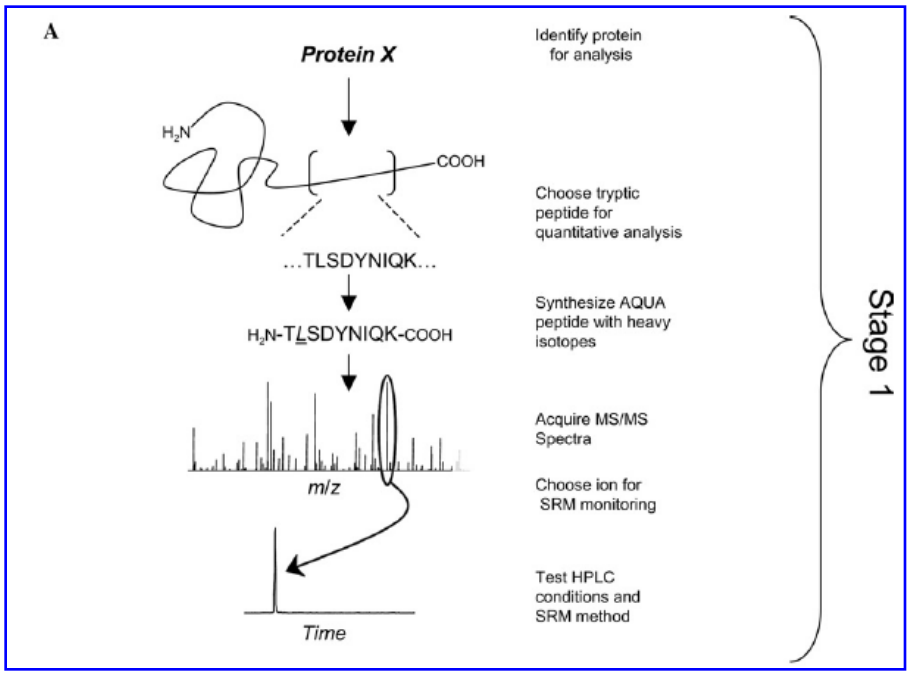
Enzymatic labeling: trypsin catalyzed O^{16} to O^{18} exchange

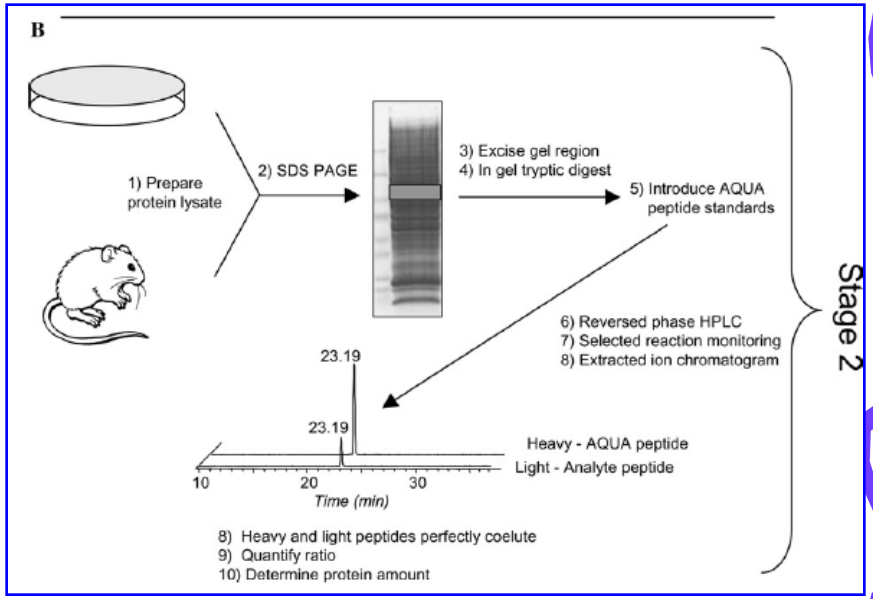
优点：①。简单，快速。②完全标记，不带倾向性。③不会丢失翻译后修饰信息。

缺点：①不稳定。②完全标记后肽段只相差4Da。

(Stemart II, et al., Rapid Commun Mass Spectrom, 2001, 15: 2456-2465)

(4) 靶蛋白、靶肽及PTM的定量：LC-MS/MS
-筛选或确证疾病相关的标记分子





Flowchart of AQUA based on LC-SRM

新趋势：临床上流行利用MALDI-TOF质谱进行靶蛋白及靶肽的绝对定量，一次性处理成千上万的临床样品，用于肿瘤等疾病的Biomarker高通量筛选和确证。

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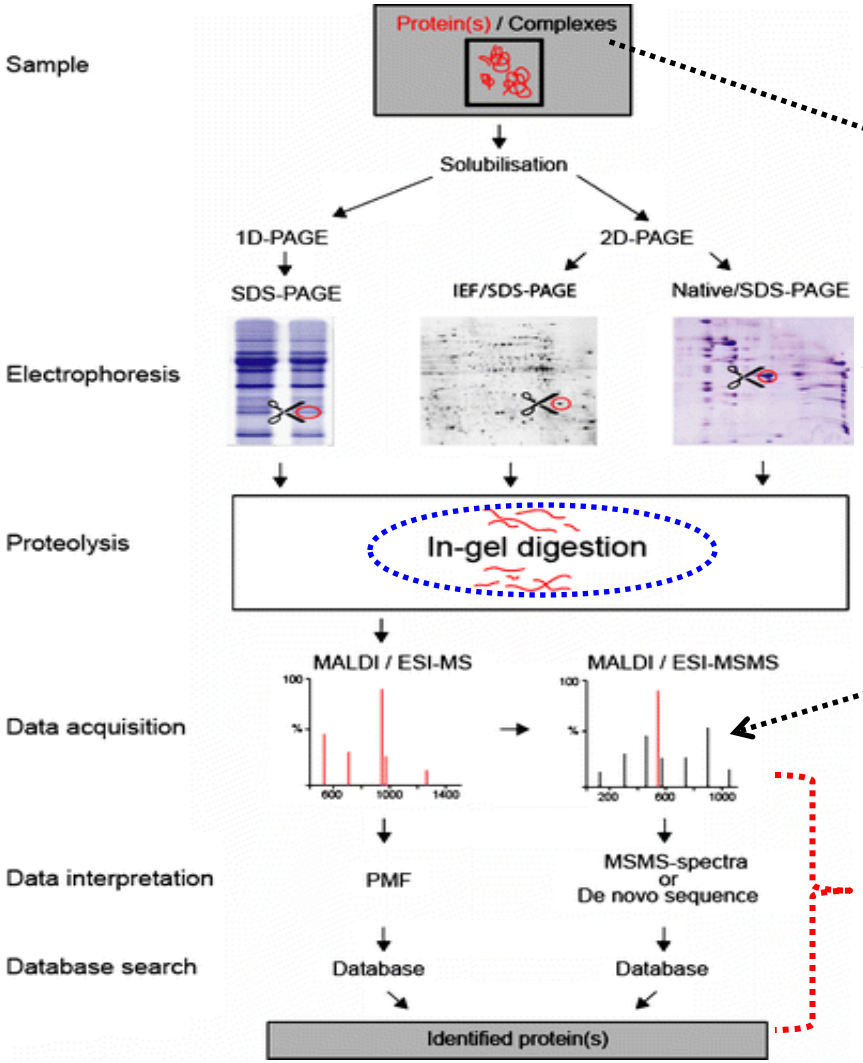


1D,2D or multidimensional liquid chromatography

In solution digestion

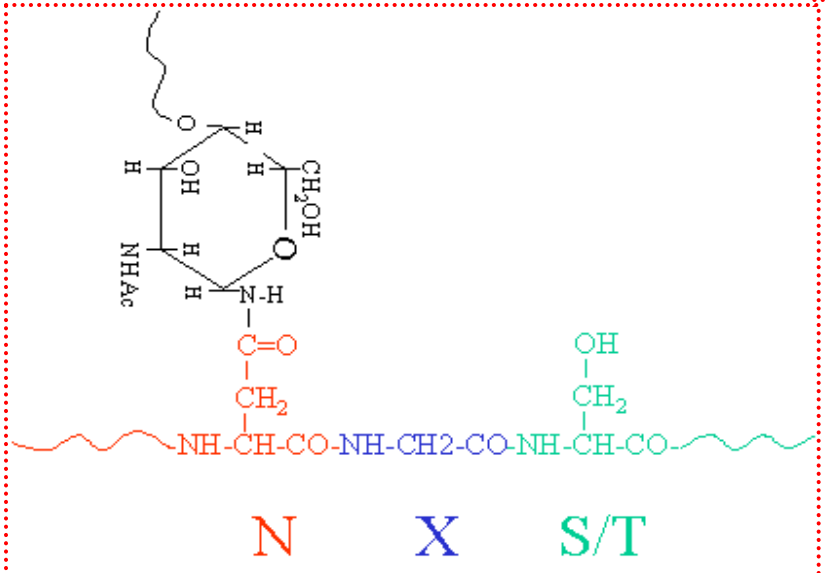
Identification of PTMs

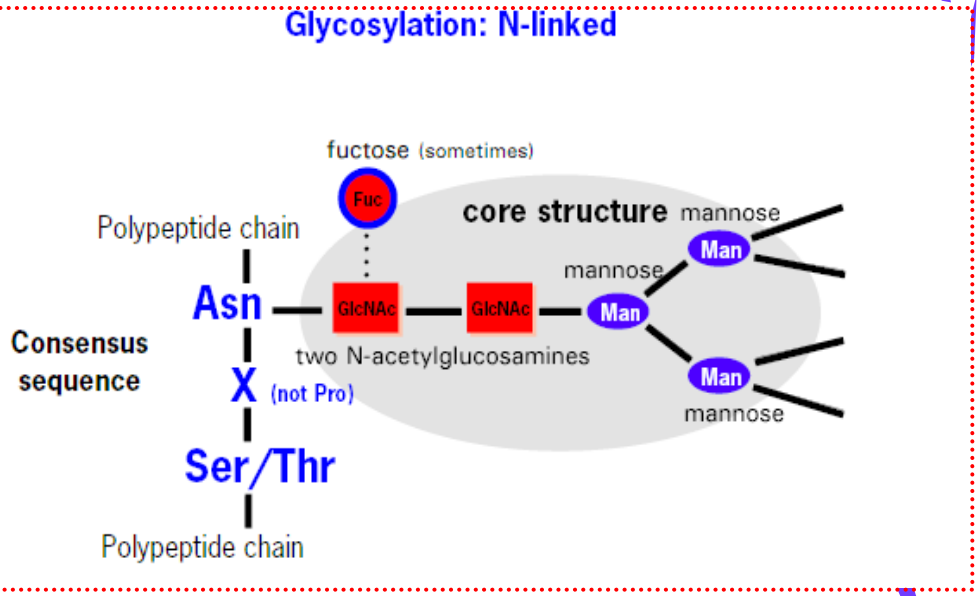
Typical workflow for bottom-up MS based proteomics

常见的蛋白翻译后修饰
Post-translational modification, PTM

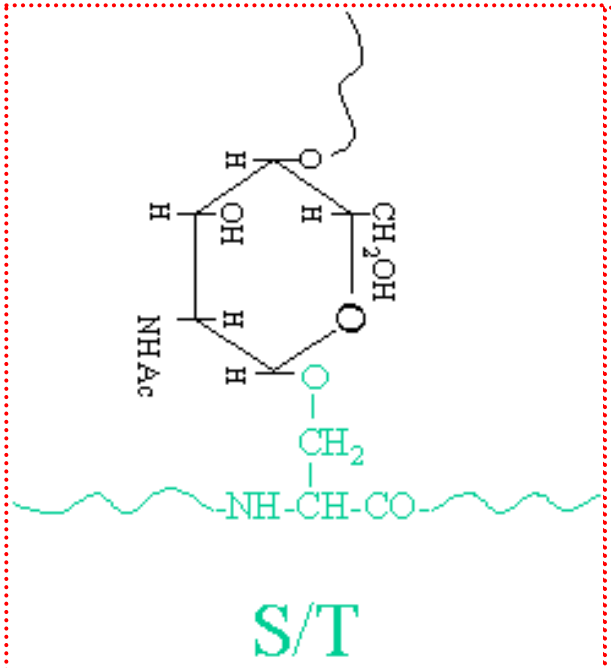
- 糖基化(Glycosylation)
- 甲基化(Methylation)
- 乙酰化(acetylation)
- 磷酸化(phosphorylation)
- 硒代半胱氨酸的鉴定(SeCys)
- 基因突变位点的鉴定
- 二硫键位点和数目的鉴定
- 泛素化研究（和蛋白降解直接相关）
- ……?

N-linked Glycosylation



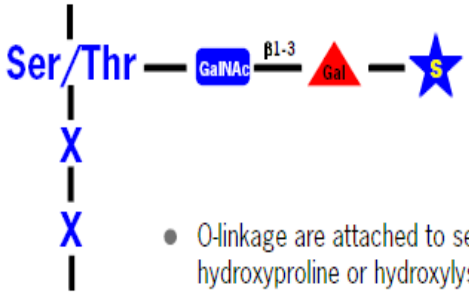


O-linked Glycosylation



Glycosylation: O-linked

O-linked core structures



1. Galβ-1,3GalNAcα-Ser/Thr
2. GlcNAcβ-1,6(Galβ1,3)-GalNAcα-Ser/Thr
3. GlcNAcβ-1,3GalNAcα-Ser/Thr
4. GlcNAcβ-1,6(GlcNAcβ-1,3)α-Ser/Thr
5. GalNAcα-1,3GalNAcα-Ser/Thr
6. GlcNAcβ-1,6GalNAcα-Ser/Thr

- O-linkage are attached to serine, threonine, tyrosine and, occasionally, to hydroxyproline or hydroxylysine
- Cores are elongated by addition of galactose, fucose, sialic acid and galactosamine residues and can carry other modifications
- O-linked glycans tend to be moderate in size and of varied structure
- O-linked glycans have been implicated in regulation processes

nature biotechnology

Identification and quantification of N-linked glycoproteins using hydrazide chemistry, stable isotope labeling and mass spectrometry

Hui Zhang, Xiao-jun Li, Daniel B Martin & Ruedi Aebersold

Zhang H, et al., Nat Biotechnol, 2003, 21(6): 660-666

nature biotechnology

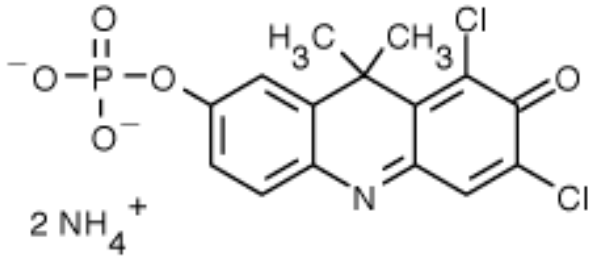
Lectin affinity capture, isotope-coded tagging and mass spectrometry to identify N-linked glycoproteins

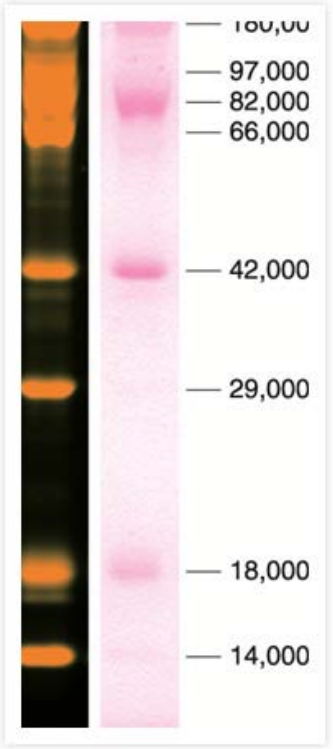
Hiroyuki Kaji[1], Haruna Saito[1], Yoshio Yamauchi[2], Takashi Shinkawa[1], Masato Taoka[1], Jun Hirabayashi[3], Ken-ichi Kasai[3], Nobuhiro Takahashi[2,4] & Toshiaki Isobe[1,2,5]

Kaji H, Saito H, et al., Nat Biotechnol, 2003, 21 (6): 667-672

Glycoprotein Gel Stain

Detection of glycoproteins and total protein on an SDS-polyacrylamide gel using the Pro-Q Fuchsia Glycoprotein Gel Stain Kit.



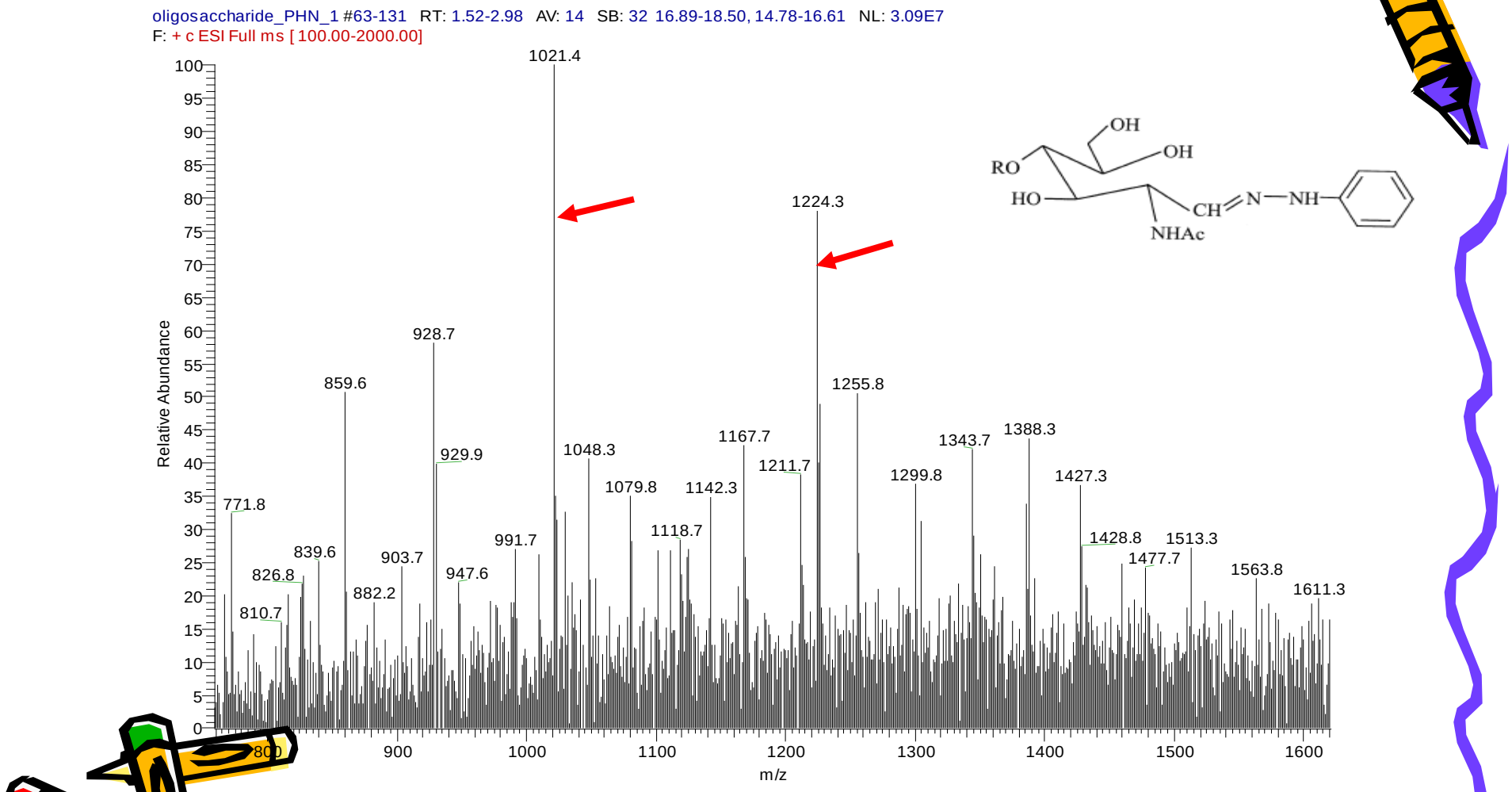


Pro-Q™ Glycoprotein Stain (DDAO phosphate)
Molecular Formula: $C_{15}H_{18}Cl_2N_3O_5P$ (MW 422.20)

Steinberg TH, Proteomics, 2001, 1, 841-855

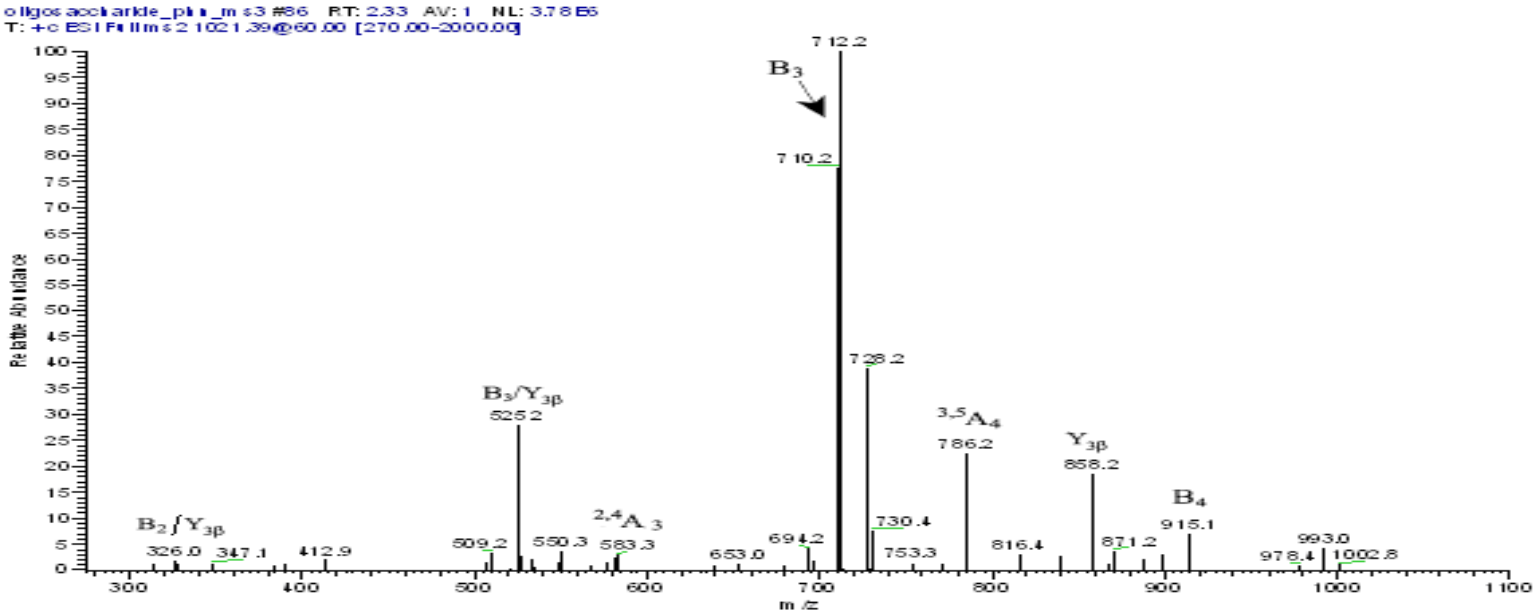
(http://www.invitrogen.com/site/us/en/home/support/Research-Tools/Image-Gallery/Image-Detail.2410.html)

苯肼(Phenylhydrazine, PHN)标记寡糖的鉴定



ESI mass spectrum of PHN-glycans released from chick ovalbumin

([M+Na]$^+$, m/Z 1021.30)

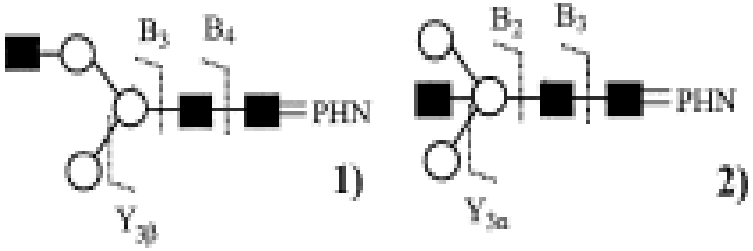


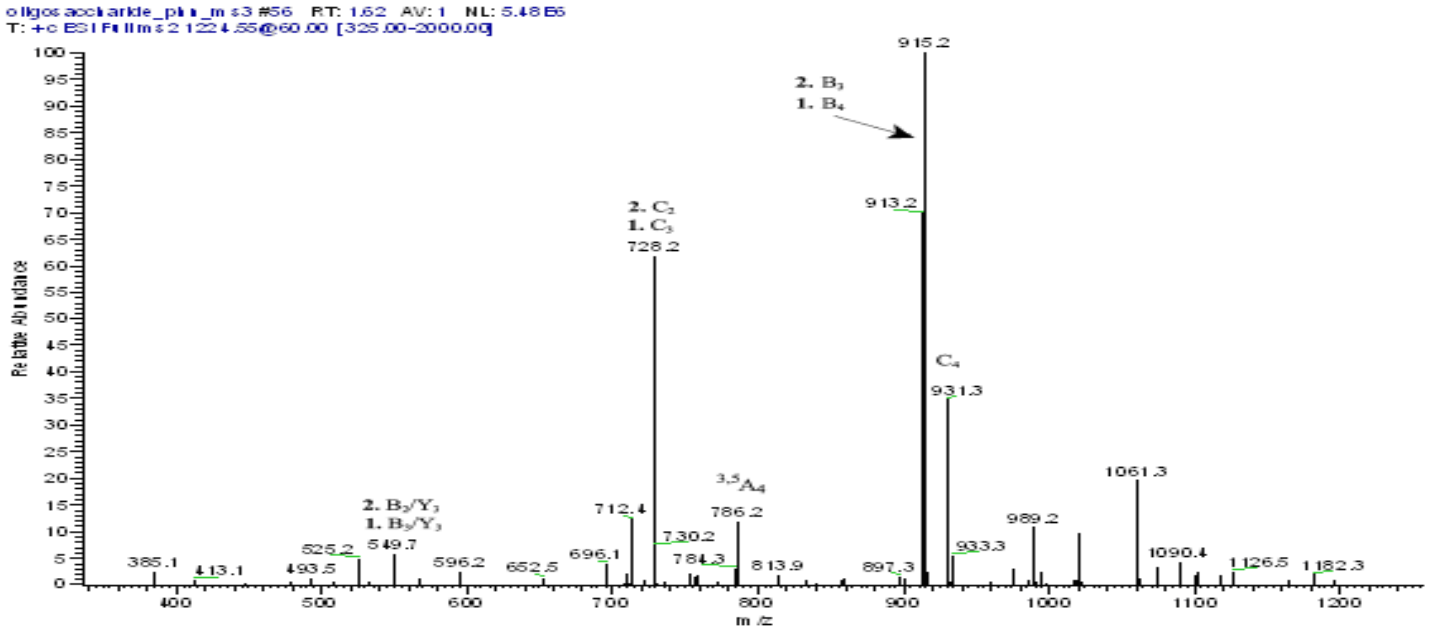
MS/MS spectrum of precursor m/z 1021.

■ N-Acetylglucosamine

○ Mannose

([M+Na]+, m/Z 1224.55)



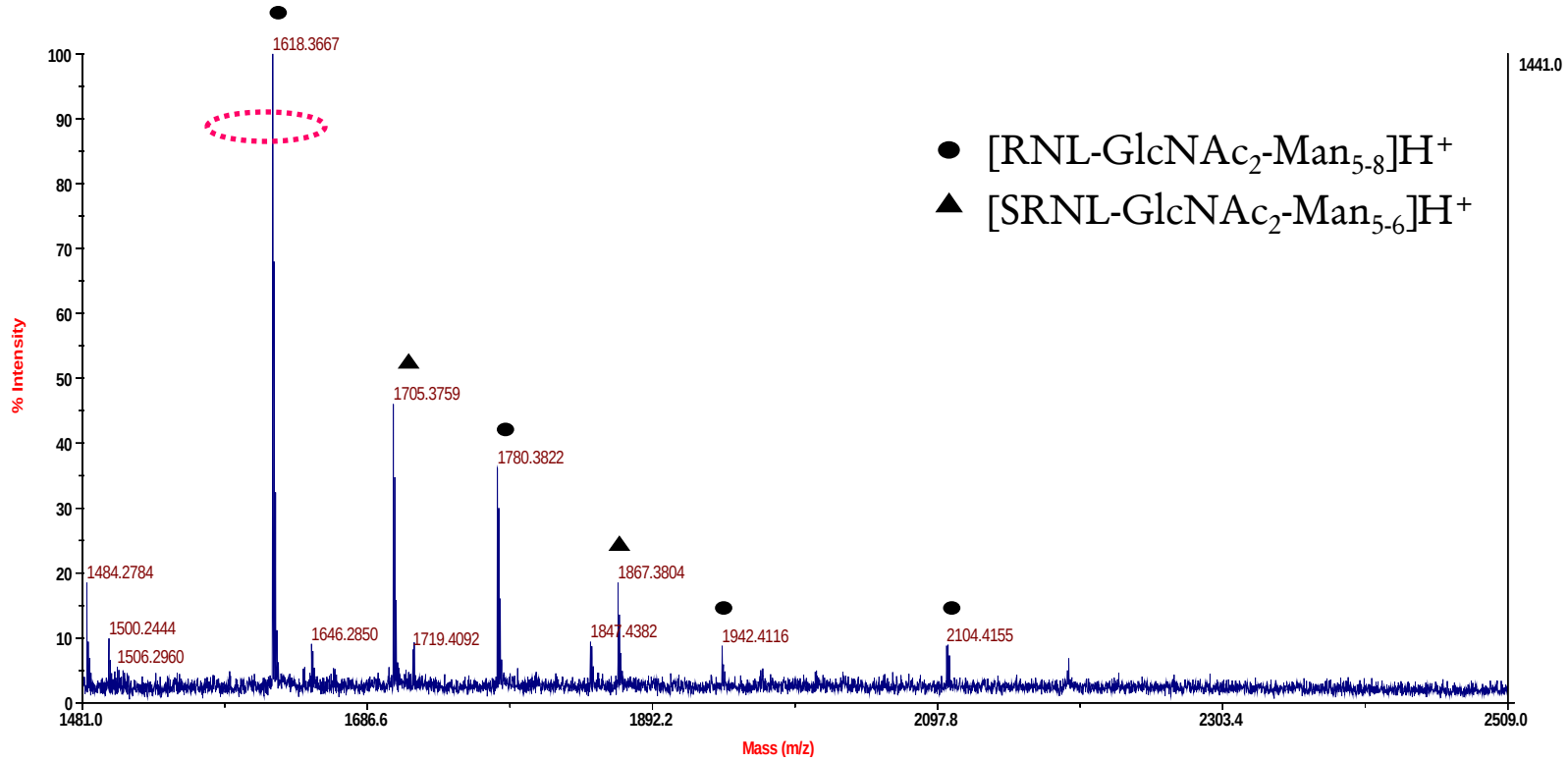


MS/MS spectrum of precursor m/z 1224.55.

N-Acetylglucosamine

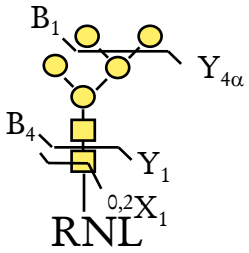
Mannose

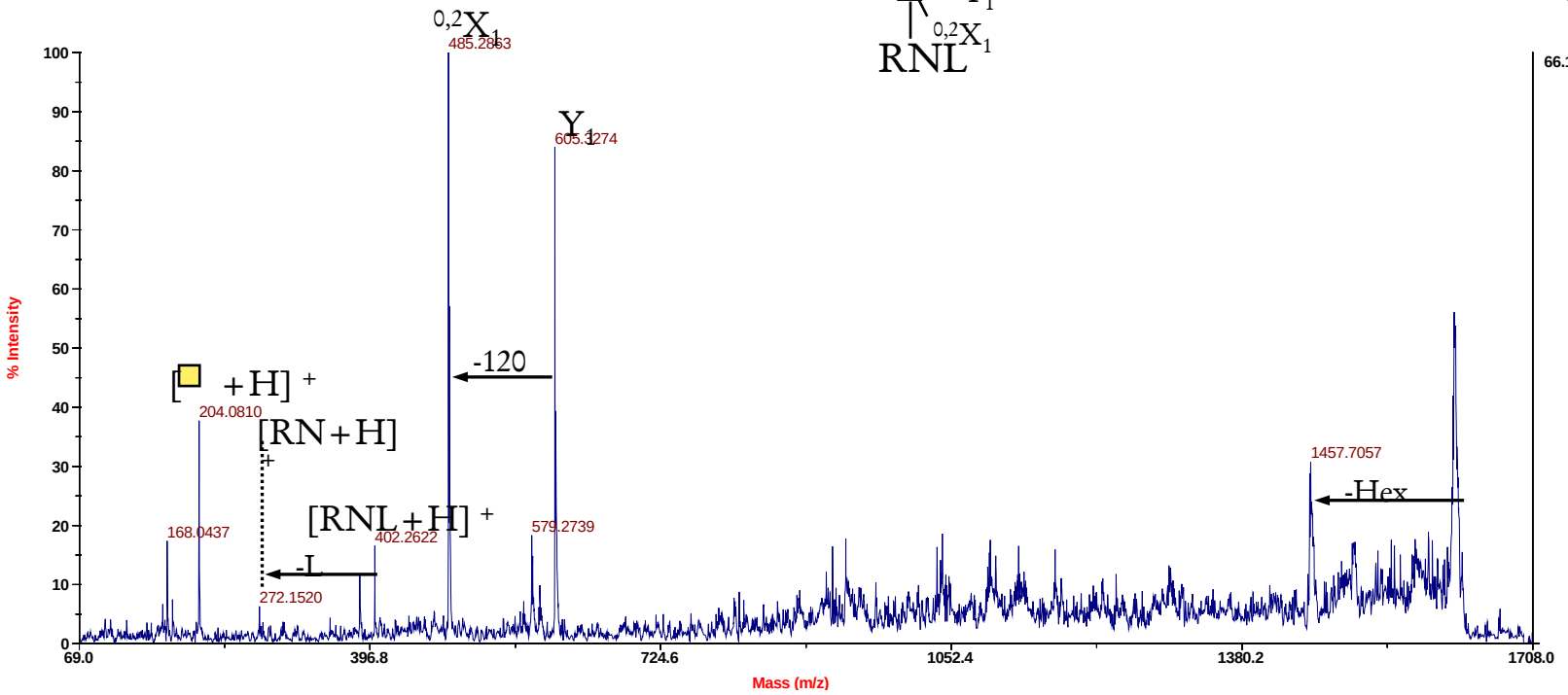
利用质谱分析非专一性酶切产生的糖肽



MS spectrum of glycopeptides produced by non-specific digestion of Rnase B.

Precursor ion: 1618.2





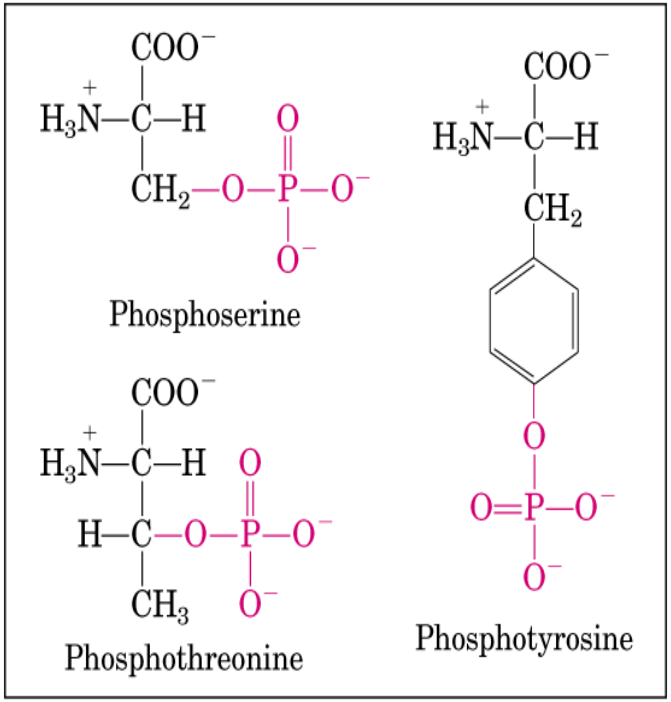
MS/MS spectrum of precursor m/z 1618.2 from non-specific digestion of RNase B.

Protein phosphorylation

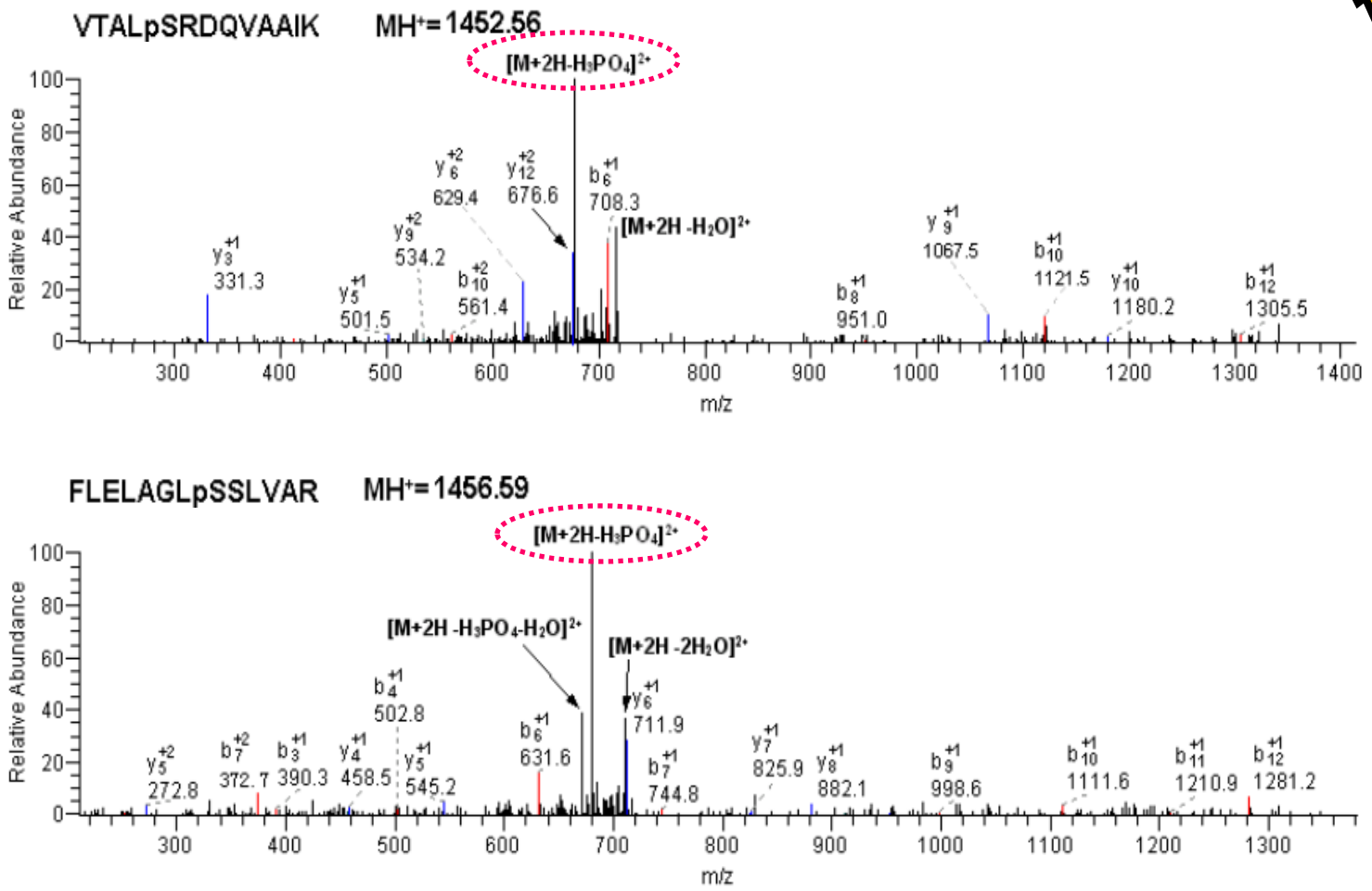
PhosphoBase v. 2.0 (http://phospho.elm.eu.org/) by EMBL

Enzymes:

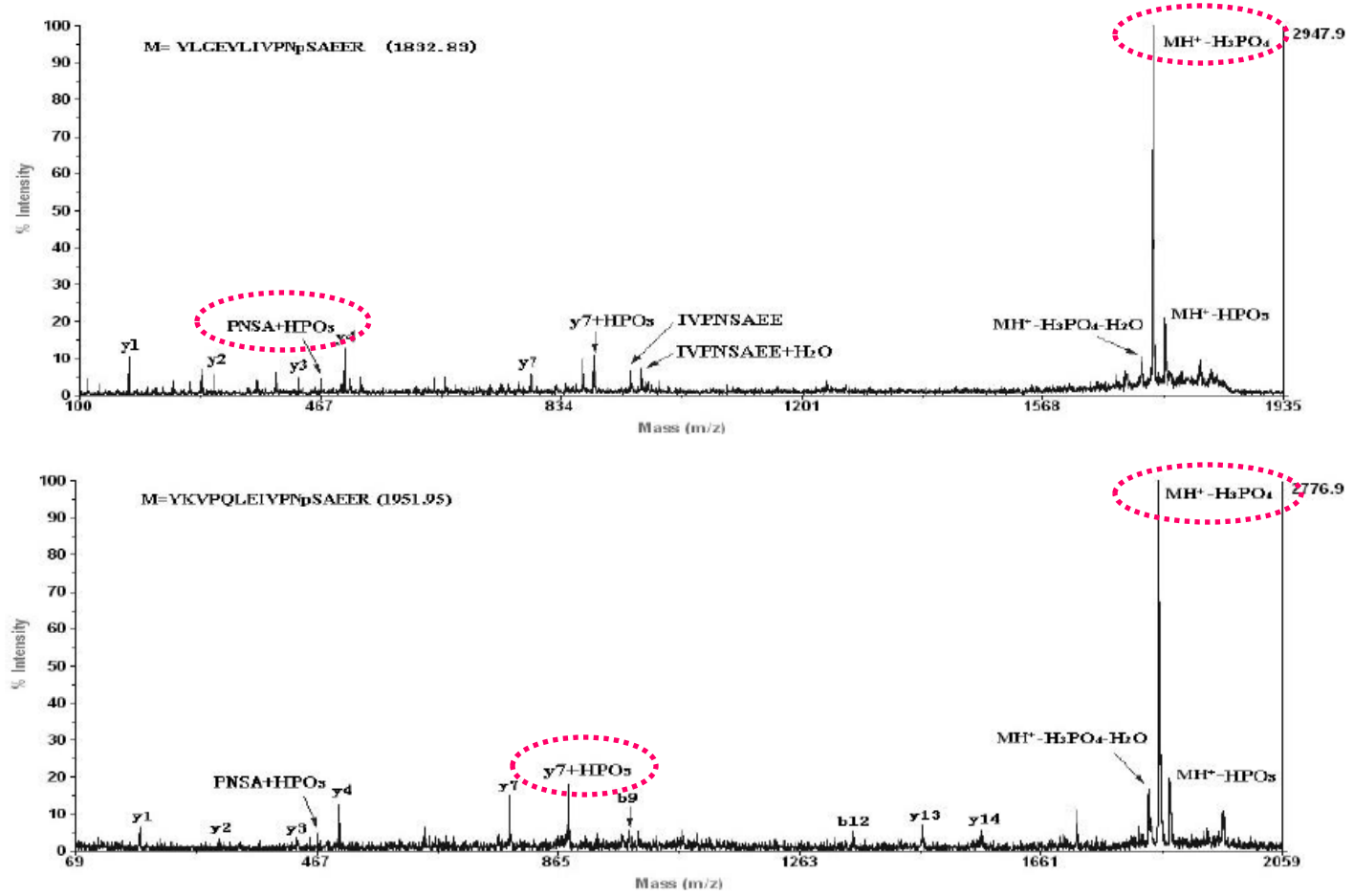
1. ***Basophilic protein kinases (e.g. protein kinase C (PKC))***
2. ***Acidophilic protein kinases (e.g. protein kinase CK2)***
3. ***Proline-directed protein kinases (e.g. protein kinase cdc2)***
4. ***Protein tyrosine kinases (e.g. epidermal growth factor receptor, EGFR)***
5. ***Protein serine/threonine kinases***



(a)



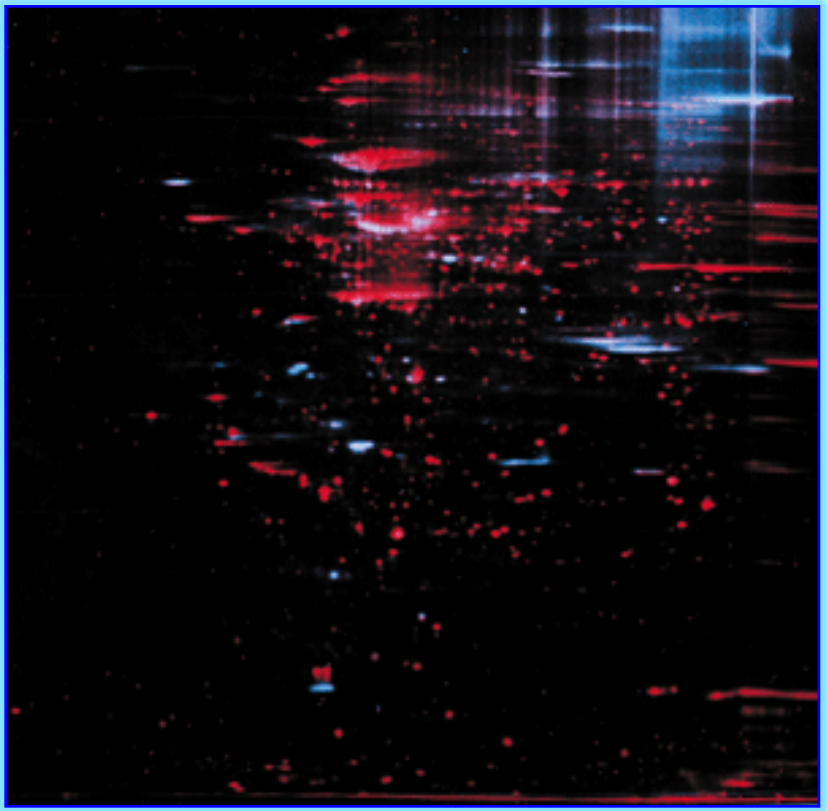
MS/MS spectra of phophorylated peptides by ESI-MS.



MS/MS spectra of phophorylated peptides by MALDI-TOF-TOF MS.

Enrichment strategies to analyze phosphoproteins/peptides

- Phosphospecific antibodies
  - Anti-pY quite successful
  - Anti-pS and anti-pT not as successful, but may be used. (Grønborg M, Kristiansen TZ, Stensballe A, et al., *Mol Cell Proteomics* 2002, 1:517–527.*)*
- Immobilized metal affinity chromatography (IMAC)
  - Negatively charged phosphate groups bind to postively charged metal ions (e.g., Fe^{3+}, Ga^{3+}) immobilized to a chromatographic support
  - Limitation: non-specific binding to acidic side chains (D, E)
    - ✓ Derivatize all peptides by methyl esterification to reduce non-specific binding by carboxylate groups. (Ficarro SB, et al., Nat Biotechnol, 2002, 20: 301-305).



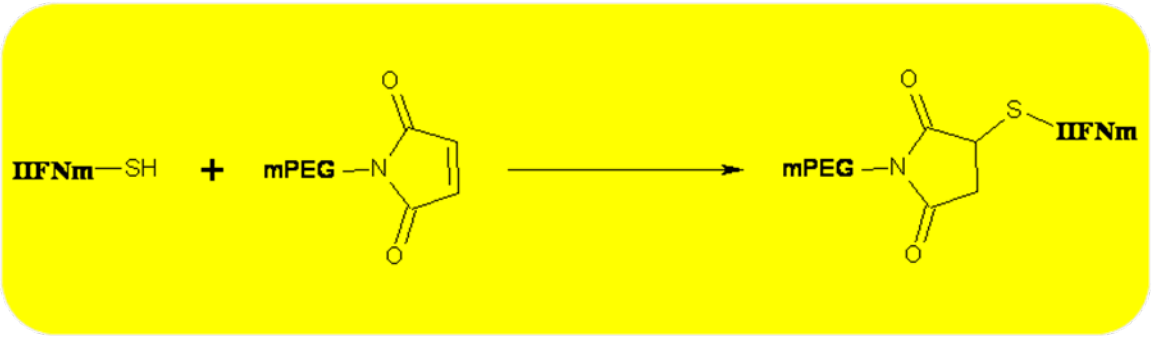
Phosphoprotein Stain. Proteins were separated by 2-D gel electrophoresis and stained with Pro-Q Diamond phosphoprotein gel stain (blue) followed by SYPRO Ruby protein gel stain (red). After each dye staining, the gel was imaged and the resulting composite image was digitally pseudocolored and overlaid.

Steinberg TH, et al., Global quantitative phosphoprotein analysis using Multiplexed Proteomics technology, Proteomics 2003, 3, 1128-1144.

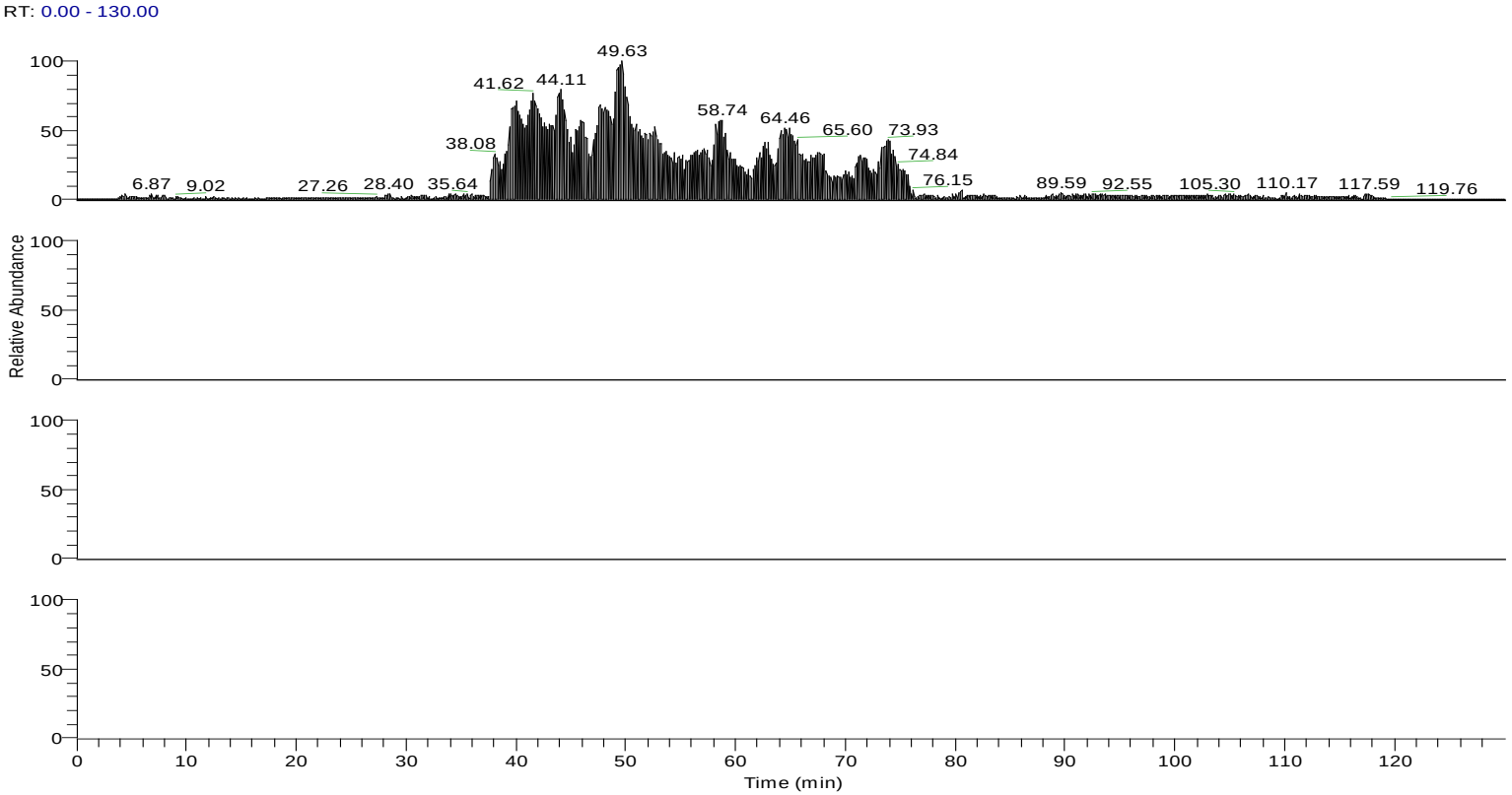
重组干扰素二硫键鉴定及定量

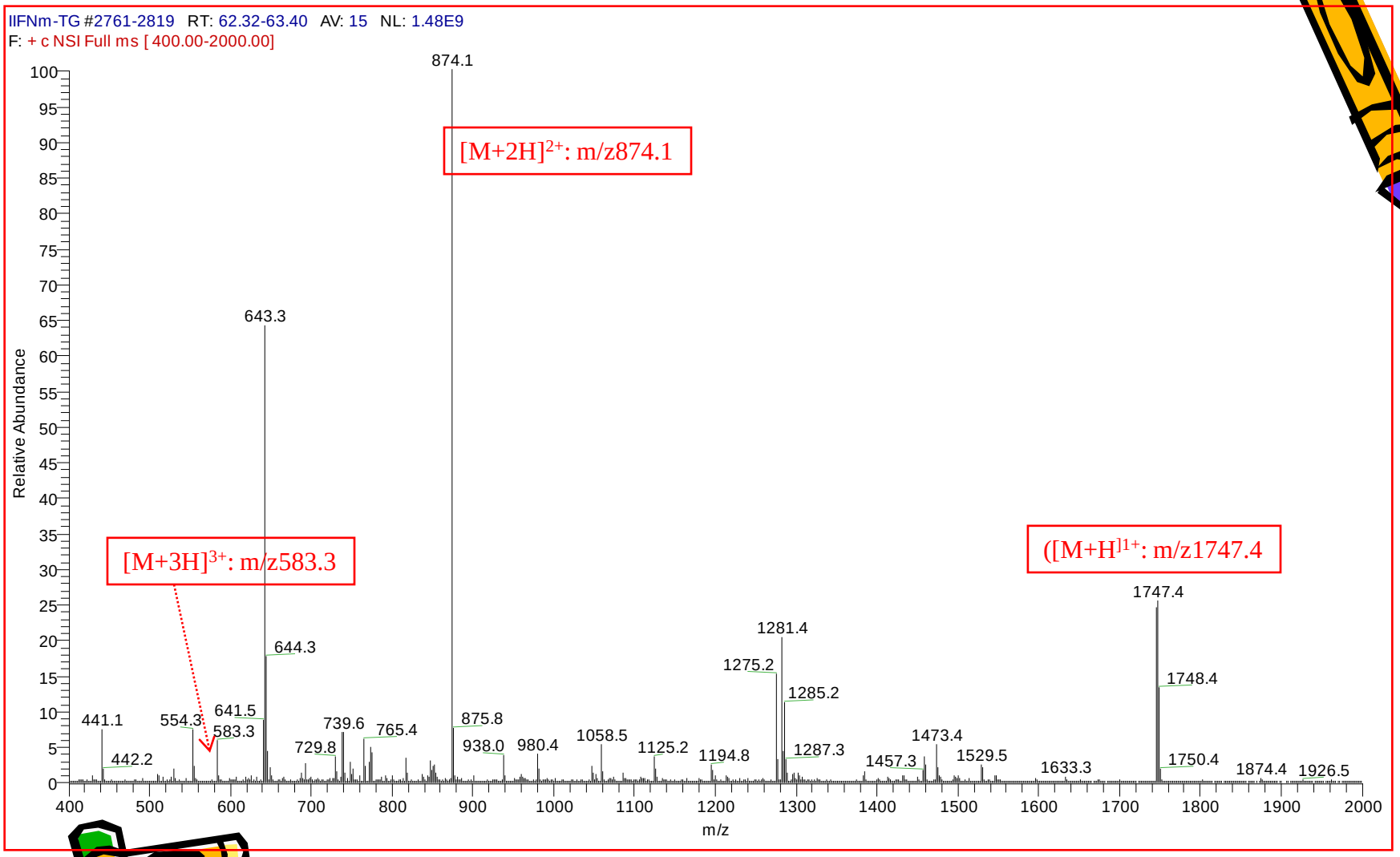
- C_1DLPQTHSLGSRRTLILLAQMRRISPFSC_{29}LKDRHDFGFPQEEFDG NQFQKAQAISVLHEMIQQTFNLFSTKDSSAAWDESLLEKFC_{86}TELY QQLNDLEAC_{99}VIQEVGVEETPLMNVDSILAVKKYFQRITLYLTEKK YSPC_{139}AWEVVRAEIMRSFSLSTNLQERLRRKD

红色标识的半胱氨酸是PEG修饰Cys_{86}，此位点原为Y，（平均分子量20KDa）的目标连接位点。PEG修饰剂与该蛋白连接反应的机理如下：

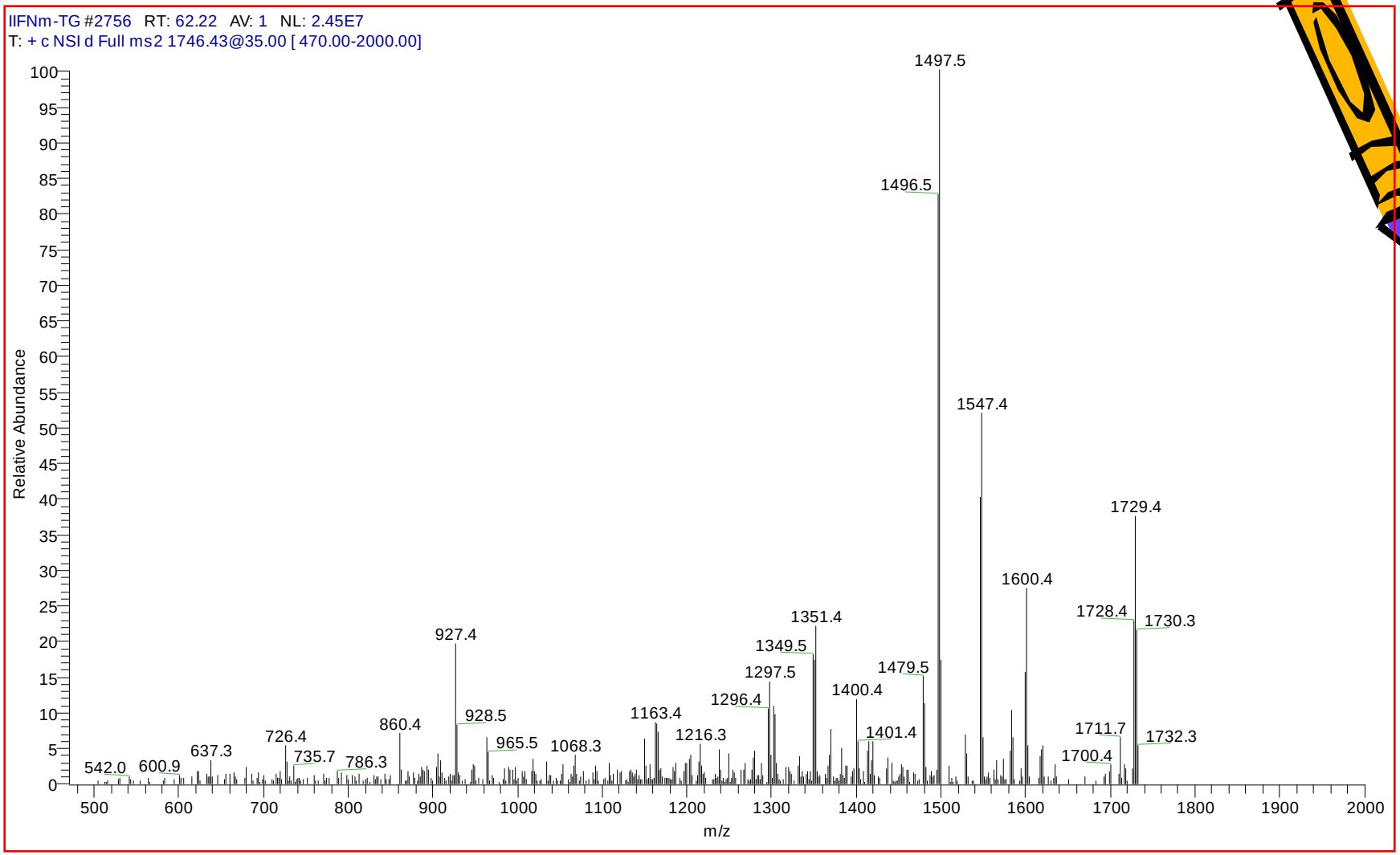


酶切条件：Trypsin+Gluc
液相条件：色谱柱：Thermo C18 (100 mm×0.18 mm，5 μm, 300Å)；流动相A:水(含0.1%甲酸)，B:乙腈 (含0.1%甲酸)；梯度：0-5min，2%B；5-90min，2%B-35%B；90-100min，35%-45%B；100-110min，45%-95%B；110-130min，95%B-2%B。流速: 0.1 ml/min，进样量10 μL。
质谱条件：离子源(ESI)喷雾电压2.2kV；毛细管温度200℃；一级质谱扫描范围m/z400-2000；二级质谱(MS/MS)扫描时碰撞能量为35%。


RT: 0.00 - 130.00
100
50
0
6.87
9.02
27.26
28.40
35.64
38.08
41.62
44.11
49.63
58.74
64.46
65.60
73.93
74.84
76.15
89.59
92.55
105.30
110.17
117.59
119.76
Relative Abundance
0
10
20
30
40
50
60
70
80
90
100
110
120
Time (min)



MS spectrum for C2-C5 : ISPFSCLK ~YSPCAWE).



2-5二硫键肽片段([M+H]$^{1+}$: m/z1747.4)的MS/MS图谱。

泛素化研究（ Ubiquitination ）

--A Proteomics Approach to Understanding Protein Ubiquitination

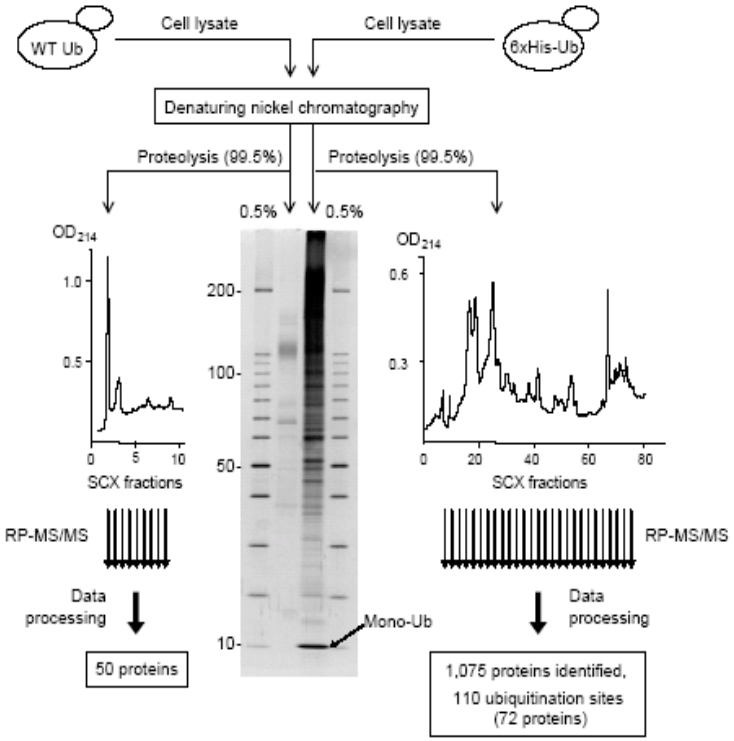


Figure 2 Isolation and sequence analysis of yeast ubiquitin conjugates. Ubiquitin conjugates were

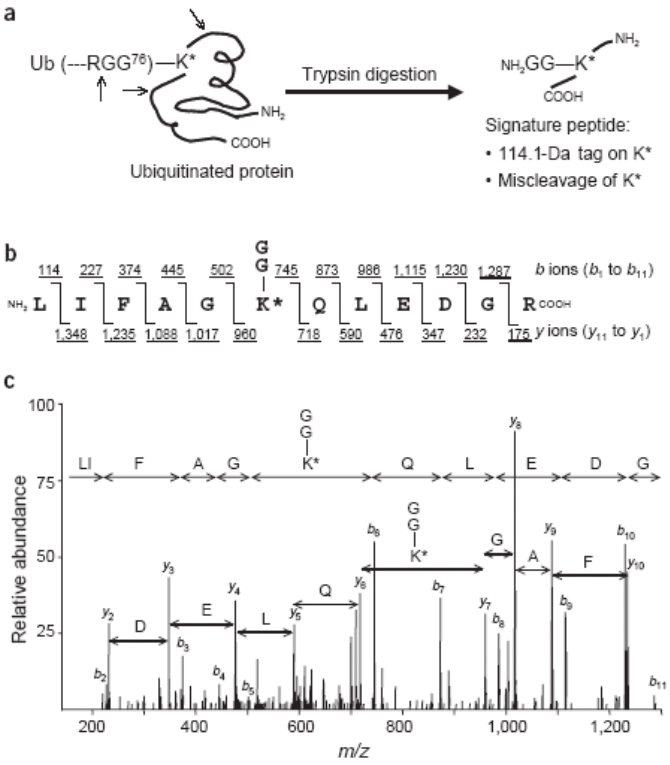
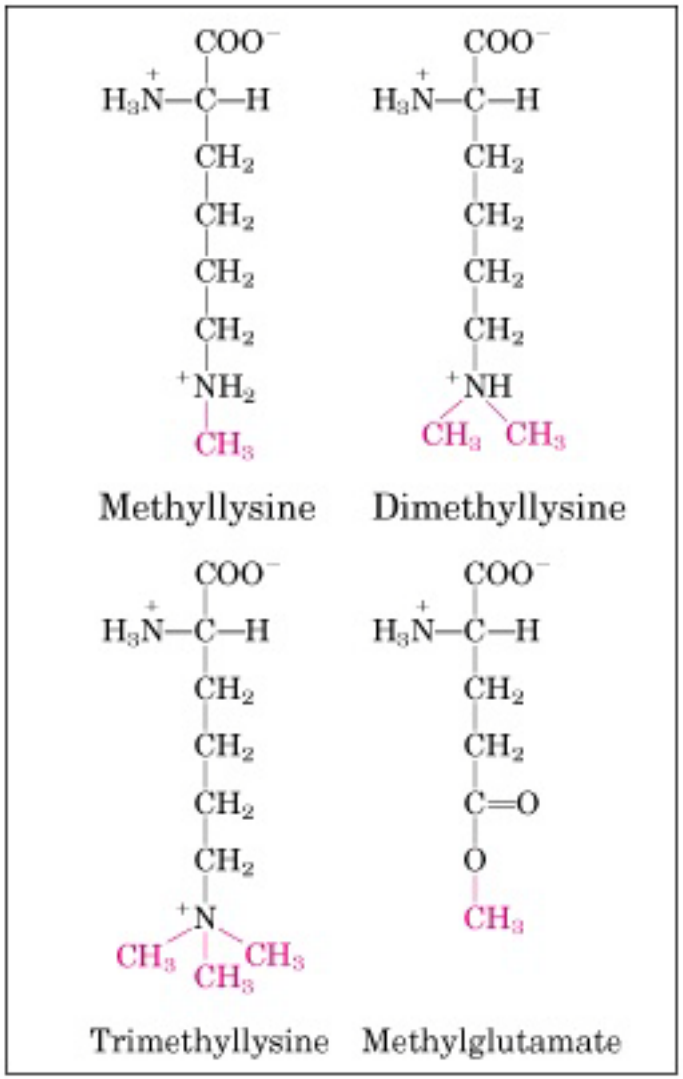


Figure 1 Strategy for identifying the precise site of ubiquitination by MS/MS. (a) After trypsin

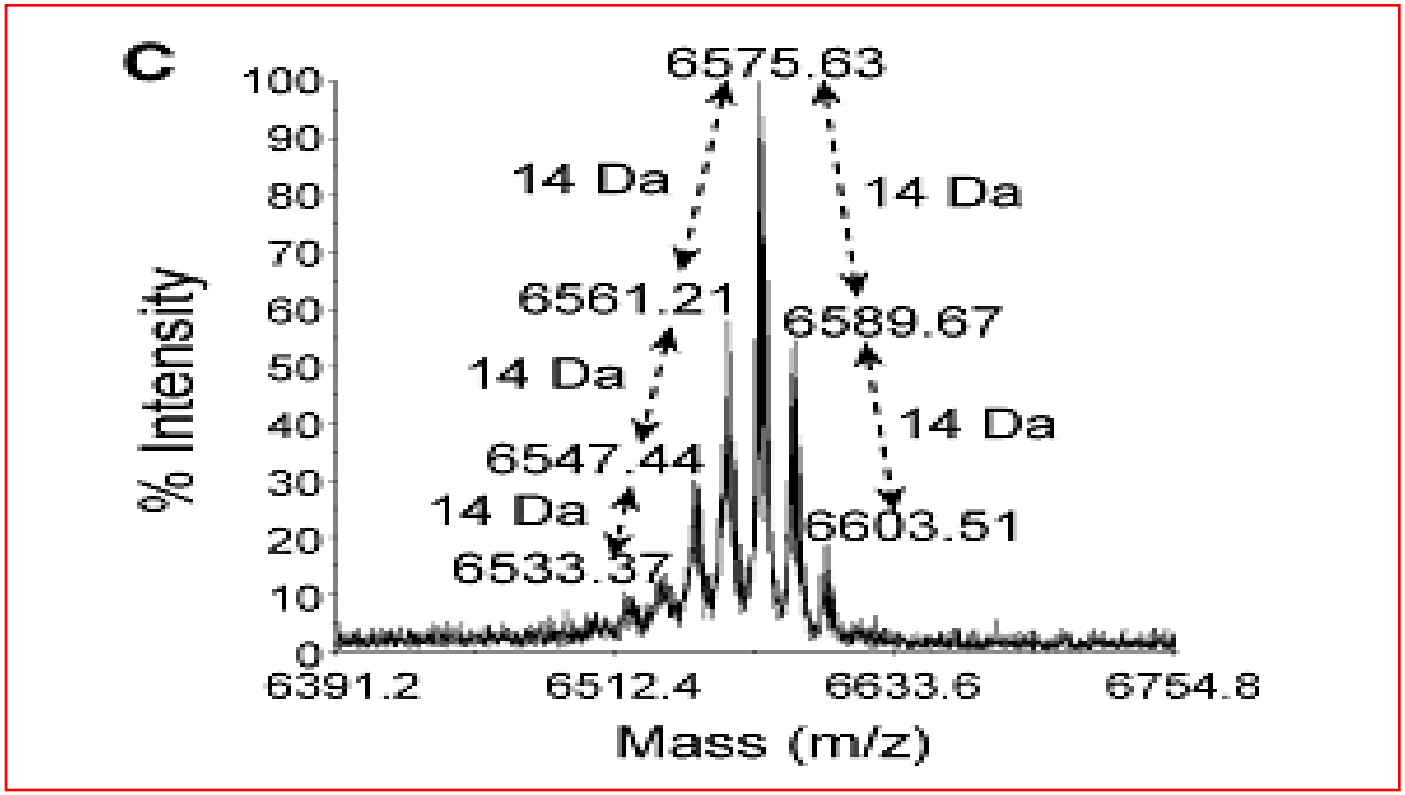
(Nat Biotechnol, 2003, 21:921-926)

蛋白质甲基化修 (Methylation)



(c)

1. Increases hydrophobicity
2. May alter the charge of the protein (e.g. if a carboxyl group of Glu is methylated)



MALDI-TOF-MS spectrum of native Cren7 from S.shibatae. A cluster of peaks with a mass difference of 14k Da between adjacent peaks are shown.

(Guo L, et al, Nucl Acids Res, 2008, 36(4): 1129-1137)

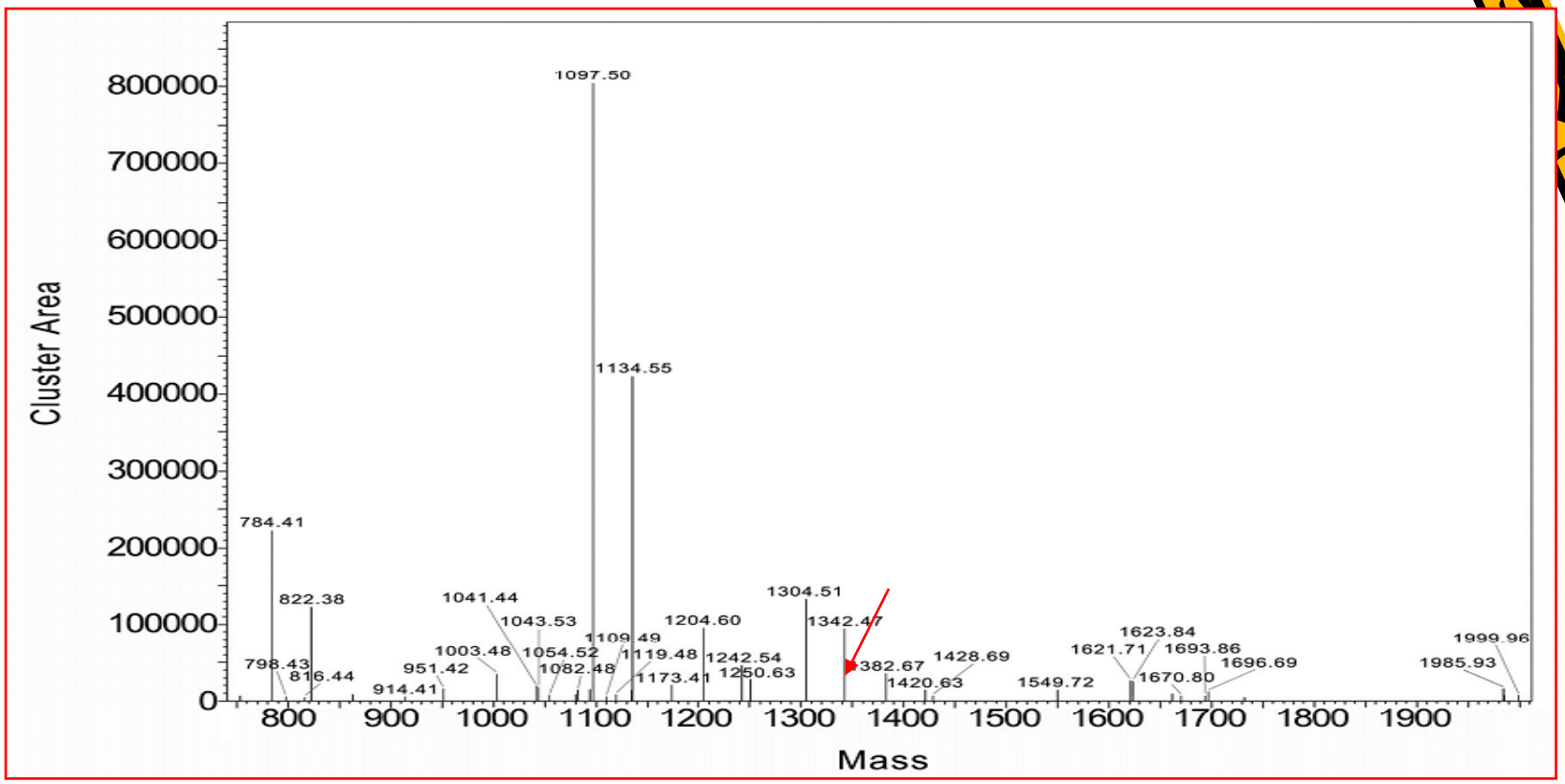


Figure S1. MALDI-TOF mass spectrometry of the tryptic digest of *S. shibatae* Cren7.

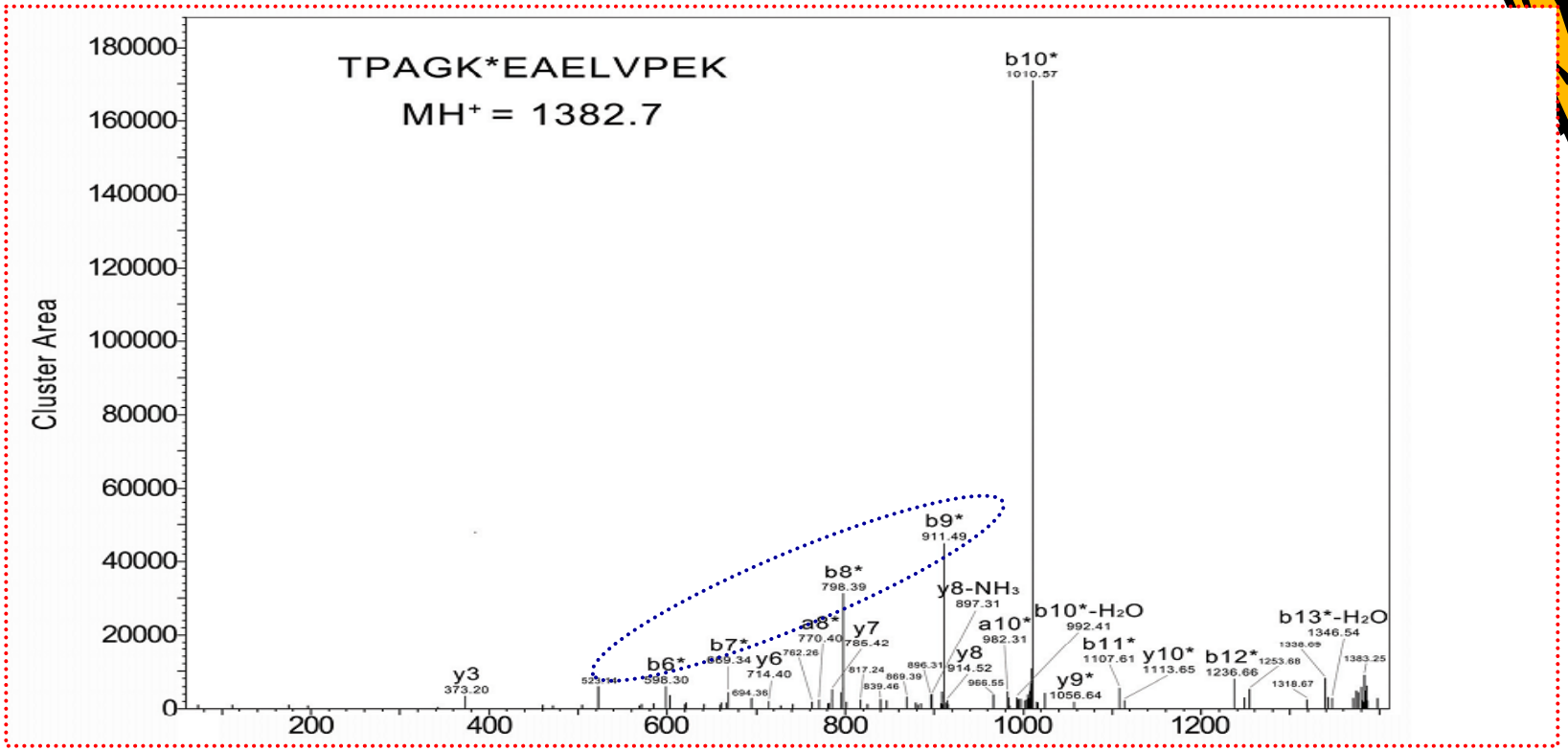


Figure S2. MALDI-TOF MS/MS spectrum of peptide TPAGK*EAELVPEK (*m/z* 1382.7) derived from the tryptic digestion of *S. shibatae* Cren7. Methylation of residue K16, or K at the 5th position from the N-terminus of the fragment, was identified. "b" and "y" ions labeled with an asterisk contained a methylated lysine.

主要内容：

一、微生物所现有仪器及检测项目简介；

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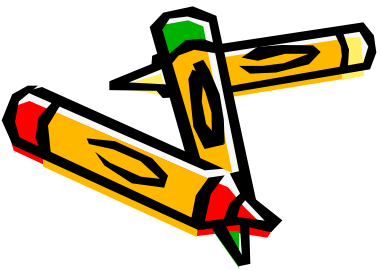
四、基于Top-down质谱的蛋白相互作用及PTM研究；

五、离子迁移质谱（IM-MS）及其应用。



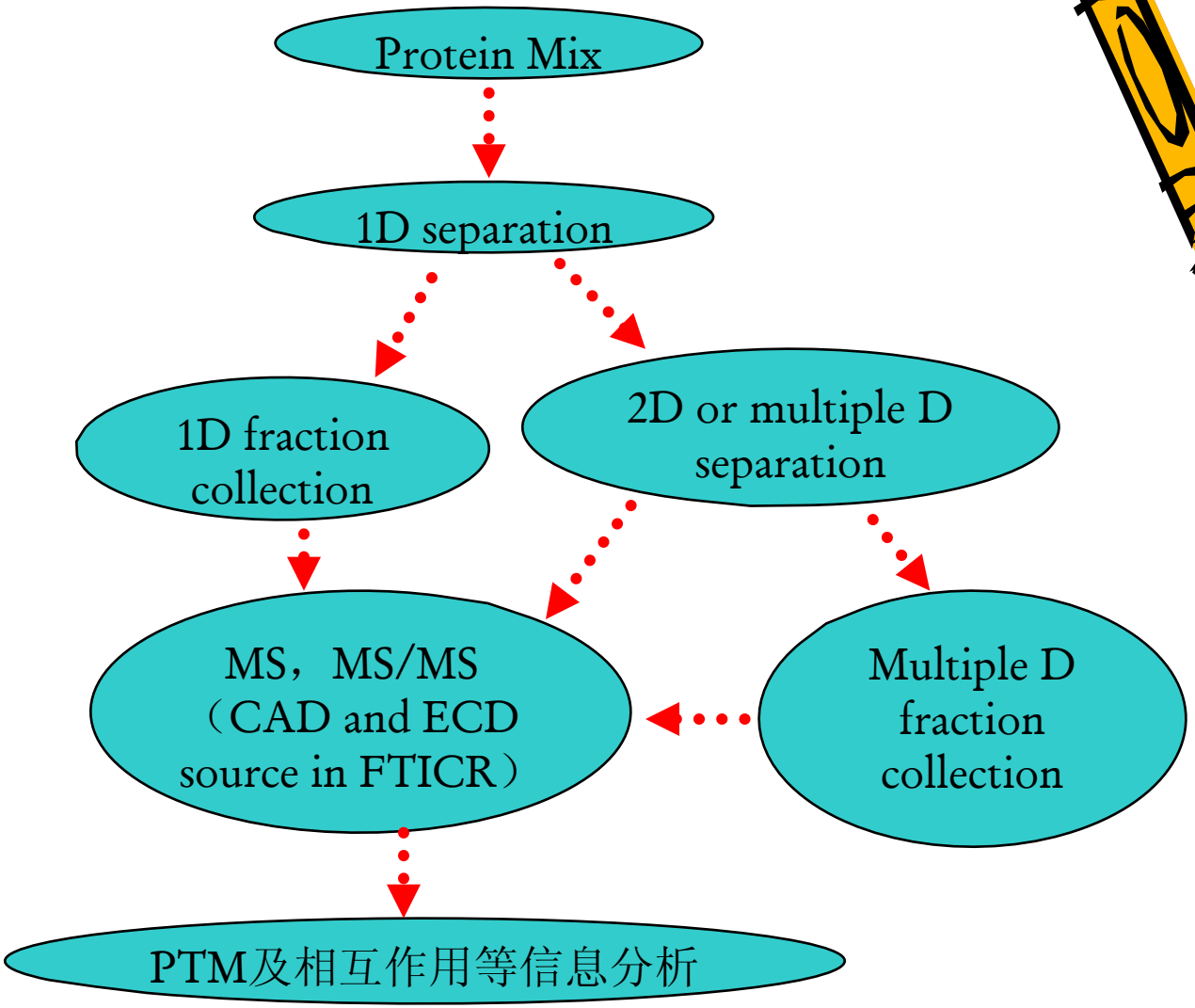
***Bottom-up* 缺点：**

样品制备时耗时劳动强度大；

较低的序列覆盖率；

酶切过程中引入认为修饰或者假阳性；

由于酶切导致真实分子量信息丢失，不能获得准确PTM以及不同PTM之间相互关系的信息。



Top-down 质谱方法学流程示意图

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Top-down质谱鉴定蛋白酶复合物中的金属离子结合位点

Top-down mass spectrometry of supercharged native protein–ligand complexes

Sheng Yin[a], Joseph A. Loo[a,b,*]

[a] Department of Chemistry and Biochemistry, University of California-Los Angeles, Los Angeles, CA 90095, United States
[b] Department of Biological Chemistry, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, CA 90095, United States

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This article is dedicated to John Fenn, who pioneered the virtues of multiple charging for protein MS analysis. He inspired us "to continue stumbling for a while along the road ahead, kicking over stones here and there, driven by curiosity to find out what may be hidden under the next one [36]." We should all strive to be as curious as John Fenn, as one can't always predict what hidden jewels can be discovered by turning over stones on the road of scientific exploration.

ABSTRACT

Tandem mass spectrometry (MS/MS) of intact, noncovalently bound protein–ligand complexes can yield structural information on the site of ligand binding. Fourier transform ion cyclotron resonance (FT-ICR) top-down MS of the 29 kDa carbonic anhydrase-zinc complex and adenylate kinase bound to adenosine triphosphate (ATP) with collisionally activated dissociation (CAD) and/or electron capture dissociation (ECD) generates product ions that retain the ligand and their identities are consistent with the solution phase structure. Increasing gas phase protein charging from electrospray ionization (ESI) by the addition of supercharging reagents, such as *m*-nitrobenzyl alcohol and sulfolane, to the protein analyte solution improves the capability of MS/MS to generate holo-product ions. Top-down proteomics for protein sequencing can be enhanced by increasing analyte charging.

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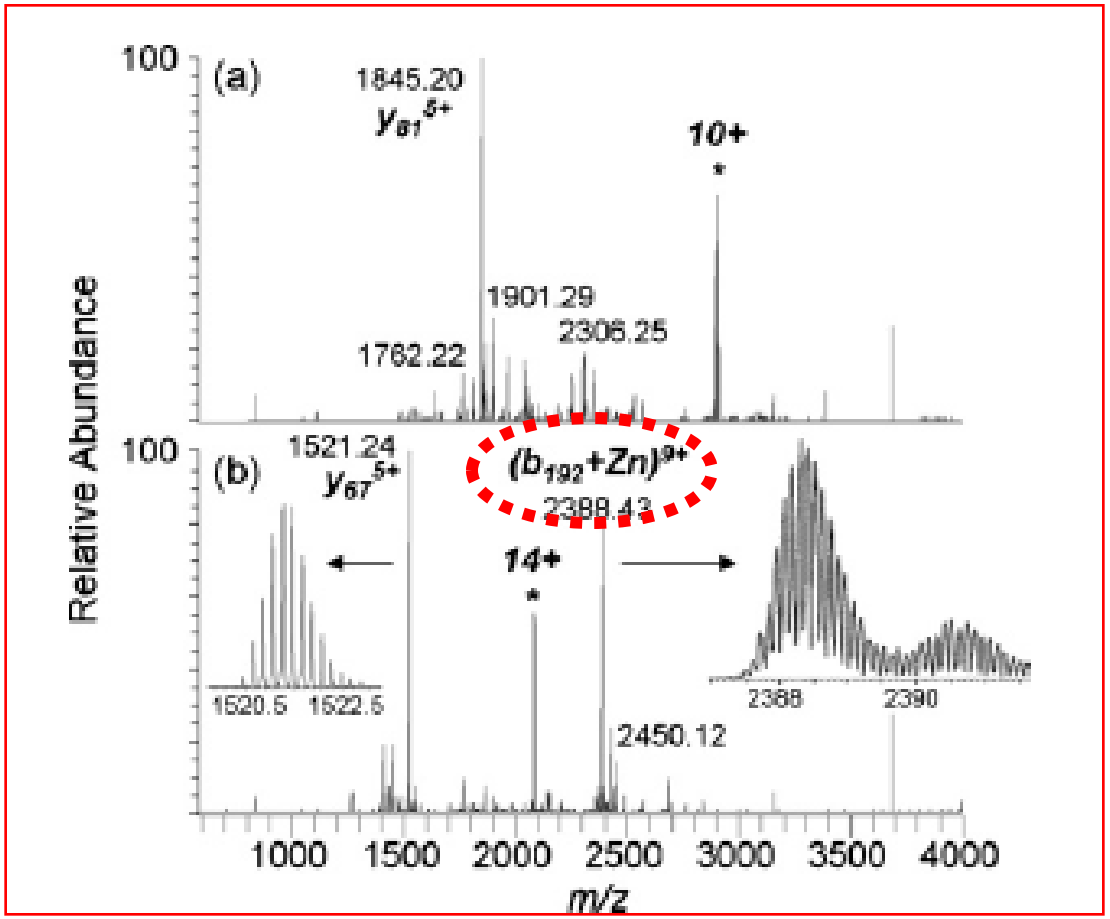
Ref: Yin S, Loo JA. Int J Mass Spectrom. 2011, 300(2-3):118-122.



ESI CAD-MS/MS mass spectra of the (a) 10+ zinc-bound CA-II and the (b) 14^{+} zinc-bound CA-II (with m-NBA). With 0.5% m-NBA, CAD of the supercharged 14+ CA-II/Zn complex generates the $y_{67}^{5+}/(b_{192} + Zn)^{9+}$ complementary product ion pair, which in sum (mass and charge) account for the entire protein molecule.

利用Top-down质谱研究心肌钙蛋白(cTnT)磷酸化修饰

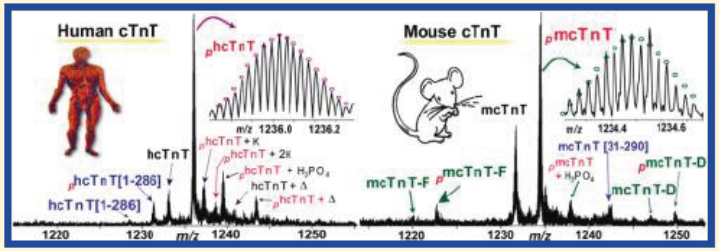
Phosphorylation, but Not Alternative Splicing or Proteolytic Degradation, Is Conserved in Human and Mouse Cardiac Troponin T

Jiang Zhang,[†,‡] Han Zhang,[†] Serife Ayaz-Guner,[†] Yi-Chen Chen,[†] Xintong Dong,[†] Qingge Xu,[†,§] and Ying Ge*[,†,§]

[†]Human Proteomics Program, School of Medicine and Public Health, [‡]School of Pharmacy, and [§]Department of Cell and Regenerative Biology, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin 53706, United States

Supporting Information

ABSTRACT: Cardiac troponin T (cTnT), the tropomyosin binding subunit of the troponin complex, plays a pivotal regulatory role in the Ca^{2+}-mediated interaction between actin thin filament and myosin thick filament. The post-translational modifications (PTMs) and alternative splicing of cTnT may represent important regulatory mechanisms of cardiac contractility. However, a complete characterization of PTMs and alternatively spliced isoforms in cTnT present in vivo is lacking. Top-down protein mass spectrometry (MS) analyzes whole proteins, thus providing a global view of all types of modifications, including PTMs and sequence variants, simultaneously in one spectrum without *a priori* knowledge. In this study, we applied an integrated immunoaffinity chromatography and top-down MS approach to comprehensively characterize PTMs and alternatively spliced isoforms of cTnT purified from healthy human and wild-type mouse heart tissue. High-resolution Fourier transform MS revealed that human cTnT (hcTnT) and mouse cTnT (mcTnT) have similar phosphorylation patterns, whereas higher molecular heterogeneity was observed for mcTnT than hcTnT. Further MS/MS fragmentation of monophosphorylated hcTnT and mcTnT by electron capture dissociation and collisionally activated dissociation unambiguously identified Ser1 as the conserved in vivo phosphorylation site. In contrast, we identified a single spliced isoform for hcTnT but three alternatively spliced isoforms for mcTnT. Moreover, we observed distinct proteolytic degradation products for hcTnT and mcTnT. This study also demonstrates the advantage of top-down MS/MS with complementary fragmentation techniques for the identification of modification sites in the highly acidic N-terminal region of cTnT.



Zhang J, Zhang H, et al.Biochemistry. 2011, dx.doi.org/10.1021/bi2006256

Top-Down MS methods:

Instumentation:

linear ion trap/Fourier transform ion cyclotron resonance (FTICR) hybrid mass spectrometer (LTQ FT Ultra, Thermo Scientific Inc., Bremen, Germany) equipped with an automated chip-based nano ESI source (Triversa NanoMate) (Advion BioSciences, Ithaca, NY).

Parameter:

1)The spray voltage was 1.21.6 kV versus the inlet of the mass spectrometer; 2)flow rate: 50-200 nL/min;

3)The resolving power of the FTICR mass analyzer: 200,000 at m/z 400;

4) For **CAD and ECD** fragmentation, individual charge states of protein molecular ions were first isolated and then dissociated using15-25% normalized collision energy (CAD) or 23% electron energy (ECD) with a duration of 25-75 ms with no delay.

Zhang J, Zhang H, et al.Biochemistry. 2011, dx.doi.org/10.1021/bi2006256

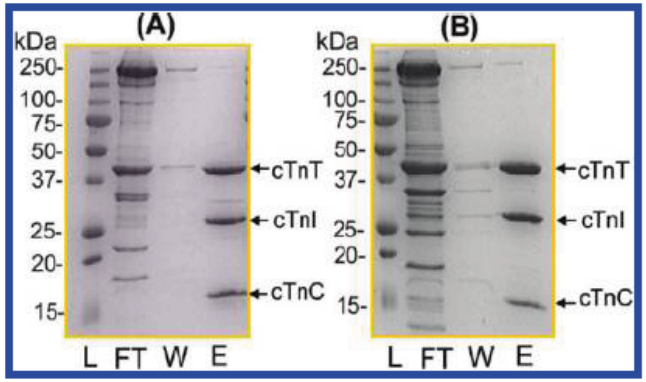


Figure 1. SDS–PAGE analysis of immunoaffinity-purified cTn complexes from (A) human and (B) mouse heart tissue samples. L, protein mass ladder; FT, sample flow through; W, wash; E, elution.

The minor degradation 28 charged peaks of hcTnT[1-286]

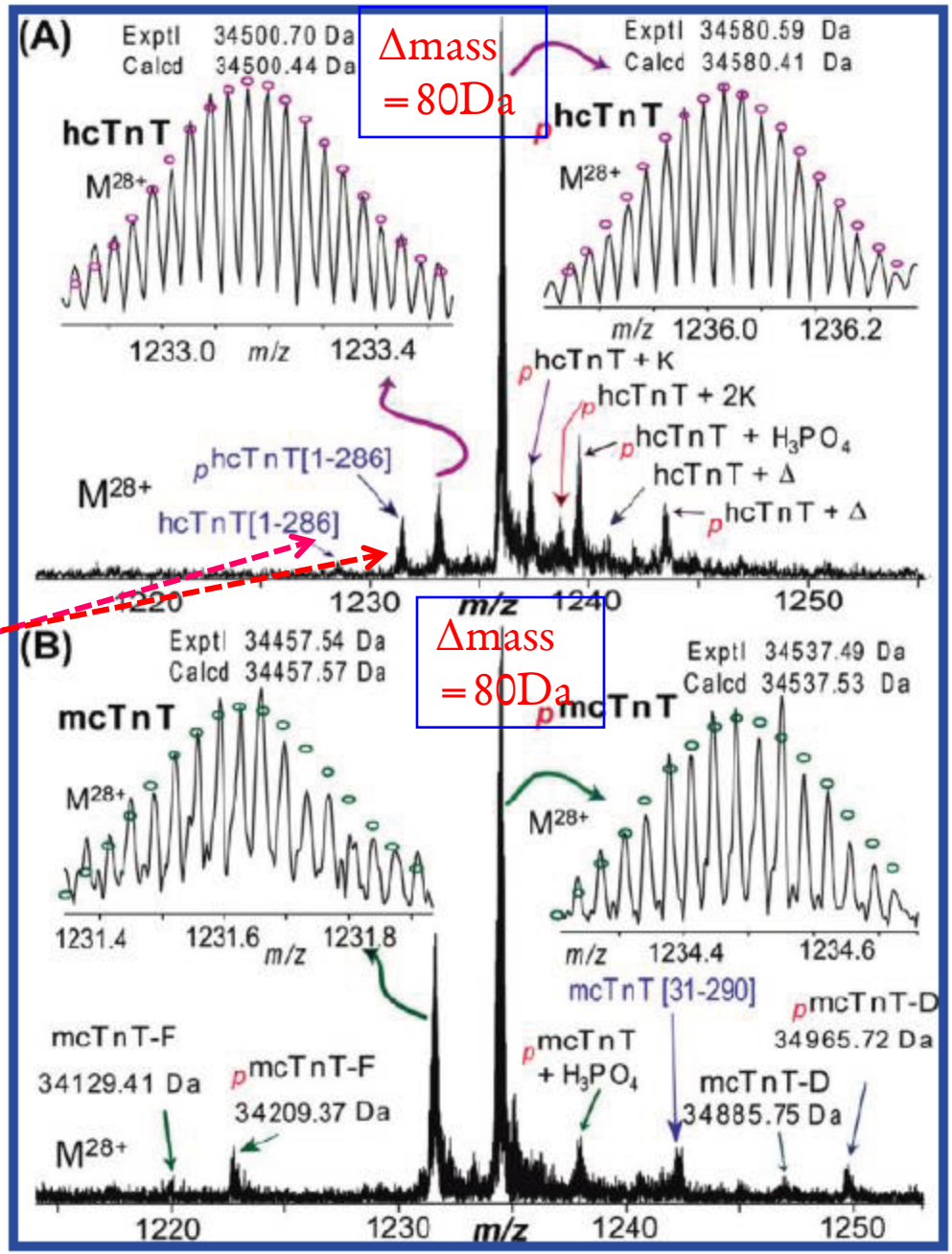


Figure 2. High-resolution ESI/FTMS spectra of intact cTnT purified from (A) human and (B) mouse heart samples. hcTnT isoform 6 (P45379-6) and mcTnT isoform E (P50752-3) were denoted as hcTnT and mcTnT, respectively. mcTnT-D and mcTnT-F stand for isoform D (P50752-4) and isoform F (P50752-2) of mcTnT, respectively. The subscript p stands for phosphorylation (80 Da), +K for the potassium adduct (38 Da), $+H_3PO_4$ for the noncovalent adduct of phosphoric acid (98 Da), and Δ for 210 Da. Insets show high-accuracy measurements of un- and monophosphorylated hTnT (A) and mcTnT (B). Circles represent the theoretical isotopic abundance distributions of the isotopomer peaks corresponding to the assigned mass. Calcd denotes the calculated most abundant mass and exptl the experimental most abundant mass.

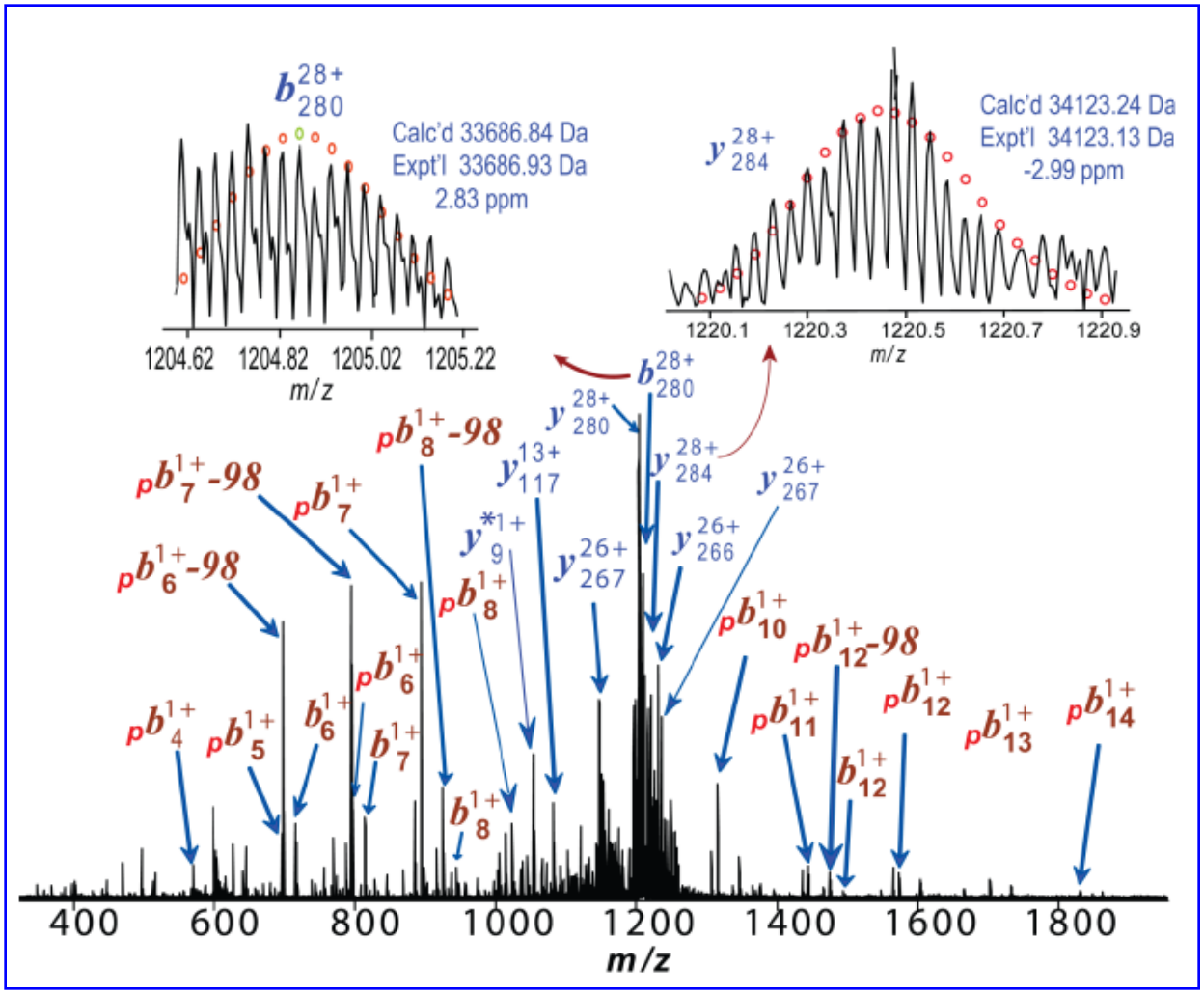


Figure. Representative CAD spectra of *phcTnT for determination of phosphorylation* site in cTnT purified from normal human heart samples. Neutral loss of phosphate moiety is evident. -98 indicates the loss of H3PO4 (-98 Da). Note that the masses of *b ions (e.g. b6)* equal to those with the loss of HPO3 (-80 Da) from their phosphorylated counterparts (e.g. *pb6).*

主要内容：

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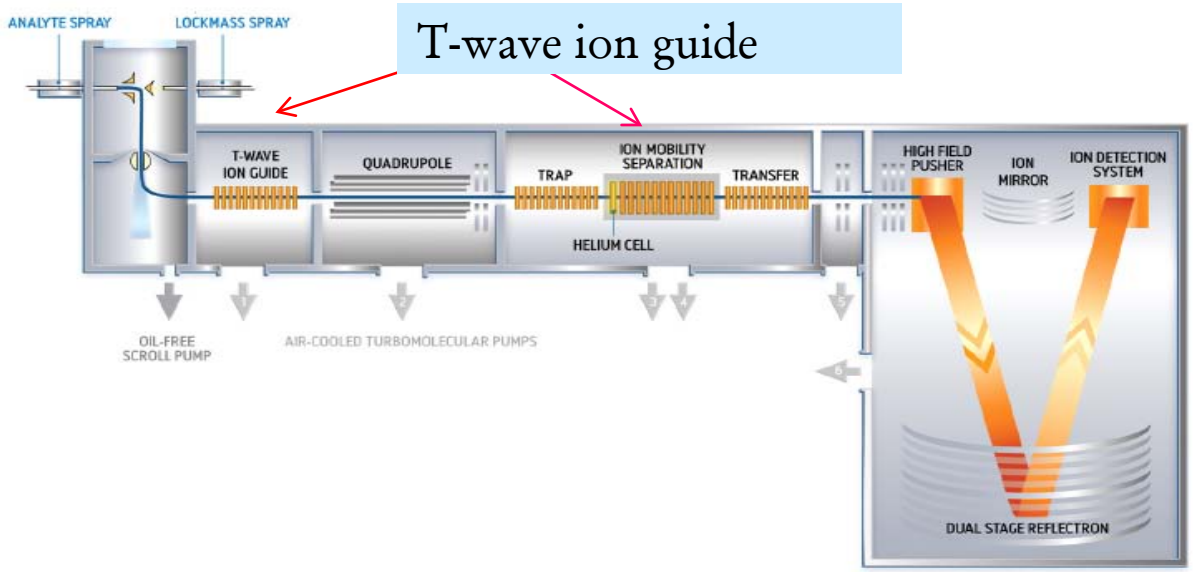
五、离子迁移质谱（IM-MS）及其应用。

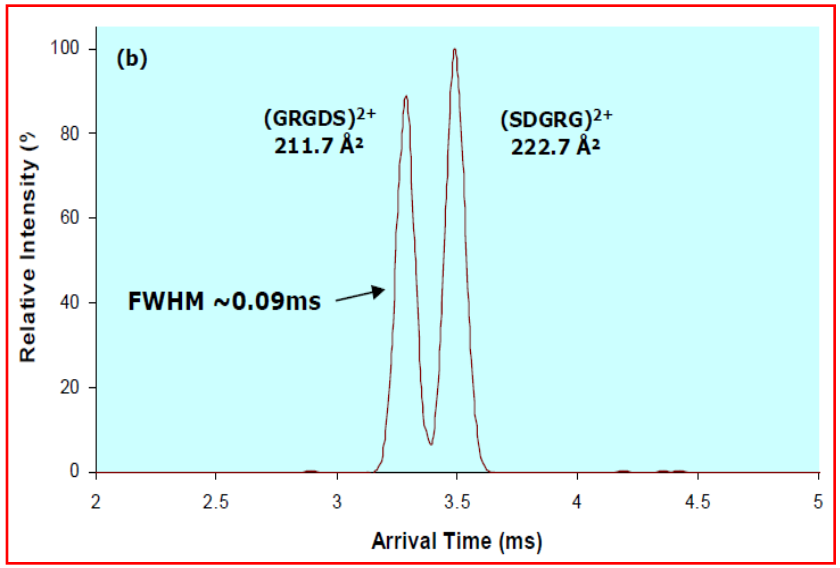


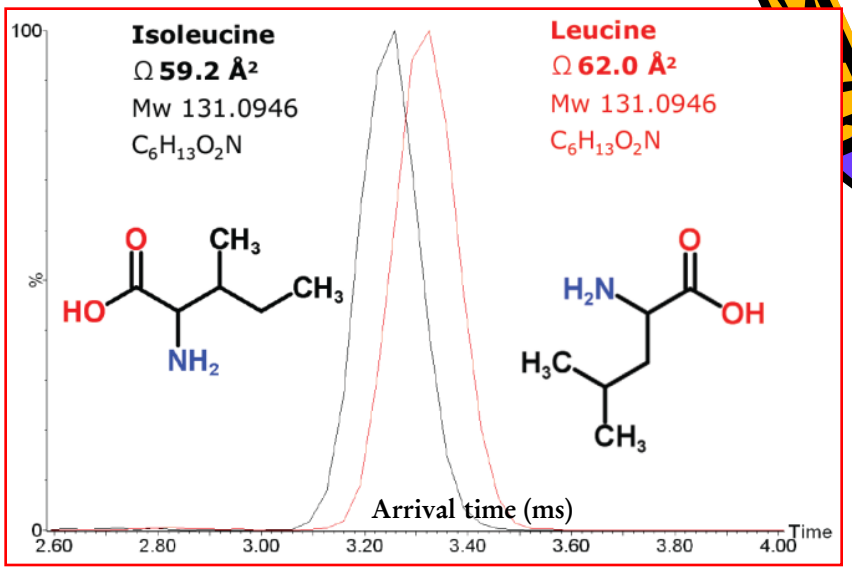
$$t_D^X = c\Omega / q \qquad (1)$$

where:

t_D is the measured drift time,
X is the empirically estimated exponent,
q is the molecular charge,
Ω is the collisional cross section, and
c is the constant that remains unchanged in a single experiment.

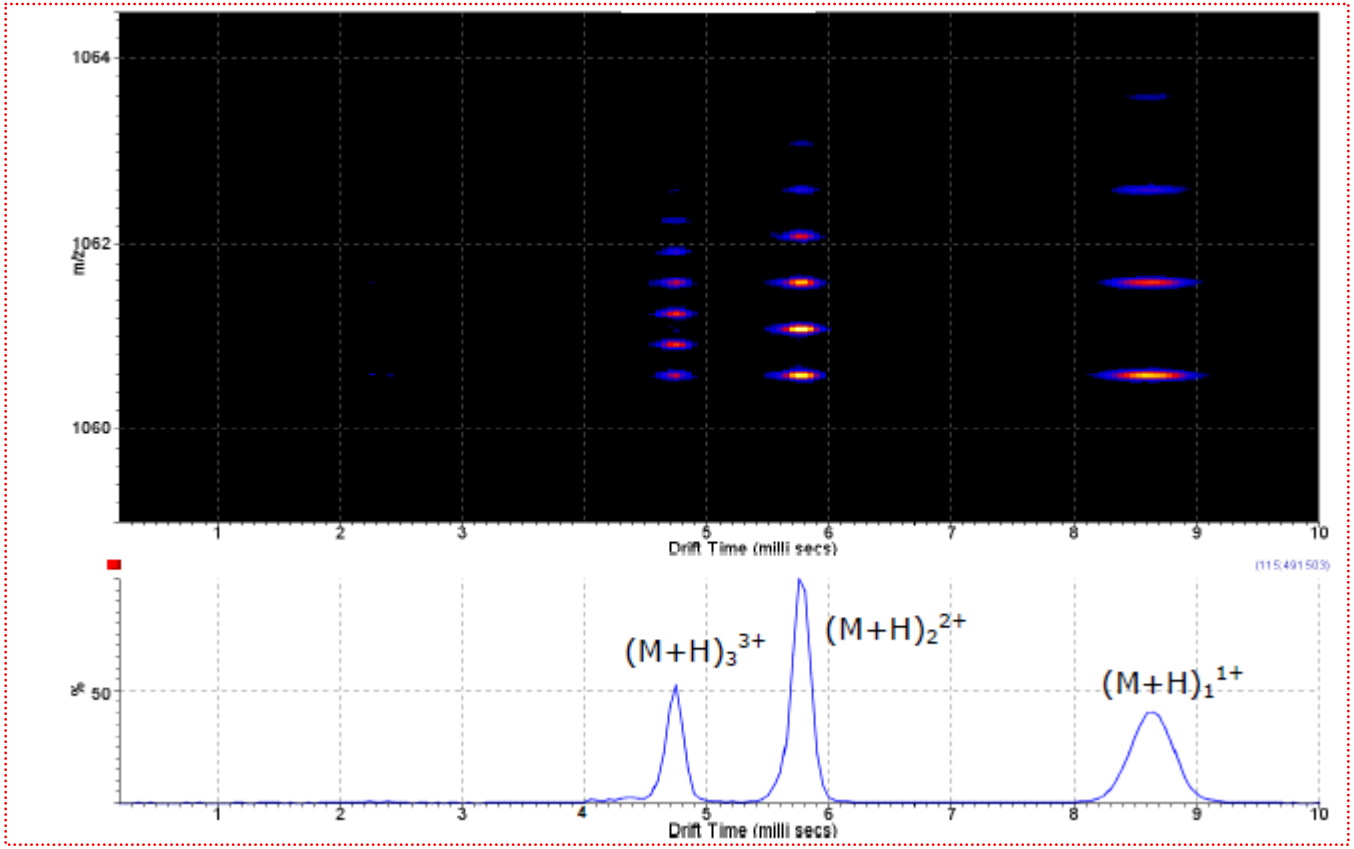
UV和圆二色谱（circular dichroism）也可研究构象变化，但只能提供蛋白结构或功能的特征信息，不能检测到由于微弱的结构变化引起的构象改变。NMR和X-ray的缺点：速度慢、通量低，要求大量高纯度蛋白样品，而且不能直接分析真正的生物学基质样品，特别是添加成分的药物蛋白。因此发展高通量、灵敏度高且可直接检测生物质样品的方法，即IM-MS （ion mobility-mass spectrometry）法。





Mobility separation of inverse sequence peptides GRGDS and SDGRG (m/z 490) with different collision cross-section (Ω).

T-wave ion mobility drift time (msec) chromatograms for isoleucine and leucine and their respective T-wave derived from collision cross-sections (Ω).



Mobility separation of multiply charged multimeric species of Bradykinin (m/z1061) with the same $[M+H]^{1+}$.

IM-MS质谱研究蛋白构象转变与平衡

Angewandte International Edition Chemie

Protein Conformation

DOI: 10.1002/anie.201101077

Detection of a Protein Conformational Equilibrium by Electrospray Ionisation-Ion Mobility-Mass Spectrometry**

*Matthew Jenner, Jacqueline Ellis, Wei-Cheng Huang, Emma Lloyd Raven, Gordon C. K. Roberts, and Neil J. Oldham**

Compact and extended forms of NADPH-cytochrome P450 reductase (CPR) showing the FMN binding domain (blue), the FAD binding and linker domains (magenta), FMN (orange), and FAD (yellow). NADPH is omitted for clarity. FAD = flavin adenine dinucleotide, FMN = flavin mononucleotide, NADPH = nicotinamide adenine dinucleotide phosphate.

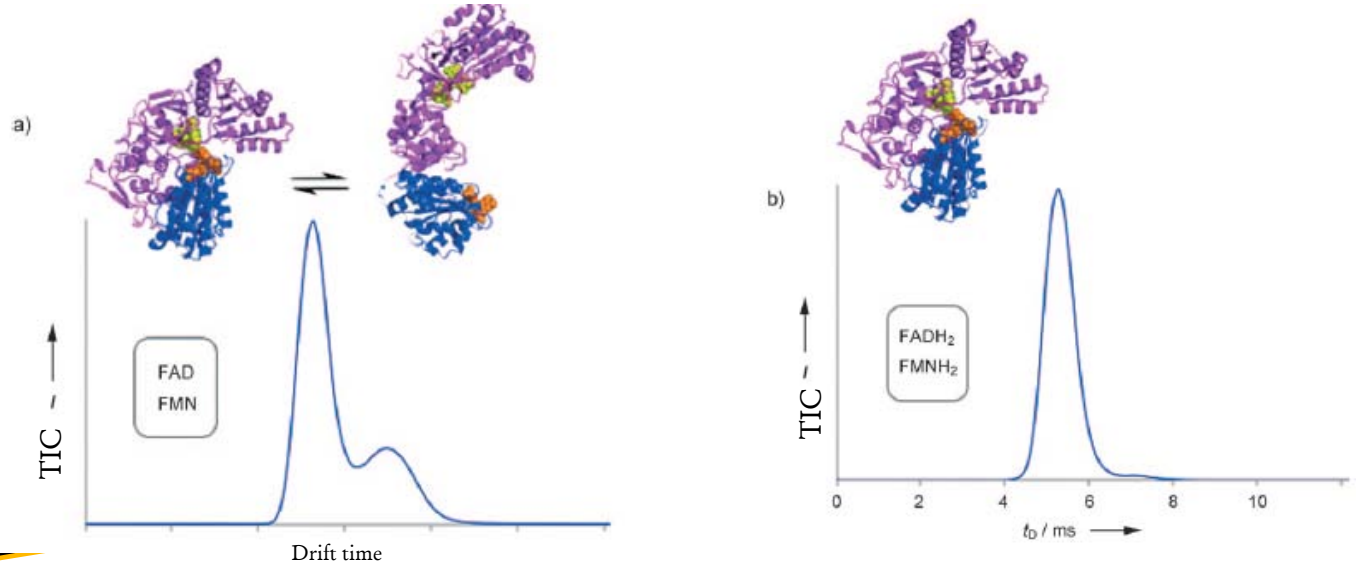


Figure . TWIMS drift traces for CPR (18+ charge state) in the presence of a) NADP+ and b) NADPH (anaerobic conditions) showing the dependence of the conformational equilibrium on the redox state of FAD and FMN (bound to CPR in both cases).

IM-MS质谱鉴定AD中Ab1-40淀粉样肽的聚合状态

doi:10.1016/j.jmb.2011.01.012 J. Mol. Biol. (2011) 407, 110–124

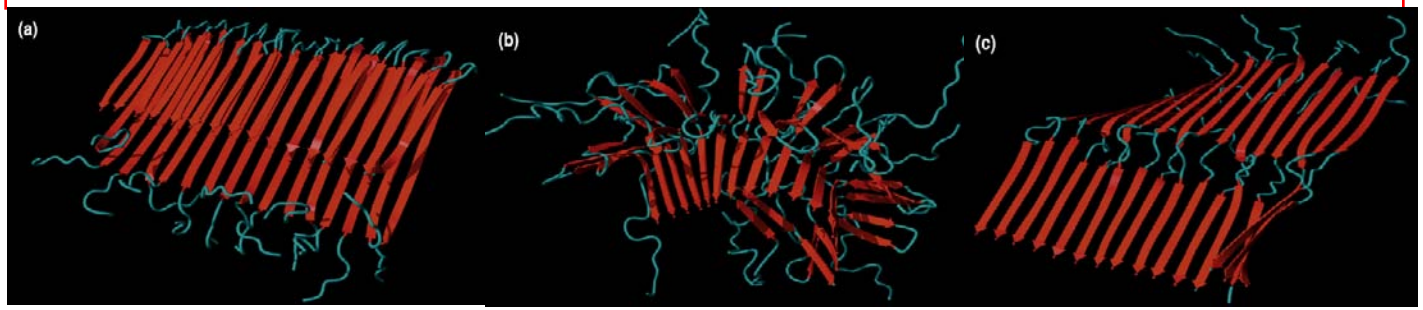
Contents lists available at www.sciencedirect.com

Journal of Molecular Biology

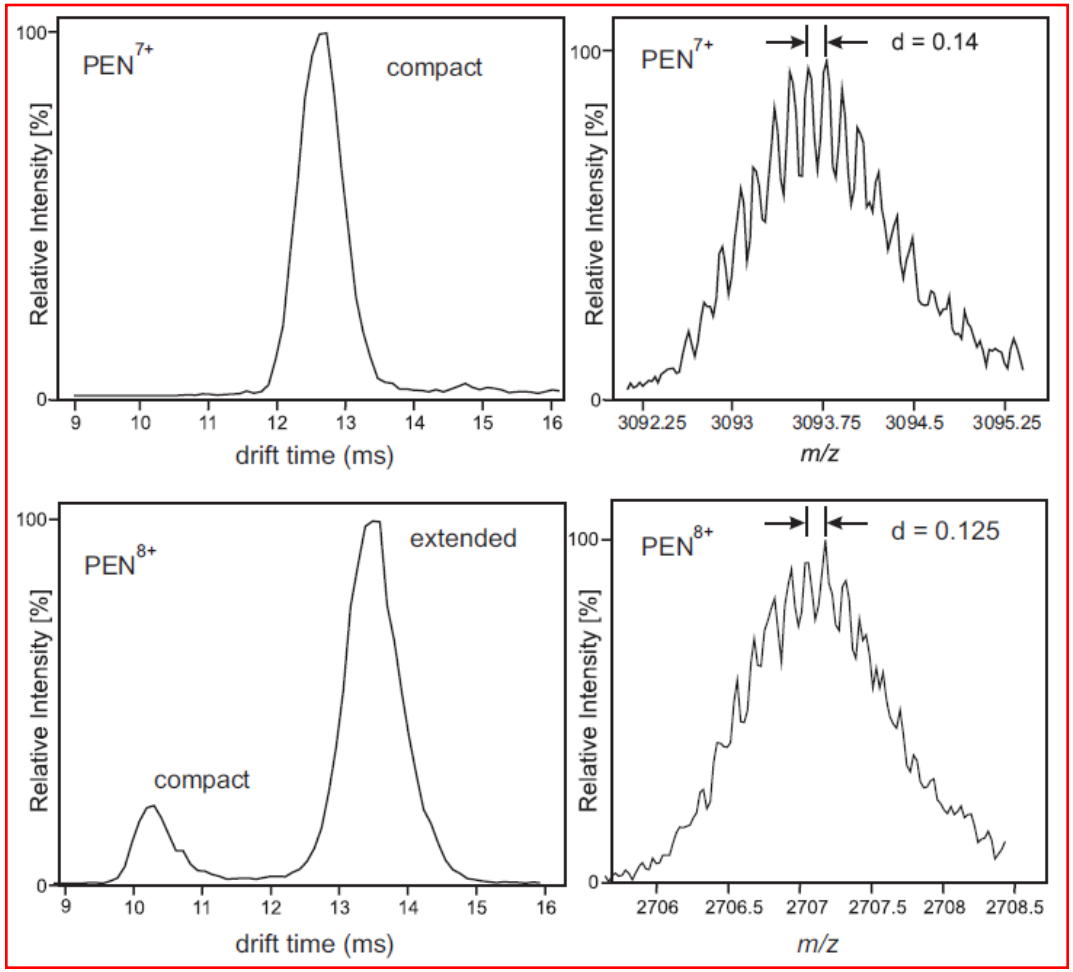
journal homepage: http://ees.elsevier.com.jmb

Ion Mobility Separation Coupled with MS Detects Two Structural States of Alzheimer's Disease Aβ1–40 Peptide Oligomers

Marcin Kłoniecki[1], Agnieszka Jabłonowska[1], Jarosław Poznański[1], James Langridge[3], Chris Hughes[3], Iain Campuzano[3], Kevin Giles[3] and Michał Dadlez[1,2*]



Model of closed (a), globulomer (b), and open (c) forms of Aβ1–40 peptide hexadecamer.



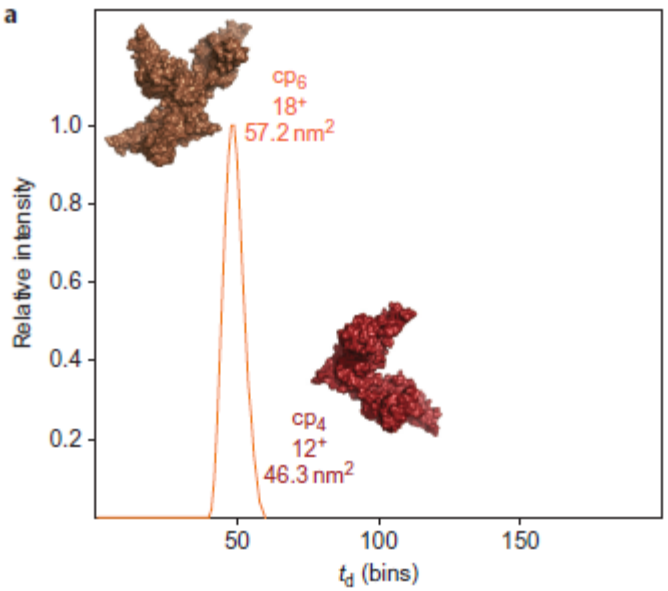
IMS spectrum signals corresponding to PEN^{7+} and PEN^{8+} species of Aβ1-40 oligomers. Drift time distribution is shown in left panels and the corresponding isotopic envelope is shown in right panels. Whereas for PEN^{7+} only one form, assigned to compact species is present in a drift time distribution spectrum, at higher charge of +8 a more extended form is prevalent (peak denoted “extended”) and the compact form is less populated

ARTICLES
PUBLISHED ONLINE: 19 DECEMBER 2010 | DOI: 10.1038/NCHEM.947
nature chemistry

IM-MS质谱监测HBV病毒颗粒的组装过程及途径

Interrogating viral capsid assembly with ion mobility–mass spectrometry

Charlotte Uetrecht[1,2], Ioana M. Barbu[1,2], Glen K. Shoemaker[1,2†], Esther van Duijn[1,2] and Albert J. R. Heck[1,2*]



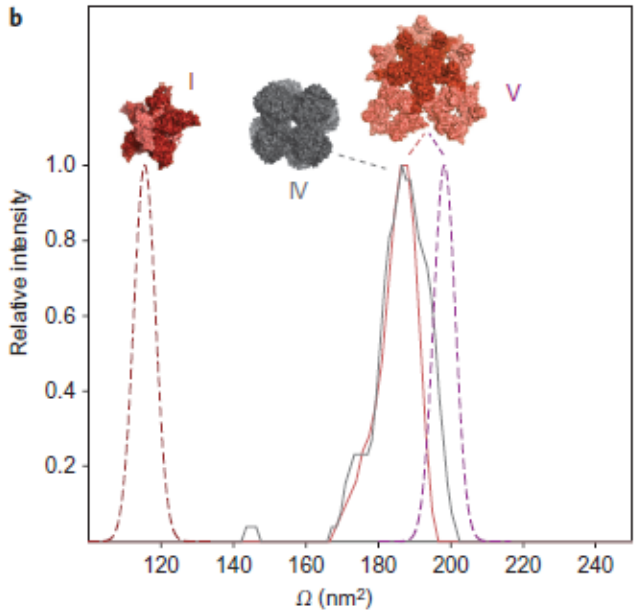


Figure 3 | Ion mobility data for selected HBV oligomers. a, Drift time trace (in bins) of HBV capsid protein (cp) at $m/z = 5{,}291$, showing two different species that are well separated: a hexamer (charge state 18^+) at ~50 bins and a tetramer (12^+) at ~60 bins. The corresponding Ω values and structures are indicated. **b**, Overlay of experimental drift time traces structures are indicated. **b**, Overlay of experimental drift time traces converted to Ω (solid lines) of the HBV 28-mer (structure V, 447 kDa) and a globular protein complex, VAO 8-mer (IV, 510 kDa), both appearing at $m/z = 10{,}400$. Their Ω, as well as the peak width, are similar, even though the VAO 8-mer has a substantially higher mass (~14%). This illustrates that globular proteins of the same mass are generally more compact than the viral intermediates. Apparently, unfolding is limited in the present experiments, because the VAO trace only shows a small shoulder around 200 nm^2 when compared to the HBV 28-mer trace. Also shown are hypothetical Gaussian traces (dashed lines) based on the Ω of the HBV 28-mer modelled from the crystal structure (V) of the virus capsid and a collapsed structure (I).

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Lipid analysis and lipidomics by structurally selective ion mobility-mass spectrometry☆

Michal Kliman [1,2], Jody C. May [1,2], John A. McLean *

IM-MS质谱在代谢组学中的应用

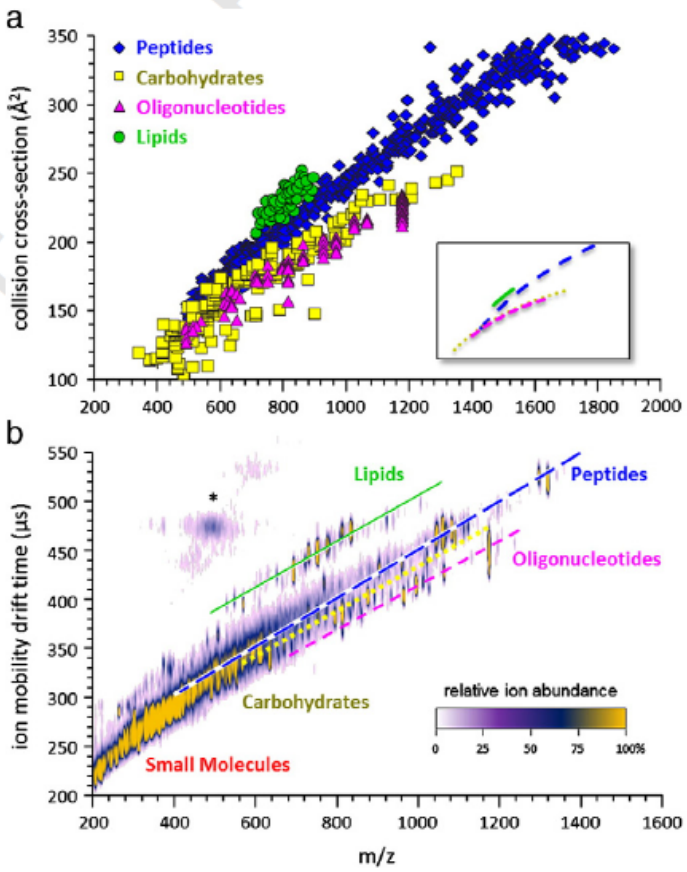
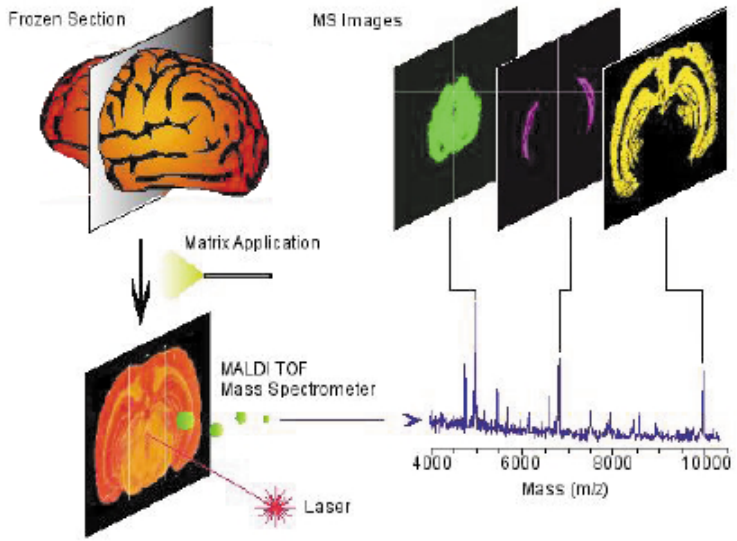


Fig. 5. (a) A plot of collision cross-section *vs.* mass-to-charge measured from a large sample of various biomolecular classes, including peptides (n = 610), carbohydrates (n = 192), oligonucleotides (n = 96), and lipids (n = 53). The average mobility-mass correlation lines are contained in the inset, which represents logarithmic regression fits to the data. (b) An empirical 2D IM-MS spectrum of ion mobility drift time *vs.* mass-to-charge obtained on a DTIM-MS instrument for a complex sample containing a mixture of different biomolecular classes. The lines are provided for visualization purposes only. Signals in the vicinity of the asterisk (*) represent limited post-IM fragmentation of the high abundant peptide signals. Note that in the 2D plots lipid signals appear significantly removed from the other chemical signals.

Panels (a) and (b) are adapted from Springer, Anal. Bioanal. Chem. 394(1) 2009, 235–244, Characterizing ion mobility-mass spectrometry conformation space for the analysis of complex biological samples, Fenn, L.S.; Kliman, M.; Mahsut, A.; Zhao, S.R.; McLean, J.A.; from Fig. 1 © Springer-Verlag 2008 with kind permission from Springer Science + Business Media B.V.

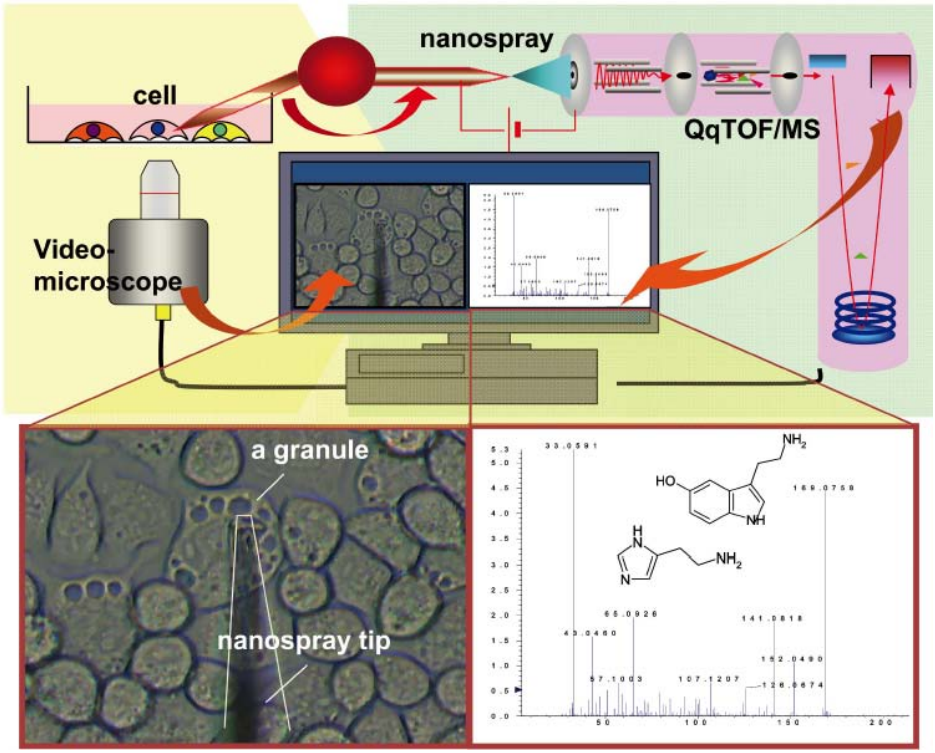
成像质谱(imaging mass spectrometry)



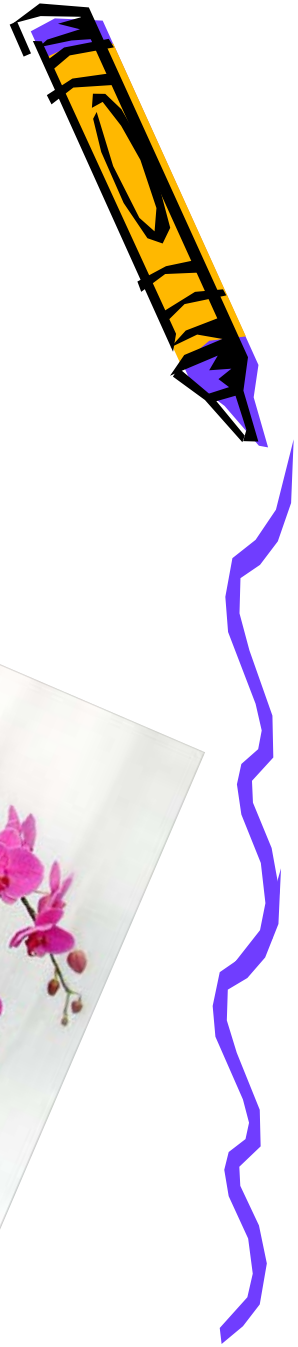
Stoeckli M, et al., Nature, 2001, 7: 493-496.

Methodology developed for the spatial analysis of tissue by MALDI mass spectrometry. Frozen sections are mounted on a metal plate, coated with an UV-absorbing matrix and placed in the mass spectrometer. A pulsed UV laser desorbs and ionizes analytes from the tissue and their m/z values are determined using a time-of-flight analyzer. From a raster over the tissue and measurement of the peak intensities over thousands of spots, mass spectrometric images are generated at specific molecular weight values.

单细胞质谱(**Single-cell** mass spectrometry)



Scheme of the Live Single-cell Mass spectrometry. The cell is observed by a video-microscope, at the moment of sampling, a nano-spray tip is inserted into a target site of a live cell and the contents are sucked into the tip. After the addition of an ionization solvent into the tip, the tip is set to the nano-spray ionization source to feed the molecular contents into the mass spectrometer to obtain MS spectrum.



Thanks !