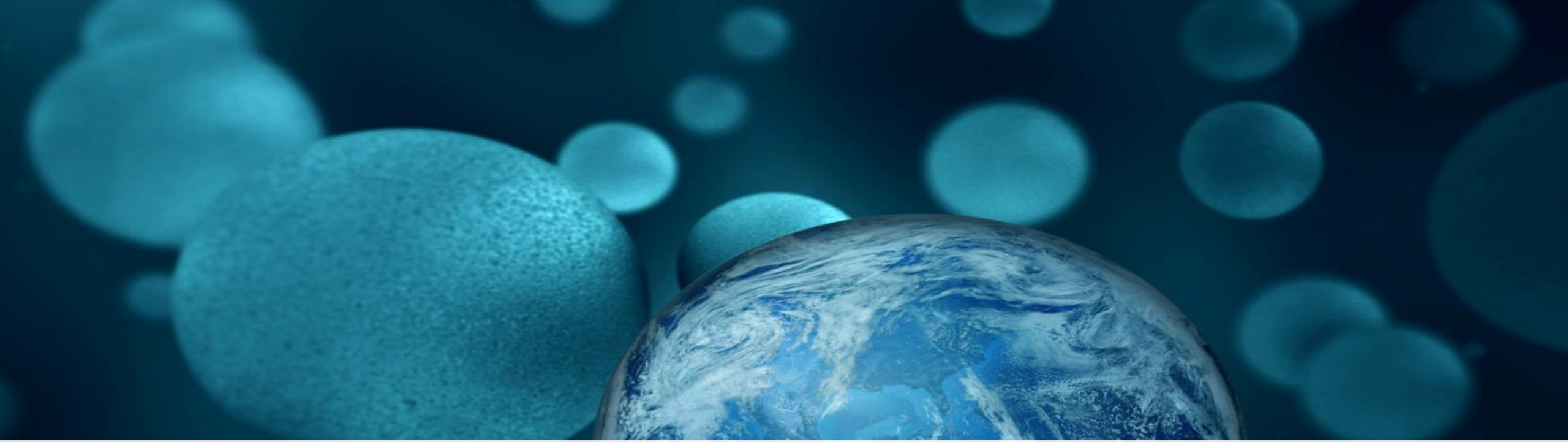


Orbitrap超高分辨质谱在蛋白质组学领域的应用

Cheng Hanxing
Application Engineer
Hanxing.cheng@thermofisher.com

大纲

- **Orbitrap 原理和技术介绍**

- Orbitrap在蛋白质组学研究的应用

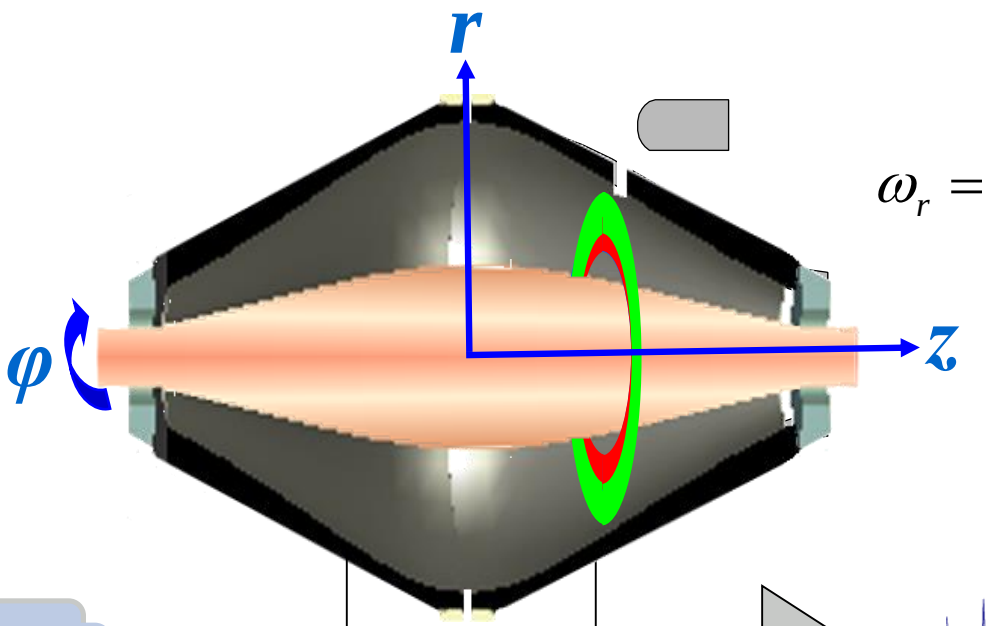
高分辨率质谱_Orbitrap质量分析器原理

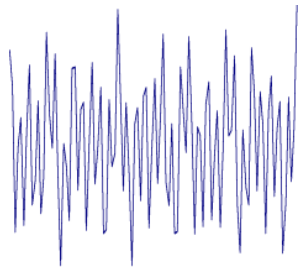
轴旋转频率 ω_ϕ
径向振荡频率 ω_r
轴向振荡频率 ω_z

$$\omega_\varphi = \frac{\omega_z}{\sqrt{2}}\sqrt{\left(\frac{R_m}{R}\right)^2 - 1}$$

$$\omega_r = \omega_z\sqrt{\left(\frac{R_m}{R}\right)^2 - 2}$$

$$\omega_z = \sqrt{\frac{k}{m/z}}$$





傅里叶变换

- 体积小
  - 真空度高 3个数量级
    - 灵敏度高 分辨率高
  - 控温容易 环境要求低
    - 质量轴稳定

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大纲

- Orbitrap 原理和技术介绍

- **Orbitrap蛋白质组学研究的应用**

蛋白质谱分析对样品的要求

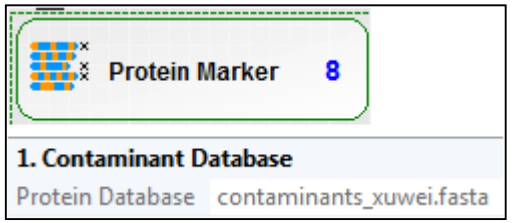
- 无盐：如NaCl, NH_4HCO_3等
  在系谱分析前充分脱盐

- 无聚合物：如PEG等
  采用高品质的EP管和其他耗材，避免手套直接接触样品

- 无去垢剂：如SDS, NP40等
  这些去垢剂不能通过C18 小柱脱盐除去，通过FASP去除

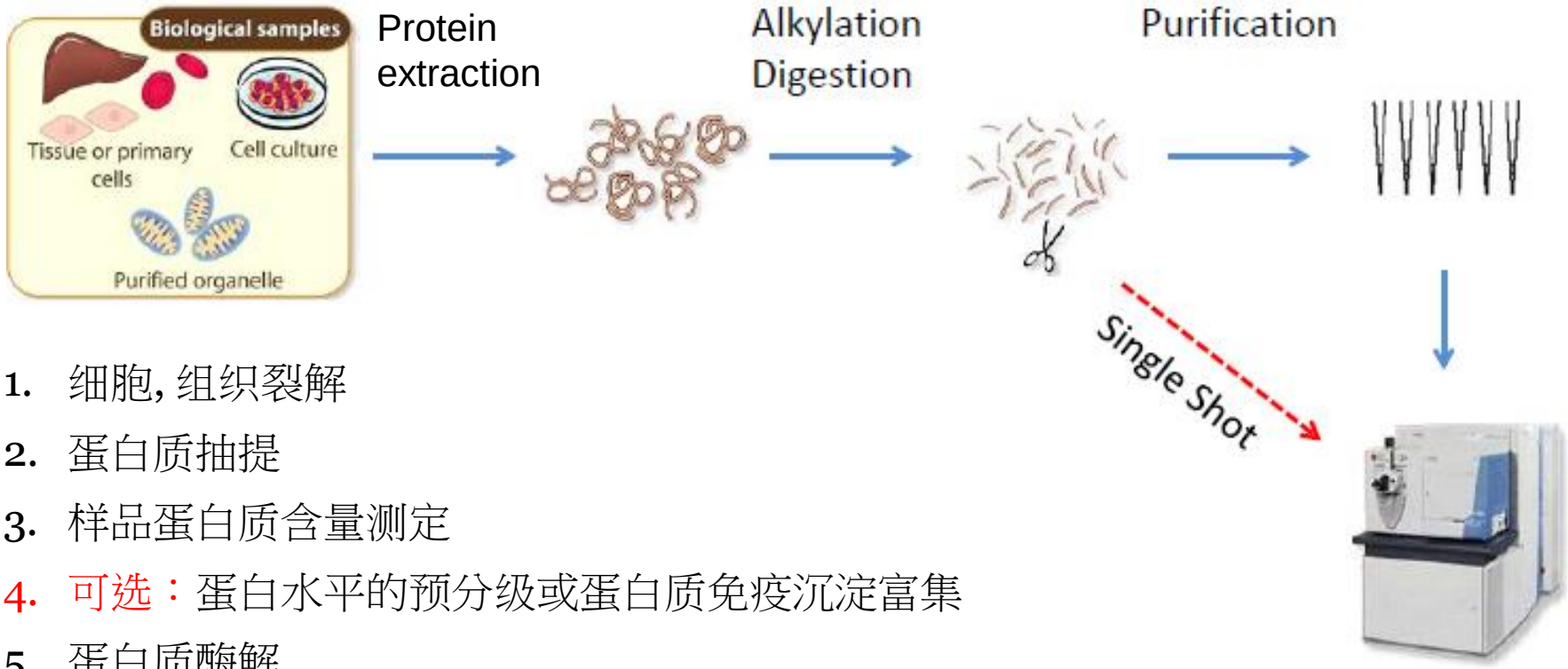
- 无沉淀或颗粒
  充分离心、过滤

- 减少常见污染蛋白（如keratin）
  在处理蛋白样品和酶解时不穿着羊毛衫
  避免裸手接触样品



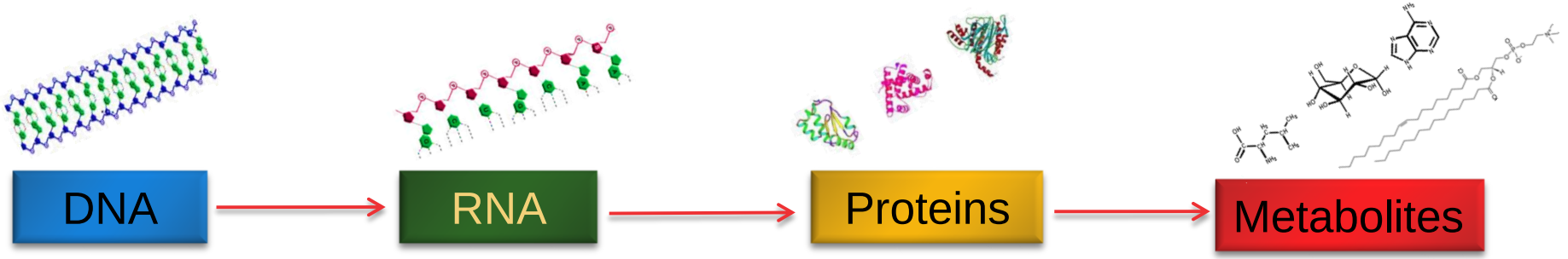
蛋白质组学样品制备的常规流程

General workflow



1. 细胞, 组织裂解
2. 蛋白质抽提
3. 样品蛋白质含量测定
4. 可选：蛋白水平的预分级或蛋白质免疫沉淀富集
5. 蛋白质酶解
6. 可选：肽段水平的预分级或翻译后修饰的富集
7. C18肽段除盐 (非常重要)

中心法则和生命组学



基因组学
22,000 基因

转录组学
100,000 transcripts

蛋白质组学
1,000,000 proteoforms

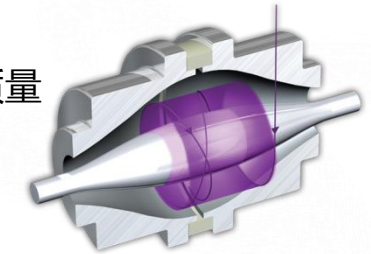
代谢组学/脂质组学
29,289 代谢物 (HMDB)
40,360 脂质(LipidMaps)

Biological potential ***Response to conditions*** ***Biological function*** ***Physiological state***

Proteomics

Metabolomics and Lipidomics

超高分辨、超高精确质量
Orbitrap 质谱



系统生物学：从DNA-RNA-蛋白-代谢物层面全面研究生物学问题
基因组学、转录组学——测序技术
蛋白质组学、代谢组学——生物质谱技术

Nature *227, 561–563*

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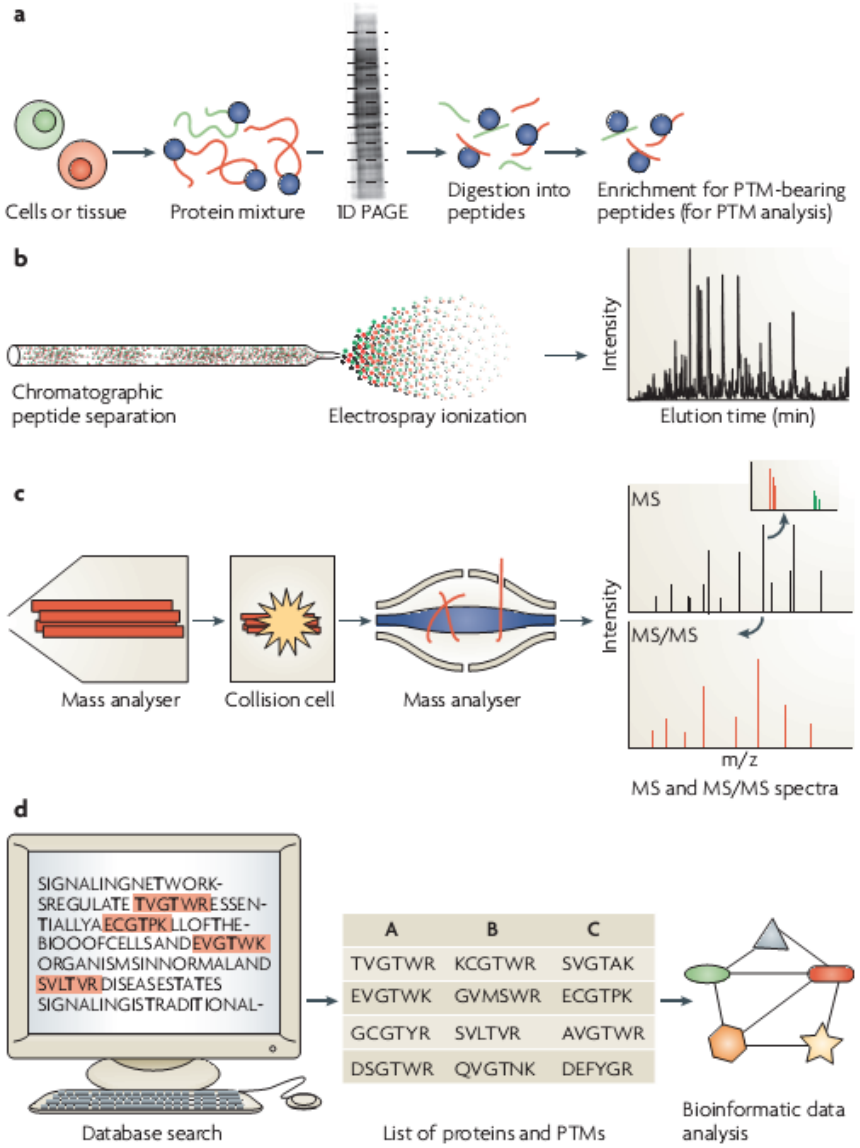
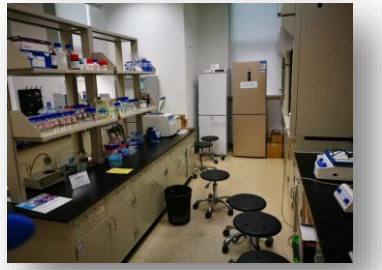
蛋白质组学常规工作流程





Sample preparation

nanoLC-MS/MS

Data analysis

Bioinformatics

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自上而下（Top-Down）解析

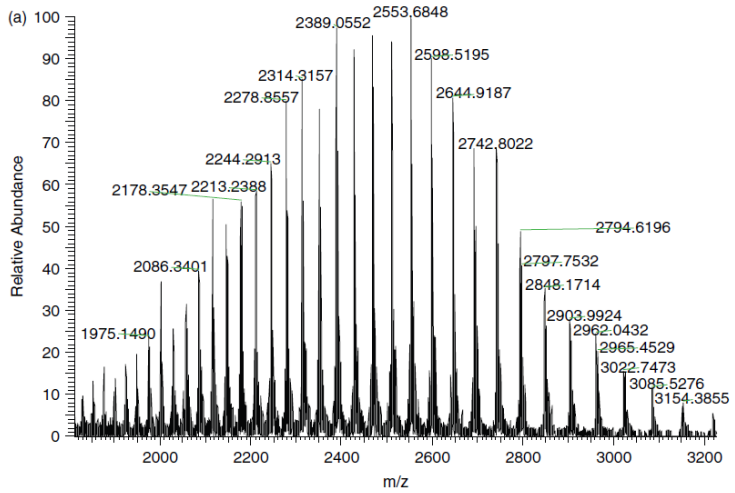
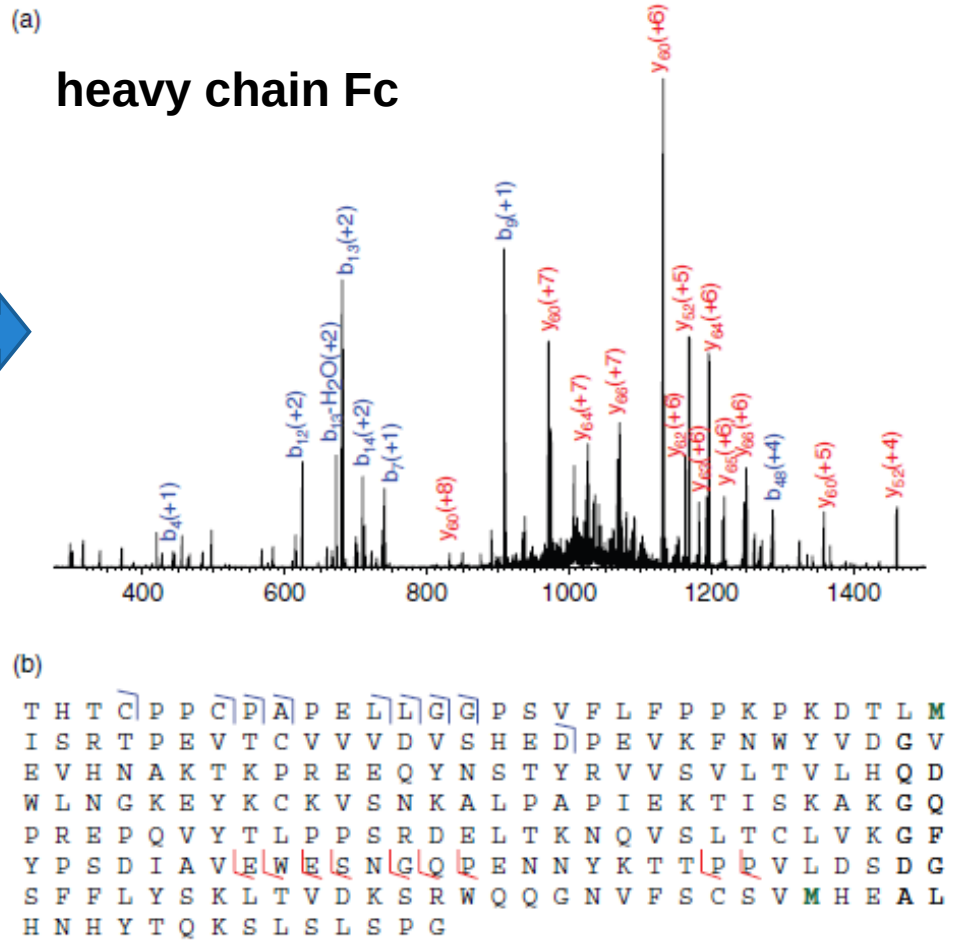


Table 1. Comparison of theoretical and measured masses from four intact MAbs

Antibody	Theoretical average mass (Da)[a]	Measured average mass (Da)	Mass accuracy (ppm)
MAb1	148058.0	148057.2	5.4
MAb2	146291.7	146291.9	1.4
MAb3	147072.7	147073.4	4.8
MAb4	148446.3	148444.3	−13.5

[a] Theoretical values correspond to the most abundant form for each MAb.



J. Mass. Spectrom. **2010**, *45*, 112-120.

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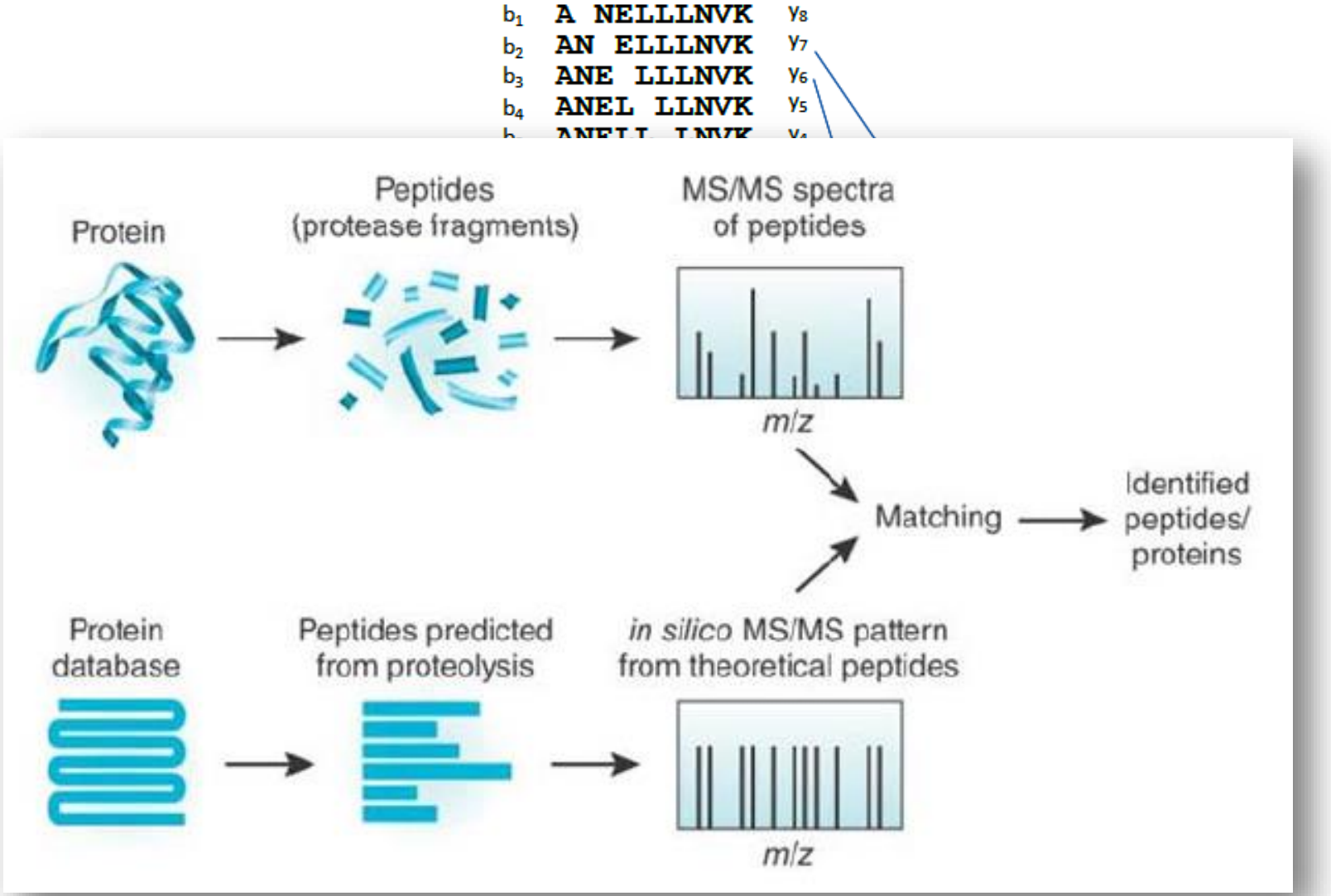
蛋白质组学技术应用领域概览

- **蛋白质鉴定表征**
  蛋白鉴定原理, 典型工作流程…
- **蛋白翻译后修饰的质谱解析**
  磷酸化, 糖基化, 泛素化…
- **定量蛋白质组学**
  LFQ, TMT, SILAC…
- **蛋白相互作用研究**
  AP-MS, XL-MS…

生物质谱进行蛋白鉴定的基本原理



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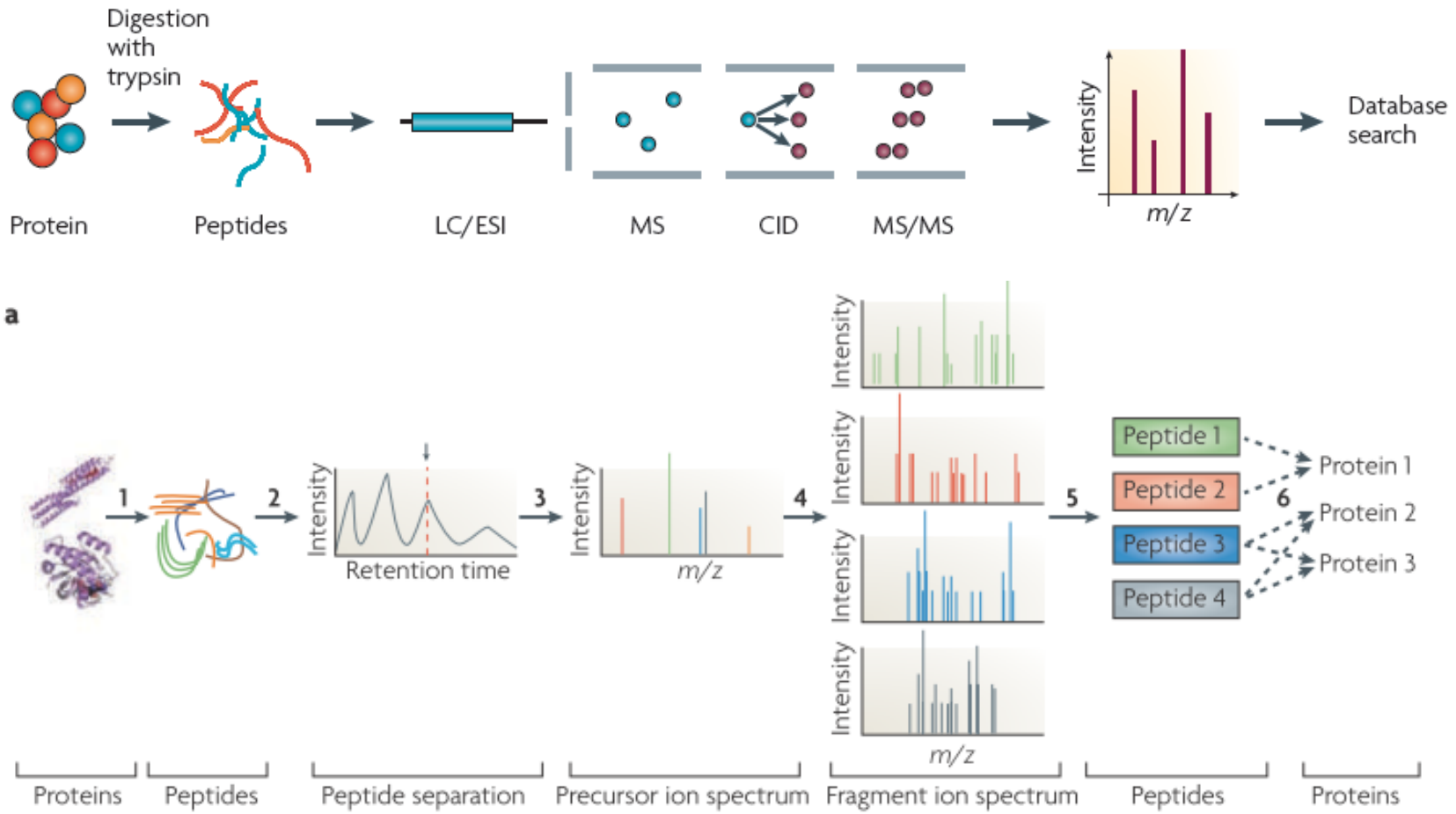
Bottom-up 蛋白质组学分析策略

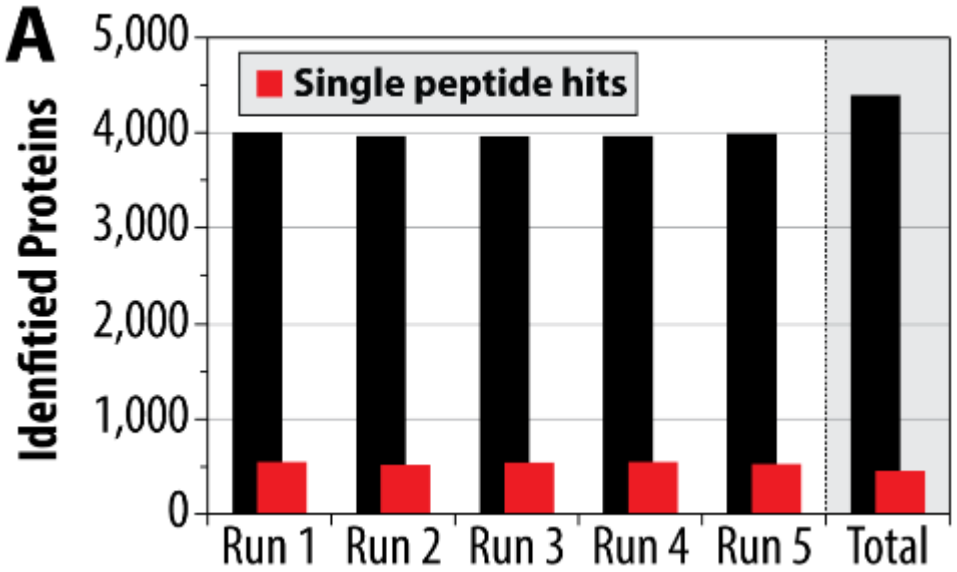


- 采用母离子的精确质量和碎片离子的信息来确定肽段序列
- 通常依赖于蛋白质序列数据库

采用 Orbitrap 超高分辨质谱对蛋白质组进行鉴定： Case 1

The One Hour Yeast Proteome

Alexander S. Hebert[1,2*], Alicia L. Richards[2,3*], Derek J. Bailey[2,3], Arne Ulbrich,[2,3]
Emma E. Coughlin[2], Michael S. Westphall[2], & Joshua J. Coon[1,2,3]



Experiment	PSMs	Peptides	Proteins
1	43,423	34,535	4,002
2	43,622	34,495	3,966
3	42,339	33,450	3,959
4	43,326	34,347	3,968
5	43,343	34,449	3,991
Total	**216,256**	**47,624**	**4,395**

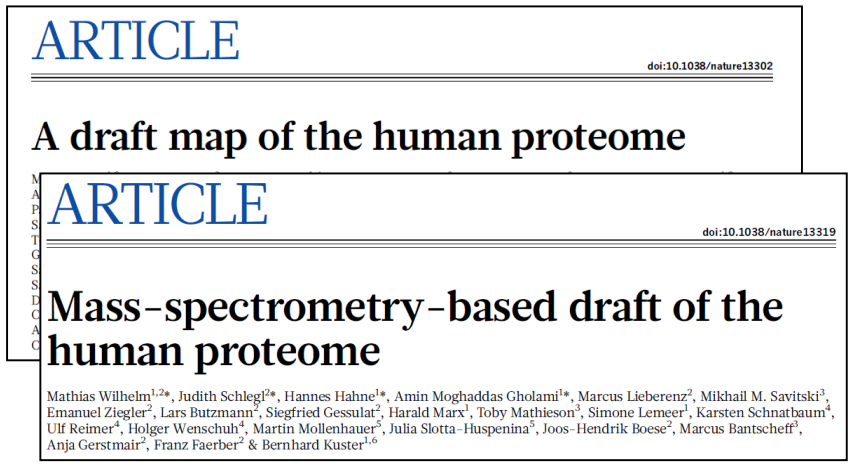
- **动态时间管理技术使得蛋白质鉴定的速度和通量显著提升**

Orbitrap Fusion

1 hour 实现对酵母全蛋白质组鉴定！

Mol Cell Proteomics, 2014,13, 339.

采用 Orbitrap 超高分辨质谱对蛋白质组进行鉴定： Case 2


ARTICLE

doi:10.1038/nature13302

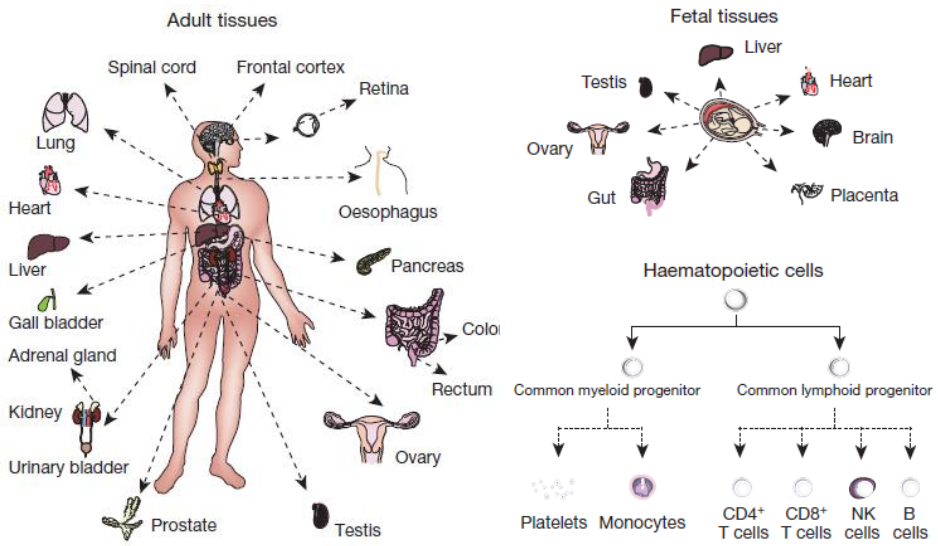
A draft map of the human proteome

ARTICLE

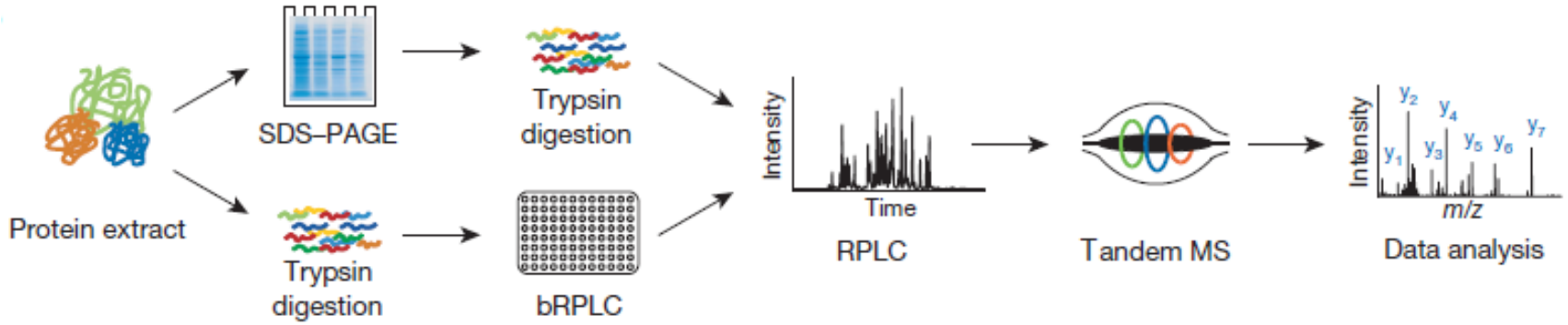
doi:10.1038/nature13319

Mass-spectrometry-based draft of the human proteome

Mathias Wilhelm[1,2*], Judith Schlegl[2*], Hannes Hahne[1*], Amin Moghaddas Gholami[1*], Marcus Lieberenz[2], Mikhail M. Savitski[3], Emanuel Ziegler[2], Lars Butzmann[2], Siegfried Gessulat[2], Harald Marx[1], Toby Mathieson[3], Simone Lemeer[1], Karsten Schnatbaum[4], Ulf Reimer[4], Holger Wenschuh[4], Martin Mollenhauer[5], Julia Slotta-Huspenina[5], Joos-Hendrik Boese[2], Marcus Bantscheff[3], Anja Gerstmair[2], Franz Faerber[2] & Bernhard Kuster[1,6]



- 17种成人组织，7种胎儿组织，6种人造血细胞
- 共鉴定**17294**非冗余蛋白，覆盖**84%**人类基因
- 人类蛋白质组实现接近完全覆盖
- 全部基于Orbitrap质谱技术



Nature, 2014, 509, 575; Nature, 2014, 509, 582

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采用 Orbitrap 超高分辨质谱对蛋白质组进行鉴定： Case 3

Journal of proteome research

Technical Note

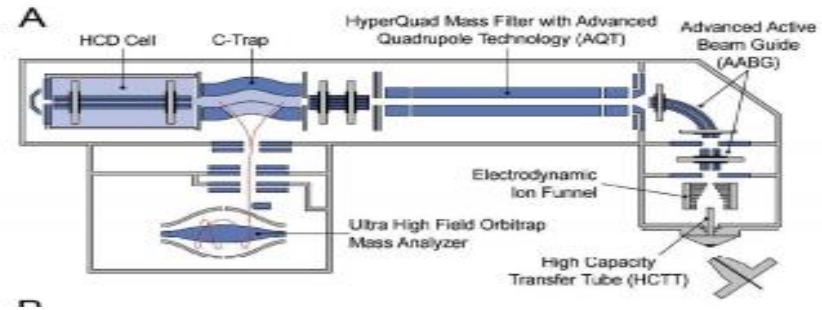
Performance evaluation of the Q Exactive HF-X for shotgun proteomics

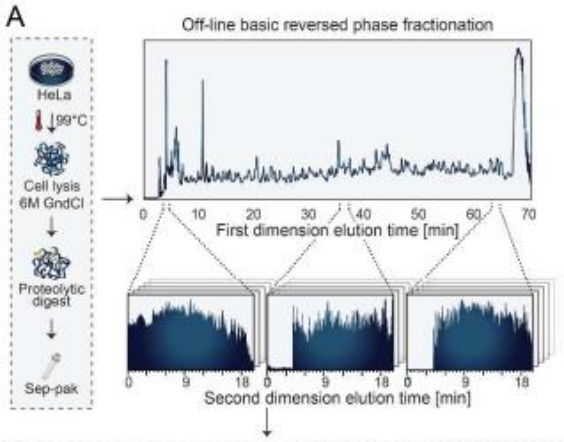
Christian D Kelstrup, Dorte B. Bekker-Jensen, Tabiwang N. Arrey,
Alexander Hogrebe, Alexander Harder, and Jesper V. Olsen

J. Proteome Res., **Just Accepted Manuscript** • DOI: 10.1021/acs.jproteome.7b00602 • Publication Date (Web): 29 Nov 2017

新型 Orbitrap 质谱仪 Q Exactive HF-X 性能测试

- 每分钟超过 1200 条unique peptides鉴定
- 60min 鉴定时间，共鉴定到 25,000 个肽段 4700 多个蛋白质
- 12h 鉴定时间，鉴定超过 140,000 个肽段, 9000 多个蛋白质
- 10 标TMT试验，6个分级条件下，共鉴定 16,700 个磷酸化肽段





46 raw files per sample, 19 min MS method with 15 min gradient
Q Exactive HF-X HCD acquisition at 28 or 41 Hz
MaxQuant protein and peptide FDR < 0.01

B

MS method	Total time	Gradient time	Amount	Peptides	Proteins
HF-X(28Hz)	28.3h	11.5h	100 µg	130,997	8,854
HF-X(41Hz)	28.3h	11.5h	100 µg	141,081	9,015

J Proteome Res. 2018, 5, 17, 727.

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蛋白质组学技术应用

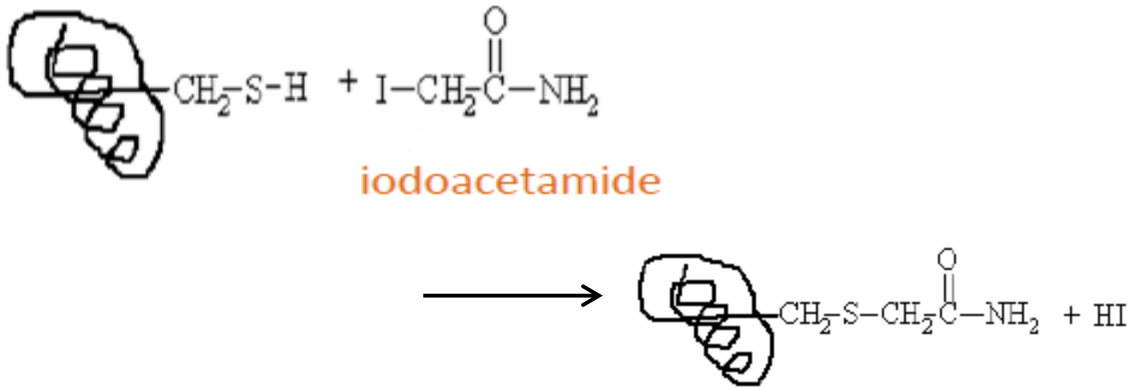
- **蛋白质鉴定表征**
  蛋白鉴定原理, 典型工作流程…
- **蛋白翻译后修饰的质谱解析**
  磷酸化, 糖基化, 泛素化…
- **定量蛋白质组学**
  LFQ, TMT, SILAC…
- **蛋白相互作用研究**
  AP-MS, XL-MS…

蛋白质酶解

胶内酶解 In-gel digestion

- 将考染后的SDS-PAGE切成小块
- 用50mM NH_4HCO_3/30%ACN 脱色
- 在胶内进行 DTT, IAA
- 在NH_4HCO_3溶液体系中加入酶，在胶内进行酶解
- 用60% ACN/0.1% TFA 从胶中抽提生成的肽段

原理：蛋白质不能从PAGE胶里被抽提出，而肽段则可以被抽提出



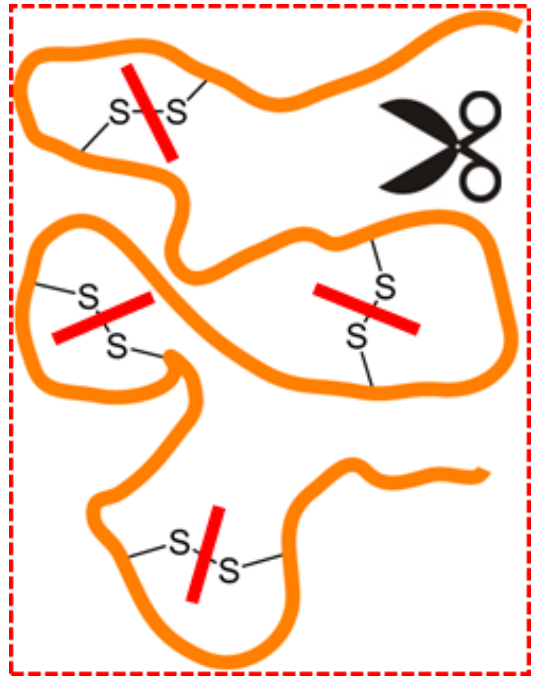
PROTOCOL

In-gel digestion for mass spectrometric characterization of proteins and proteomes

Andrej Shevchenko[1,3], Henrik Tomas[1], Jan Havliš[1], Jesper V Olsen[2] & Matthias Mann[2,3]

[1]Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany. [2]Max Planck Institute for Biochem 18, 82152 Martinsried, Germany. [3]Correspondence should be addressed to A.S. (shevchenko@mpi-cbg.de) or M.M. (mmann@biochem.mpg.de

还原烷基化



打断蛋白质的二硫键，破坏蛋白质二级结构，是蛋白质结构松散，增加蛋白质酶切效率

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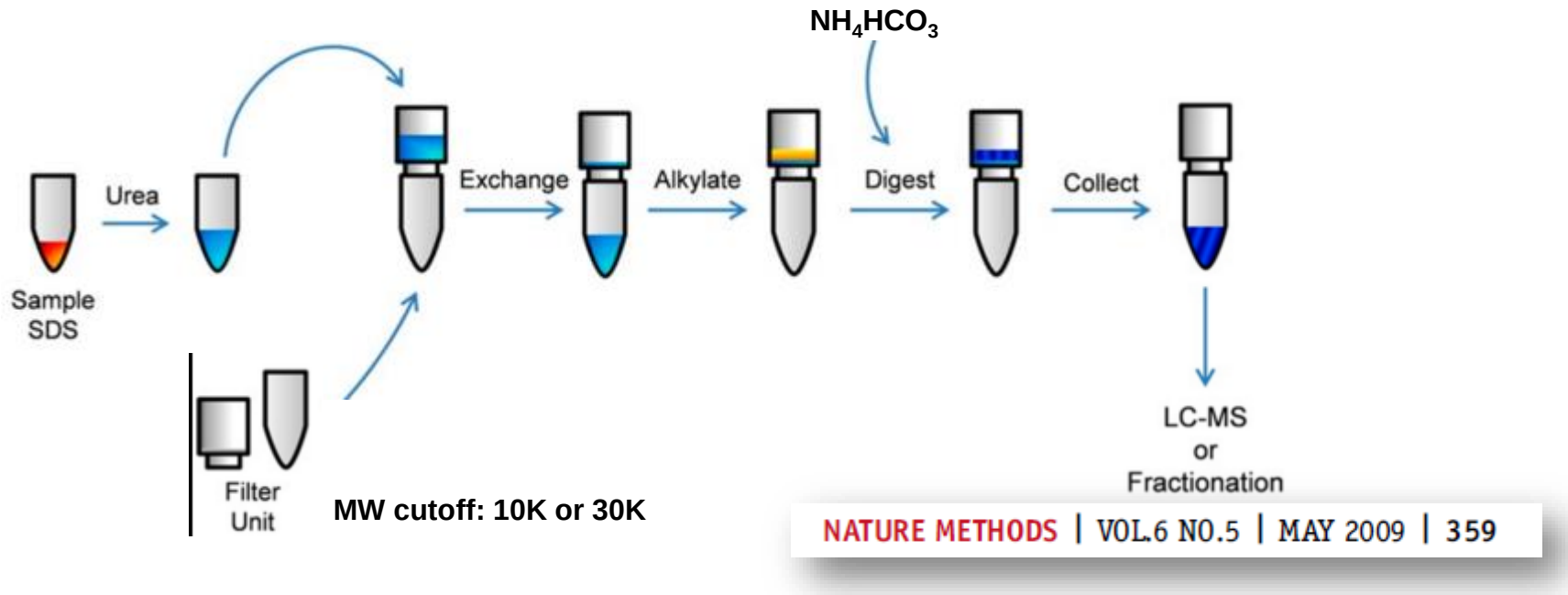
蛋白质酶解

FASP: Filter aided sample preparation

- 采用滤膜装置进行溶液置换（10K, 20K, 30K）
- 使用尿素可以更有效的除去溶液体系中的去垢剂（如SDS）
- 最后置换至NH4HCO3 or TEAB 溶液体系进行蛋白质酶切（pH 在8.0左右）
- 酶解完成后收集滤膜的流穿组分得到肽段

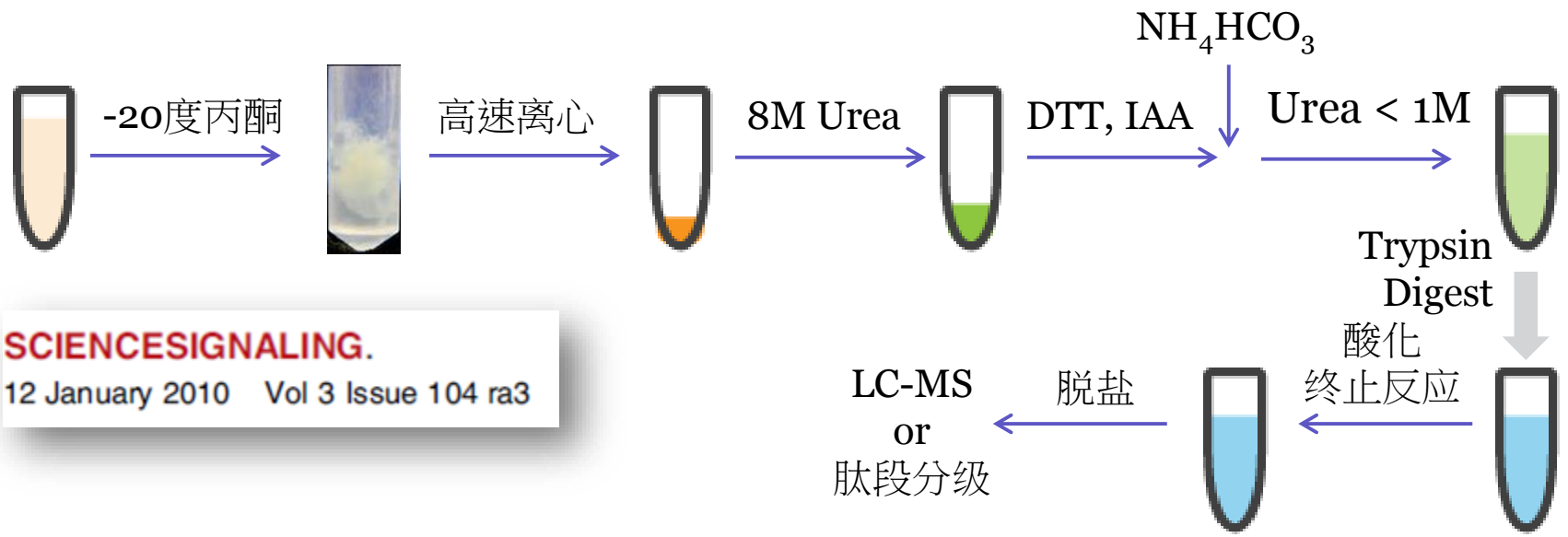


原理：蛋白质不能通过滤膜，而酶解生成的肽段则可以通过

蛋白质酶解

丙酮沉淀酶解

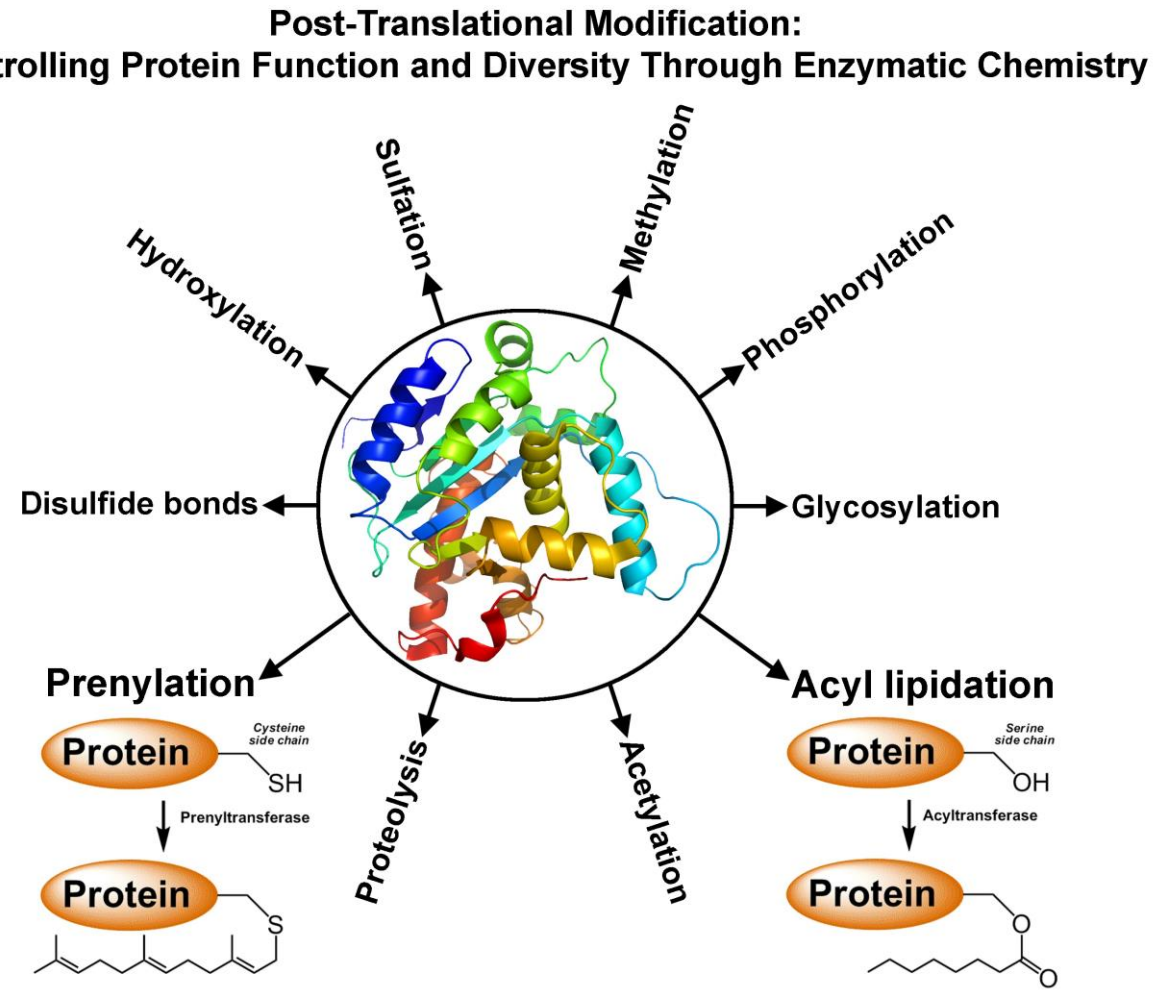
- 4-6倍体积的冷丙酮用于沉淀蛋白质
- 使用8M尿素使得蛋白质更容易复溶
- 必须将尿素稀释至1M以下，以保证Trypsin的活性, LysC可适应高浓度尿素体系
- 酶解完成后必须对体系进行酸化以终止酶解反应
- 由于体系中不挥发性盐浓度极高，上质谱以及分级前均需要脱盐



SCIENCESIGNALING.
12 January 2010 Vol 3 Issue 104 ra3

原理：冷丙酮可以沉淀蛋白质，而去垢剂则保留在上清中

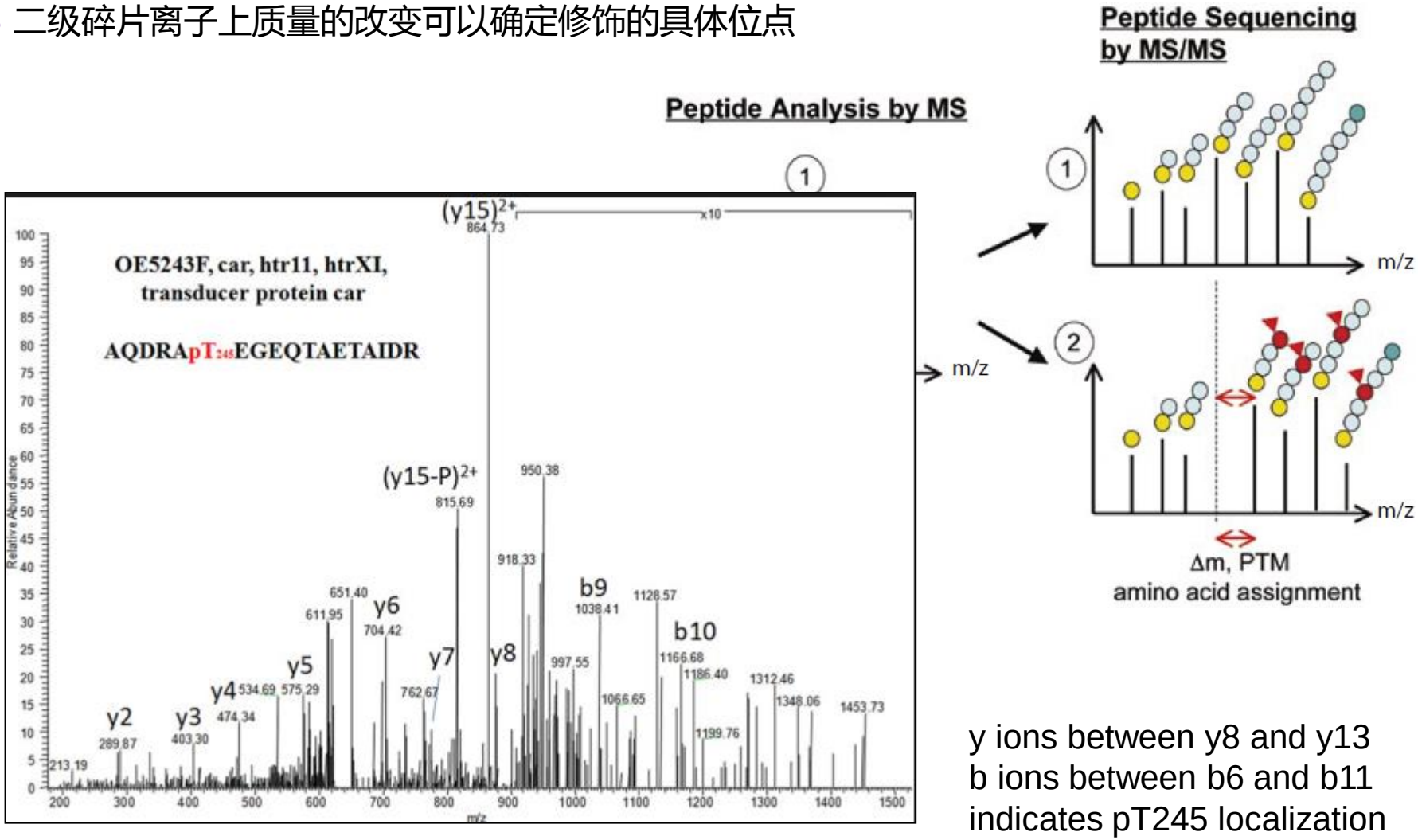
翻译后修饰是蛋白质组学研究的重要组成部分



翻译后修饰丰度通常较低（<5%），对仪器灵敏度要求更高！

翻译后修饰 (PTM) 的质谱分析

- 母离子上质量的改变可以确定修饰的类型
- 二级碎片离子上质量的改变可以确定修饰的具体位点



y ions between y8 and y13
b ions between b6 and b11
indicates pT245 localization

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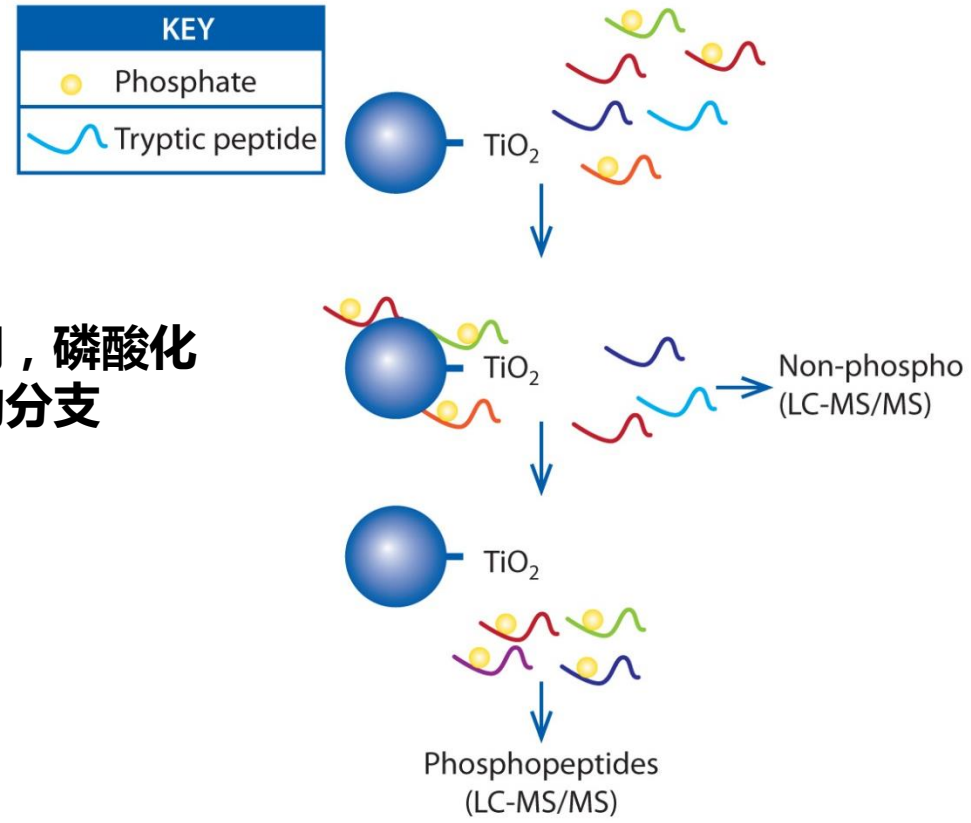
翻译后修饰：磷酸化

翻译后修饰的肽段由于丰度较低，通常在质谱检测前需要富集
磷酸化肽段的主要富集手段

- IMAC: Immobilized Metal-Affinity Chromatography (固定化金属亲和层析)
- TiO_2
- 抗体(Phospho-Tyr)



由于磷酸化在信号转导过程中的重要作用，磷酸化蛋白质组一直是蛋白质组学领域最重要的分支

细胞样品收集

裂解液: SDT buffer, 2% SDS, 0.1M DTT, 0.1M Tris/HCl, pH 7.6
另加入蛋白酶抑制剂cocktail和磷酸酶抑制剂cocktail
或RIPA, M-PER from Thermo
不同的裂解液后处理方式不同（详见前处理初级班）

- Cold PBS wash
- 加入裂解液裂解, 用细胞刮刀刮下, 吸入Ep管
- 沸水浴 2min
- 超声破碎细胞
- 沸水浴2min
- 离心, 取上清
- 荧光法测定蛋白浓度

样品中蛋白质含量测定

- Bradford assay

  在酸性条件，蛋白质与考马斯染料G-250结合，颜色从棕色变为蓝色，595nm测吸光值

  染料主要结合碱性氨基酸（特别是精氨酸），芳香族氨基酸残基

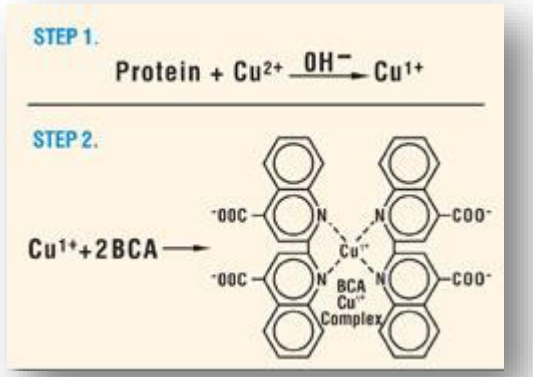
  干扰物: 一些去垢剂

- BCA (bicinchoninic acid) assay

  在碱性条件下，蛋白将Cu^{2+}还原为Cu^{+}，Cu+与BCA试剂

  形成紫色络合物，562nm测吸光

  干扰物: 还原剂



- Fluorescent assay

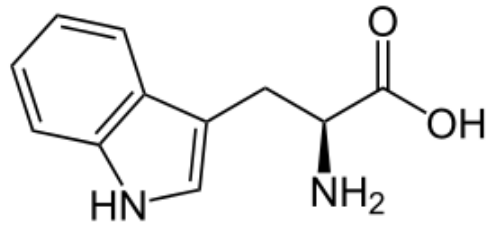
  测定样品中色氨酸的含量，除以1.3%得到蛋白质含量

  Excitation wavelength (nm) 295.00

  Emission wavelength (nm) 350.00

  一般无干扰物，但只能对复杂体系定量，血浆尿液等样品不适合

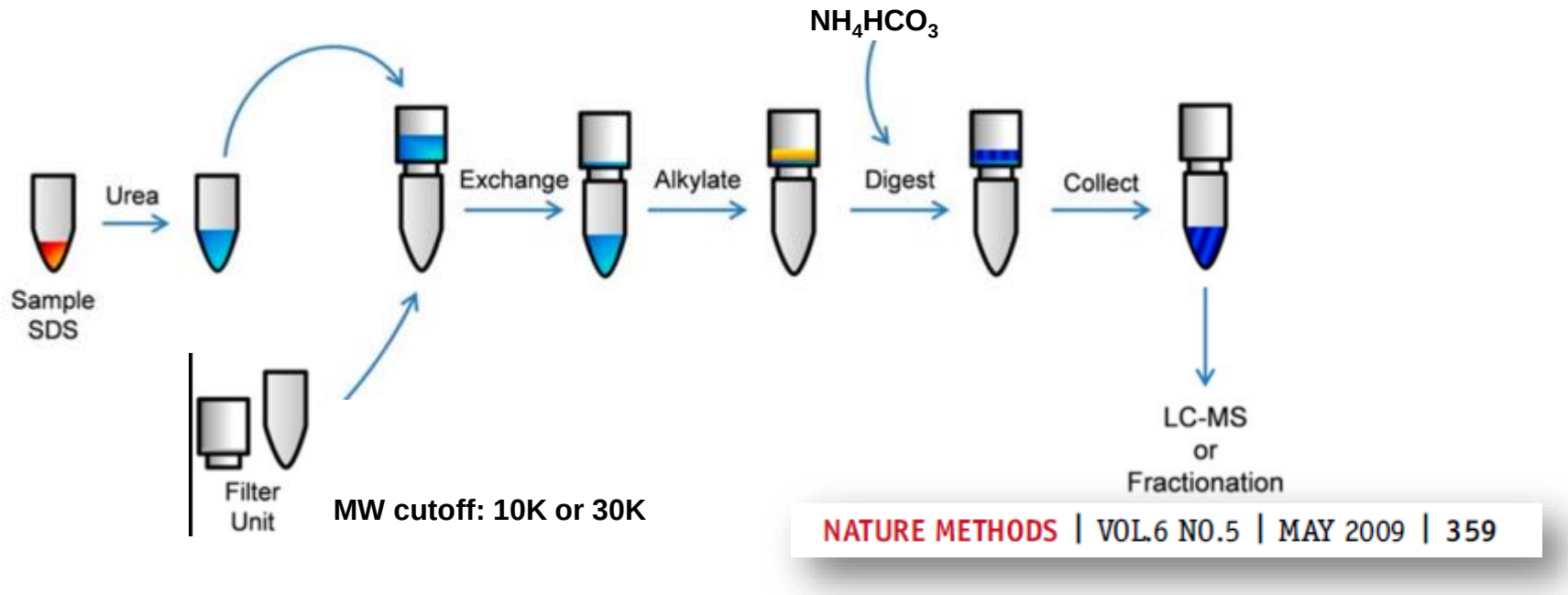
tryptophan



蛋白质酶解

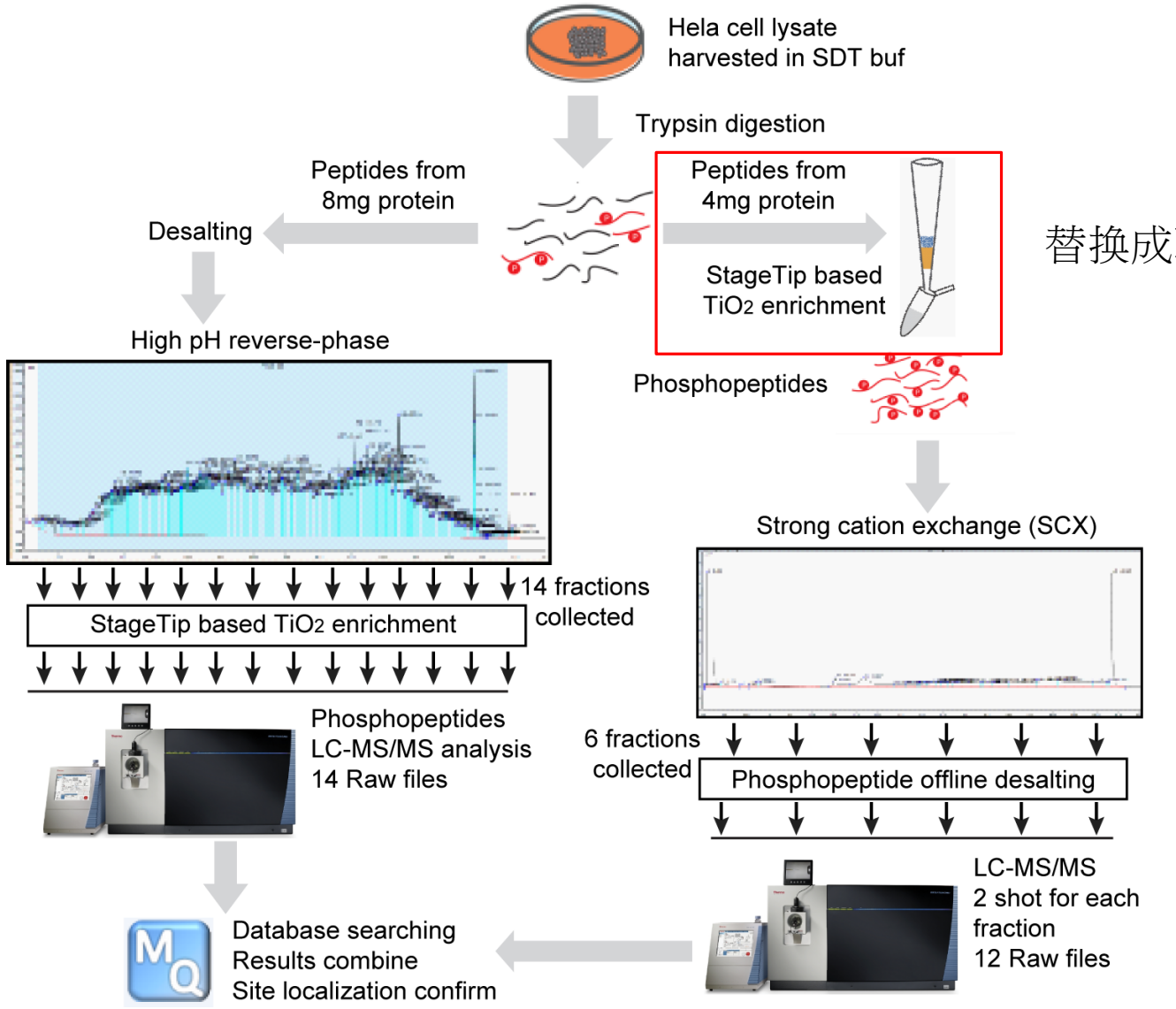
FASP: Filter aided sample preparation

- 采用滤膜装置进行溶液置换（10K, 20K, 30K）
- 使用尿素可以更有效的除去溶液体系中的去垢剂（如SDS）
- 最后置换至NH4HCO3 or TEAB 溶液体系进行蛋白质酶切（pH 在8.0左右）
- 酶解完成后收集滤膜的流穿组分得到肽段



原理：蛋白质不能通过滤膜，而酶解生成的肽段则可以通过

磷酸化肽段富集workflow

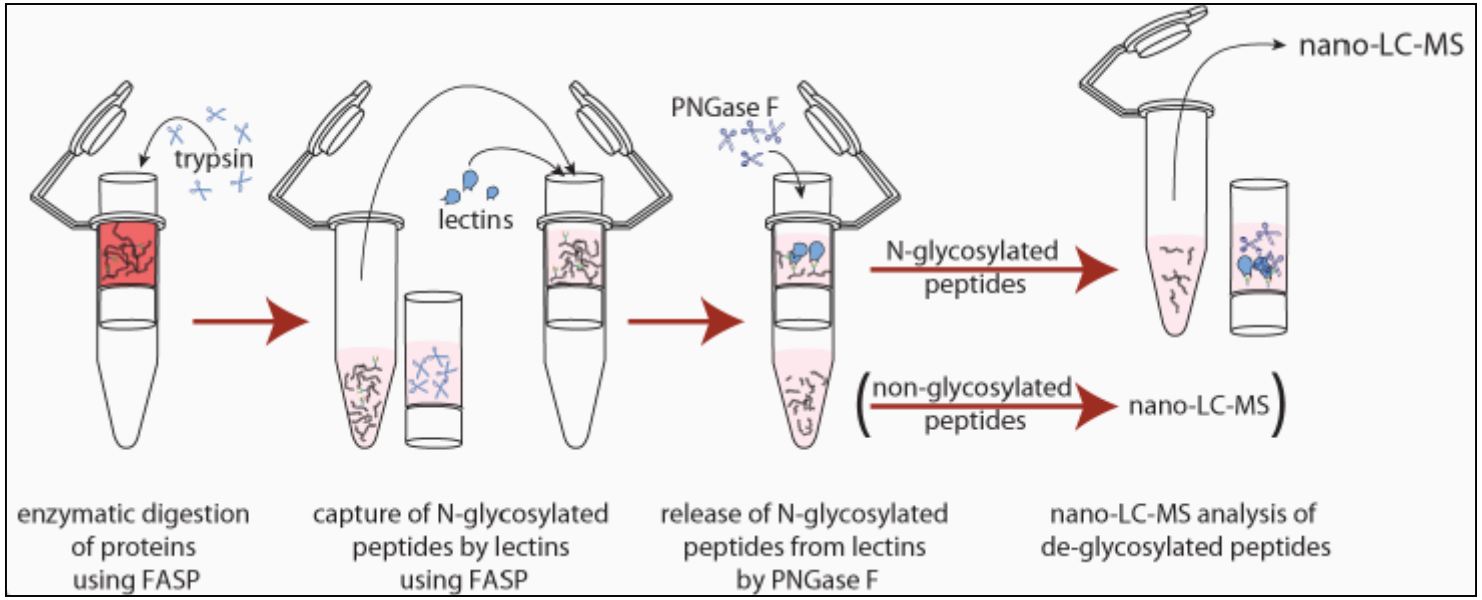


替换成Fe-NTA kit

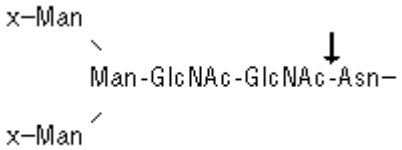
翻译后修饰：糖基化

糖基化位点分析

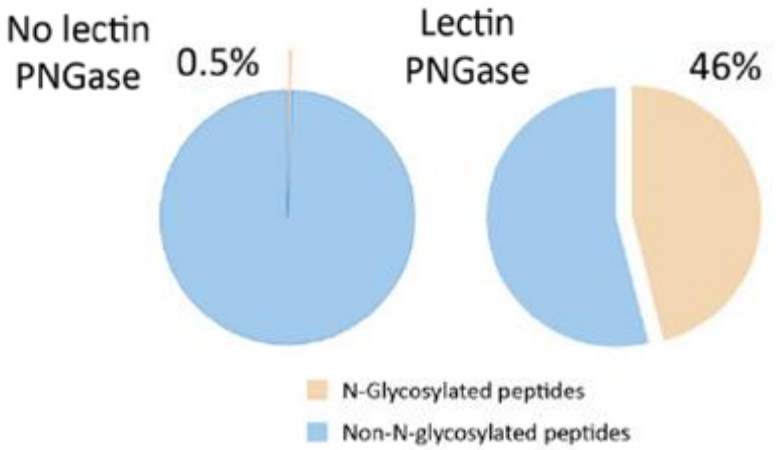
Lectin 富集
PNGase F
释放糖链



Peptide release: PNGase F, *N*-Glycosidase



Database search: (Asn)+0.98406

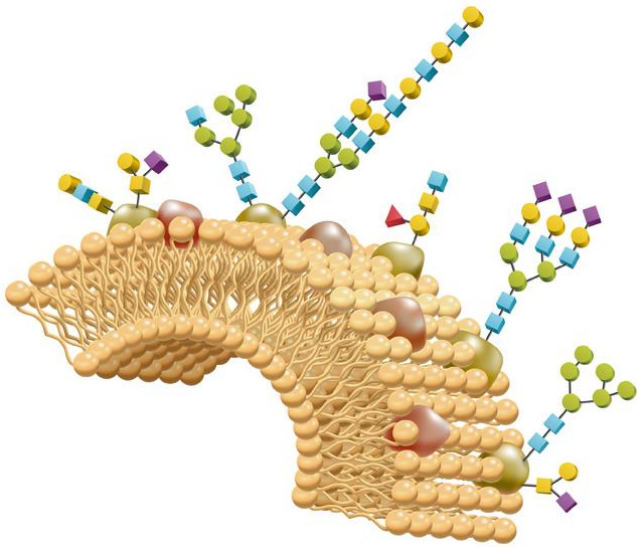


Cell, 2010, 141, 897.

ThermoFisher SCIENTIFIC

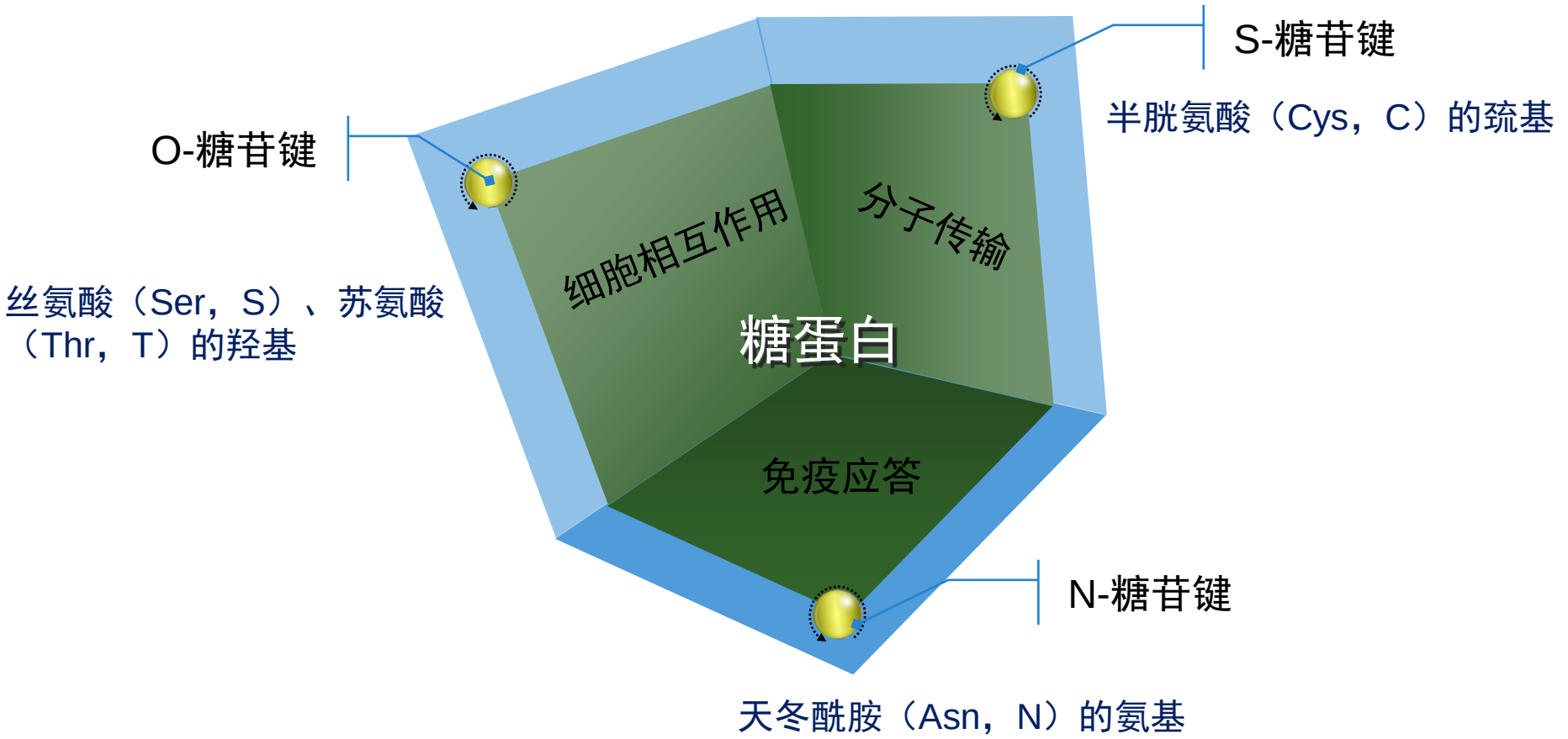
糖蛋白质组学简介

- 糖基化蛋白质组学
  - 研究生物体系内的糖蛋白信息和分布情况（超过50%的蛋白质，尤其是膜蛋白、分泌蛋白和外泌体等）
    - 糖基化位点和对应糖链表达的异常导致代谢紊乱
    - 生物标志物和药物作用靶标
  - 以蛋白质为研究中心
    - 糖基化蛋白的类型
    - 糖基化类型和位点
    - 特定位点的糖链组成



糖基化蛋白质组学简介

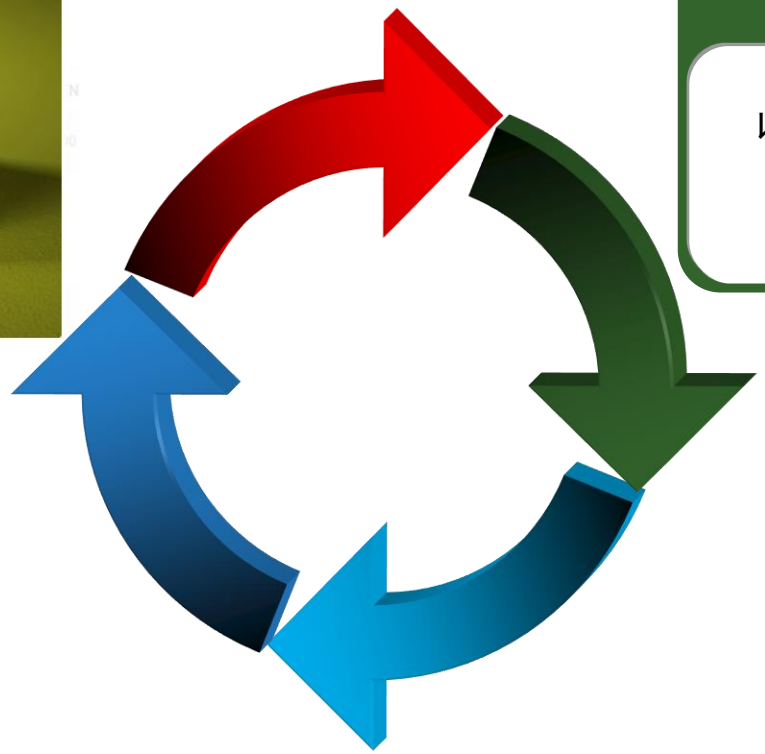
- 糖蛋白的类型——糖链（还原端半缩醛羟基）与氨基酸（侧链活性基团）键合位点



富集方法

Pierce™ Glycoprotein Isolation Kit, WGA

Man和Glc——伴刀豆球凝集素（Con A）
核心Fuc——扁豆凝集素（LCA）
NeuNAc——黑接骨木凝集素（SNA）
$GlcNAc_2$以及NeuNAc——麦胚凝集素（WGA）



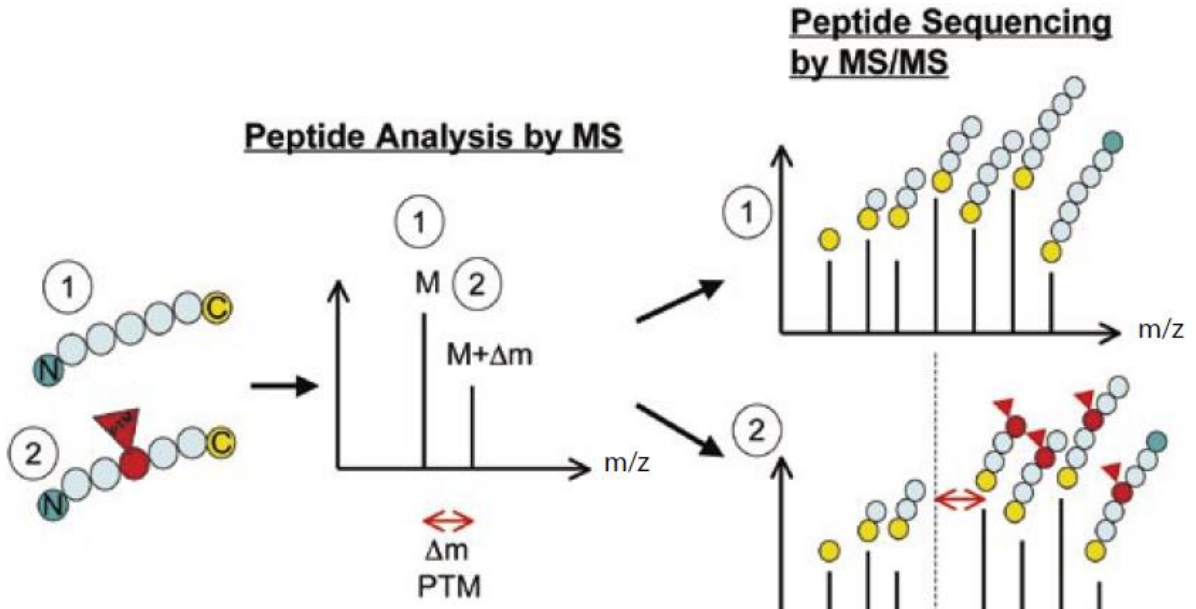
凝集素亲和

以特定糖链为亲和靶标
特异性强
亲和力较弱

- 以糖蛋白或糖肽上的糖链作为靶标，采用具有特定糖链结构亲和力的凝集素蛋白质进行捕获，通常需要用特定的高浓度单糖进行竞争性洗脱糖蛋白或糖肽

糖基化位点鉴定

- 糖基化位点暴露
  - 酶切法
  - 化学修饰
- 形成特定的分子量差异
- 通过二级质谱碎裂找到发生差异的位置即可判断修饰位点



Resource

> 6300个N-糖基化位点

Cell

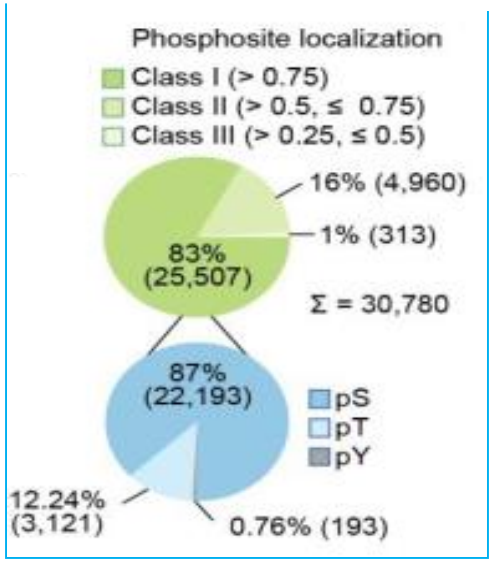
Precision Mapping of an In Vivo N-Glycoproteome Reveals Rigid Topological and Sequence Constraints

Dorota F. Zielinska,[1,3] Florian Gnad,[1,2,3] Jacek R. Wiśniewski,[1,*] and Matthias Mann[1,*]

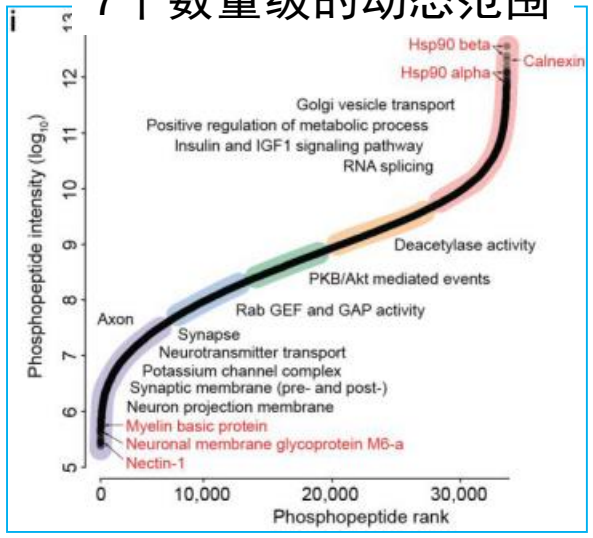
ThermoFisher SCIENTIFIC

磷酸化蛋白质组进展:胰岛素信号通路动态变化

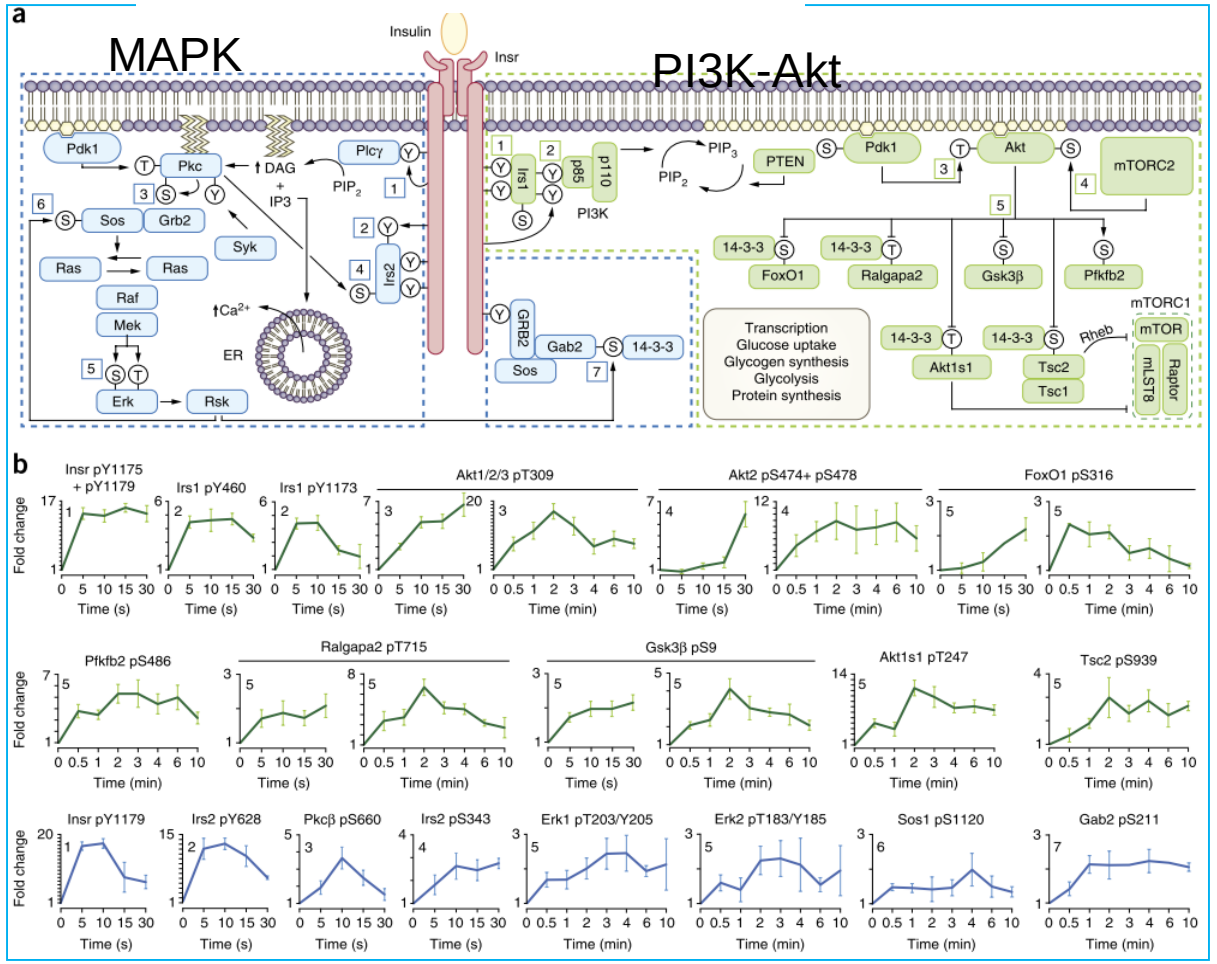
30,780个磷酸化位点定量



7个数量级的动态范围



胰岛素信号转导通路动态变化



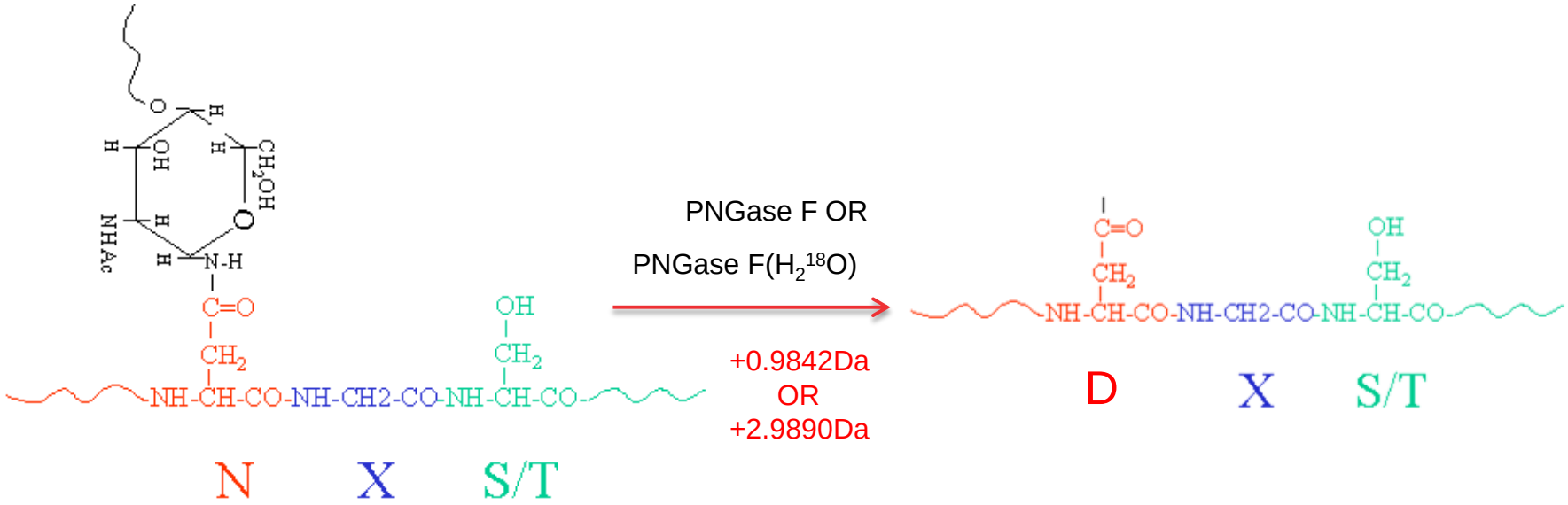
糖基化位点鉴定——酶切法

- N-糖酰胺酶（PNGase F）：特异性地识别N-糖基化位点，有效的切除N-糖基化蛋白质或肽段上的糖链，非常适用于大规模的N-糖基化位点的鉴定和糖链结构的分析

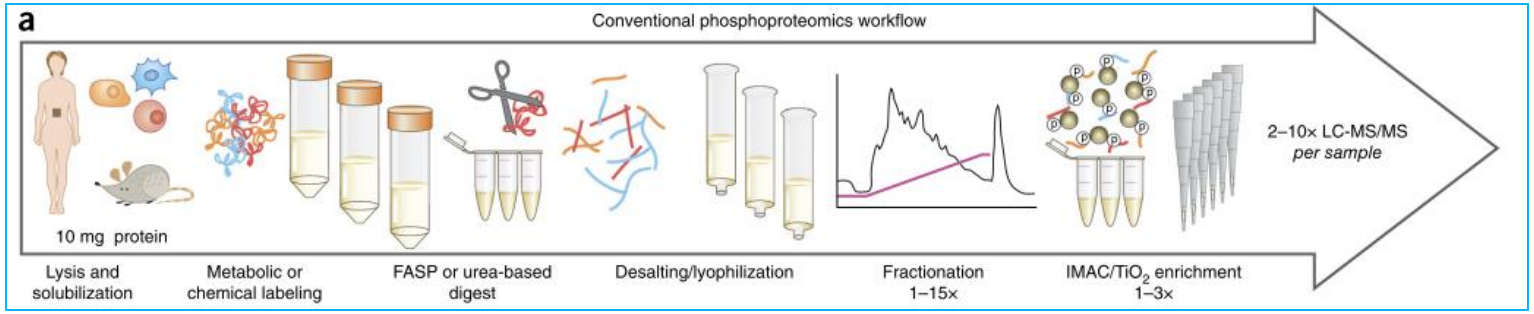
+0.9842 Da的质量差异

  - 鉴定假阳性——加入^{18}O水标记糖基化位点(（排除deamidation）

+2.9890 Da的质量差异



Other PTMs



PTM	Enrichment	MS/MS	特殊碎片离子
磷酸化	TiO2	CID/HCD	中性丢失的b，y离子
乙酰化	抗体	CID/HCD	无
泛素化	抗体	CID/HCD	无
甲基化	抗体	CID/HCD	无

磷酸化蛋白质组学的研究方法研究思路可以推广至乙酰化、泛素化、甲基化等修饰

翻译后修饰研究新进展得益于 Orbitrap 质谱的超高灵敏度

深度解析经典翻译后修饰

> 6300 个 N-糖基化位点

Cell

Precision Mapping of an In Vivo N-Glycoproteome Reveals Rigid Topological and Sequence Constraints

Dorota F. Zielinska,[1,3] Florian Gnad,[1,2,3] Jacek R. Wiśniewski,[1,*] and Matthias Mann[1,*]

> 20000 个泛素化位点

Research

American Society for Biochemistry and Molecular Biology, Inc. available on line at http://www.mcponline.org

Proteomic Analyses Reveal Divergent Ubiquitylation Site Patterns in Murine Tissues*

Sebastian A. Wagner‡§**, Petra Beli‡**, Brian T. Weinert‡, Christian Schölz‡, Christian D. Kelstrup‡, Clifford Young‡, Michael L. Nielsen‡, Jesper V. Olsen‡, Cord Brakebusch¶, and Chunaram Choudhary‡‖

> 40000 个磷酸化位点

OPEN ACCESS CellPress

Ultradeep Human Phosphoproteome Reveals a Distinct Regulatory Nature of Tyr and Ser/Thr-Based Signaling

Kirti Sharma,[1] Rochelle C.J. D'Souza,[1] Stefka Tyanova,[1] Christoph Schaab,[1] Jacek R. Wiśniewski,[1] Jürgen Cox,[1,*] and Matthias Mann[1,*]

[1]Department of Proteomics and Signal Transduction, Max-Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany

*Correspondence: cox@biochem.mpg.de (J.C.), mmann@biochem.mpg.de (M.M.)

http://dx.doi.org/10.1016/j.celrep.2014.07.036

发现新型翻译后修饰

Cell

Resource

Identification of 67 Histone Marks and Histone Lysine Crotonylation as a New Type of Histone Modification

Minjia Tan,[1,6] Hao Luo,[1,6] Sangkyu Lee,[1,6] Fulai Jin,[2] Jeong Soo Yang,[1] Emilie Montellier,[3] Thierry Buchou,[3] Zhongyi Cheng,[1] Sophie Rousseaux,[3] Nisha Rajagopal,[2] Zhike Lu,[1] Zhen Ye,[2] Qin Zhu,[4] Joanna Wysocka,[5] Yang Ye,[4] Saadi Khochbin,[3] Bing Ren,[2] and Yingming Zhao[1,*]

nature chemical biology

ARTICLE

PUBLISHED ONLINE: 12 DECEMBER 2010 | DOI: 10.1038/NCHEMBIO.495

Identification of lysine succinylation as a new post-translational modification

Zhihong Zhang[1,2], Minjia Tan[1,2], Zhongyu Xie[1], Lunzhi Dai[1], Yue Chen[1] & Yingming Zhao[1*]

Regulation of Cellular Metabolism by Protein Lysine Acetylation

Shimin Zhao,[1,2] Wei Xu,[1,2*] Wenqing Jiang,[1,2*] Wei Yu,[1,2] Yan Lin,[2] Tengfei Zhang,[1,2] Jun Yao,[3] Li Zhou,[4] Yaxue Zeng,[4] Hong Li,[5] Yixue Li,[6] Jiong Shi,[6] Wenlin An,[7] Susan M. Hancock,[7] Fuchu He,[3] Lunxiu Qin,[5] Jason Chin,[7] Pengyuan Yang,[3] Xian Chen,[3,4] Qunying Lei,[1,2,8] Yue Xiong,[1,2,4]† Kun-Liang Guan[1,2,8,9]†

- 大多数的翻译后修饰都依靠特异抗体进行富集

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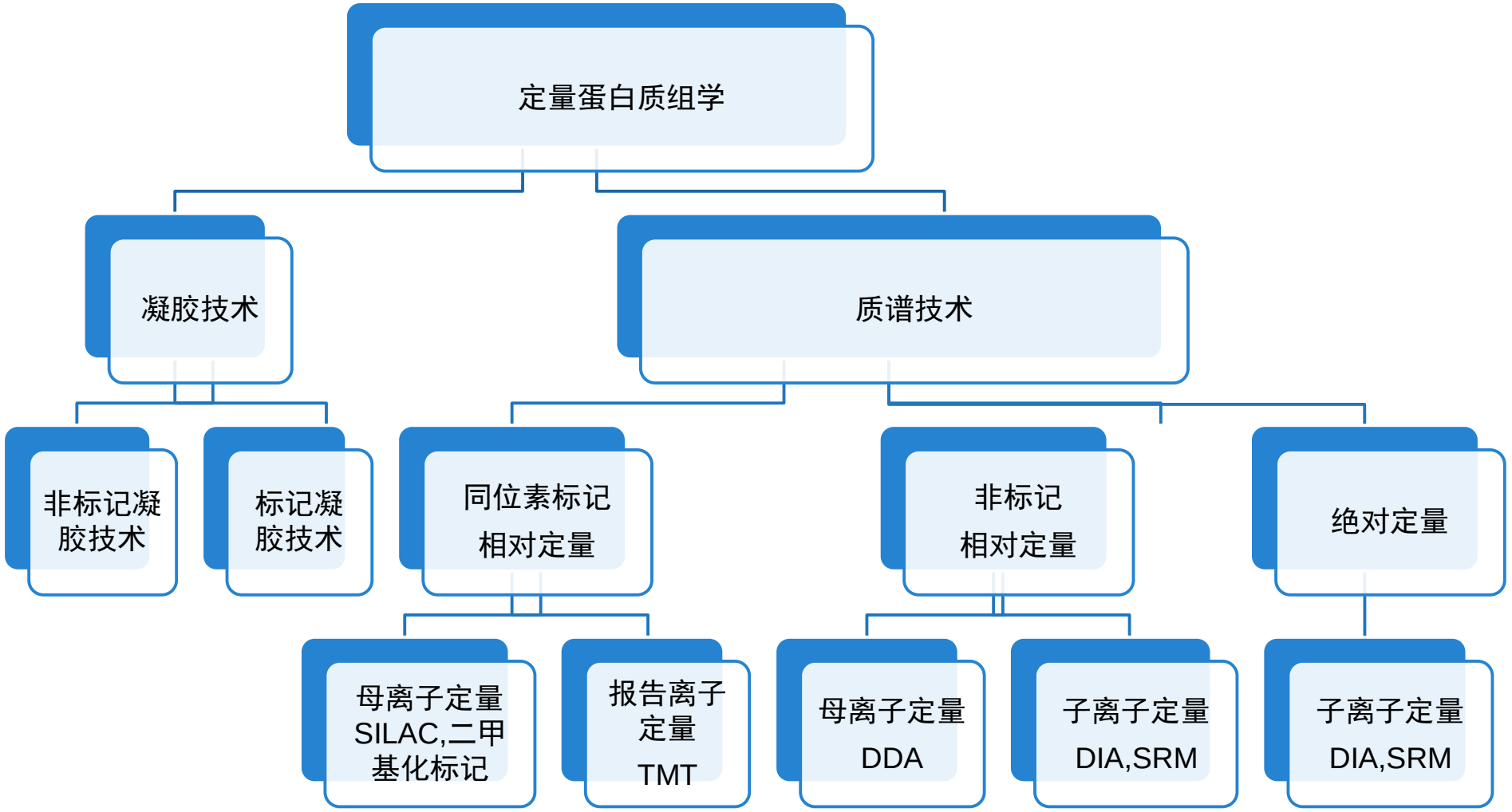
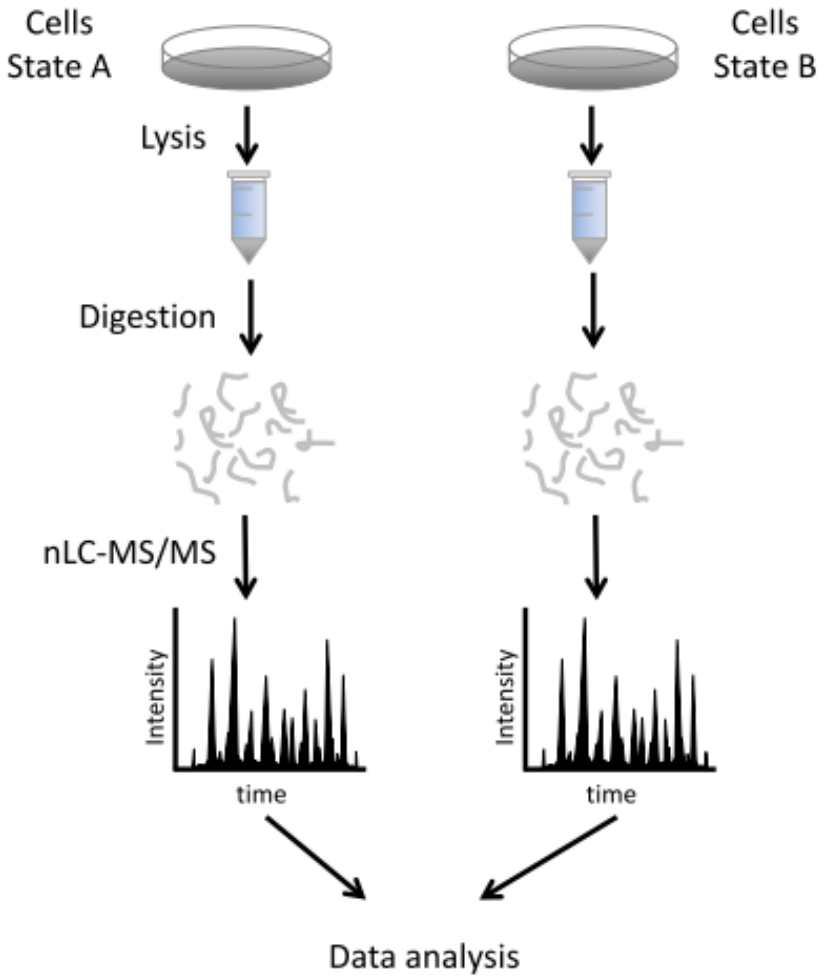
蛋白质组学技术应用领域概览

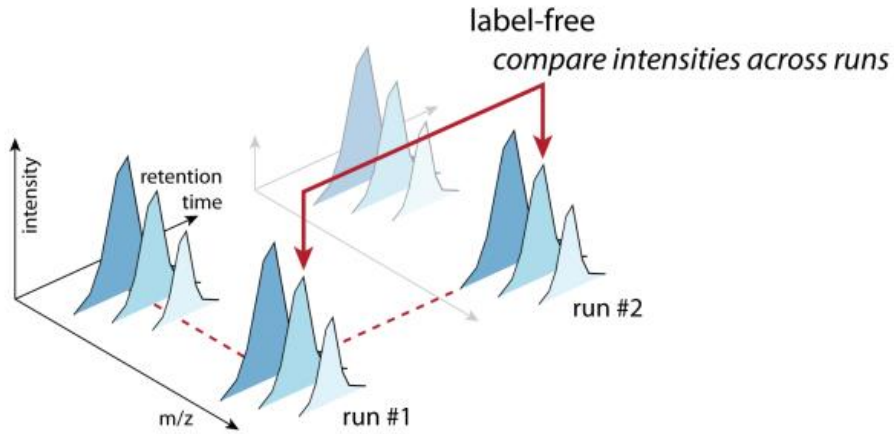
- **蛋白质鉴定表征**
  蛋白鉴定原理, 典型工作流程…
- **蛋白翻译后修饰的质谱解析**
  磷酸化, 糖基化, 泛素化…
- **定量蛋白质组学**
  LFQ, TMT, SILAC…
- **蛋白相互作用研究**
  AP-MS, XL-MS…

定量蛋白质组学主要方法



非标记定量（LFQ）





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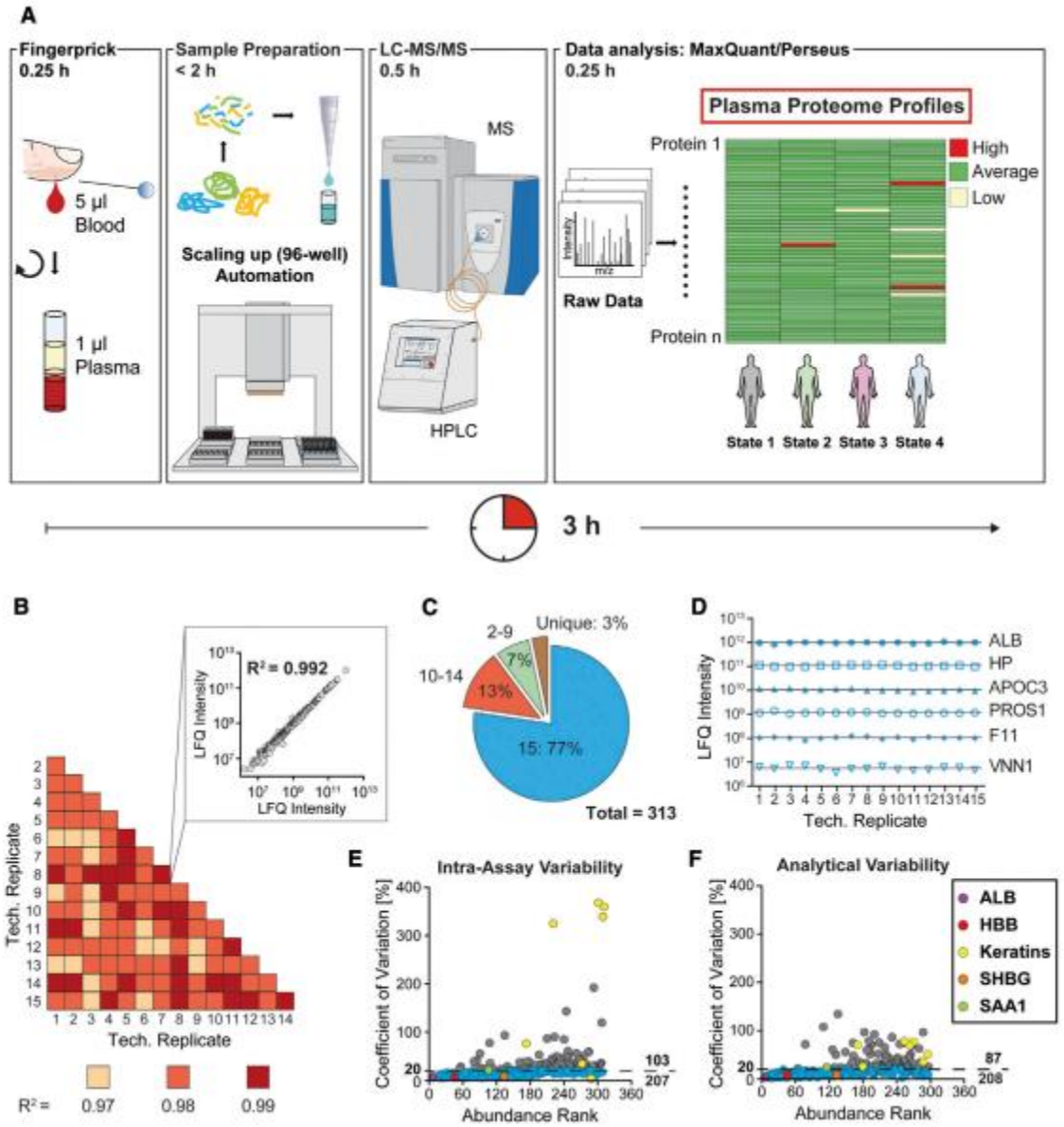
非标记定量（LFQ）

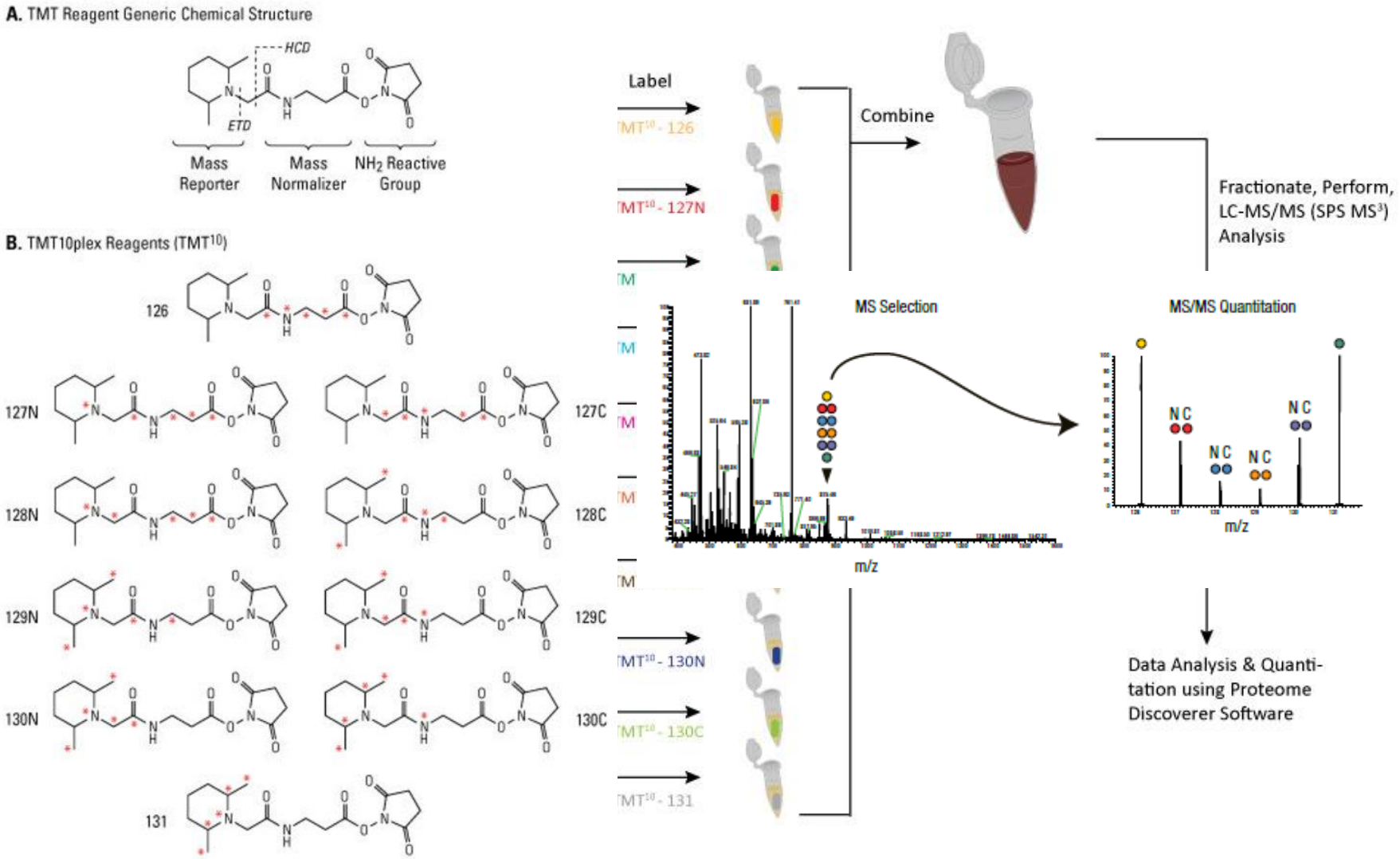


- ~300 proteins in 3h from blood to result
- >1000 proteins with 8 fractions and 100min gradient

- 得益于 Orbitrap 超高分辨质谱的极佳稳定性，可获得高可靠性的定量结果 (CV<20%)

Cell Syst, 2016, 2,185

体外化学标记（TMT）



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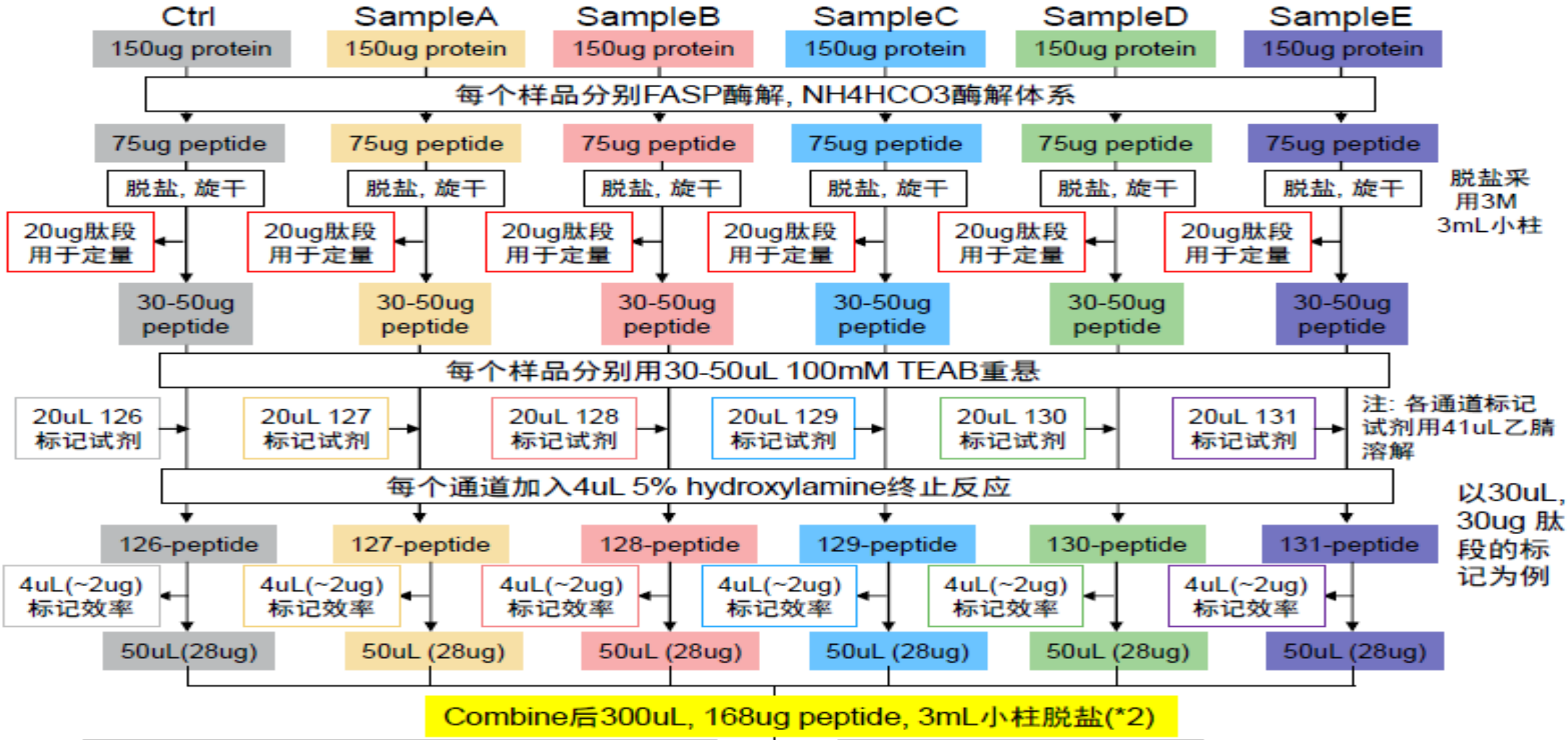
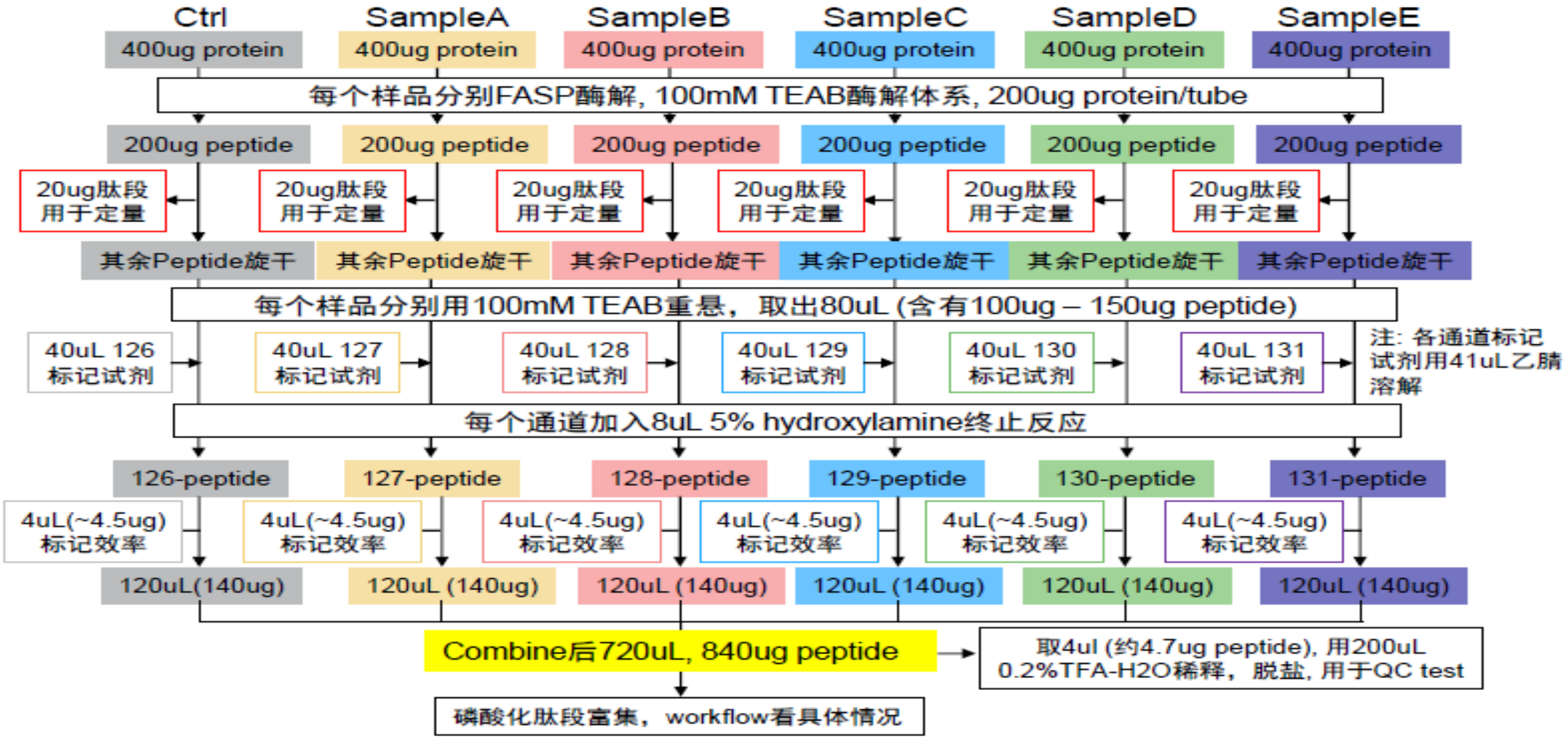
全蛋白质组TMT深度定量实验流程



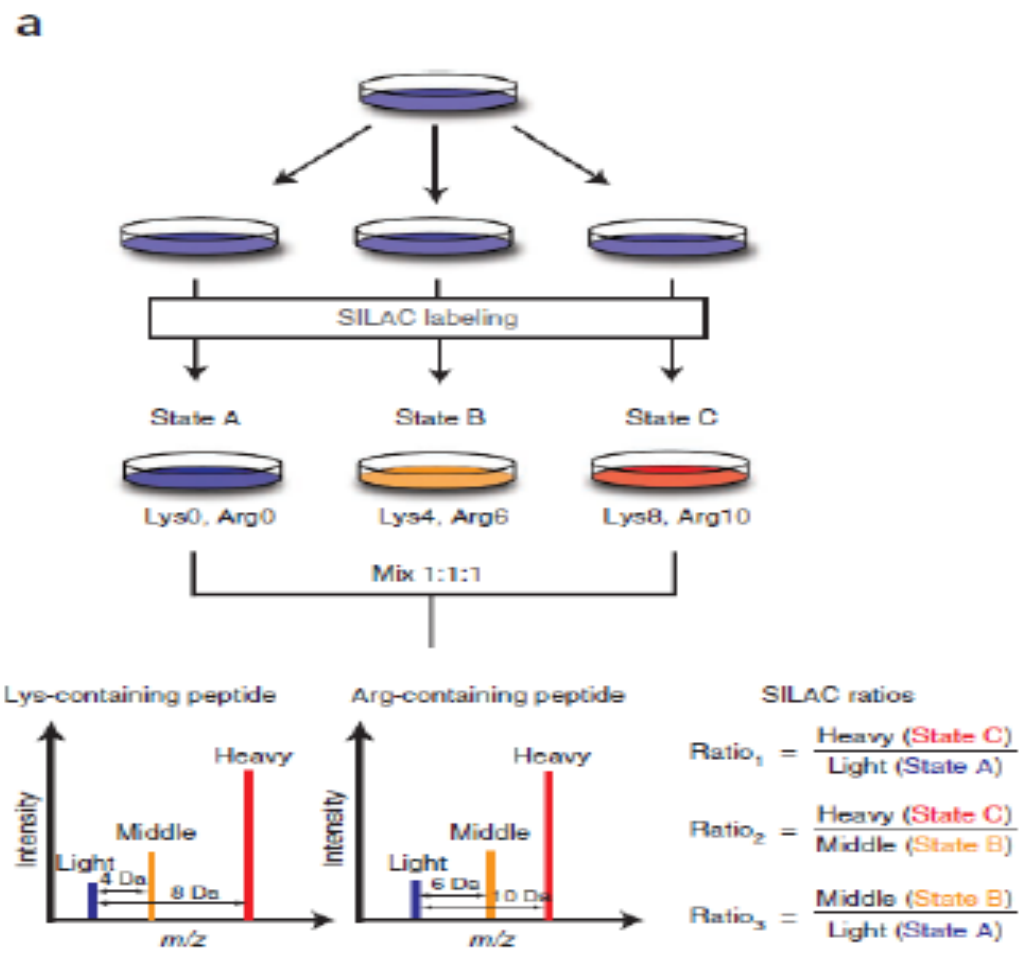
ThermoFisher SCIENTIFIC

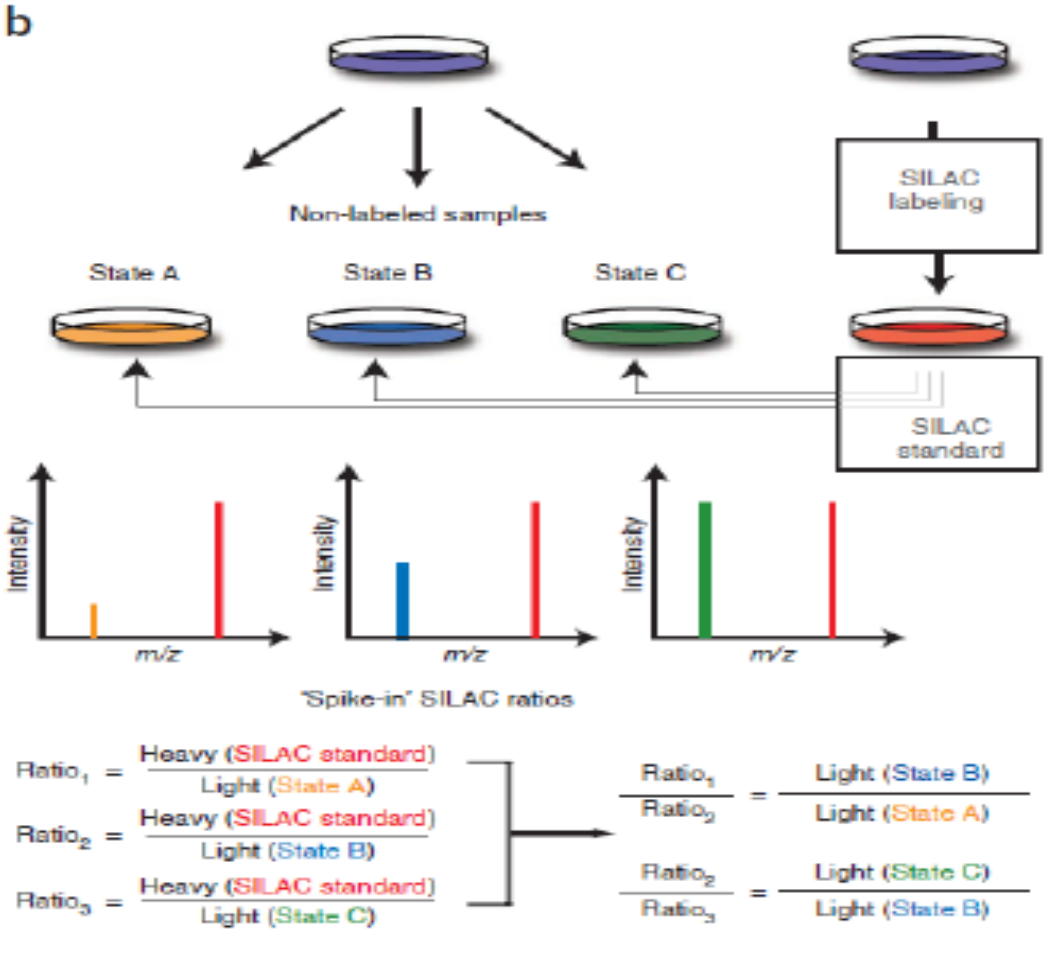
磷酸化蛋白质组TMT定量实验流程



体内代谢标记：SILAC

Stable Isotope Labeling by Amino acid in Cell culture





- 我们通常标记lysine, arginine, why?

Nat Rev Mol Cell Biol. 2006 Dec;7(12):952-8

ThermoFisher SCIENTIFIC

重标培养基培养

- 在SILAC培养基中，我们需要将Lysine, Arginine替换成重同位素标记的Lysine和Arginine
- 需要以下三样试剂:
  a. SILAC缺失培养基
  b. 稳定同位素标记的Lysine and Arginine
  c. 透析血清

DMEM for SILAC

	货号	单位规格	单价 (CNY)	数量
☆	A33822	6 x 500 mL	3,168.00	

Features of DMEM for SILAC:
- Flexible—liquid media deficient in both L-lysine and L-arginine, allowing for more complete proteome coverage through multiple isotopic amino acid labeling
- High quality—medium is sterile, endotoxin-free, and cell culture-compatible

RPMI 1640 Medium for SILAC

	货号	单位规格	单价 (CNY)	数量
☆	A33823	6 x 500 mL	3,168.00	

Features of RPMI 1640 Medium for SILAC:
- Flexible—liquid media deficient in both L-lysine and L-arginine, allowing for more complete proteome coverage through multiple isotopic amino acid labeling
- High quality—medium is sterile, endotoxin-free, and cell culture-compatible

SILAC DMEM Flex Media,

	货号	单位规格	单价 (CNY)	数量
☆	A2499901	500 mL	814.00	

This DMEM is modified as follows:

With	Without
	• Glucose
	• Phenol red
	• L-arginine
	• L-glutamine
	• L-lysine
	• Sodium pyruvate
	• HEPES

SILAC RPMI 1640 Flex Media,

	货号	单位规格	单价 (CNY)	数量
☆	A2494201	500 mL	814.00	

This RPMI 1640 Medium is modified as follows:

With	Without
	• Glucose
	• Phenol red
	• HEPES
	• L-arginine
	• L-glutamine
	• L-lysine

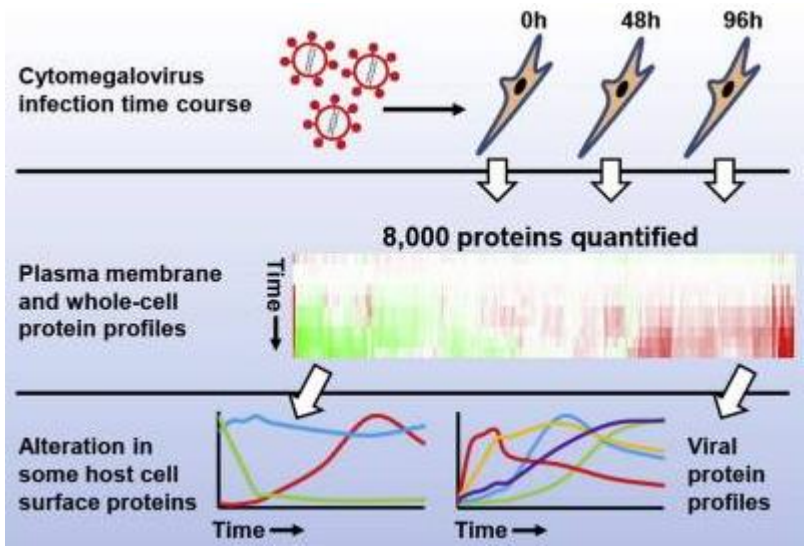
体外化学标记（TMT）

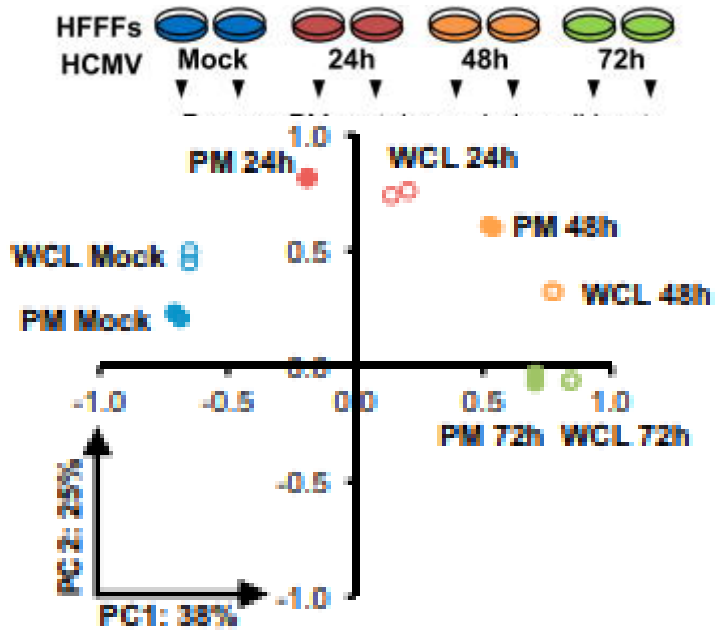
Cell

Resource

Quantitative Temporal Viromics: An Approach to Investigate Host-Pathogen Interaction

Michael P. Weekes,[1,3,4,*] Peter Tomasec,[2,4] Edward L. Huttlin,[1] Ceri A. Fielding,[2] David Nusinow,[1] Richard J. Stanton,[2] Eddie C.Y. Wang,[2] Rebecca Aicheler,[2] Isa Murrell,[2] Gavin W.G. Wilkinson,[2] Paul J. Lehner,[3] and Steven P. Gygi[1,*]

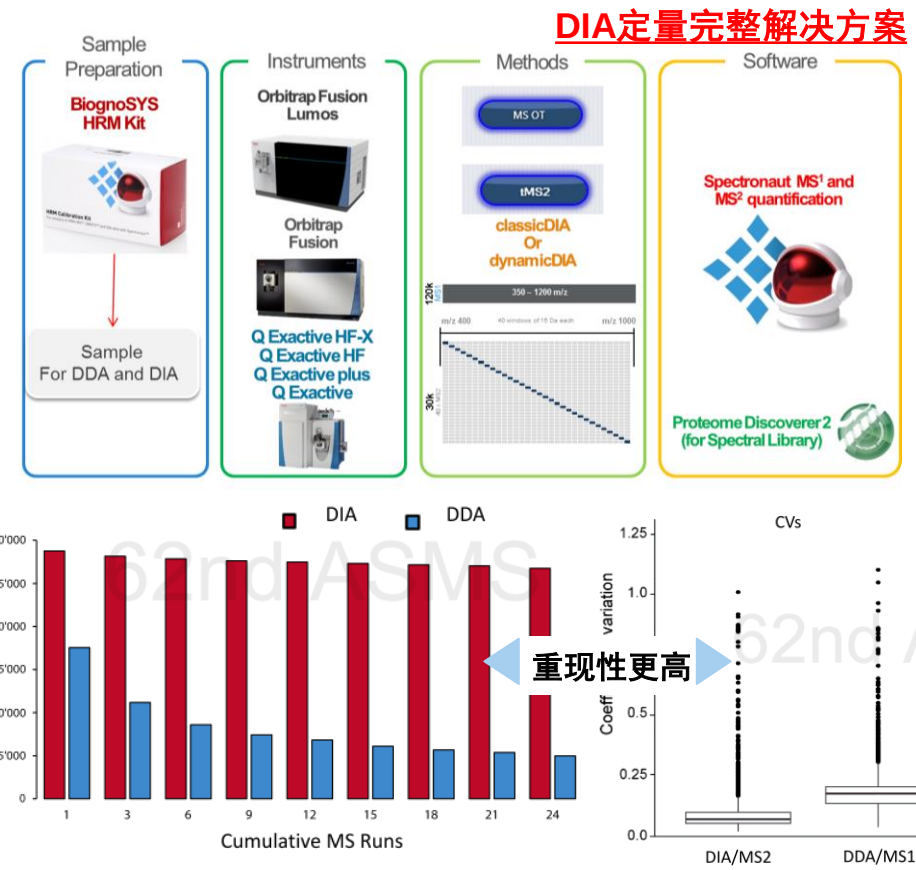


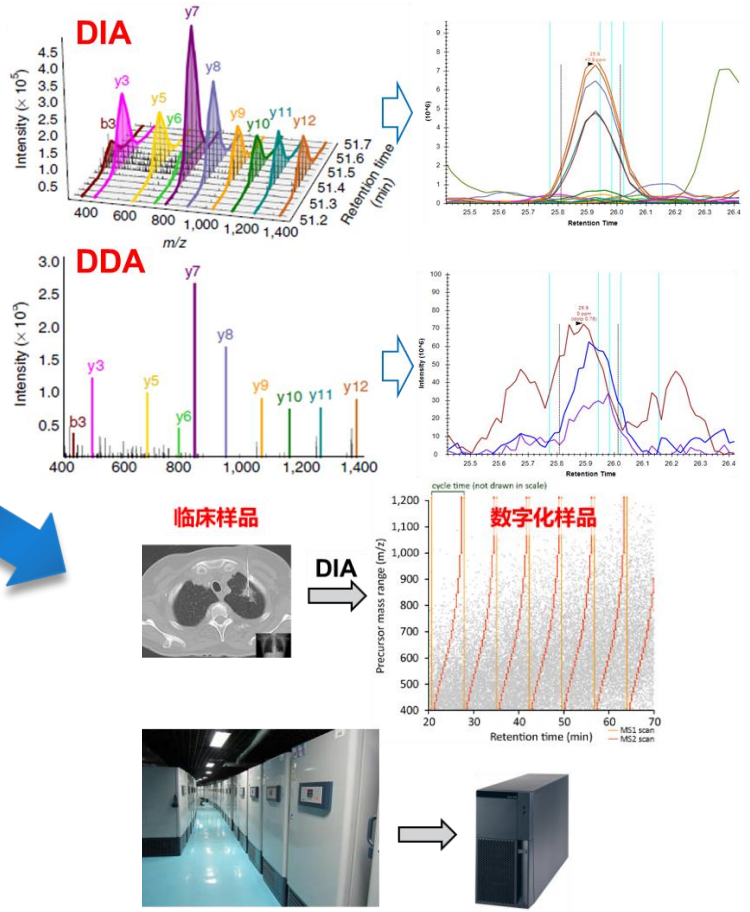


Cell, 2014, 157, 146

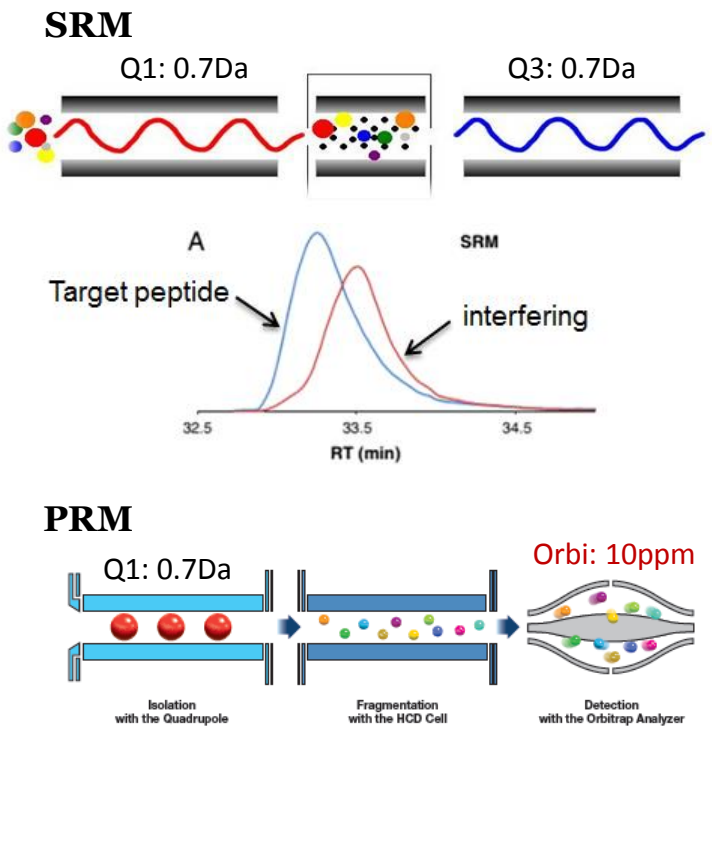
ThermoFisher SCIENTIFIC

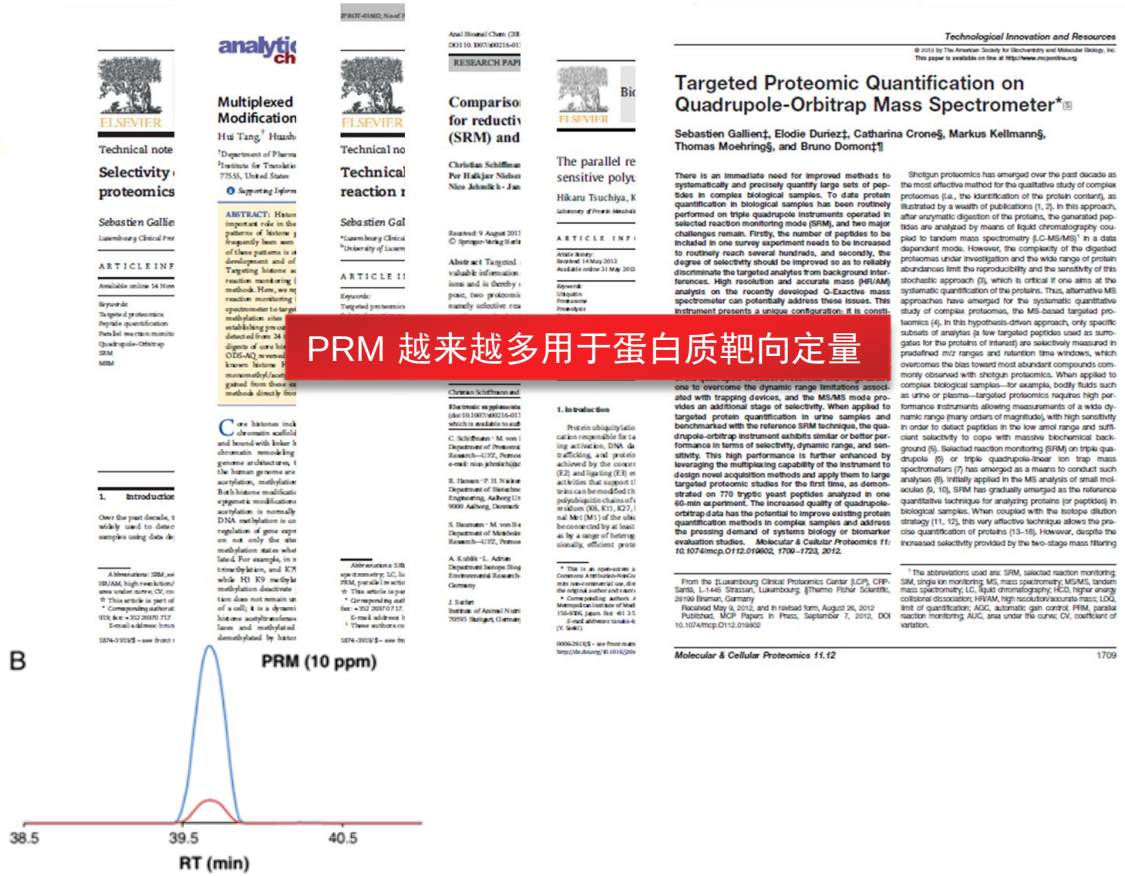
DIA定量蛋白组学


DIA定量完整解决方案
Sample Preparation
BiognoSYS HRM Kit
Sample For DDA and DIA
Instruments
Orbitrap Fusion Lumos
Orbitrap Fusion
Q Exactive HF-X
Q Exactive HF
Q Exactive plus
Q Exactive
Methods
MS OT
tMS2
classicDIA Or dynamicDIA
Software
Spectronaut MS1 and MS2 quantification
Proteome Discoverer 2 (for Spectral Library)
DIA
DDA
Full peptide profiles
Cumulative MS Runs
重现性更高
CVs
Coeff variation
DIA/MS2
DDA/MS1


DIA
DDA
Intensity
m/z
Retention time (min)
临床样品
数字化样品
DIA
Precursor mass range (m/z)
Retention time (min)

平行离子反应监测PRM（Parallel Reaction Monitoring）定量技术





PRM 越来越多用于蛋白质靶向定量

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PRM定量应用实例

研究背景：

- **SAA** (Serum amyloid A, 血清淀粉样蛋白)是一种由104个氨基酸组成的急性期反应蛋白，分为SAA1与SAA2两种亚型，序列同源度高达90%，商品化的抗体无法有效区分上述两种亚型。SAA1和SAA2同时存在基因**单核苷酸多态性(SNP)**，包含多个不同的等位基因编码，分别包括SAA1 α/β/γ与SAA2 α/β。
- 血浆中SAA不同亚型和不同等位基因的表达量可能与恶性肿瘤的发生和转移密切相关，本实验开发了SAA的PRM定量流程，**用于27例非小细胞肺癌和20例正常人血浆样品中的标志物验证。**

实验结果：

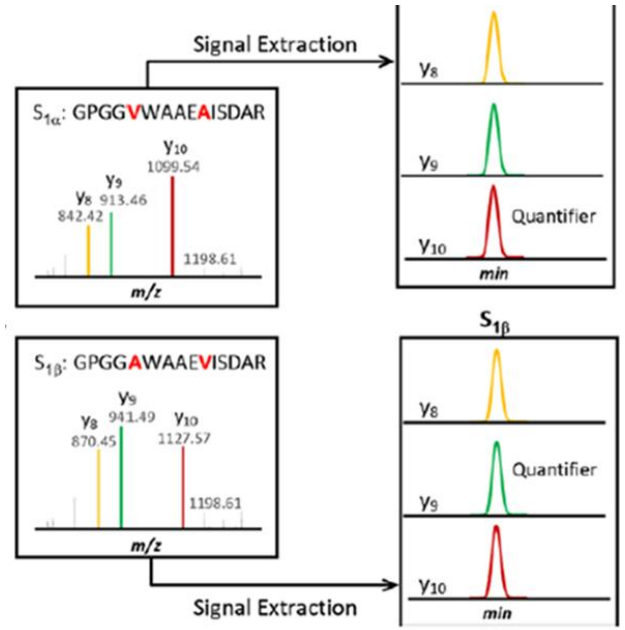
- **定量方法：**选择强度最高的子离子最为Quantifier（定量离子），其他5个子离子用于和谱图库匹配进行定性确证
- **定量结果：**9.6-104.2ng/mL (LOQ)

3116 DOI 10.1002/pmic.201400382 *Proteomics* 2015, *15*, 3116–3125

RESEARCH ARTICLE

Quantification of SAA1 and SAA2 in lung cancer plasma using the isotype-specific PRM assays

1α	SFFSFLGEAFDGAR	GPGGVWAAEAISDAR	FFGHGAEDSLADQAANEWGR
1β	SFFSFLGEAFDGAR	GPGGAWAAEVISDAR	FFGHGAEDSLADQAANEWGR
1γ	SFFSFLGEAFDGAR	GPGGAWAAEAISDAR	FFGHGAEDSLADQAANEWGR
2α	SFFSFLGEAFDGAR	GPGGAWAAEVISNAR	LTGHGAEDSLADQAANK
2β	SFFSFLGEAFDGAR	GPGGAWAAEVISNAR	GAEDSLADQAANK



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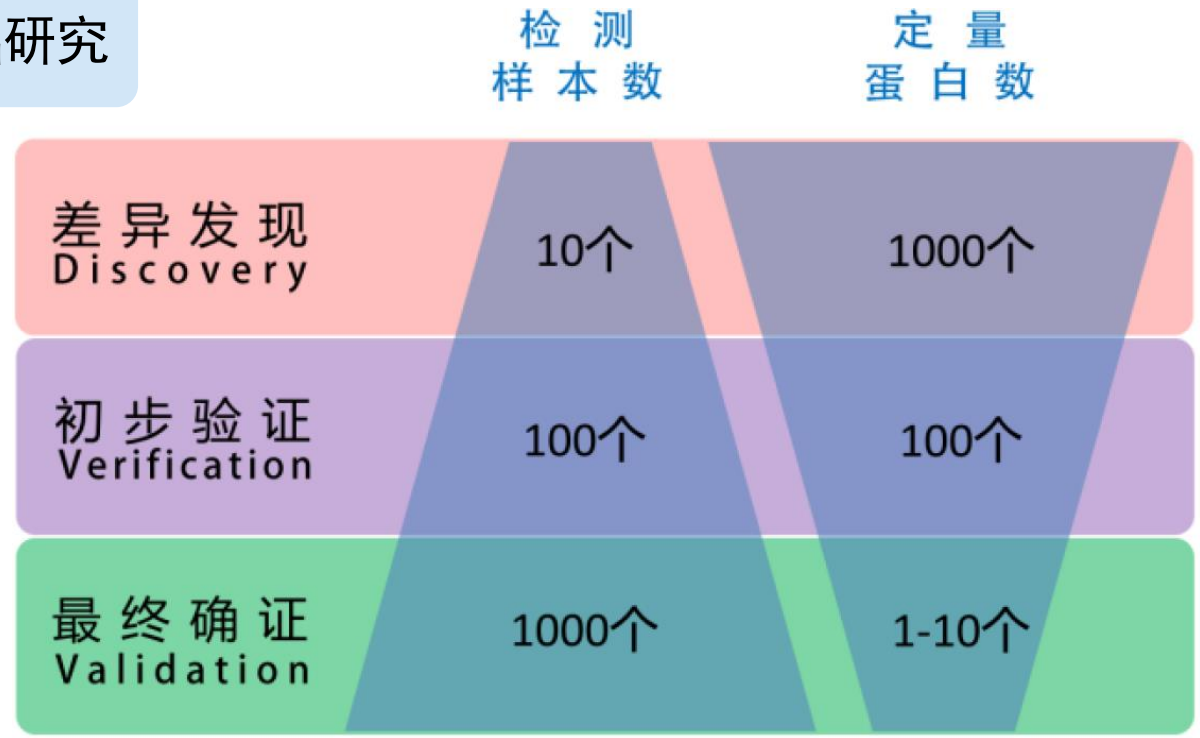
定量蛋白质组学技术技术路线

基础研究



TMT-11 (标记)

DIA (非标记)

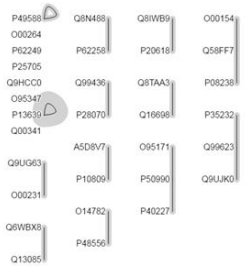
PRM (目标蛋白追踪)

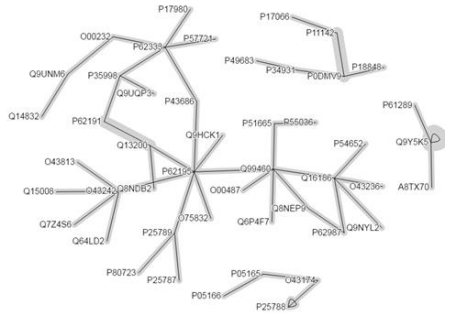
蛋白质组学技术应用领域概览

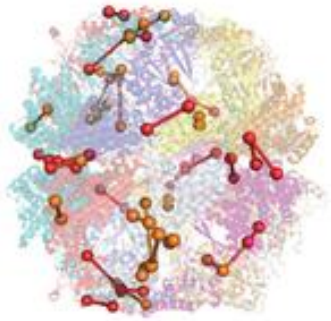
- **蛋白质鉴定表征**
  蛋白鉴定原理, 典型工作流程…
- **蛋白翻译后修饰的质谱解析**
  磷酸化, 糖基化, 泛素化…
- **定量蛋白质组学**
  LFQ, TMT, SILAC…
- **蛋白相互作用研究**
  AP-MS, XL-MS…

蛋白相互作用形成的蛋白复合物对蛋白功能起到重要影响

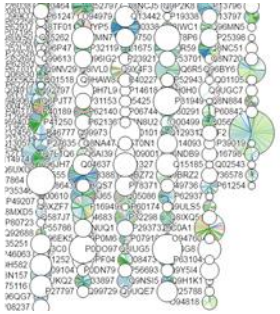
- **Protein Complex**

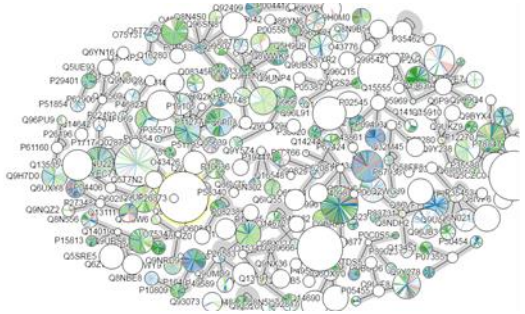


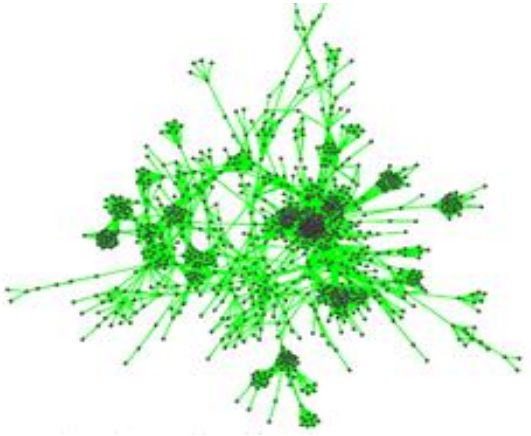




- **Interactomics**

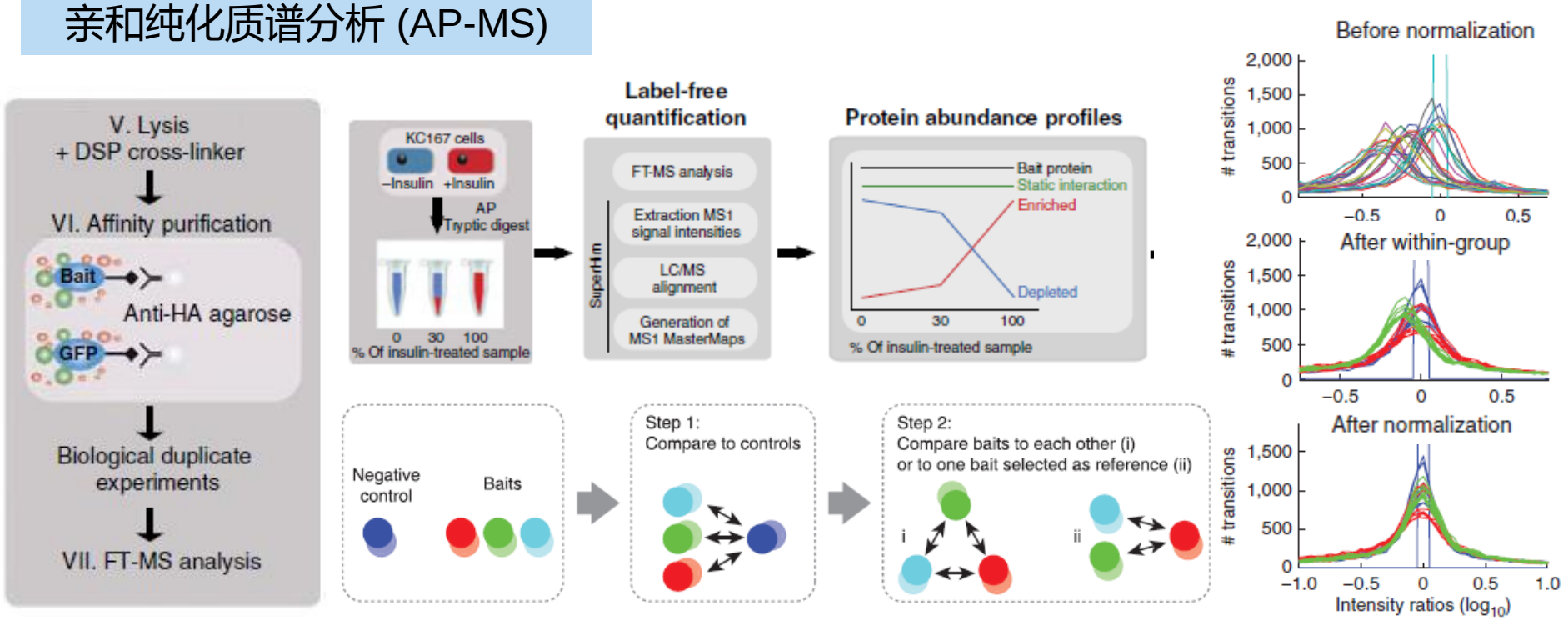






蛋白质相互作用动态分析

亲和纯化质谱分析 (AP-MS)



- 确定相互作用蛋白
  引入一个无关蛋白（如GFP）
  作为 negative control
- 蛋白酶解形式
  SDS-PAGE, 胶内酶解
  直接溶液内酶解
- 互作蛋白的定量比较
  Label free, SILAC, Isobaric…
- 分析方法
  DDA, DIA, targeted…
- Normalization
  Bait protein, most likely ratio normalization…

Mol Syst Biol. 2011, 7,547. Nat Methods. 2009, 6, 203.

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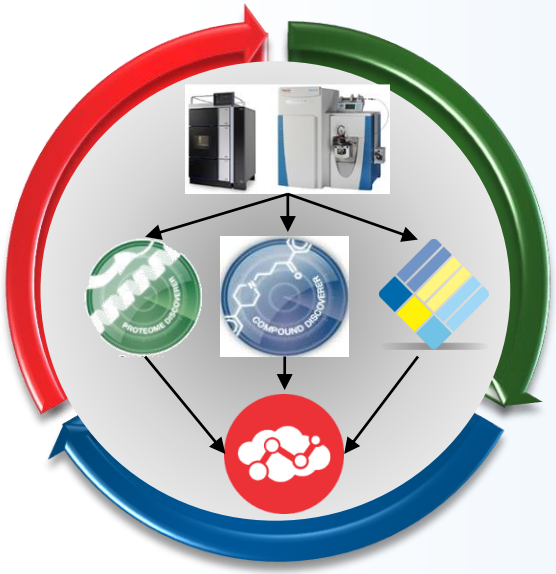
iOmics Cloud: 高通量组学数据的生物信息分析解决方案



Closed-Loop
HW + SW + Cloud account

iOmics Cloud: accelerate the transformation from chemical data to biological knowledge

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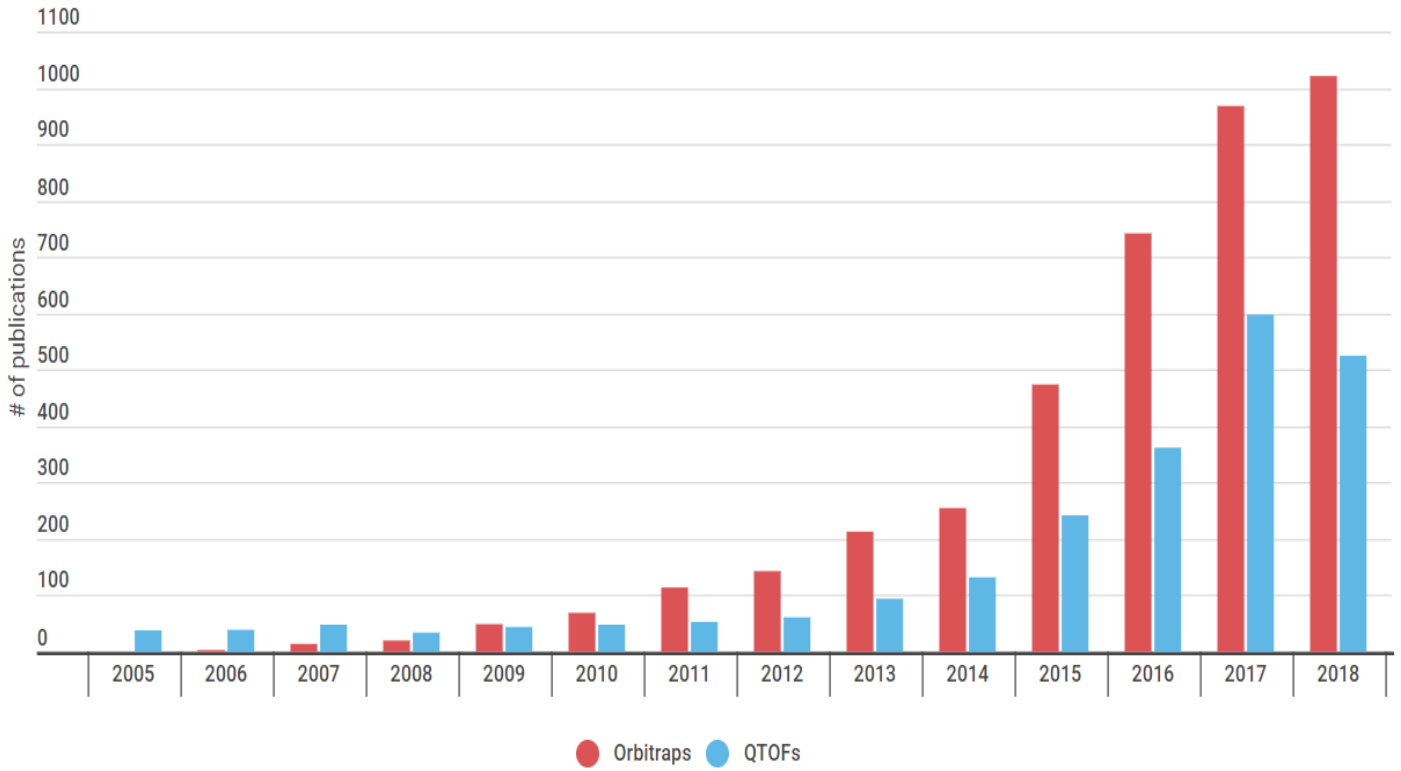
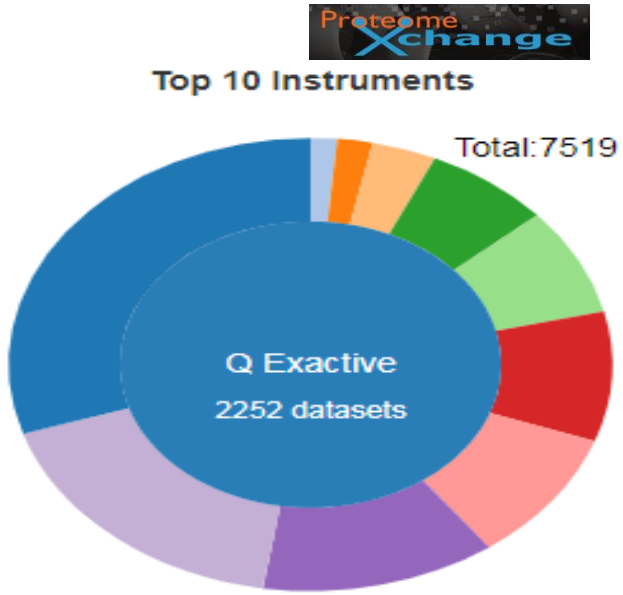
Orbitrap成为蛋白质组学研究的唯一选择

Orbotrap VS TOF 发表Nature、Science系列杂志文章对比



原始数据上传网站使用仪器对比



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