武漢大學
Wuhan University

第五讲
蛋白质组学研究方法和技术
2015－11

一 基本概念

1. 基因组(Genome)：单倍体细胞核、细胞器或病毒粒子所含的全部DNA或RNA。

真核细胞基因组：指一个物种的单倍体染色体组上所含有的一整套基因。

真核生物细胞器中叶绿体DNA→叶绿体基因组；

线粒体DNA→线粒体基因组。

原核生物其环状DNA分子上所含有的基因为一个基因组。

2 基因组学（Genomics）：研究生物体基因组的组成、结构与功能的学科。

- 结构基因组学（structure genomics）：研究基因组的结构并构建高分辨的遗传图、物理图、序列图和转录图以及蛋白质组成与结构的学科。
- 功能基因组学（functional genomics）：

  利用结构基因组学研究所得到的各种信息在基因组水平上研究编码序列及非编码序列生物学功能的学科。

3 蛋白质组（proteome）：

PROTEOME is the PROTEins expressed by a genOME or a tissue.

由一个细胞的基因组所表达的全部相应的蛋白质；
或某种细胞或组织的基因组中表达的所有蛋白质。

- 功能蛋白质组（functional proteome）：细胞在一定阶段或与某一生理现象相关的所有蛋白质。

4 蛋白质组学（proteomics）：研究细胞内全部蛋白质（蛋白质组）的组成、结构与功能的学科。

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The emergence of proteomics

Historical review for proteomics

mass spectrometry

chip-based approaches

complex mixture analysis LC-MS(/MS)

2D gel electrophoresis

proteomics

time

1975 | 1990 | 1994 | 1996 | 2002

algorithms

nucleotide sequencing ESTs/genome scale

genetic approaches

1958 → 1981 → 1987 → 1990 → 2000 →

1981	1987	1990
Ionization technology Fast atom bombardment (FAB) ***Peptide sequence analysis***	MALDI ⋯→ ESI ⋯→ ***Biomolecule analysis***	MALDI-TOF ESI-MS/MS ***Protein 2-DE analysis***

Computer science

(Swiss-Prot, PC/GENE, GCG, NCBI)

Proteomics and the Proteome

Central Dogma in macroscopic

Genome

Transcriptome

Proteome

Metabolome

DNA

Transcription

RNA

Translation

Protein

Function

Juang RH (2006) Proteomics

Genome

DNA

1

Transcriptional control

2

RNA editing control

pre-mRNA

NUCLEUS

CYTOSOL

inactive mRNA

3

RNA proccessing control

5

mRNA degradation control

Transcriptome

mRNA mRNA

4

mRNA

RNA transport control

6

mRNA translational control

8

Physiologic effect

7

active protein

Protein activity control

Proteome

protein

inactive protein

Figure 1. Overview of the transfer of information from the sequence in the genes to the functioning proteins of the cell (the central dogma) with the possible control mechanisms indicated. A gene (DNA, red) is transcribed (step 1) to pre-mRNA that may be edited (step 2) and then processed (step 3) to one mRNA or by alternative splicing to several forms of mRNAs (blue). The mRNAs are transported (step 4) out of the nucleus to the cytosol. In the cytosol, the mRNA may be degraded (step 5), or translated (step 6) into protein (green). Protein activity is controlled (step 7). Proteins may be synthesized as inactive forms that are later reversibly or irreversibly activated or, alternatively, they may be synthesized as active proteins that are later inactivated. Proteins are the ultimate operating molecules producing the physiologic effect (step 8) in virtually every mechanism in the cell. Reprinted with permission from Honoré B & Østergaard M. Transcriptomics and proteomics: integration? Nature Encyclopedia of the Human Genome, Vol. 5, 579–584 (2003) Nature Publishing Group.

与基因组的关系

Flow	Control	
Genome sequence		GENOMICS
↓	Transcriptional control	GENOMICS
mRNA		GENOMICS
↓	Translational control	
Primary protein products		PROTEOMICS
↓	Post-translational control	PROTEOMICS
Functional protein products		PROTEOMICS
↓		
Determination of gene		

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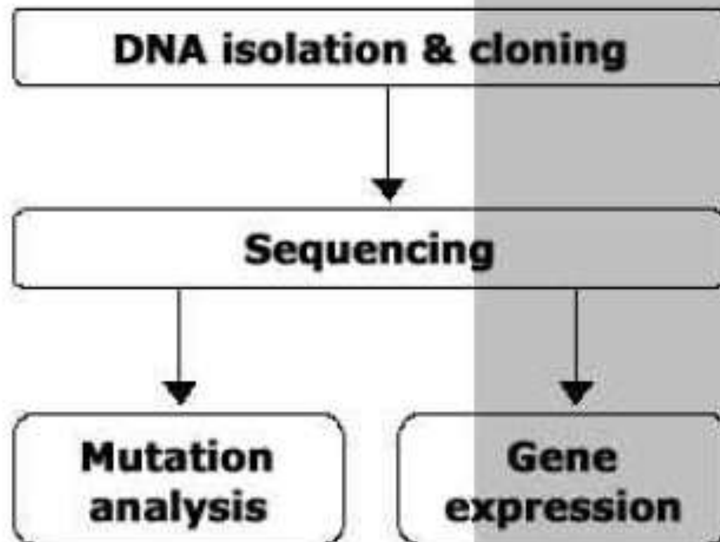
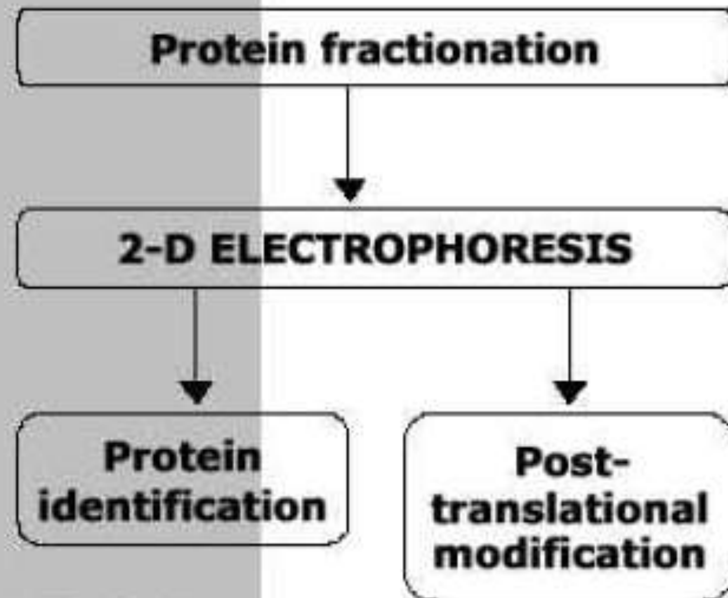
与功能基因组的关系





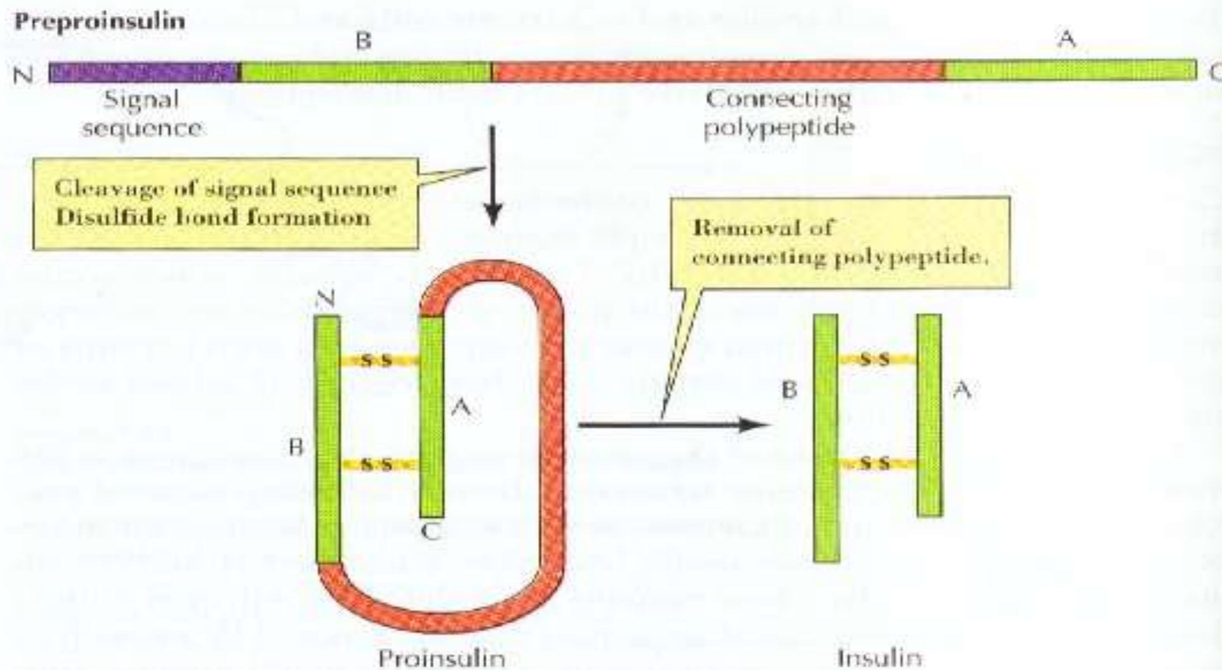
与传统蛋白质研究的区别

- 个体→→ 整体
- 分析静态性质→→ 比较动态变化
- 孤立个体→→ 相互作用
- 结构→→ 功能
- 小规模、非连续→→ 高通量、自动化

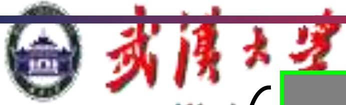
蛋白质组与基因组的特点比较：

基因序列不能反映蛋白质的功能，蛋白质远比核酸复杂。

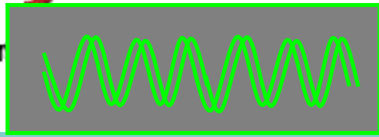
①与DNA修饰不同，蛋白质的修饰有磷酸化、糖基化、乙酰化、巯基化等。



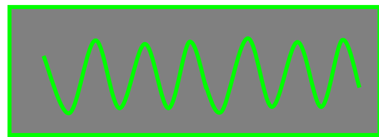
Proteomics: Systematic Analysis of Proteins for their Identity, Quantity and Function



Genomics

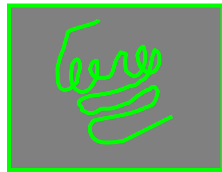


Genome



Transcriptome

Proteomics



Proteome

~ 200 Modifications

Lipidation

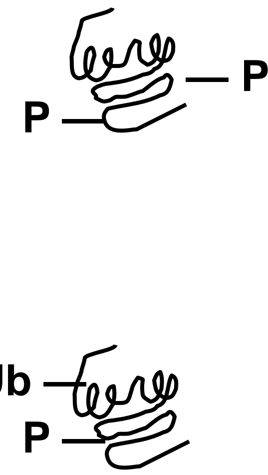
Lipid

Glycosylation

Sugar

Phosphorylation

P



Ubiquitination

Ub

Cleavage

Many more

PTM

Laboratory of plant molecular cytogenetics

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②在形成成熟的**mRNA**的过程中，一个基因由于拼接方式的不同能编码多个不同的蛋白。

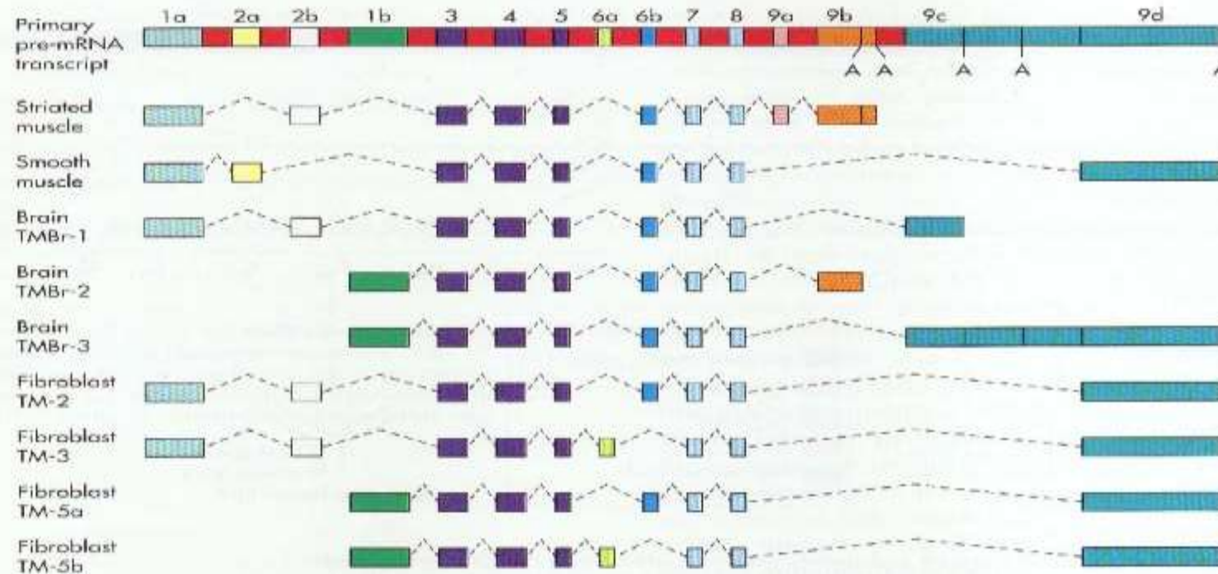


FIGURE 8-8
Complex patterns of eukaryotic mRNA splicing. The pre-mRNA transcript of the α-tropomyosin gene is alternatively spliced in different cell types. The red boxes represent introns; the other colors represent exons. Polyadenylation signals are indicated by an A. Dashed lines in the mature mRNAs indicate regions that have been removed by splicing. TM, tropomyosin. (After J. P. Lees, et al., 1990.)

③蛋白在细胞的定位可以改变；

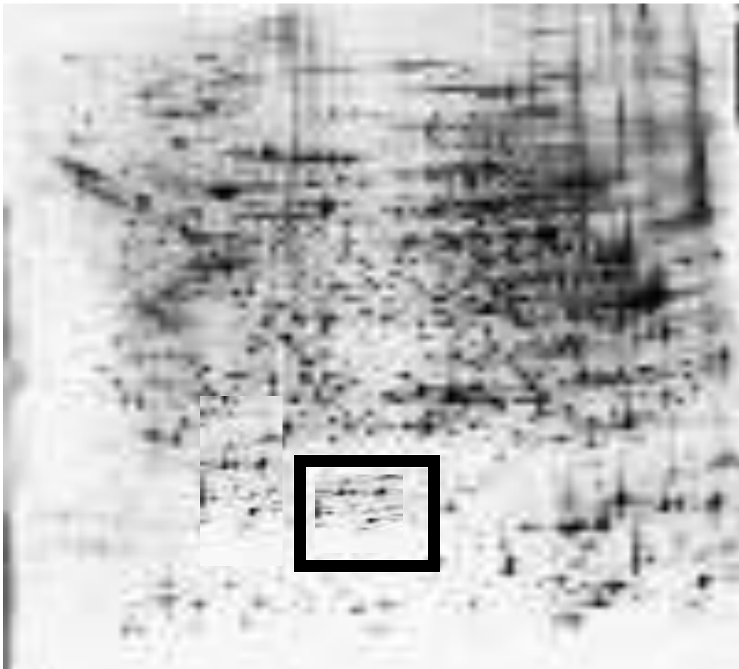
④蛋白对环境的改变也会产生反应；

⑤mRNA水平常常不反映蛋白质表达的水平，而且即使存在一个开放阅读框也不保证只代表一个蛋白；

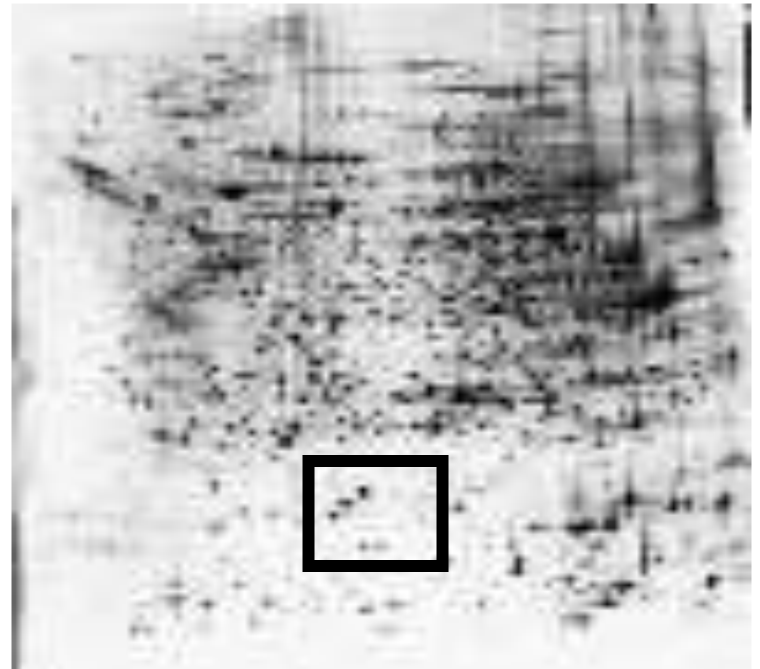
⑥基因表现不一定完全反映在蛋白质，由基因组较难预测蛋白质的修饰及调控，也无法预测蛋白质间的交互作用；

⑦蛋白质组是一个动态的概念，同一细胞，处在不同的发育阶段，细胞中的蛋白质也会发生相应的变化，从而导致不同的蛋白质组。

Expressional Proteomics



Prostate tumor



Normal

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Structural Proteomics

- Separation
- Identification
- Identification of the PTM (post translation modification)
- Comparison of the Proteome in different states. (health/disease;drug treated/untreated)

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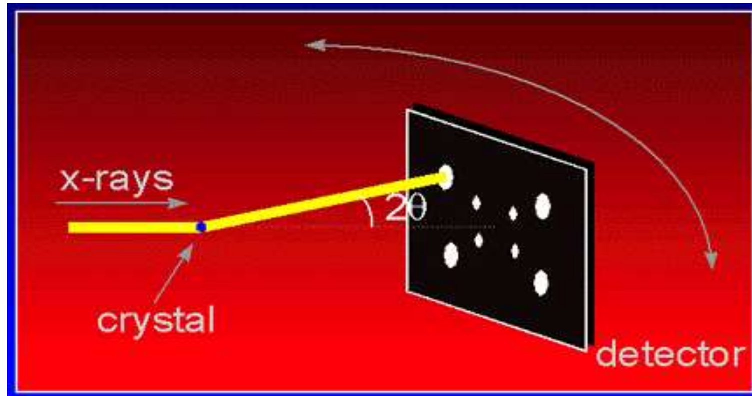
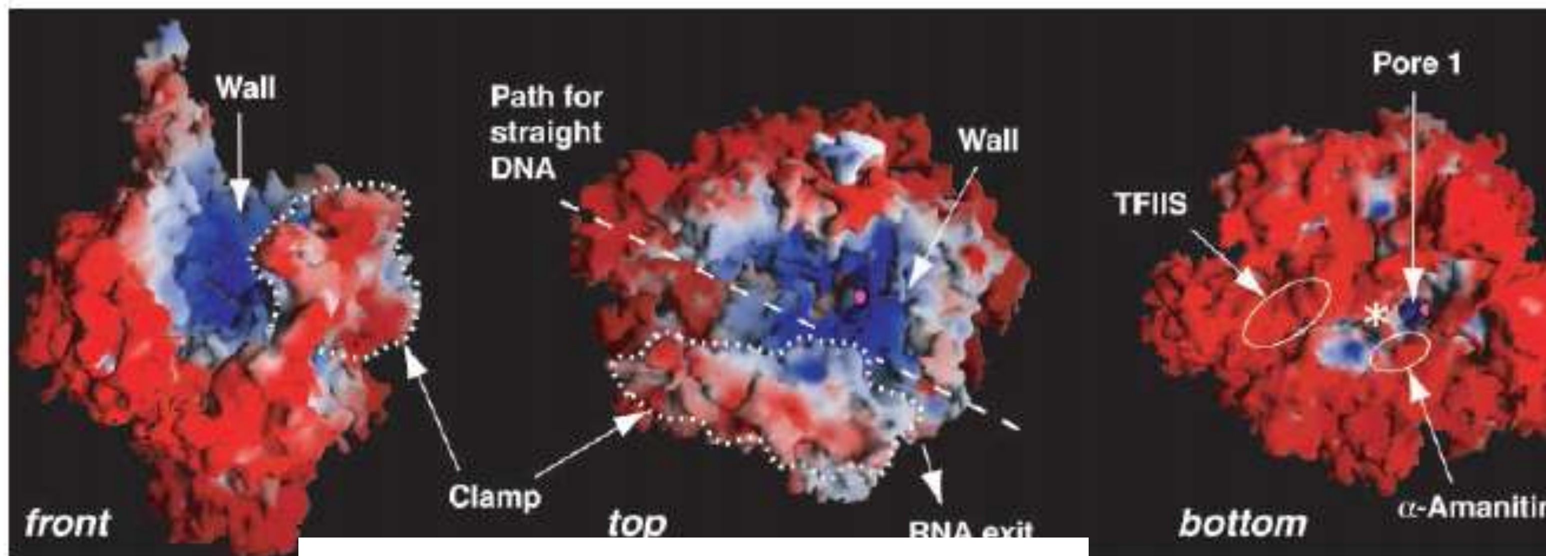
Structural Proteomics



- **High Throughput protein structure determination via X-ray crystallography, NMR spectroscopy or comparative molecular modeling**

Fig. 6. Surface charge distribution and factor binding sites. The surface of Pol II is colored according to the electrostatic surface potential (84), with negative, neutral, and positive charges shown in red, white, and blue, respectively. The active site is marked by a pink sphere. The asterisk indicates the location of the conserved start of a fragment of *E. coli* RNA polymerase subunit β′ that has been cross-linked to an extruded RNA 3′ end (54).



Structural Basis of Transcription: RNA Polymerase II at 2.8 Ångstrom Resolution

Patrick Cramer,* David A. Bushnell, Roger D. Kornberg†

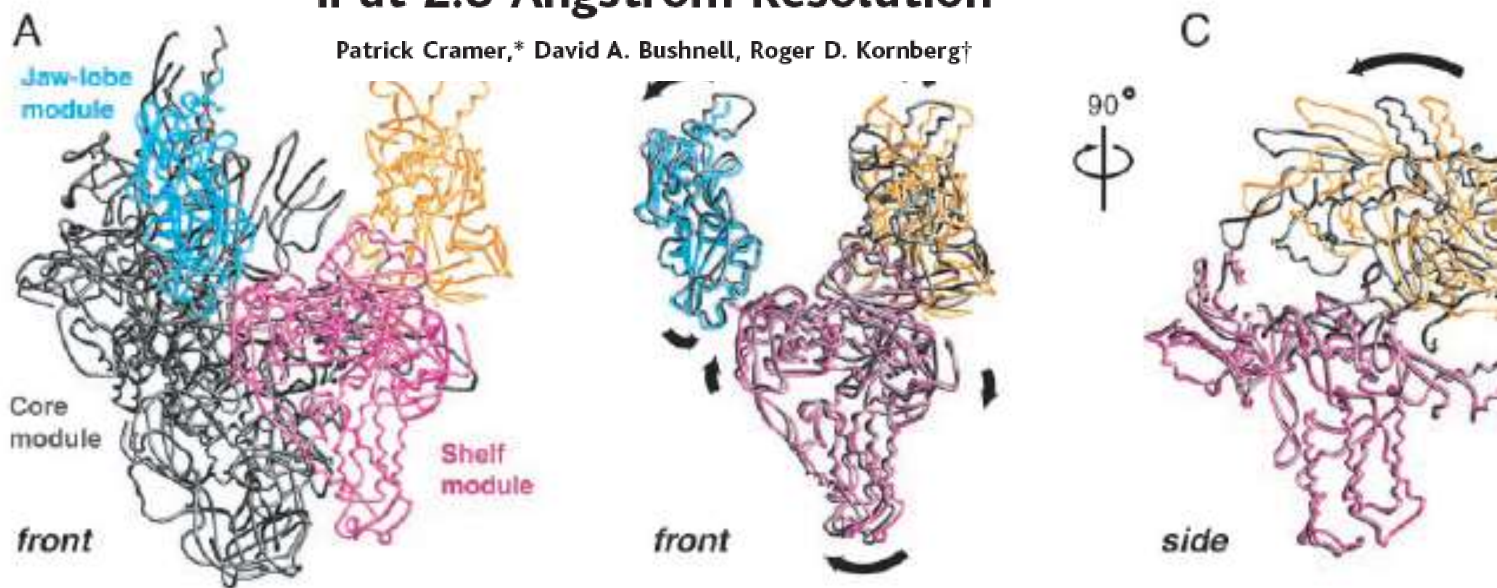


Fig. 7. Four mobile modules of the Pol II structure. (**A**) Backbone traces of the core, jaw-lobe, clamp, and shelf modules of the form 1 structure, shown in gray, blue, yellow, and pink, respectively. (**B**) Changes in the position of the jaw-lobe, clamp, and shelf modules between form 1 (colored) and form 2 structures (gray). The arrows indicate the direction of charges from form 1 to form 2. The core modules in the two crystal forms were superimposed and then omitted for clarity. (**C**) The view in (B) rotated 90° about a vertical axis core and jaw-lobe modules are omitted for clarity. In form 2, the clamp has swung to the left, opening a wider gap between its edge and the

Functional Proteomics

- Localization within the cells
- Protein complex determination
- Protein-Protein Interaction / Interaction Network
- Function the proteins involved in.

  (Metabolic and regulatory pathway;

  Signal transduction pathway)

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Functional Proteomics

Growth factor

Cell membrane

p21ras GTP

PI3K

p110

p85

ras pathway

P

kinase

SH2

p21ras GDP

p21ras GTP

p21ras GDP

Grb2

Sos

Raf-1

PLCγ

Dynamin

DAG

PKC's

PI4,5P

Ca

MEK

MAPK

Transcription

Nucleus

c-Fos

c-Myc

c-Jun

Laboratory of plant molecular cytogenetics

二.蛋白质组分析的基本方法和技术

1 蛋白质鉴定方法

质谱技术出现以前　最成功的方法是Edman化学降解法

1950年由Edman首先提出

Edman化学降解法所使用的试剂是异硫氰酸苯酯

（phenylisothiocyanate,PITC）

肽与PITC在pH8－9的条件下反应生成PTC－肽（苯异硫甲氨酰肽）。在强酸作用下，可使靠近PTC基的氨基酸环化，肽键断裂形成噻唑啉酮苯胺（Thiazolinone,ATZ）衍生物和一个失去末端氨基酸的新生肽链。新生肽链有一个新的N端，可以重复上述反应。

图 20－2 Edman 降解法反应示意图

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- 如此不断循环，可依次使构成肽链的氨基酸，逐一水解，形成ATZ一衍生物(ATZ-氨基酸极易被有机溶剂抽提，并在水溶液中转化成稳定的乙酰苯硫脲氨基酸phenylthiohydantoin-AA,PTH-氨基酸)
- 根据上述Edman降解原理进行肽链顺序分析的方法可分为手工测序与自动化测定。

利用质谱进行蛋白质鉴定主要有两种方法

（1）肽质量指纹谱（Peptide mass fingerprint,PMF）法：是在经过蛋白质分离后（如利用2DE技术分离蛋白质），将蛋白质取出，用特定的酶进行酶切，获得肽段混合物，而后进入质谱进行肽段质量的测定，从而获得该蛋白质各切割肽段的质量，通过这些质量搜索已有的数据库，得以进一步识别出该蛋白质

（2）串联质谱（Tandem mass spectrum）法：是在蛋白质酶解后，对酶解肽段进一步裂解，并对其裂解产物（各种碎片离子）进行质谱检测，最后利用这些测定的质量反推肽段的氨基酸序列

二.蛋白质组分析的基本方法和技术

2.双向凝胶电泳

（Two-dimensional gel electrophoresis, 2-DGE 或 2-DE ）蛋白质组技术的核心。

第一向：等电聚焦凝胶电泳

(Isoelectrifocusing gel electrophoresis, IEF)

第二向：SDS-PAGE

(Sodium dodecyl sulfate-polyacrylamide gel electrophoresis)十二烷基硫酸钠聚丙烯酰胺凝胶电泳

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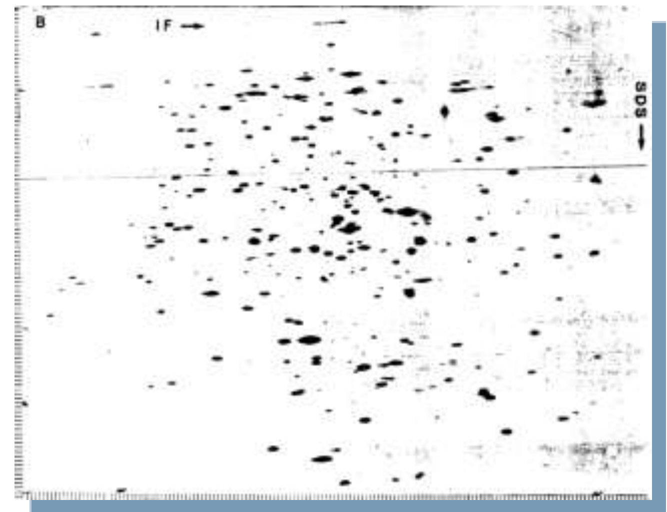
2-DE: In the beginning

- 1975: P.H. O'Farrell, J. Klose independently invent a 2-D protein electrophoresis system
  - (1) Isoelectric focusing in urea and carrier ampholytes in a tube gel
  - (2)Size separation in SDS on a vertical slab gel
  - – detection by radio-labeling or Coomassie blue staining



E. coli extract O'Farrell, *J Biol Chem*, 1975

High Resolution Two
of Proteins*

Th
Vol. 25(

SUMMARY

A technique has been developed for the separation of proteins by two-dimensional polyacrylamide gel electrophoresis. Due to its resolution and sensitivity, this technique
. of proteins
separated
ising in the
t by sodium
dimension.
possible to
spots across
solved 1100
should be
s. A protein
in of either
. A protein
tein can be
e reproduci-
ach spot on
a different
r estimation
of proteins
can resolve
quently can
resulting in
changed by
description of the methods as well as the characteristics of this system are presented.

IF →
SDS ↓

Fig. 2. Separation of *Escherichia coli* proteins. *E. coli* (1100) ... mixer. The total volume of the gel was 16 ml. At this exposure, 8

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2-D Electrophoresis: Traditional Method

First Dimension:

Isoelectric Focusing in presence of urea, Nonidet NP-40 in vertical gel rod

⊖ sample

pH 10

pH 3

⊕

gel rod rebuffered in SDS buffer

Second Dimension:

SDS Polyacrylamide Gel Electrophoresis in discontinuous gradient gel

pH 3 ⊖ pH 10

⊕

Separation acc. to Isoelectric Points (charge)

Separation acc. to Molecular Weight (mass)

Principle according to P.H. O'Farrell and J. Klose (1975)

(1) IEF 原理：

蛋白质是两性物质，在不同pH溶液下所带净电荷不同，当其净电荷为零时的pH值即为该蛋白的等电点pI。蛋白质在具有pH梯度的电场中，当pH<pI时，向负极移动；pH>pI时，向正极移动。随着移动，蛋白质的净电荷逐渐减小，直至为零，此时pH＝ pI ，蛋白质不再移动而被聚焦。

由此可知，等电聚焦是利用具有不同等电点的蛋白质在线形pH梯度下泳动，并聚焦于其 pI值相同的pH位置，达到蛋白质混合物分离的方法。

线形pH梯度的形成:

1) .载体两性电解质pH梯度

常用两性电解质**Ampholine™**，一种多氨基多羟基两性化合物的混合物。MW：**300—1000Da**, 直流电场下，从正极→负极连续pH梯度，分辨率可达**0.01pH**单位（图示原理）

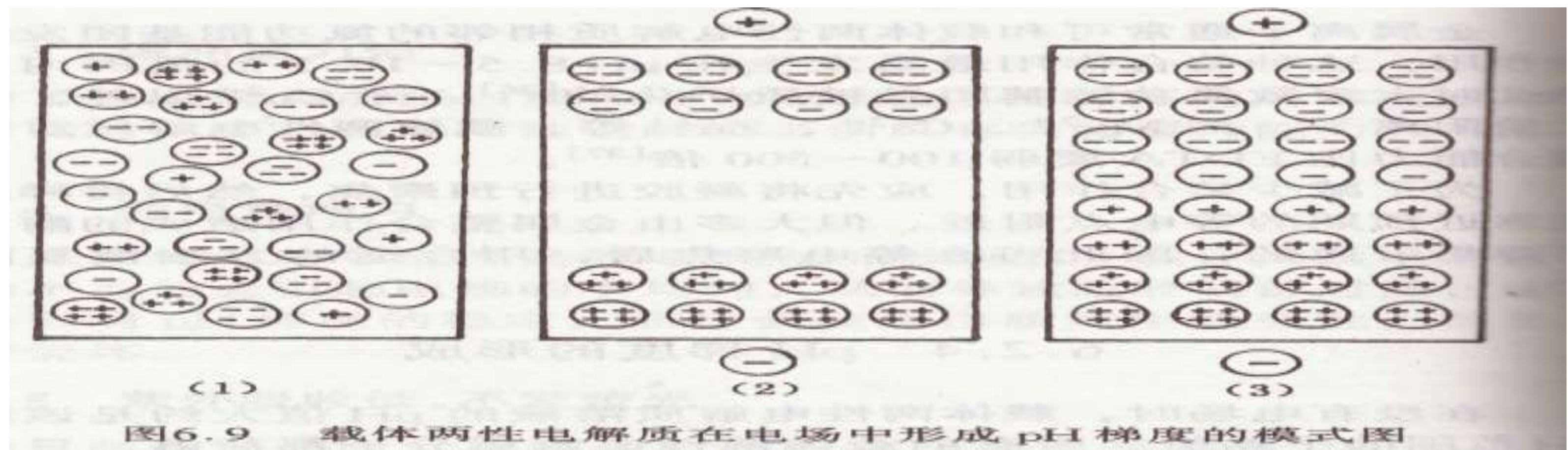
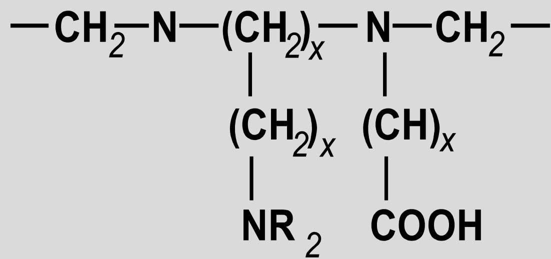


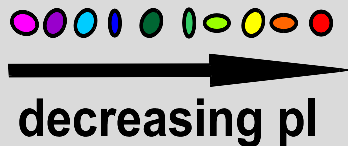
图6.9　载体两性电解质在电场中形成pH梯度的模式图

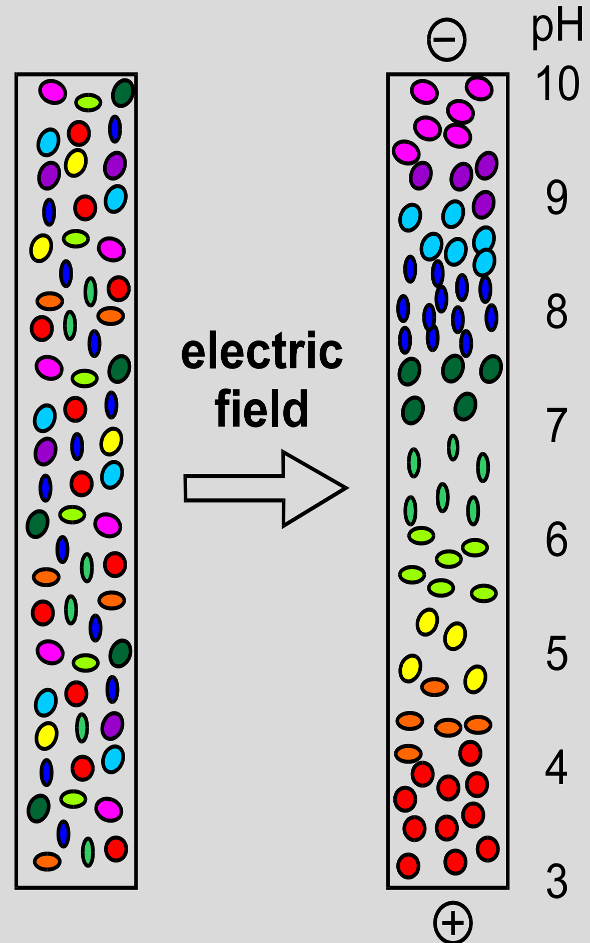
IEF with Carrier Ampholytes

Pharmalytes
Ampholines



where R = H
or - $(CH_2)_x$ - COOH,
x = 2 or 3





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2-D Electrophoresis

+ −
pH 3 pH 7.5 pH 10

+ −
pH 3 pH 7.5 pH 10

+ −
pH 3 pH 7.5 pH 10

+ −
pH 3 pH 7.5 pH 10

The 1st D: Isoelectric Focusing

2).固相pH梯度

固相pH梯度：Immobiline 由7种丙烯酰胺衍生物组成，可与丙烯酰胺和甲叉双丙烯酰胺共同聚合，使pH梯度固定于凝胶中→Immobiline干胶条→IPG（Immobilized pH gradients），分辨率可达0.0001pH单位。

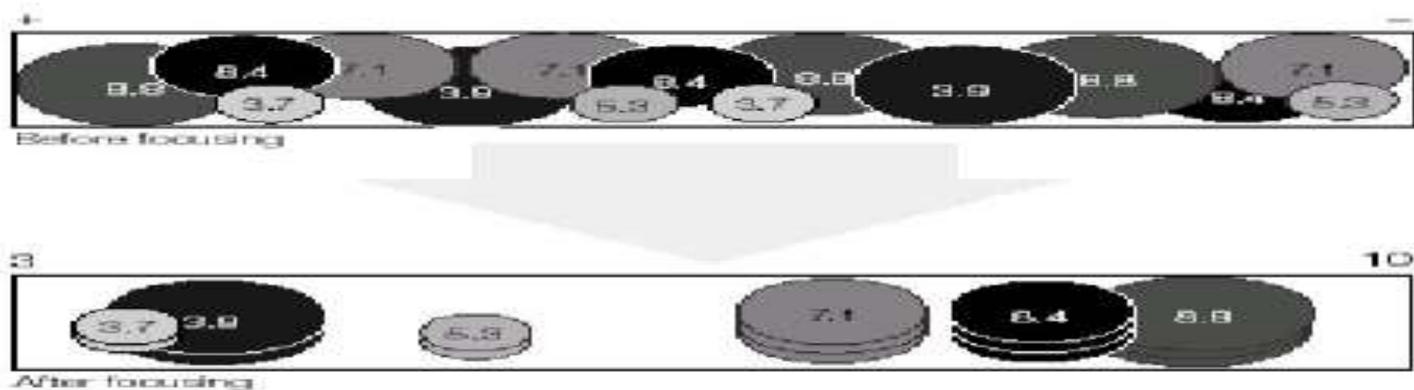


Fig. 3.1. A mixture of proteins is resolved on a pH 3–10 IPG strip according to each protein's pI and independently of size, as described in the IEF section.

IPG （immobilized pH gradient）的优点

- pH梯度稳定，不受溶液及电场影响，电泳重复性好
- 分辨率高，可达0.0001pH
- pH范围灵活

  窄带IPG用于特定pH范围的二次分离

  宽带IPG可提高碱性蛋白质的分辨率
- 上样量大，平衡时蛋白质不易洗脱，易转移至SDS胶

ReadyStrip™ IPG Strips

3–10

4–7

3–6

5–8

7–10

High-purity monomers

Narrow ranges for resolution of low-abundance proteins

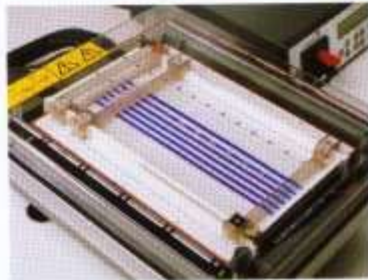
Overlapping gradients for “cyber gels”

Three lengths

--- 7 cm, 11 cm and 17 cm

--- 0.5 mm x 3.3 mm

Immobiline 干胶条确保电泳重复性



第一向等电聚焦(IEF)电泳分离需要以最高分辨率对目标蛋白质群进行分离，以保证在第二向电泳中尽可能多地识别和定量更多的蛋白质。IPGphor是一个完全一体化的电泳系统，它是为快速、简便、高重复性并专用固相pH梯度干胶条电泳而设计的。

选择与所需pH梯度相匹配的Immobiline干胶条和IPG缓冲液，并用IPGphor走电泳。分辨率极高，重复性极好，无需考虑样品容许量，重复性或上样量都能得到保证。

每批预制Immobiline干胶条都具有高重复性，保证得到可重复的实验结果。

能运行所有规格的Immobiline干胶条：7、11、13、18和24厘米。

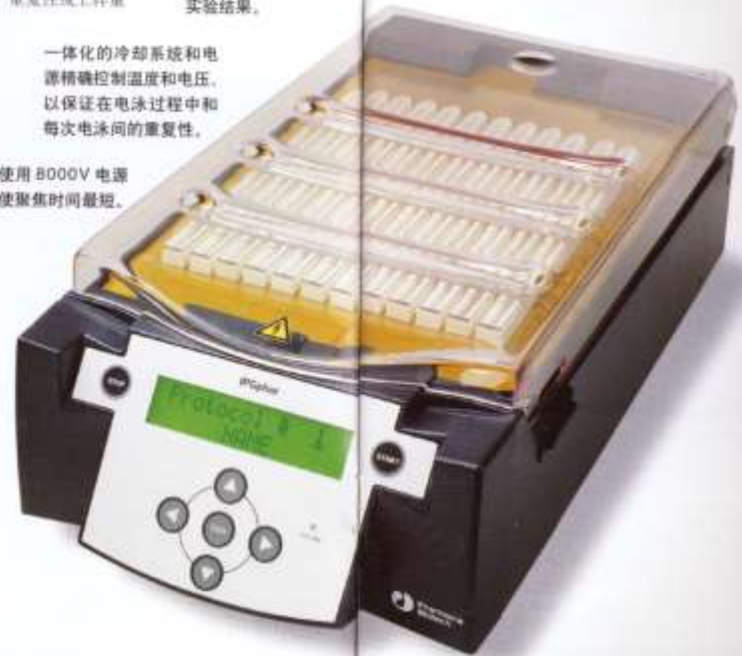
最多可同时走

一体化的冷却系统和电源精确控制温度和电压，以保证在电泳过程中和每次电泳间的重复性。

使用8000V电源使聚焦时间最短。

陶瓷IPGphor胶条槽系列(7, 11, 13, 18和24厘米)

胶条槽保存盒



IPGphor 胶条槽

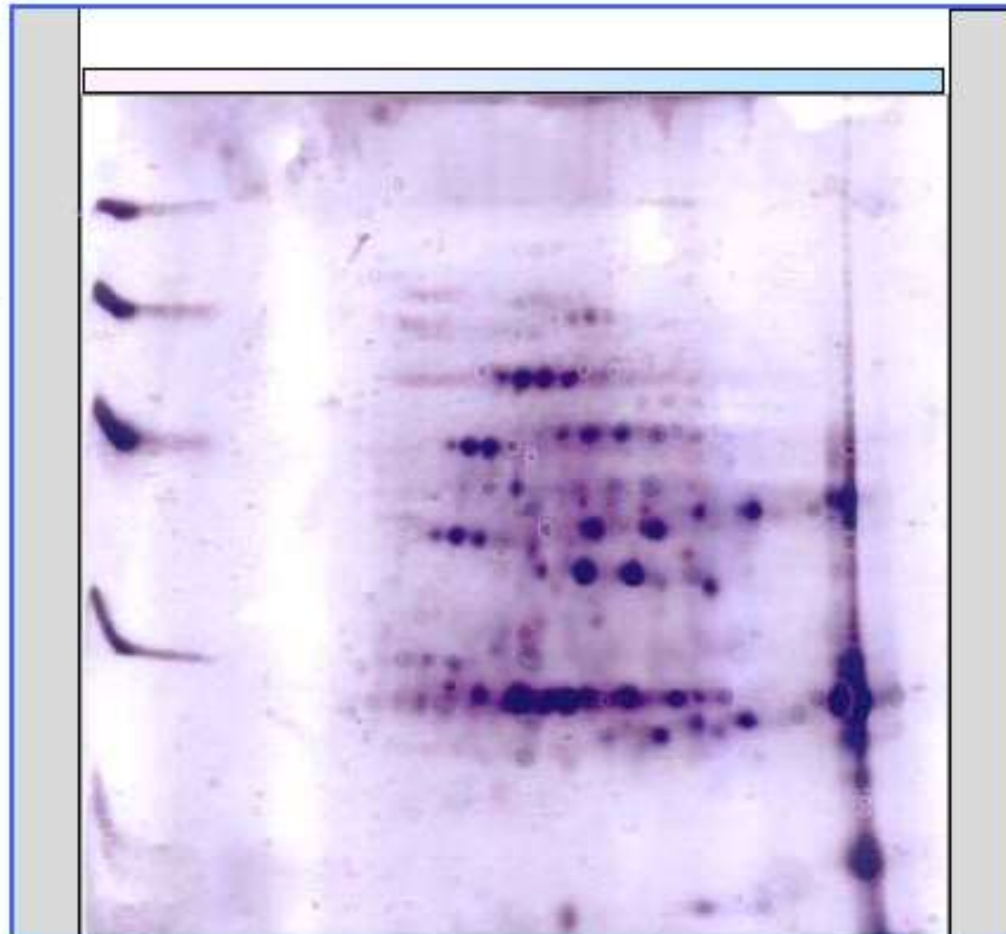
- 使用简便：用缓冲液和样品溶胀胶条并开始电泳
- 陶瓷胶条槽，散热快速，避免"热斑"(hot spots)，表面涂层处理，消除蛋白质吸附和污染
- 适用于所有Immobiline干胶条胶

NEW 平衡Immobiline干胶条用的平衡试管

其中，IPGphor等仪器，重复性好，自动化高

The operation of 2-DE analysis

(1) IEF
等電焦集電泳



(2)
SDS-PAGE
分離膠體

(3) Staining
染色脫色

Juang RH (2006) Proteomics

武汉大学 Wuhan University

(2) 第二向 SDS-PAGE

SDS与蛋白质的疏水部分结合，破坏蛋白质的折叠结构，因此，蛋白质亚基的电泳迁移取决于亚基分子量的大小，而电荷因素可被忽略，由于分子筛作用按蛋白质分子量大小进行分离。

常规电泳装置或自动化装置。

The 2nd D: SDS-PAGE

Proteins migrate through the gel at a rate proportional to their size.

Smallest proteins travel the furthest distance

+ −

pH 3 pH 7.5 pH10

size

charge

在第二向SDS-聚丙烯酰胺凝胶电泳中效率最高的Ettan DALT II系统

NEW

对于那些要求规模最大，分辨率最高，又不能降低重复性的电泳来说，新的Ettan DALT II系统加上Ettan DALT II预制胶，是最为理想的。Ettan DALT II是目前能提供的使用最大尺寸凝胶和最大容量的电泳系统。

在第二向分离流程设计中的每一细节都是为了提高效率。和24厘米长的Immobiline干胶条组合使用，使Ettan DALT II系统成为一种迄今为至能更多地鉴别分离可定量蛋白质斑点的系统。

不需灌胶

- 直接使用的预制胶 Ettan DALT II (26 X 20 厘米)
- 均匀胶(12.5%)使用稳定的缓冲液系统
- 每批产品都具高重复性

所设计的电泳槽体积小，缓冲液消耗最少。

分辨率最高：可用新的24厘米Immobiline干胶条走电泳。

规模最大：在6-10小时内最多可同时走12块胶 。

温度控制精确：一体化的珀尔帖(Peltier)冷却装置。

每次电泳之间具有高重复性：可编程的电源和温度控制。

装配不费时

- 使用合页式凝胶夹使装入、取出和保存胶非常简便

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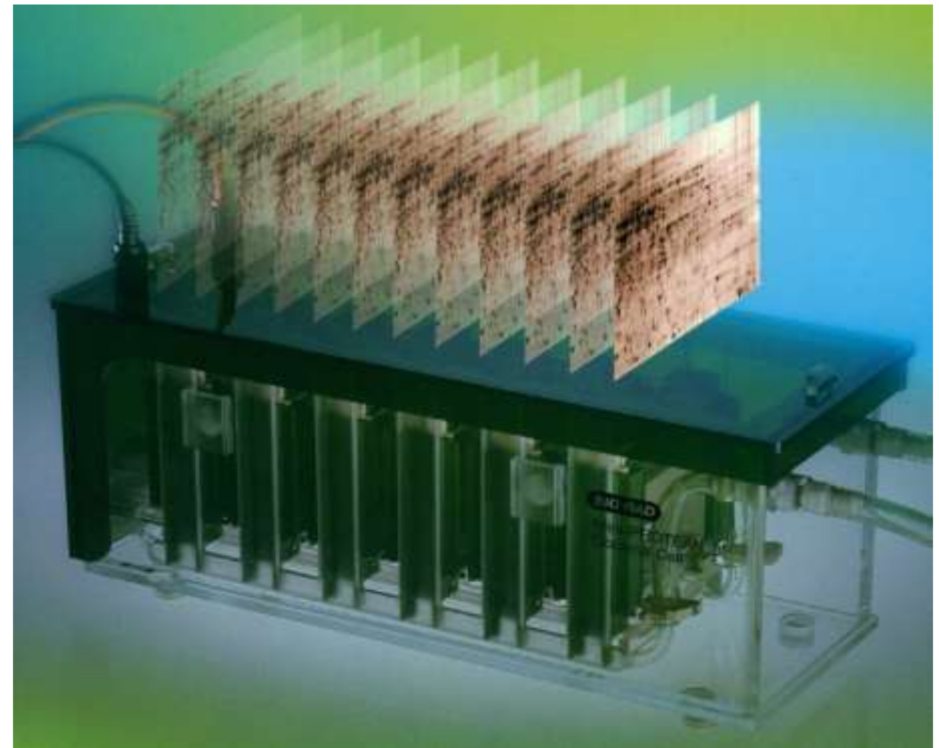
Mini-PROTEAN™ 3 Dodeca™ Cell

"2-D in a day"

- High-throughput 12 gel capacity
- Rapid results with 35 minute run times
- High resolution separations with gel to gel reproducibility
- Simplified assembly and space saving design



2 蛋白质的检出

对蛋白（肽）点检出的方法有：

(1) 考马斯亮蓝染色(Coomassie blue staining):
方法简单易操作、可重复性好；灵敏度低（比银染低50－100倍）、斑点有限（<300spot）；要求样品的蛋白含量高。

(2) 银染(silver staining)：最常用、最敏感的非放射性方法，灵敏度高(0.1ng)；可重复性差，线性范围狭窄。步骤繁琐，易污染环境。

(3) 放射性标记（autoradiography）： ^{32}P/ ^{35}S放射性标记（radio labeling）灵敏度最高(<0.1ng)；但操作繁琐，周期长，污染环境。

(4) 荧光染料染色（fluorescent dyes staining）：灵敏度接近sliver银染高于考马斯亮蓝染色，线性范围宽，多种波长；价格昂贵；不污染环境。

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3 图象扌

蛋白质组数据库(proteome database)被认为是蛋白质组知识的储存库，包含所有鉴定的蛋白质信息，如蛋白质的顺序、核苷酸顺序、2-D PAGE、3-D结构、翻译后的修饰、基因组及代谢数据库等

对2—

分析软件自动准确地进行斑点检测和 析，确定每个蛋白质点地等电点和分子量 → 蛋白质组数据库

中国蛋白质组图谱数据库：**http：//202.127.18.190 (2002年)**。标志着我国加入了蛋白质组研究的世界大舞台。

重要蛋白质数据库的下载地址

数据库	地址
NCBI's Entrez proteins	http://www.ncbi.nlm.nih.gov
Swiss-Prot	ftp://ftp.ebi.ac.uk/pub/databases/uniprot/
RefSeq	http://www.ncbi.nlm.nih.gov/RefSeq/
TrEMBL	ftp://ftp.ebi.ac.uk/pub/databases/uniprot/
GenPept	ftp://ftp.ncifcrf.gov/pub/genpept/
Ensemble	http://www.ensemble.org/
Hinv-DB ORF	http://www.jbir.aist.go.jp/hinv/index.jsp
UniRef100	ftp://ftp.ebi.ac.uk/pub/databases/uniprot/
IPI(International protein index)	ftp://ftp.ebi.ac.uk/pub/databases/IPI/current/

蛋白质组研究的技术路线

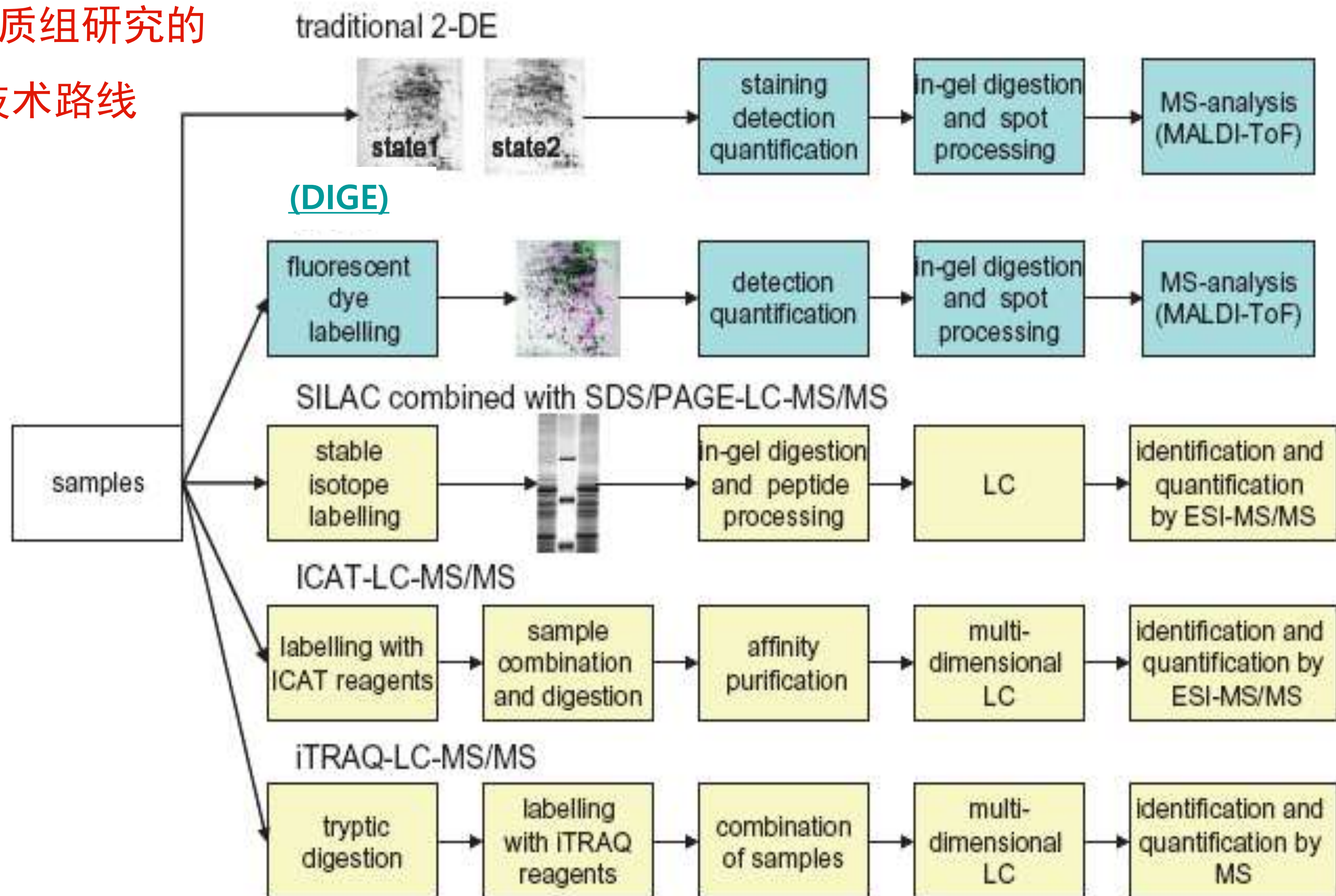

Fig. 1. Schematic presentation of the workflow of gel-based and non-gel-based proteomics approaches.

Proteomics DIGE Workflow

Sample Prep

Sample labeling

2D Separation

Image acquisition

LWS
Laboratory workflow system

Image analysis

Automated spot picking

Spot digestion

MALDI spotting

Laboratory of plant molecular cytogenetics

4 研究蛋白质组的质谱技术

　　质谱是将样品分子离子化后，根据离子间质荷比（m/z）的差异来分离并确定分子量，它是一种高灵敏度、高特异性的快速鉴定生物分子的技术。

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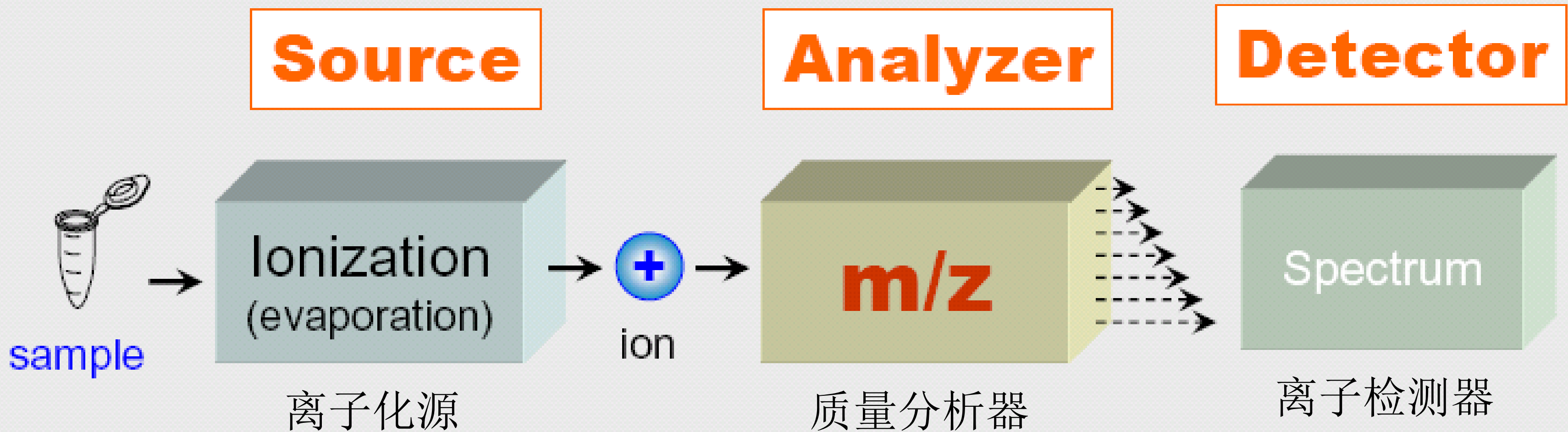
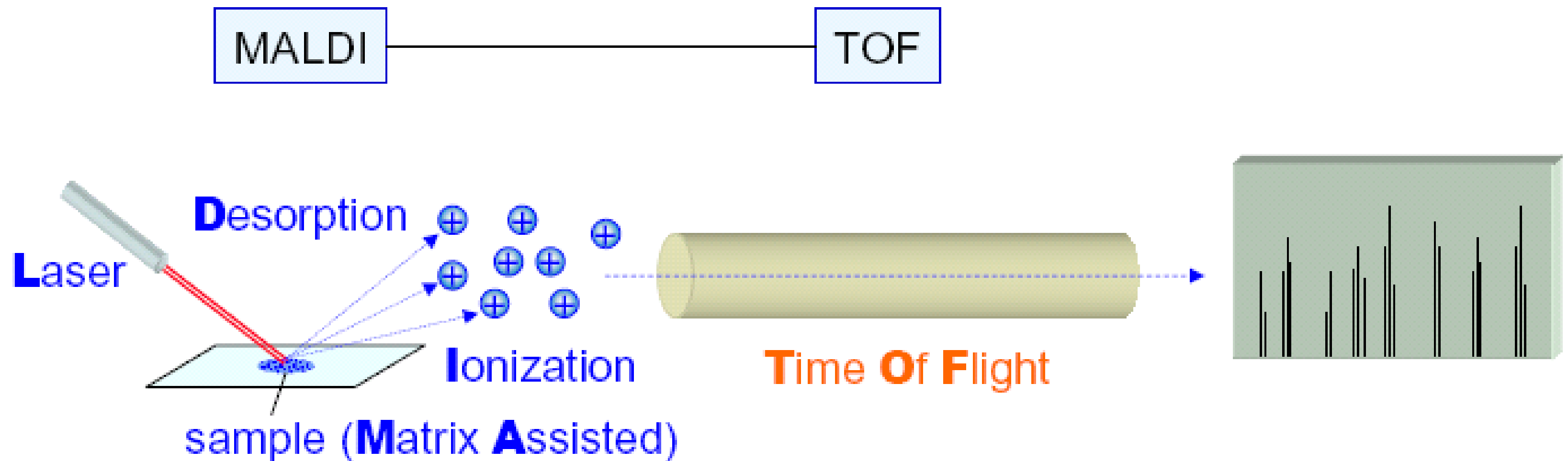
蛋白质组研究的质谱技术具体做法是：

双向电泳 → 分离到蛋白质点 → 切割胶内的酶解或转移到PVDF膜上酶解 → 质谱分析

质谱仪由进样装配、离子化源、质量分析器、离子检测器和数据分析系统组成

Mass spectrometer has three essential parts





Sample

2-D electrophoresis

Pure protein

Proteolytic digestion

Proteolytic fragments

MALDI-TOF

Capillary Electrophoresis, HPLC

Mass spectrum

GCG

Database mining

LC-MS/MS (ESI-MS)

Amino acid sequencing

N-

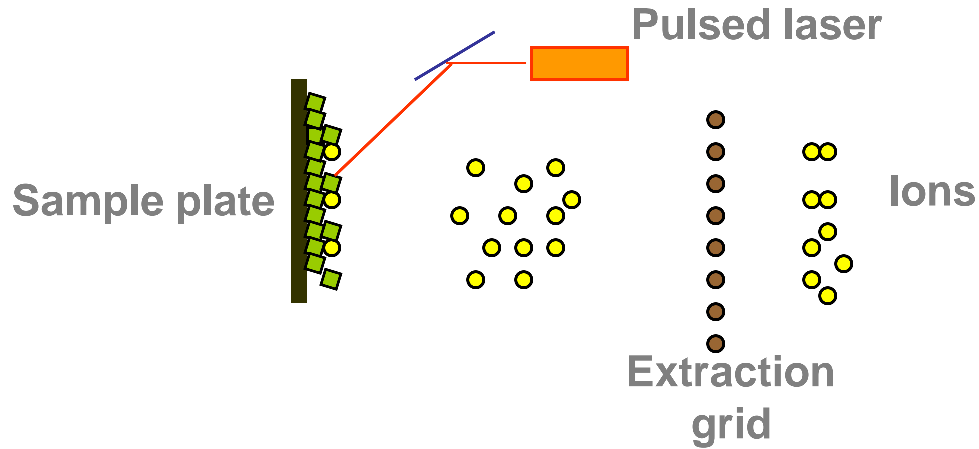
1）MALDI-TOF-MS

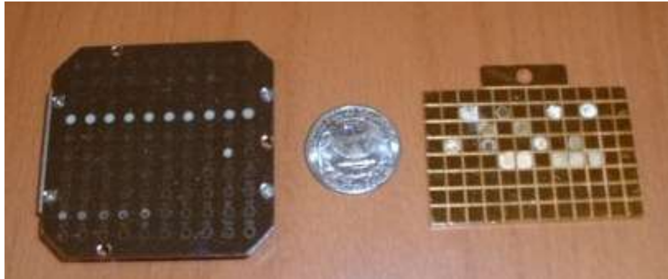
(Matrix-assisted laser desorption/ionizarion–time-of-flight mass spectrometry)

基质辅助激光解吸离子飞行时间质谱

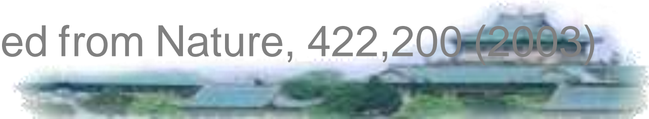
该技术是采用固相进样，样品蛋白质在检测前通过还原、烷基化、酶解、脱盐后以一定比例与基质混合，点样于点样板上，在空气中自然干燥后置于质谱仪上，用一定波长的激光打在样品上，使样品离子化，然后在电场力作用下飞行，通过检测离子的飞行时间计算出其质量电荷比，从而得到一系列酶解肽段的分子量或部分肽序列等数据，最后通过相应的数据库搜索来鉴定蛋白质。

Matrix-assisted laser desorption/ionization (MALDI) 基质辅助激光解吸电离





Adopted from Nature, 422,200 (2003)



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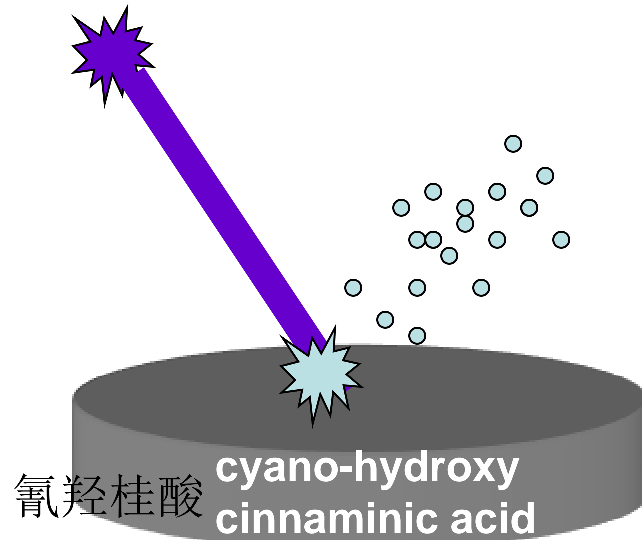
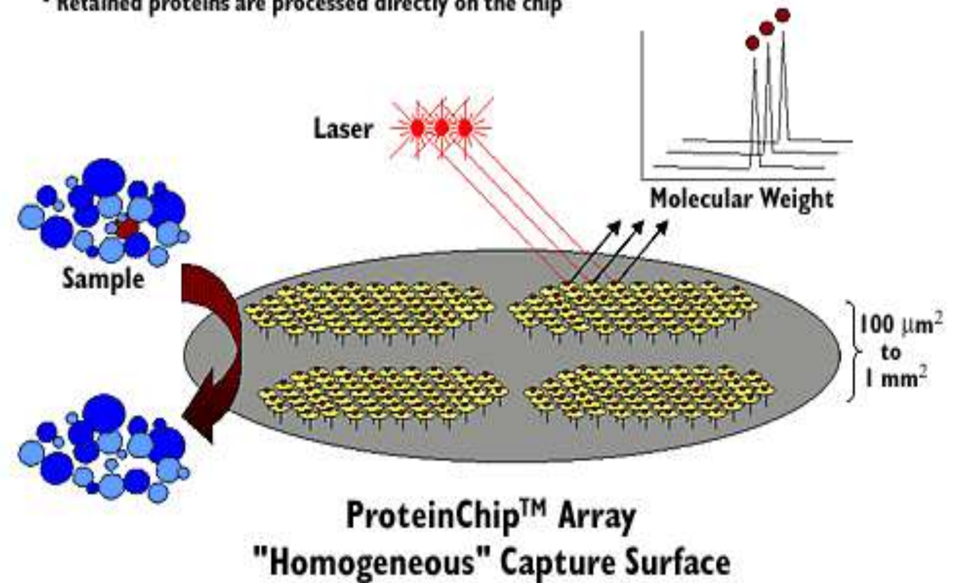
表面增强的激光解吸离子（Surface Enhanced Laser Desorption/Ionization）技术来捕获、检测和测量复杂生物样品中的肽段和蛋白质的分子量。

MALDI = SELDI





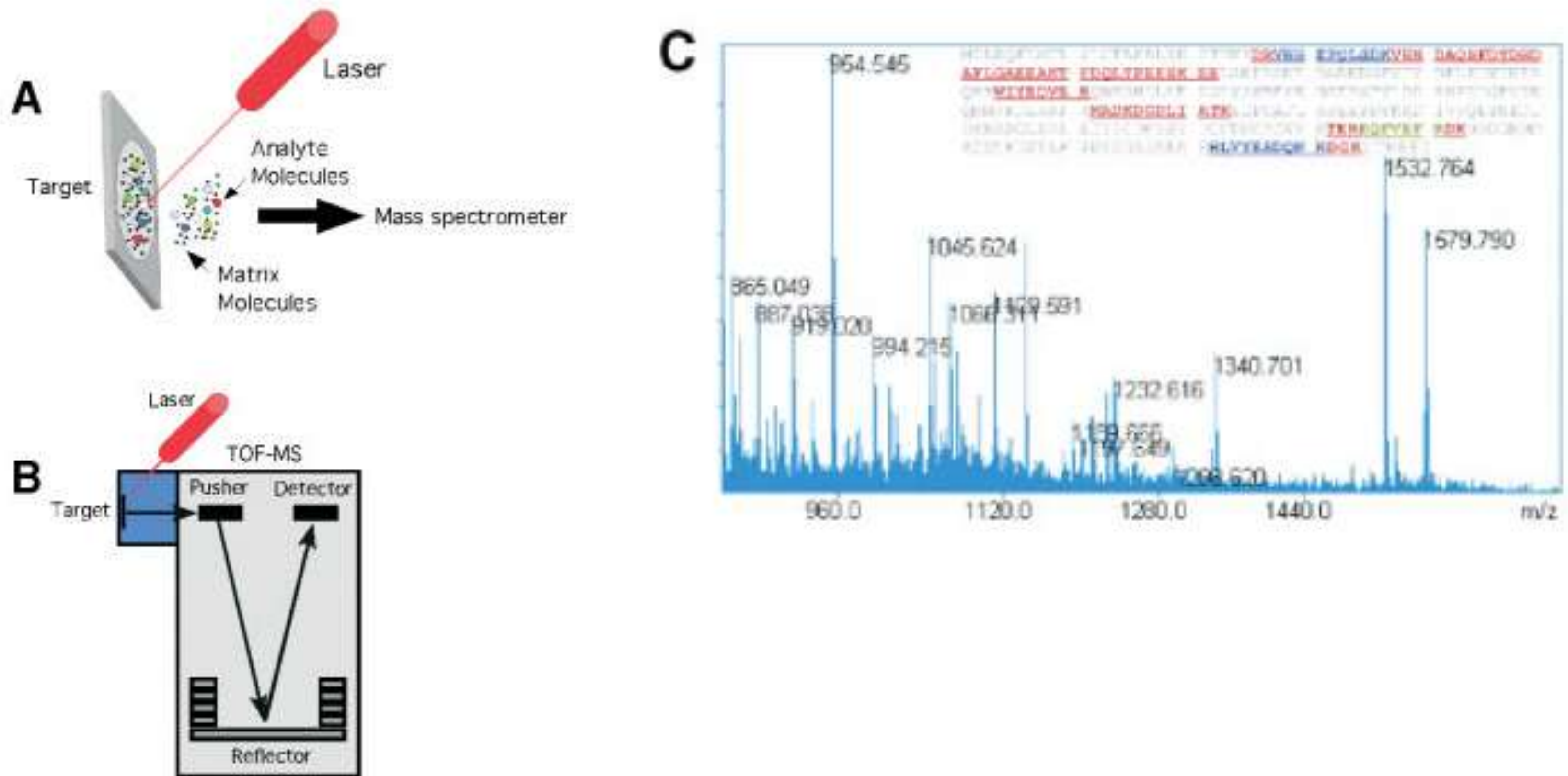


Figure 3. Principles of mass spectrometry. **A:** MALDI–TOF mass spectrometer. The sample is co-crystallized with matrix molecules as a dry sample on the plate. The peptides are brought to an ionized gas phase by a laser pulse. **B:** The ionized peptides are analyzed in the time-of-flight (TOF) unit in the mass spectrometer giving a peptide mass fingerprint. **C:** If the sample is pure enough, the peptide mass fingerprint can be used to search DNA and protein databases for identification. **D:** Tandem mass spectrometry (MS/MS) as obtained by a Q-TOF mass spectrometer. The sample is ionized at atmospheric pressure by electrospray ionization (ES source). The ions enter the vacuum system through the sampling cone and, in the quadrupole section ions, of a particular *m/z* are selected and fragmented in the collision cell. **E:** In the collision cell, peptides are mainly fragmented at the peptide bonds producing b type (blue) and y type (red) ions. The masses of the resulting peptide fragments are measured in the TOF unit. **F:** Example of a collision-induced spectrum with the amino acid sequence given as detected from the N-terminal (b type ions) and from the C-terminal (y type ions). The mass fingerprint (C) is reprinted with permission from Honoré B. Genome- and proteome-based technologies: status and applications in the postgenomic era. Expert Rev Mol Diagn 2001;1:265–274, Copyright (2001) Future Drugs Ltd.

Protein Identification by MS/MS

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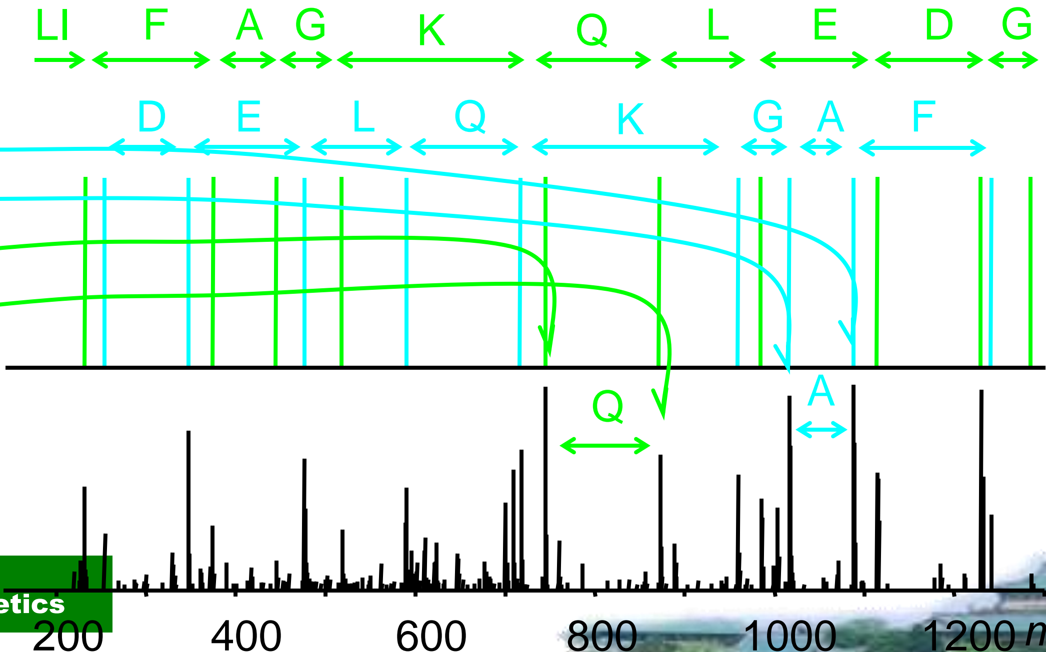
Protein digestion → Peptides → Ionization → MS spectrum (m/z) → Fragmentation → MS/MS spectrum (m/z) ↔ Database searching ↔ Theoretical Spectrum (m/z) → Peptide/protein identification

LIFAGKQLEDGR

b ions		y ions
1: L	IFAGKQLEDGR	:11
2: LI	FAGKQLEDGR	:10
3: LIF	AGKQLEDGR	:9
4: LIFA	GKQLEDGR	:8
5: LIFAG	KQLEDGR	:7
6: LIFAGK	QLEDGR	:6
7: LIFAGKQ	LEDGR	:5
8: LIFAGKQL	EDGR	:4
9: LIFAGKQLE	DGR	:3
10: LIFAGKQLED	GR	:2
11: LIFAGKQLEDG	R	:1



iron superoxide dismutase (FeSOD, 24 483 u), urease alpha subunit (UreA, 26 675 u), translation elongation factor Ef-Tu (EF-Tu, 43 400 u) and urease beta subunit (UreB, 61 700 u).

pylori strain used in this investigation.

Enzymatic digestion, peptide mass mapping and database

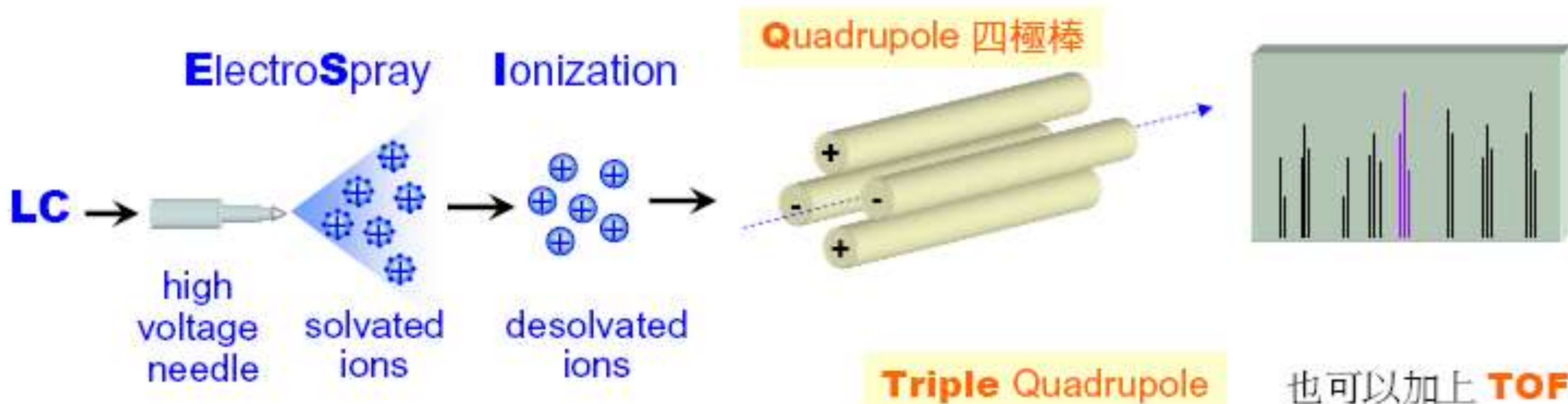
武漢大學 Wuhan University

2）在离子化方法上，还有一种称为电喷雾离子化（ESI electrospray ionization mass spectrometry(ESI-MS)）。ESI质谱仪与MALDI-TOF质谱仪在蛋白质组研究中应用较广。它们都是一种“软电离”的方法，即样品分子离子化时，不会形成碎片离子，保留了整个分子的完整性。

ESI-MS的特点是液相进样，可较好的与现有的蛋白质分析方法如HPLC、蛋白质测序技术相匹配。

ESI-MS/MS can analyze smaller fragments

Source **Analyzer** **Detector**



Triple Quadrupole 也可以加上 TOF

Adapted from Liebler (2002) Introduction to Proteomics, p.68 ,70

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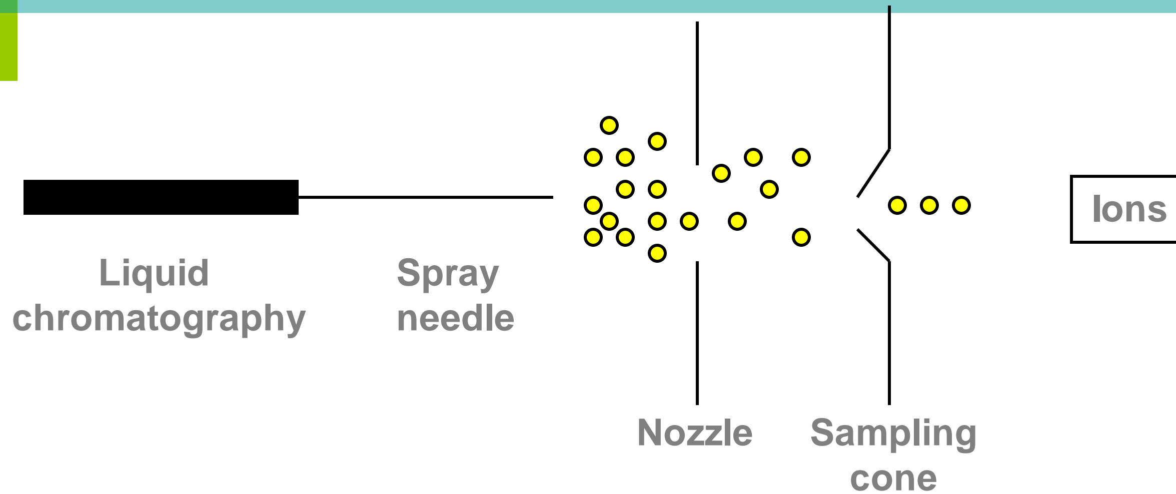
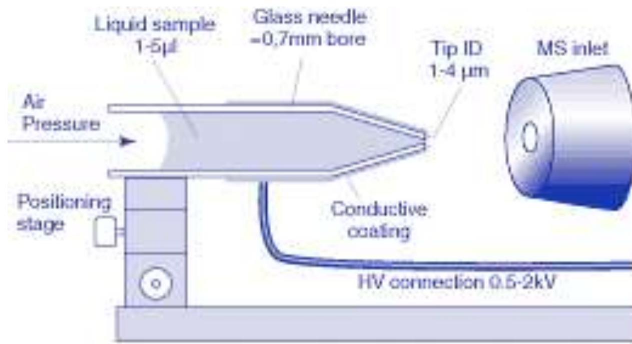
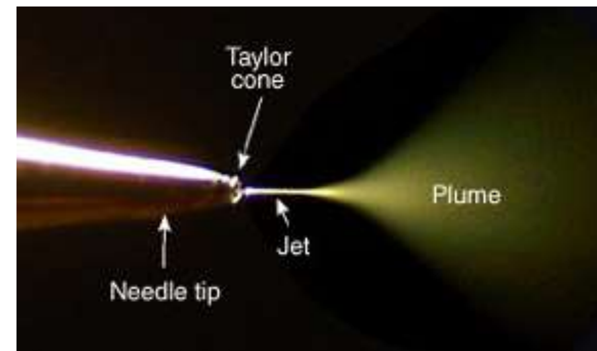
Electrospray Ionization (ESI)



Prof. John B. Fenn
The 2002
Nobel Prize Winner
in Chemistry

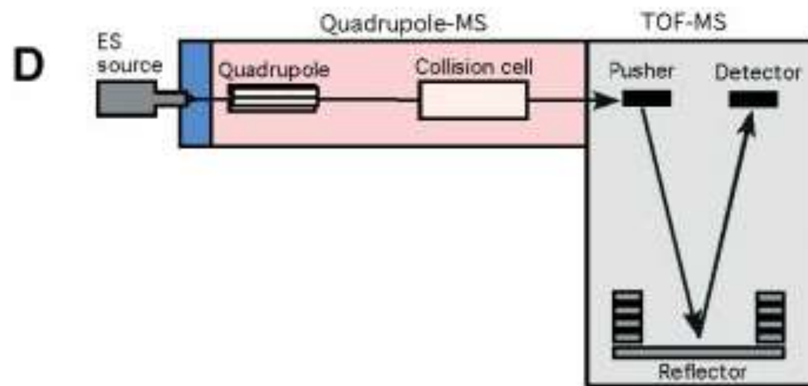




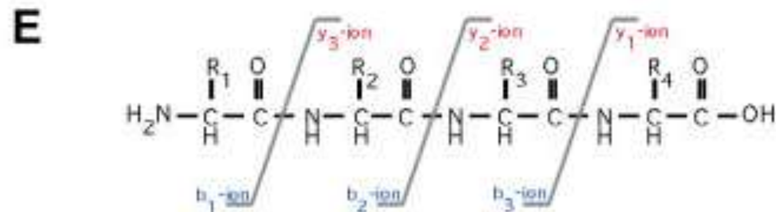
Adopted from Nature, 422:200 (2003)

ESI Q-TOF

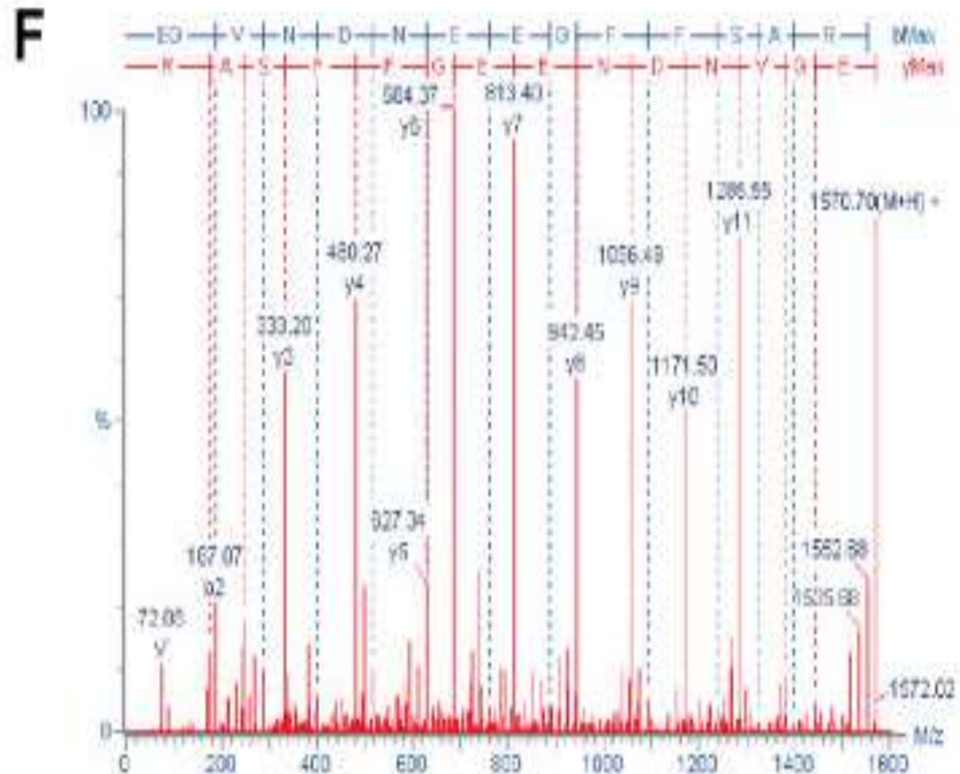
D



E



F



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Protein Identification by LC-MS/MS

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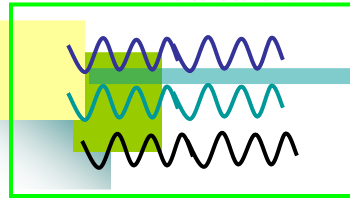
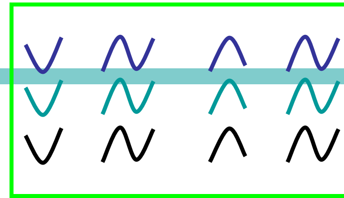


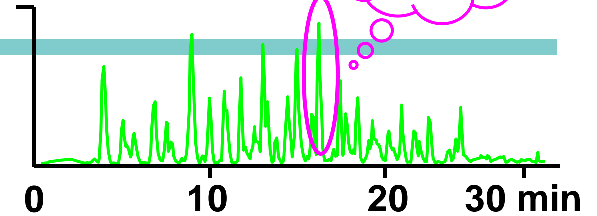
Protein mixture

Digestion

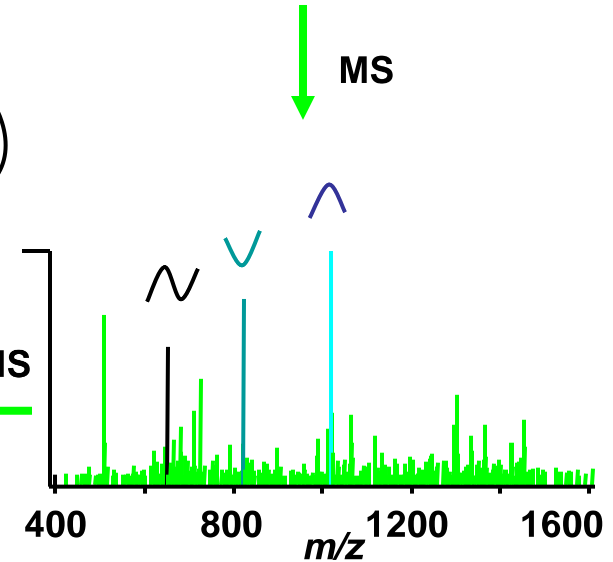


Peptides

HPLC



MS



MS/MS

Database Searching

LLTTIADAAK

SAGGNYVVFGEAK

EDDVEEAVQAADR

1 sequencing attempt per 2 sec.
900 sequencing attempts in 30 min.

All peptide sequences → Identification of many proteins

Laboratory of plant molecular cytogenetics

Automated LC-MS/MS Using "V-column" Technique

武汉大学

Wuhan University

Autosampler

Capillary column: 75 µm
Sensitivity: low femtomole (10^{-15})

Pumps at 5 µl/min

Pumps at 75 µl/min

色谱质谱联用包括气相色谱质谱联用(Gas Chromatography-mass spectrometry ， GC-MS)和液相色谱质谱联用(Liquid chromatography - mass spectrometry，LC-MS)，液质联用与气质联用互为补充，分析不同性质的化合物。

液质联用与气质联用的区别:

气质联用仪(GC-MS)是最早商品化的联用仪器，适宜分析小分子、易挥发、热稳定、能气化的化合物；用电子轰击方式（EI）得到的谱图，可与标准谱库对比。

液质联用(LC-MS)主要可解决如下几方面的问题：不挥发性化合物分析测定；极性化合物的分析测定；热不稳定化合物的分析测定；大分子量化合物（包括蛋白、多肽、多聚物等）的分析测定；没有商品化的谱库可对比查询，只能自己建库或自己解析谱图。

Mass spectrometry applications to proteomics

MS Proteomics Applications

- Protein identification/confirmation
- Determine protein molecular weight
- Detection of post-translational modifications
- De novo peptide sequencing
- Determination of disulfide bonds (# & status)
- Monitoring protein folding (H/D exchange)
- Monitoring protein-ligand complexes/struct.
- 3D Structure determination
- And many more……

Proteomic pattern changes during growth

綠竹筍

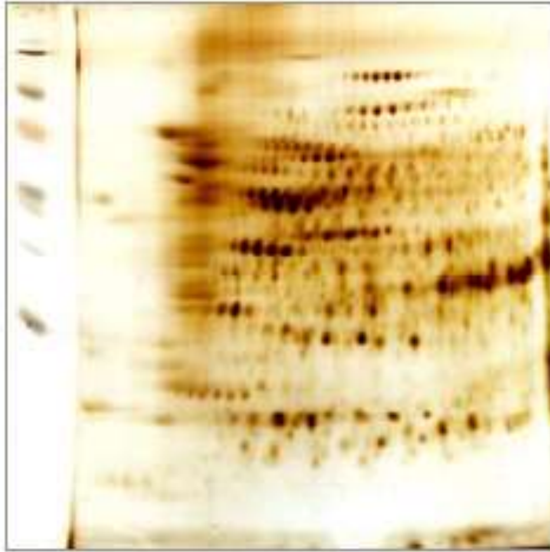
Cellulose synthesis

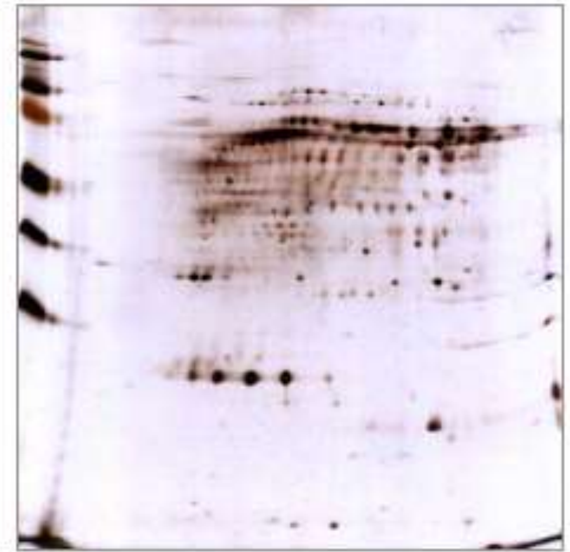
Juang RH (2006) Proteomics (Wu YJ)

	Protein ID	Accession no.	Calculated Mr (kD) / pI	Sequence coverage (%)	Score (MASCOT)	Match
79	Sucrose synthase	AAV64256 (*Bambusa oldhamii*)	92.8 / 6.03	35	402	14
80	Sucrose synthase	AAV64256 (*Bambusa oldhamii*)	92.8 / 6.03	35	245	7
82	Sucrose synthase	AAV64256 (*Bambusa oldhamii*)	92.8 / 6.03	35	1112	45
8	UDP-glucose-pyrophosphorylase	BAB69069 (*Oryza sativa*)	51.6 / 5.4	18	302	26
9	UDP-glucose-pyrophosphorylase	BAB69069 (*Oryza sativa*)	51.6 / 5.4	17	359	20
10	UDP-glucose-pyrophosphorylase	BAB69069 (*Oryza sativa*)	51.6 / 5.4	21	408	38
11	UDP-glucose-pyrophosphorylase	BAB69069 (*Oryza sativa*)	51.6 / 5.4	20	377	35

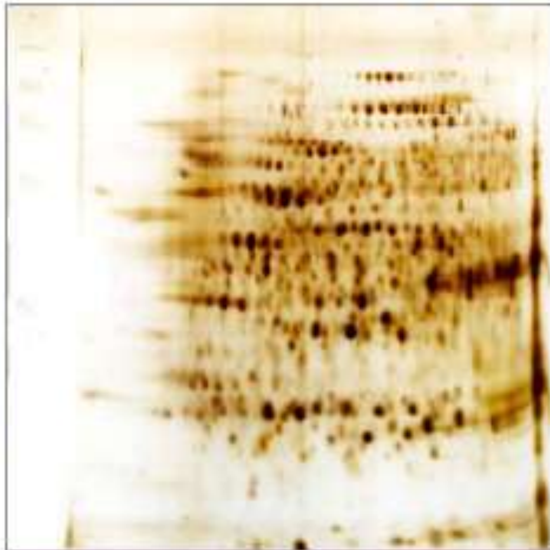
Total proteins extracted by different methods

Underground shoot





60-cm shoot



Water soluble proteins | Non-polar proteins | Glycoproteins

Juang RH (2006) Proteomics (Wu YJ)

2.DIGE技术

技术简介：DIGE（ **Differential in-gel electrophoresis** ）即荧光差异凝胶电泳，用荧光染料（Cy2, Cy3, Cy5）将蛋白质标记上，标记后蛋白质的等电点和分子量基本不受影响，等量混合标记好的蛋白质后进行双向电泳，蛋白质表达量的变化即可通过不同荧光的强度来体现。由于引入了Cy2作为内参，使得在定量效果方面大大强于传统双向电泳。

DIGE技术特点：

（1）定量精确，采用内标而消除了胶与胶之间的实验误差；

（2）检测动态范围更大；

（3）同一张凝胶上可以电泳两个样品，减轻了工作量；

（4）ImageMaster7.0（DIGE）软件可以得到统计学可信的结果，降低了操作者之间的偏差。

1多色荧光标记

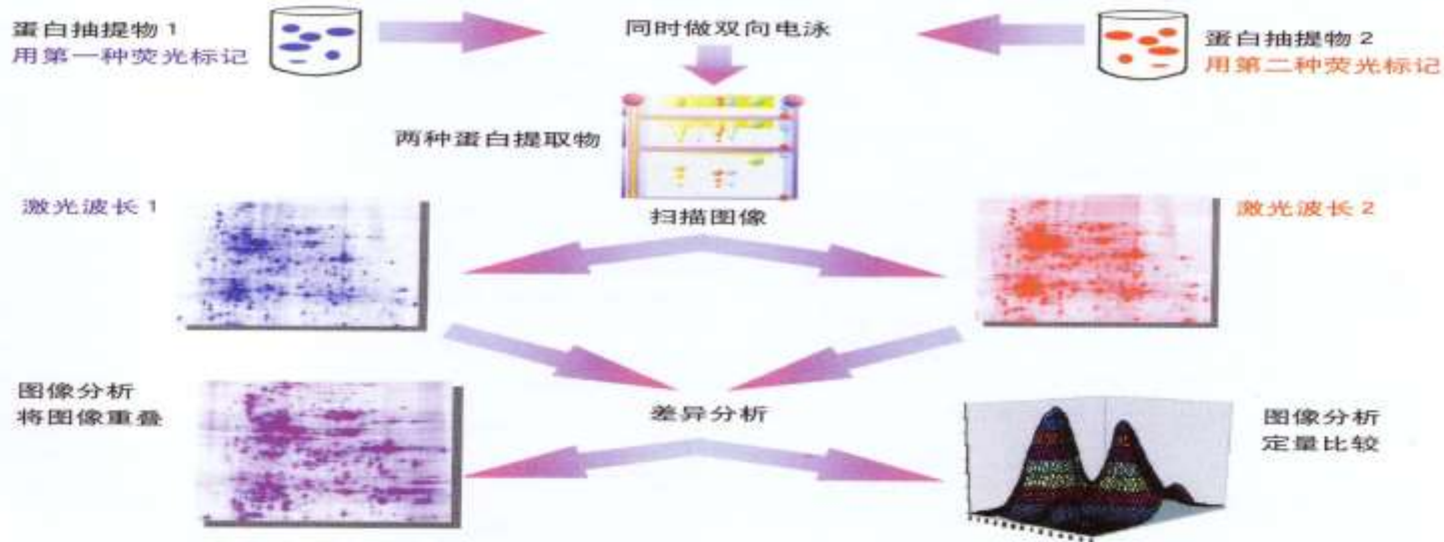
2D-DIGE专利技术和Typhoon

Differential in-Gel Electrophoresis
荧光差异凝胶电泳

双向荧光差异… 泳(2D-DIGE Two-Dimensional Differertial In-Gel Electrophoresis)

双向荧… 前，将多个需要进行比较的蛋白抽提物先用不同的荧光染料进行标… 该方法与质谱兼容，线性范围宽(> 10^5)，提供更准确的定量数据… 简化了图像分析的步骤，提高了比较的准确度。

2-D DI… 最多三个样品的蛋白表达差异，减少蛋白差异比较时潜在人为误… 括蛋白多种荧光标记的试剂(CyDye™)和方法，Typhoon多色荧光成…



Typhoon 9000 系列

- 自动检测四种荧光信号；
- 高能量的激光和Confocal的光路设计使得检测荧光信号灵敏度增强；
- 采用磷屏技术检测多种同位素信号(^{32}P, ^{35}S, ^{33}P, ^{14}C, ^{125}I, ^{28}F, ^{3}H 等)；
- 高灵敏度的检测器可直接检测化学发光信号；
- 可用于微阵列芯片信号的检测；
- 根据样品的不同，选择适合的聚焦位置。

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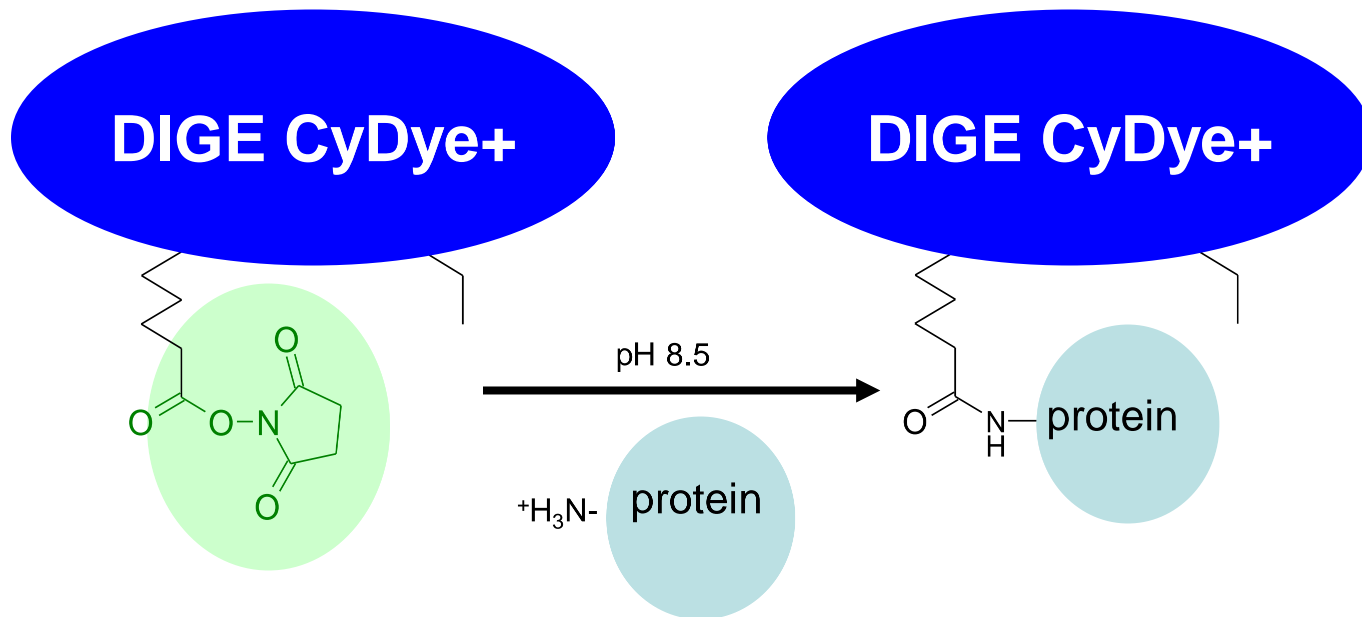
DIGE Labeling Chemistry

DIGE CyDye minimal labeling fluors



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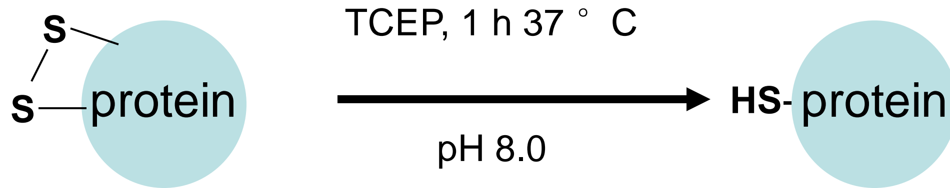
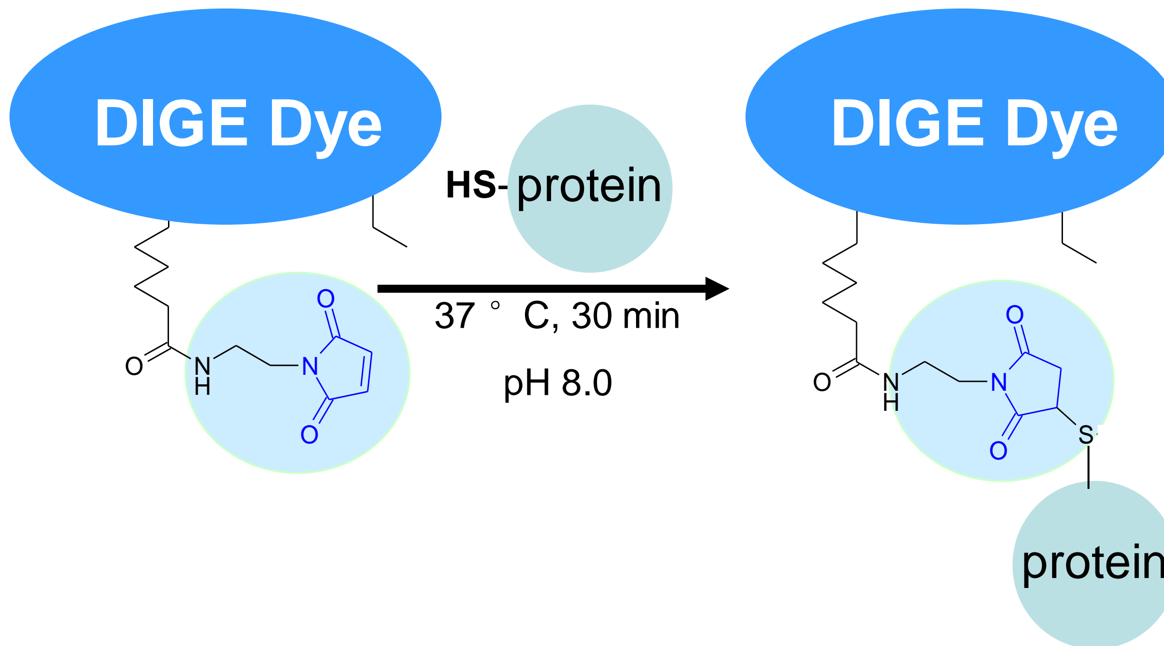
DIGE CyDye scarce sample labeling kit





A

Homogenized cells from normal tissue biopsy

Homogenized cells from cancer tissue biopsy

Proteins labeled with Cy3

Proteins labeled with Cy5

Combined proteins from the two samples

2D gel electrophoresis

B

Imaging

Figure 2. Principle of the two-dimensional difference in-gel electrophoresis system (2D-DIGE). **A:** Normal tissue is labeled with Cy3 and pathological tissue (e.g. cancer) is labeled with Cy5. The dyes label 1–2% of the proteins present. The labeled solutions are mixed and analyzed on the same 2D gel. After gel electrophoresis, the gels are scanned with a fluorescent scanner able to detect either the Cy3- or the Cy5-staining patterns. **B:** By superimposing the two images, it is possible to visualize differentially expressed proteins. Proteins upregulated in one sample may appear as red or reddish (Cy3, black arrowheads), those upregulated in the other sample as green or greenish (Cy5, white arrowheads) and those that are at the same level as yellow (white arrows). Panel B is modified with permission from Van den Bergh G et al. Fluorescent two-dimensional difference gel electrophoresis and mass spectrometry identify age-related protein expression differences for the primary visual cortex of kitten and adult cat. J Neurochem 2003;85:193–205, Copyright (2003) Blackwell Publishing.

Image Analysis

■ 图像捕捉

- 平板扫描仪（Flat-bed scanner）
- 电荷耦合数码相机CCD(charge coupled device Camera)
- 激光密度仪(Laser densitometers)
- 荧光或磷光成像仪（Fluoro-/Phospho- Imaginers）

■ 图像处理与分析(software)

- 背景消减与条纹消除(background reduction /streak removal)
- 斑点探测（detection）、图形匹配（pattern matching）
- 定量（quantitation)
- 数据分析(data analysis)
- 数据库构建(database construction)

Proteomics Initiative

2D electrophoresis
Investigator System™

Visualize Gels
Staining/SYPRO Ruby

Document Gels
ProImage Camera System

Analyze Gels & Select Spots
HT Analyzer Software

Pick Protein Spots
ProPic

Sample Prep For MS
ProPrep / ProGest / ProMS

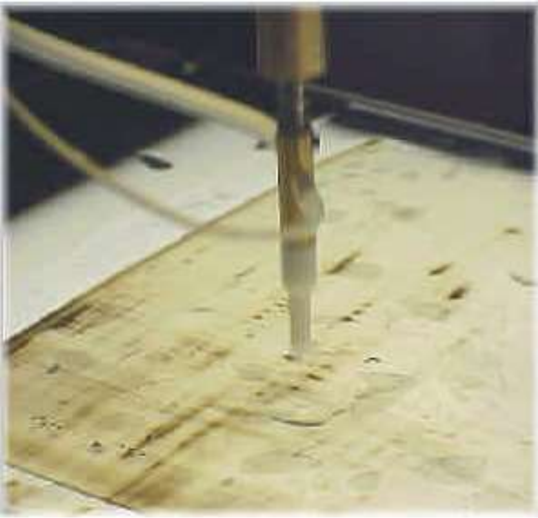
Mass Spec
(Any manufacturer)

ProFound™
(Search Engine)

Protein Warehouse/RADARS/PWRHouse
GNSL database software

Large Great Gel Format Capability

- **25 x 25 cm platform**
- **22 x 20 cm cutting area –**
  accommodates the protein resolving area of any large gel format



Spot Picker & In gel Digest

用于制备肽谱的酶需具以下特点：

酶的水解位点专一;

酶切产生的蛋白质肽段大小适合质谱分析并利于数据库检索;

酶自身稳定,不易自降解;

常用蛋白酶：

胰蛋白酶（Trpsin）

酶水解位点在赖氨酸(K)和精氨酸(R)的羧基端

Glu C蛋白酶

Digestion Protocol

De-stain
100 mM ammonium bicarbonate and acetonitrile

Dehydrate
Acetonitrile

Reduce
10 mM dithiothreitol in 100 mM ammonium bicarbonate, 30 minutes

Alkylate
55 mM iodoacetamide in 100 mM ammonium bicarbonate, 20 minutes

Wash
100 mM ammonium bicarbonate

Dehydrate
Acetonitrile

Digest
6 ng/µL Trypsin in 50 mM ammonium bicarbonate (25 µL), 5 hours at 37ºC

Extract
1% formic acid / 2% acetonitrile (30 µL)

Spot
96-spot MALDI target (2 x 2.5 µL)

Efficient spot picking

A protein pick list is generated using predefined selection criteria or by personal selection using, for example Ettan Progenesis™ Software. Protein spots are automatically excised from stained or destained gels with greater than 99% efficiency. The picker head ensures a clean, precise cut followed by transfer of the plug to the microplate. Hydrophobic coatings on the picker head and automatic washing prevent carry-over of gel or liquid and minimize the risk for cross-contamination.

Controlled digestion

Default digestion protocols are optimized and compatible with common staining systems. Standard methods are available for SYPRO™ ruby; silver (MS compatible); and Coomassie™ blue. The method editor lets you run customized methods. Low-salt digestion buffers eliminate the need for desalting. The digester needles are specially designed to minimize plug loss and eliminate cross-contamination.

Precise temperature control of all steps plus rapid drying

An incubator ensures precise temperature control throughout digestion. A dryer module effectively manages special tasks, like rapid drying of gel plugs and peptide extract solutions.

Automatic preparation for MALDI-ToF MS analysis

Automatically mixes and spots digested proteins and matrix onto MALDI-ToF MS sample trays with a high precision. A method editor lets you use customized methods.

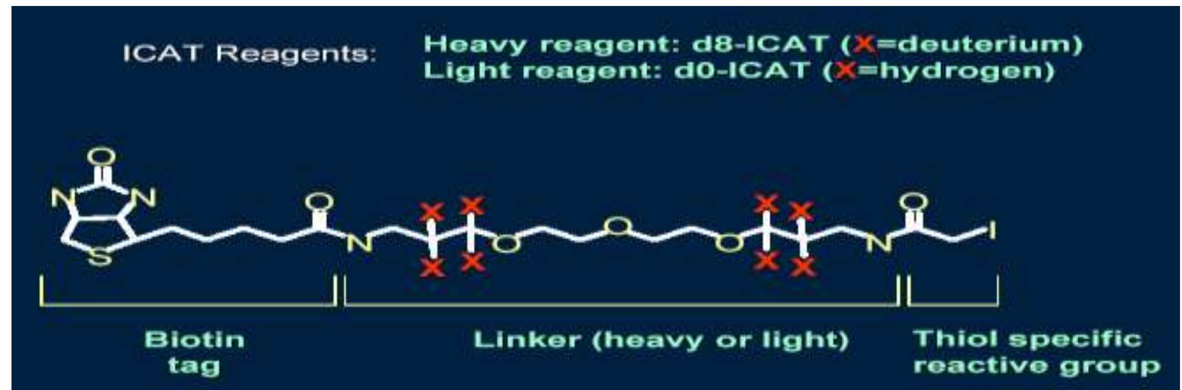
武汉大学

3、ICAT, 同位素编码亲和标记技术

同位素标记的亲和标签（isotope-coded affinity tag，ICAT）

四大基本元素

- 特异的化学反应
- 同位素编码的联接子
- 亲和标签
- 硫醇活性反应试剂



主要的特点

- 不需要凝胶分离过程
- 容易实现自动化操作
- 质谱兼容- μLC-MS/MS

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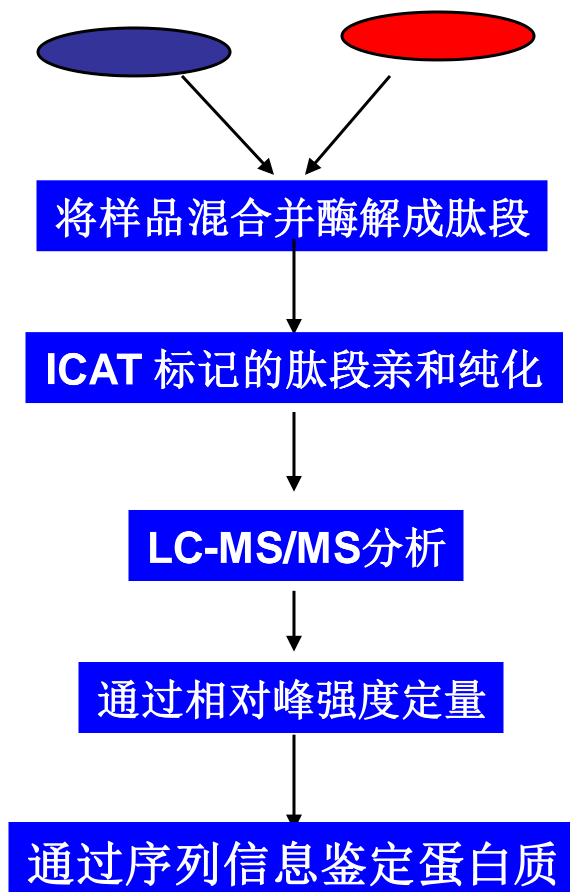
ICAT 分析流程



1.标记半胱氨酸残基

2. 混合样品并将蛋白酶解

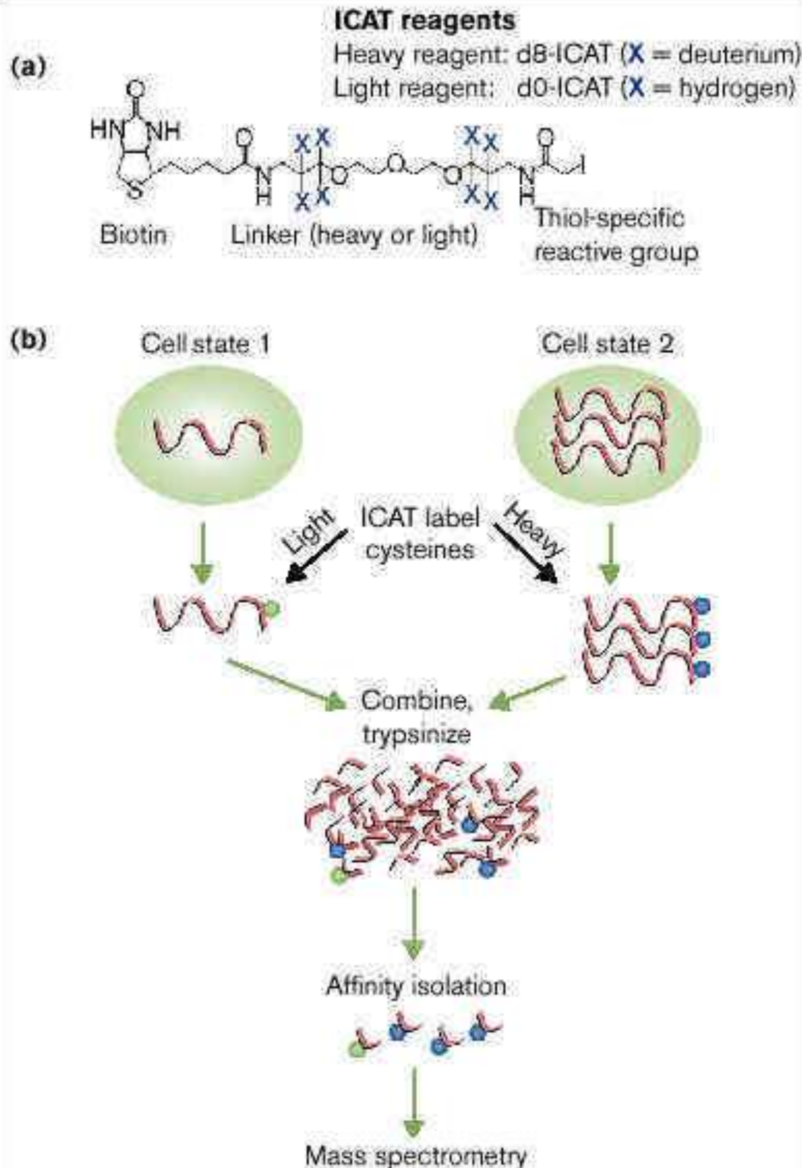
3.生物素被用于选择半胱氨酸肽段，如酵母 proteins = 6113 peptides = 344,855含半胱氨酸的 peptides = 30,619

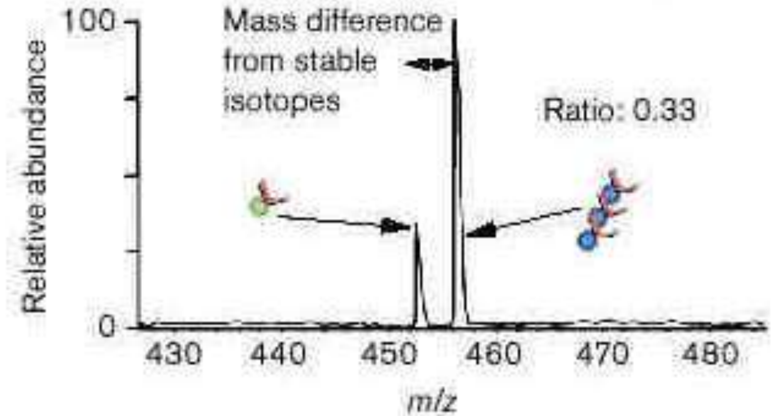
4.在两种操作模式下的m/s 的两步法分析-定量&测序

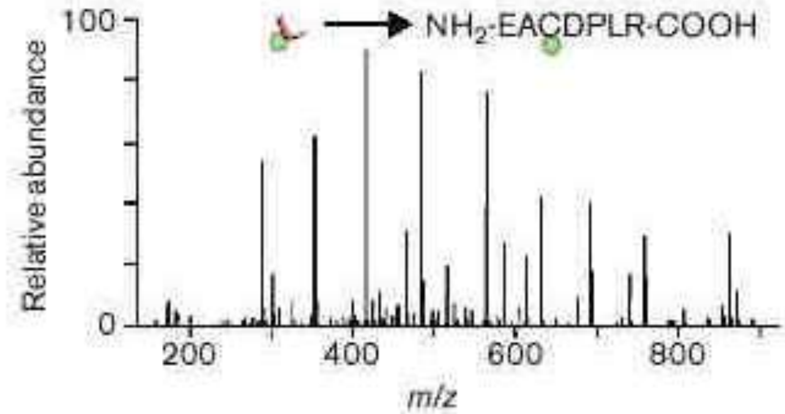
5. 计算相对定量，相对信号强度

6. 通过CID—碰撞诱导碎片化 MS/MS 扫描的序列信息鉴定蛋白质

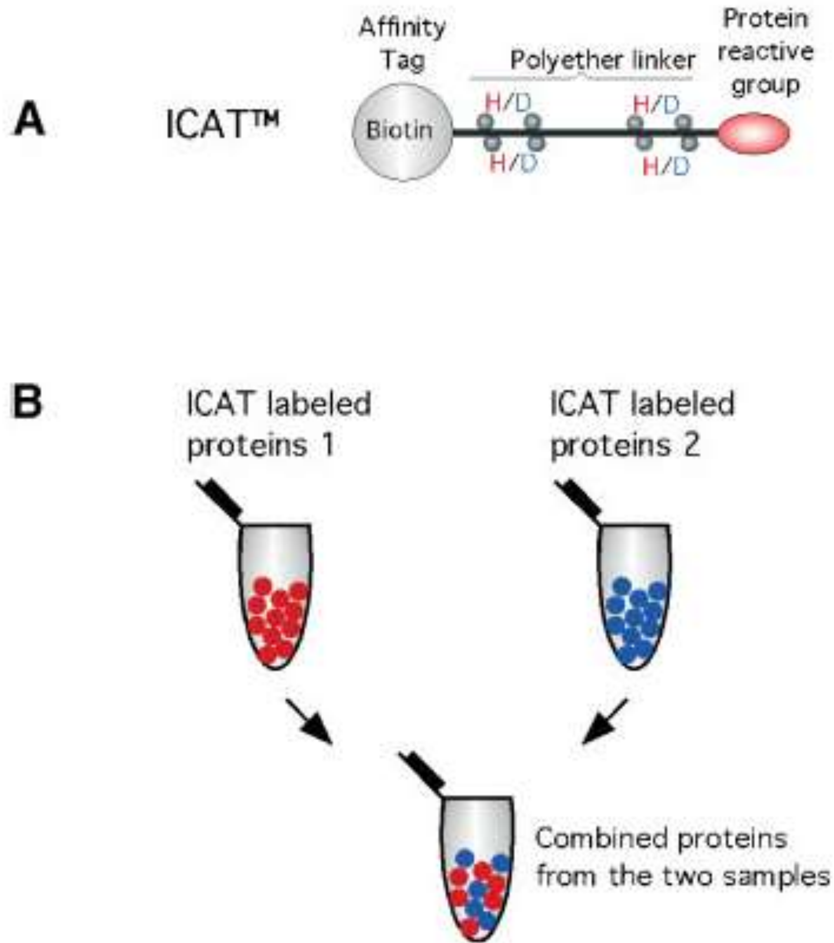
ICAT MS Quantification







Laboratory of plant molecular cytogenetics



Proteolysis and affinity purification

MS analysis

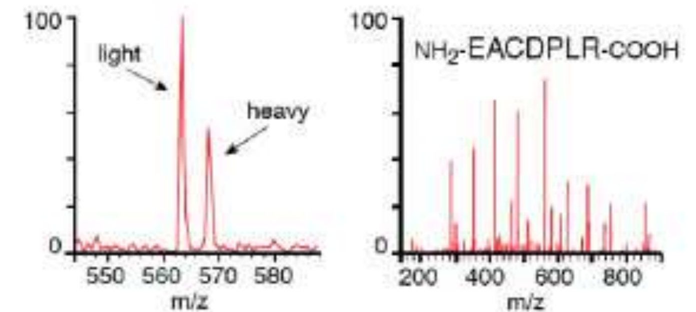


Figure 4. Quantitative mass spectrometry using ICAT reagents. **A:** The ICAT reagent consists of an affinity tag (such

4、蛋白质组学定量研究技术-iTRAQ

相对和绝对定量的等量异位标签(isobaric tags for relative and absolute quantitation,iTRAQ)是由美国应用生物系统公司ABI研发的一种多肽体外标记技术。该技术是一种新的蛋白质组学定量研究技术，具有较好的定量效果、较高的重复性，该技术采用4种或8种同位素编码的标签,通过特异性标记多肽的氨基基团,尔后进行串联质谱分析,可同时比较4种或8种不同样品。

蛋白组学中的方法一直在不断提高。基于高度敏感性和精确性的串联质谱方法，不需要凝胶，就可以获得相对和绝对定量的蛋白质结果。iTRAQ和iCAT是这些新进展中的两大主力。

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Wuhan University

iTRAQ 试剂盒包括四种同量的胺活性试剂，能对蛋白质水解的肽段进行标记，因此采用串联质谱方法，可以对肽段进行精确的鉴别和定量。

iTRAQ 的操作程序一般如下。将蛋白质裂解为肽段，然后用iTRAQ试剂进行差异标记。再将标记的样本相混合，这样就可以对其进行比较。与样本结合后，通常用 MudPIT多维蛋白质鉴定技术进行下一步的操作，用2D液相色谱串联质谱进行分析。在质谱分析鉴定特殊肽离子片断结构的基础上，采用美国应用生物系统公司的软件包MASCOT和Protein Pilot对每一个肽段进行鉴定。

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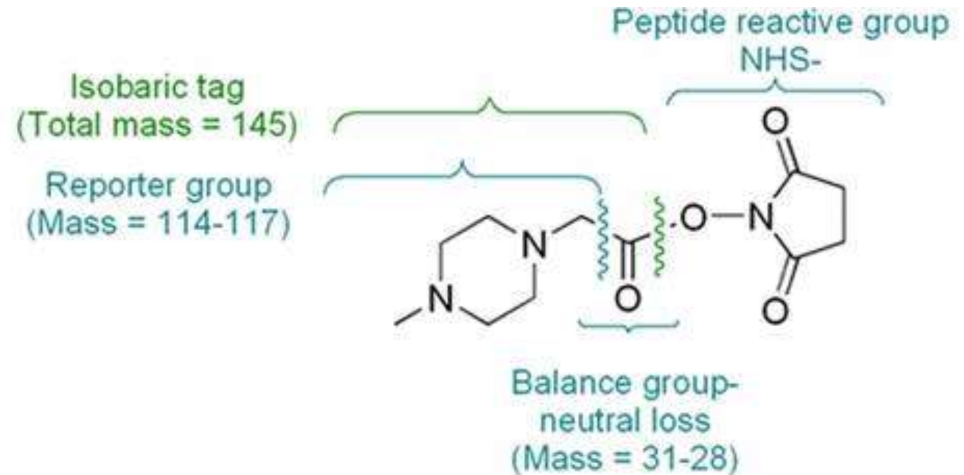
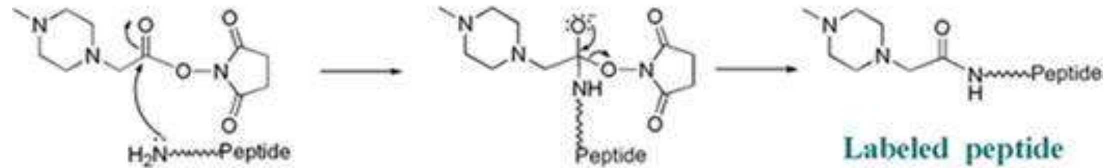
iTRAQ labeling principle

- Reporter group
- Balance group
- Peptide reactive group





4-plex

or

8-plex

Reporter Ions (Da)		Mass Balance Region (Da)		
113	—	192	—	peptide
114	—	191	—	peptide
115	—	190	—	peptide
116	—	189	—	peptide
117	—	188	—	peptide
118	—	187	—	peptide
119	—	186	—	peptide
121	—	184	—	peptide

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Simple procedure

Sample1 → digest → 114
Sample2 → digest → 115
Sample3 → digest → 116
Sample4 → digest → 117

→ mix → MS → Data analysis

iTRAQ characteristics

- Amino-groups are modified (N-terminus, Lys)
- A set of four or eight reagents, allowing simultaneous comparison of four or eight samples.
- All reagents attach nominally same mass tag, giving one peak in the MS, but produce different ‘reporter ions’ in MSMS, allowing relative quantitation.

Ross *et al*. *Mol Cell Proteomics* (2004) **3**, 1154.

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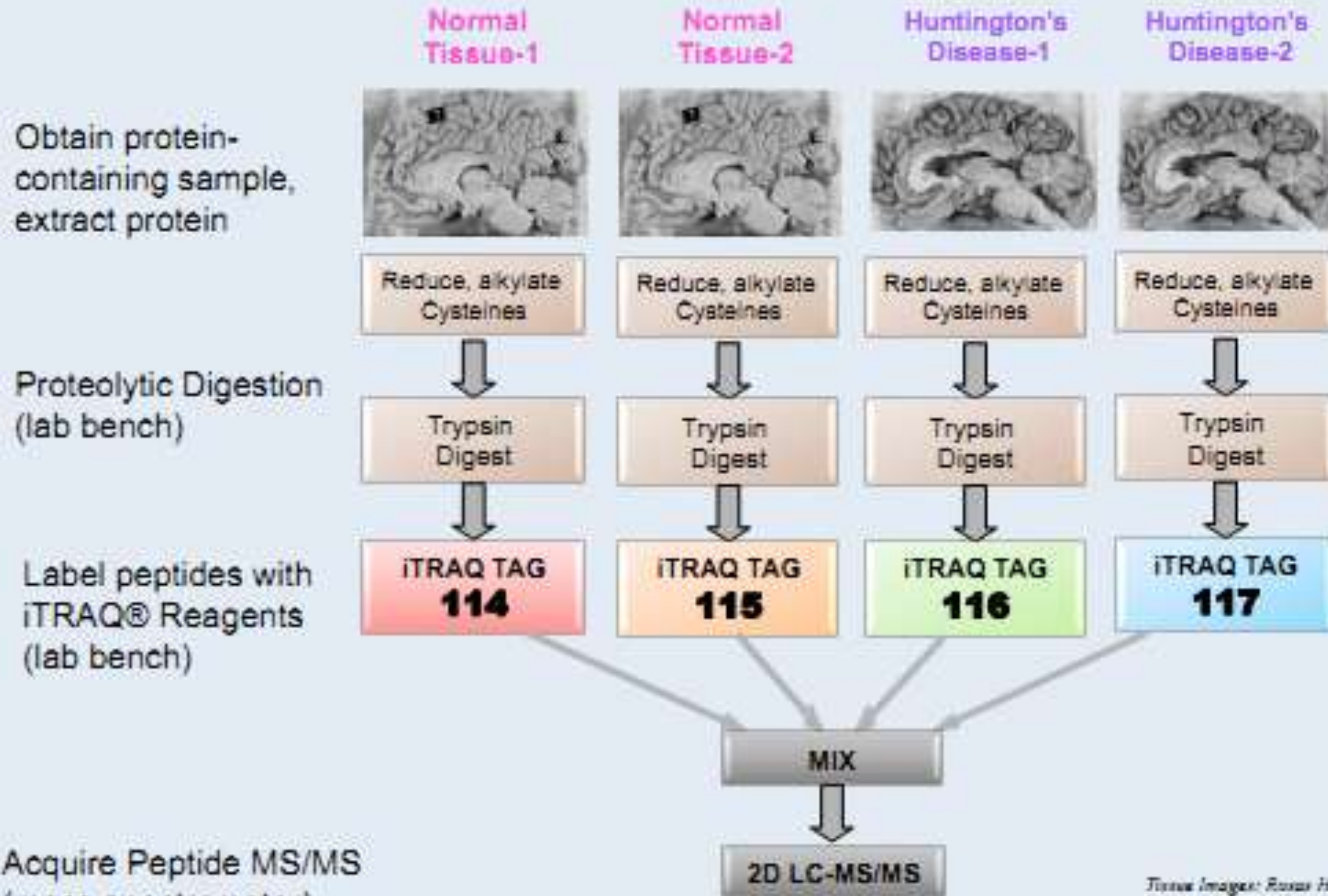
Experimental Methodology for 4-plex iTRAQ® Experiment

Example: Compare Relative Protein Expression Levels in Healthy vs Disease



Tissue Images: Rosas HD et al, (2002) Neurology, 58, 695

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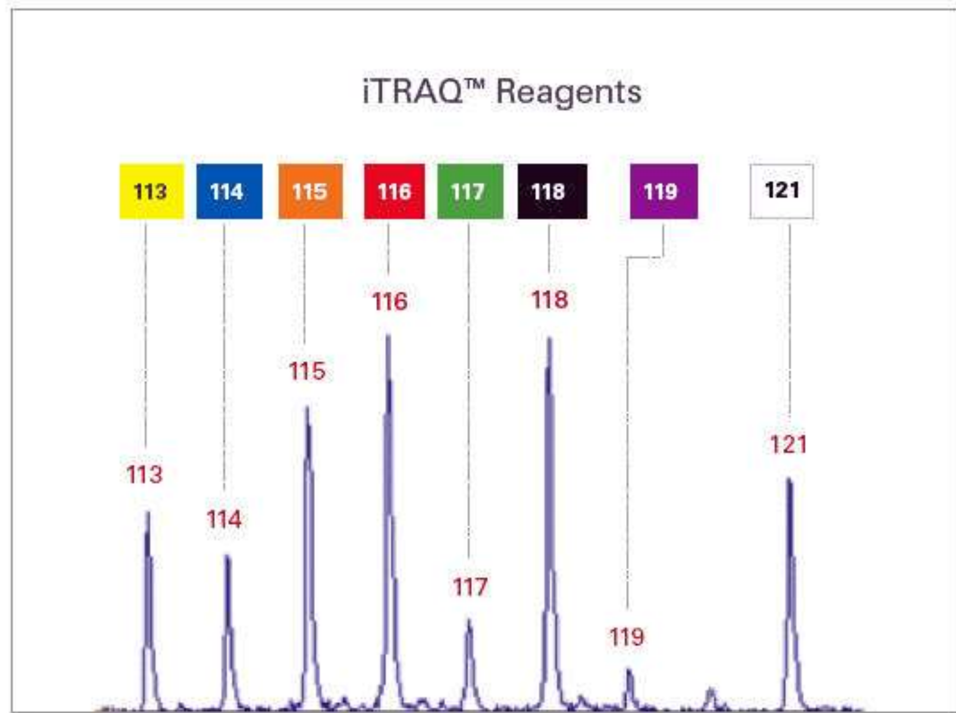
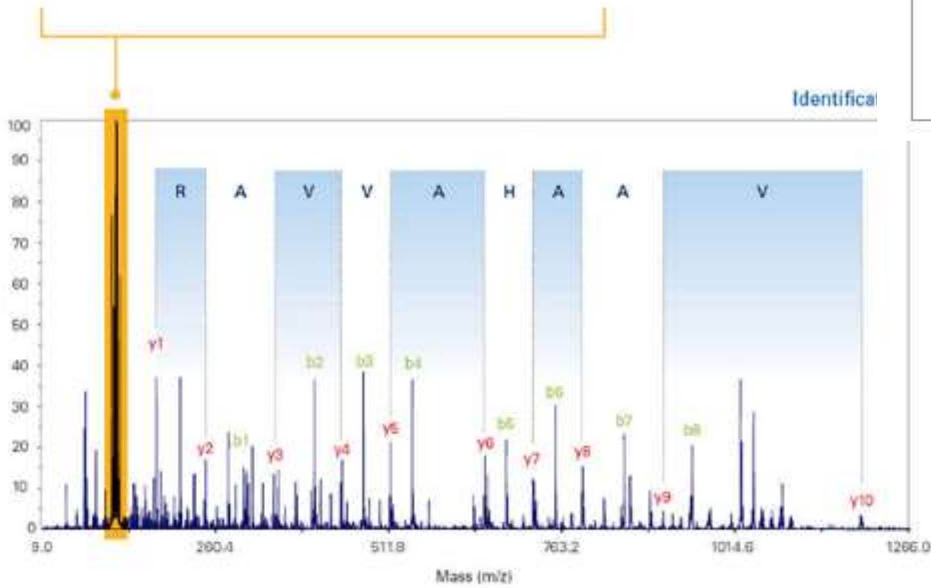


iTRAQ定量



iTRAQ鉴定

iTRAQ papers

武漢大學 Wuhan University

1. Comprehensive comparison of **iTRAQ** and label-free LC-based quantitative **proteomics** approaches using two Chlamydomonas reinhardtii strains of interest for biofuels engineering.
   Wang H, Alvarez S, Hicks LM.
   J Proteome Res. 2011 Nov 7. [Epub ahead of print]
   PMID: 22059437 [PubMed - as supplied by publisher]
   Related citations

2. DIGE and **iTRAQ** as biomarker discovery tools in aquatic toxicology.
   Martyniuk CJ, Alvarez S, Denslow ND.
   Ecotoxicol Environ Saf. 2011 Nov 4. [Epub ahead of print]
   PMID: 22056798 [PubMed - as supplied by publisher]
   Related citations

3. Plasma membrane proteomes of differentially matured dendritic cells identified by LC-MS/MS combined with **iTRAQ** labelling.
   Ferret-Bernard S, Castro-Borges W, Dowle AA, Sanin DE, Cook PC, Turner JD, Macdonald AS, Thomas JR, Mountford AP.
   J **Proteomics**. 2011 Oct 25. [Epub ahead of print]
   PMID: 22040742 [PubMed - as supplied by publisher]
   Related citations

4. Electrostatic Repulsion-Hydrophilic Interaction Chromatography (ERLIC) versus SCX for Fractionation of **iTRAQ**-Labeled Peptides.
   Hao P, Qian J, Ren Y, Sze SK.
   J Proteome Res. 2011 Oct 20. [Epub ahead of print]
   PMID: 22014306 [PubMed - as supplied by publisher]
   Related citations

5. CysTRAQ - A combination of **iTRAQ** and enrichment of cysteinyl peptides for uncovering and quantifying hidden proteomes.
   Tambor V, Hunter CL, Seymour SL, Kacerovsky M, Stulik J, Lenco J.
   J **Proteomics**. 2011 Oct 8. [Epub ahead of print]
   PMID: 22008608 [PubMed - as supplied by publisher]
   Related citations

Journal of proteome research

ARTICLE

pubs.acs.org/jpr

Analysis of Differential Proteomes of Induced Pluripotent Stem Cells by Protein-Based Reprogramming of Fibroblasts

Jonghwa Jin,[†,#] Yoo-Wook Kwon,[§,⊥,#] Jae Seung Paek,[§] Hyun-Jai Cho,[‡,§,∥,⊥] Jiyoung Yu,[†] Ji Yoon Lee,[¶] In-Sun Chu,[⊗] In-Hyun Park,[◆] Young-Bae Park,[‡,§,∥,⊥] Hyo-Soo Kim,[*,‡,§,∥,⊥,○] and Youngsoo Kim[*,†]

Journal of proteome research

ARTICLE

pubs.acs.org/jpr

Proteomic Profiling of Human Plasma by iTRAQ Reveals Down-Regulation of ITI-HC3 and VDBP by Cigarette Smoking

James D. Bortner, Jr.,[†,‡] John P. Richie, Jr.,[†,§] Arunangshu Das,[†,‡] Jason Liao,[†,∥] Todd M. Umstead,[†,⊥] Anne Stanley,[†,#] Bruce A. Stanley,[†,#] Chandra P. Belani,[†,¶] and Karam El-Bayoumy[*,†,‡]

iTRAQ-based analysis of changes in the cassava root proteome reveals pathways associated with post-harvest physiological deterioration

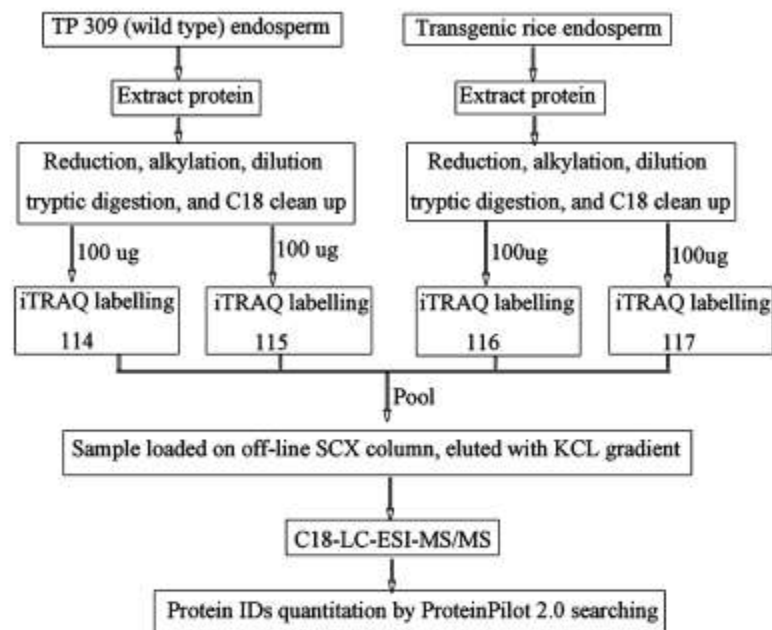
research articles Journal of proteome research

Proteomic Analysis of Rice Endosperm Cells in Response to Expression of hGM-CSF

Junling Luo,† Tingting Ning,† Yunfang Sun,† Jinghua Zhu,‡ Yingguo Zhu,† Qishan Lin,‡ and Daichang Yang*,†

Center of Engineering Research of Plant Biotechnology and Germplasm Utilization, Ministry of Education, Department of Genetics, College of Life Sciences, Wuhan University, 430072, P.R. China, and UAlbany Proteomics Facility, Center for Functional Genomics, University at Albany, 1 Discovery Drive, Rensselaer, New York 12144

Received April 17, 2008



Laboratory of plant molecul

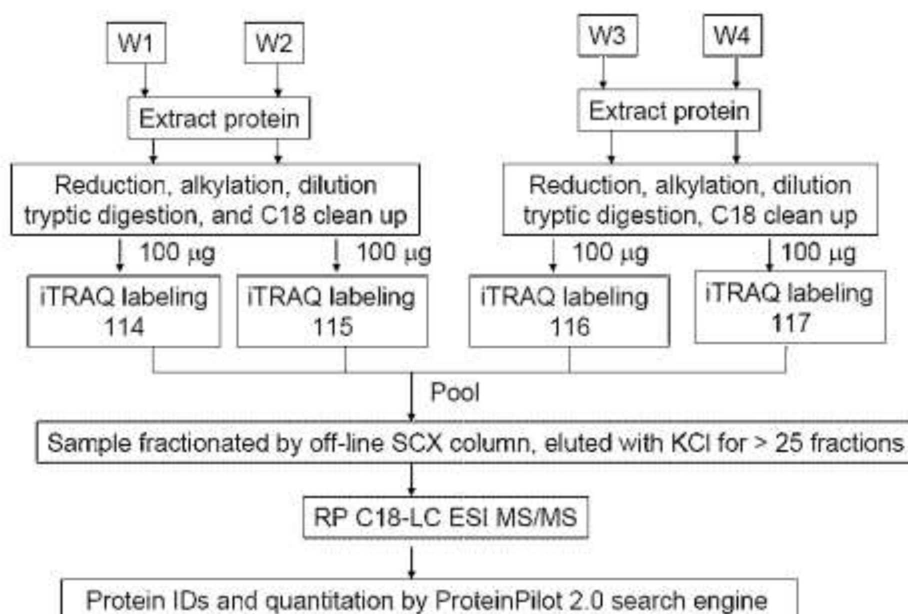
DOI 10.1002/pmic.200800840

Proteomics 2009, *9*, 2798–2808

RESEARCH ARTICLE

Understanding rice plant resistance to the Brown Planthopper (*Nilaparvata lugens*): A proteomic approach

Zhe Wei[1], Wei Hu[1], Qishan Lin[2], Xiaoyan Cheng[1], Mengjie Tong[1], Lili Zhu[1], Rongzhi Chen[1] and Guangcun He[1]



Laboratory of

Work Flow chart

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M - Maintainer liner

A - Abortive lines

Extract protein from pollen or spikelet

Reduction, alkylation, tryptic digestion, and Discovery DSC-18 desalted with 0.1%Formic acid, lyophilized and resuspend in TEAB

100ug/each

100ug/each

iTRAQ 114 TAG

iTRAQ 115,116,117 TAG

Pool

Sample fractionated by off-line Zorbax 300-SCX column(Agilent 1200 HPLC), eluted with KCl for 15 fractions, dried by speed-vac

LC-MS/MS

Protein IDs and Quantitation by ProteinPilot 4.0 search engine

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Protein Interaction

■免疫沉淀法（immuno-precipitation）：

new protein + epitope tag

■亲和层析法(affinity chromatography)：

GST tag fusion

■ 酵母双杂交法(**yeast two-hybrid**)：

Yeast two-hybrid system

- 真核转录因子

  DNA结合结构域(DNA binding domain, BD)

  转录激活结构域(activation domain, AD)

  常用转录因子：**Gal4、LexA**

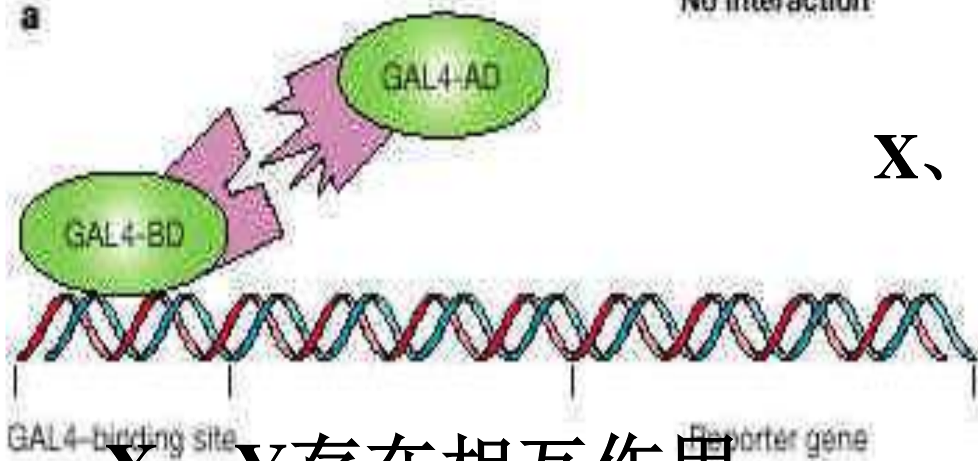
- 当两个功能域独立存在时，并不能激活转录，但两者只要相互接近，即可恢复转录激活功能。

- 杂交体融和蛋白：

  BD+X→BD-X (bait)

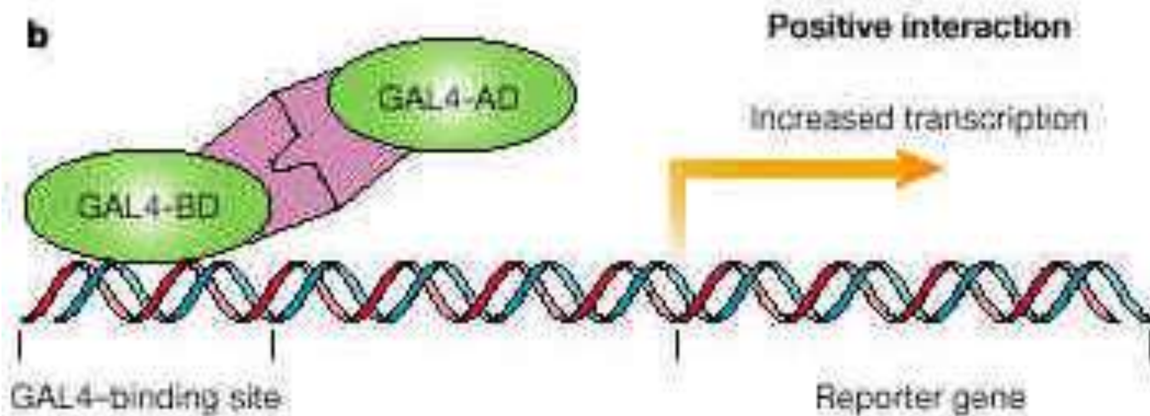
  AD+Y→AD-Y (prey)

  共转化/ 接合→→ 报告基因是否激活



X、Y不存在相互作用

X、Y存在相互作用



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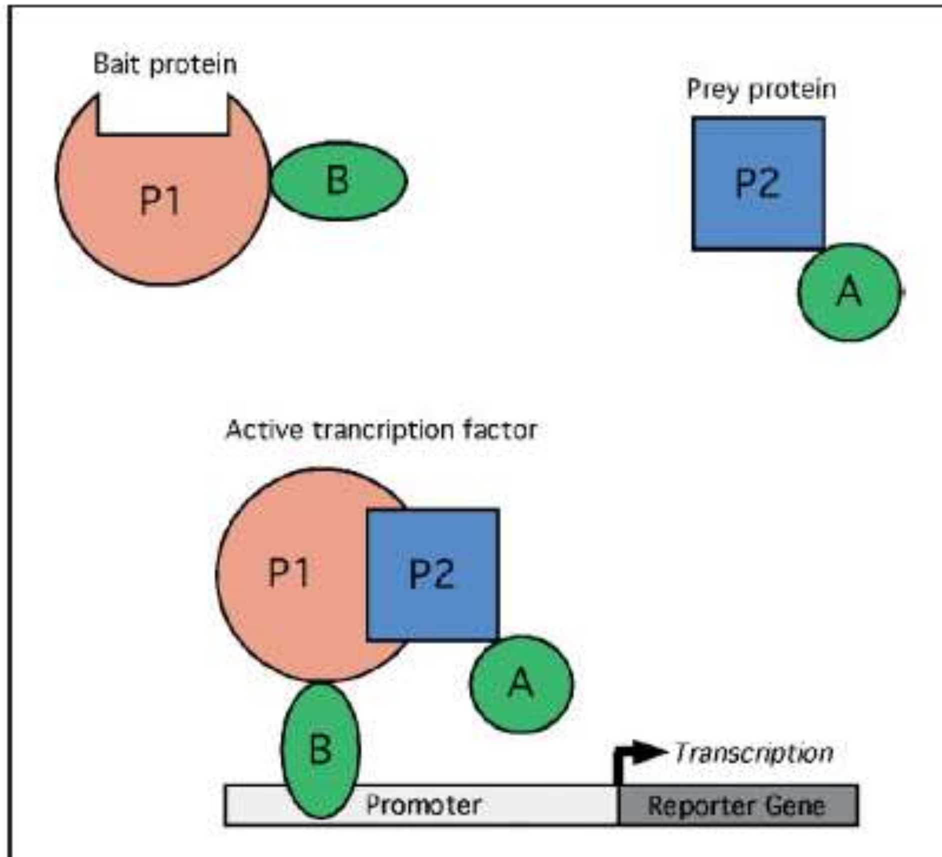


Figure 7. Yeast two-hybrid technique for in vivo detection of protein-protein interactions. The technique is based on the interaction of a 'bait' protein with a 'prey' protein inside the nucleus of a yeast cell. The bait protein consists of a target protein (P1) fused to the DNA-binding domain (B) of a transcription factor. The 'prey' protein consists of a binding protein (P2) fused to the transcriptional activator domain (A) of a transcription factor. By the interaction of the bait with the prey, a functional transcription factor is created that turns on transcription of the reporter gene. The method requires that the protein–protein interaction can occur in the nucleus of the yeast cell. A false positive reaction may occur if the prey protein in itself is a functional transcription factor.

特点

- 基于核酸进行操作，不需要纯化蛋白质
- 真核表达系
- 一个酵母菌可兼容几个不同的质粒
- 是一种在细胞内检测相互作用的方法，免去体外洗涤，灵敏度高
- 酵母生长迅速、易于操作
- 可利用酵母的生殖特性：a / α 单倍体接合（mating）

5、Chip-based methods

- specific probe placed at defined position in array
- signals indicate the interactions of the probe with its target molecule
- Identify the probe molecules and their quantity

武漢
Wu

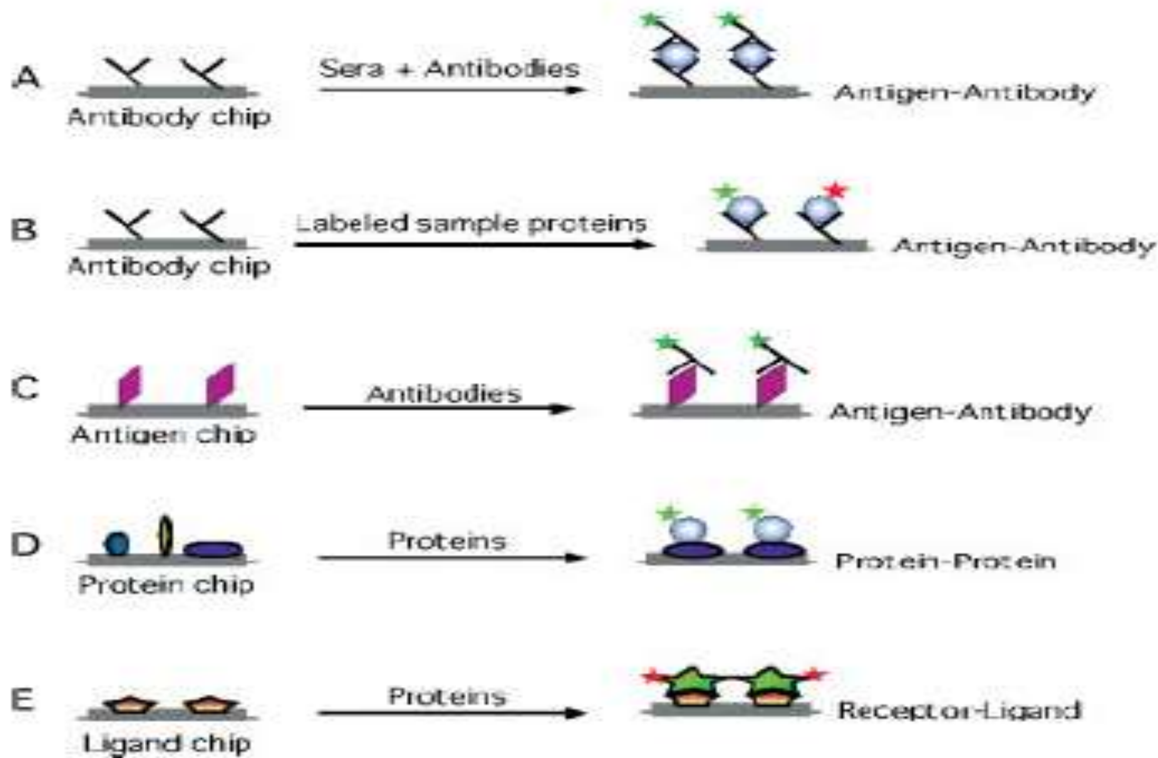


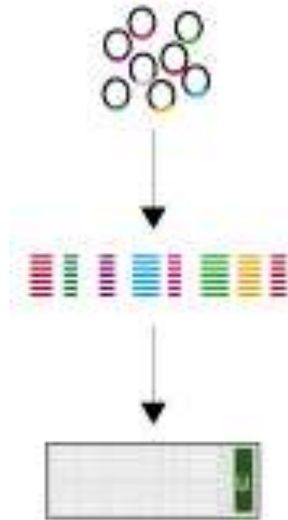
Figure 5. Protein arrays may be produced either by immobilizing antibodies on chips (**A,B**); antigens (**C**), purified or recombinant proteins (**D**) and even small molecules for studying protein ligand interactions (**E**). **A:** The antibody captures the antigen in the sample and is detected with a fluorescently labeled second antibody. **B:** In a two-colour labeling strategy the sample is labeled with one dye, e.g. Cy 5 and then mixed with a reference sample that is labeled with Cy3. **C:** Labeled antibodies are used to detect specific immobilized proteins from a sample. **D:** By immobilizing specific proteins on a chip, protein-protein interactions may be demonstrated using labeled proteins. **E:** By immobilizing small molecules (ligands) on the chip, it is possible to study binding activities of macromolecules to ligands. The challenges with the array technique are the production of high-quality

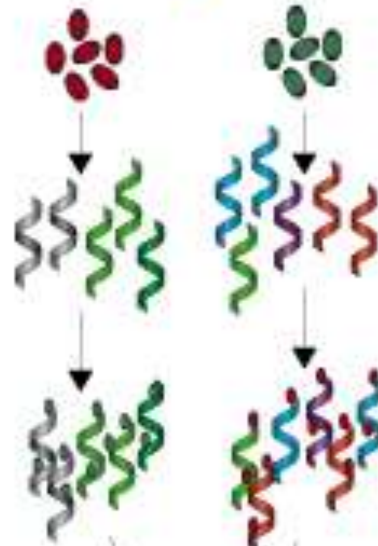
Laborator

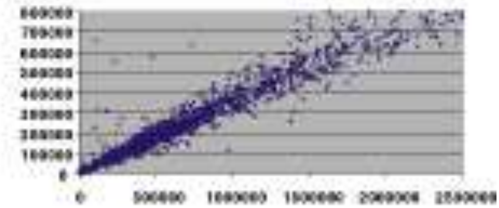
Target preparation

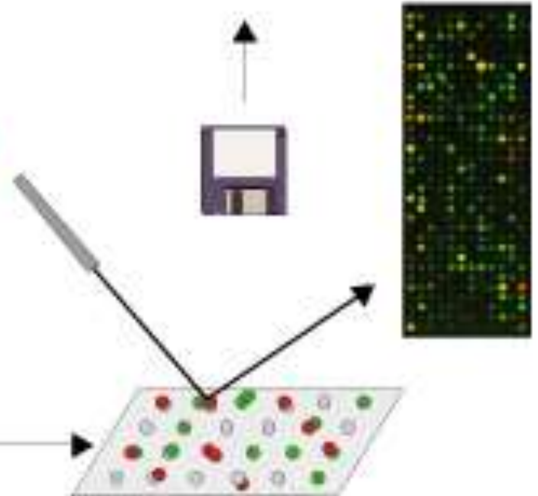
Probe preparation

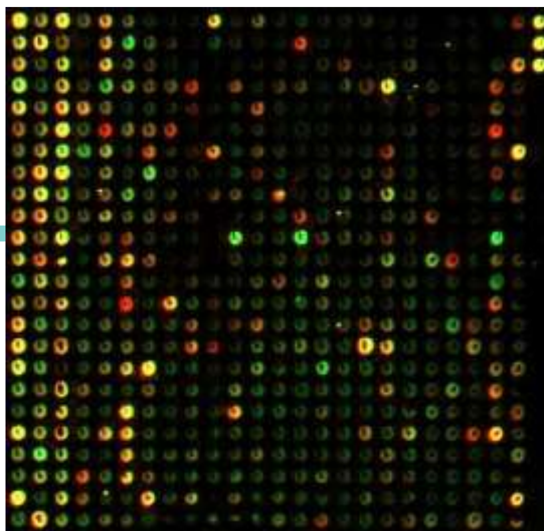
Data analysis



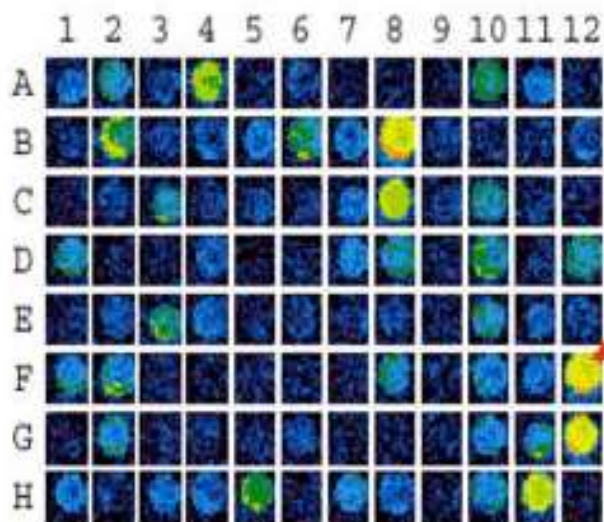




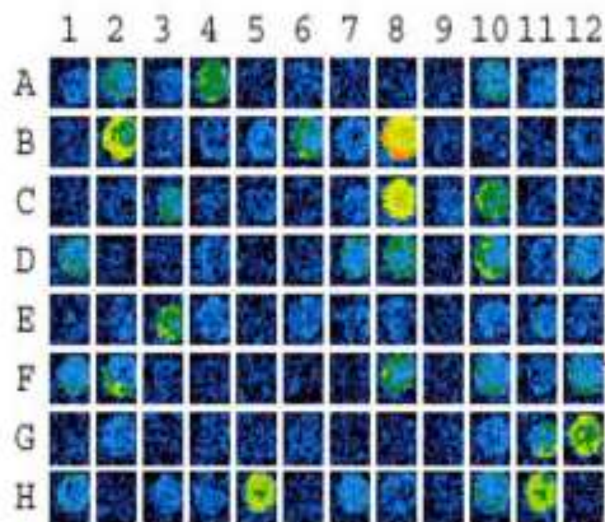




(I)



(II)



- 蛋白质组学研究进展

水稻蛋白质组研究技术路线

Rice tissues

Protein extraction

2D-PAGE (IEF and IPG)

Image analysis

Western blotting

Deblocking

N-terminal sequencing
by protein sequencer

Protein spots cutting

Electroelution

Cleveland peptide mapping

Western blotting

Internal sequencing
by protein sequencer

Destaining

Alkylation

Trypsin digestion

Elution/Desalt

Internal sequencing
by mass spectrometry

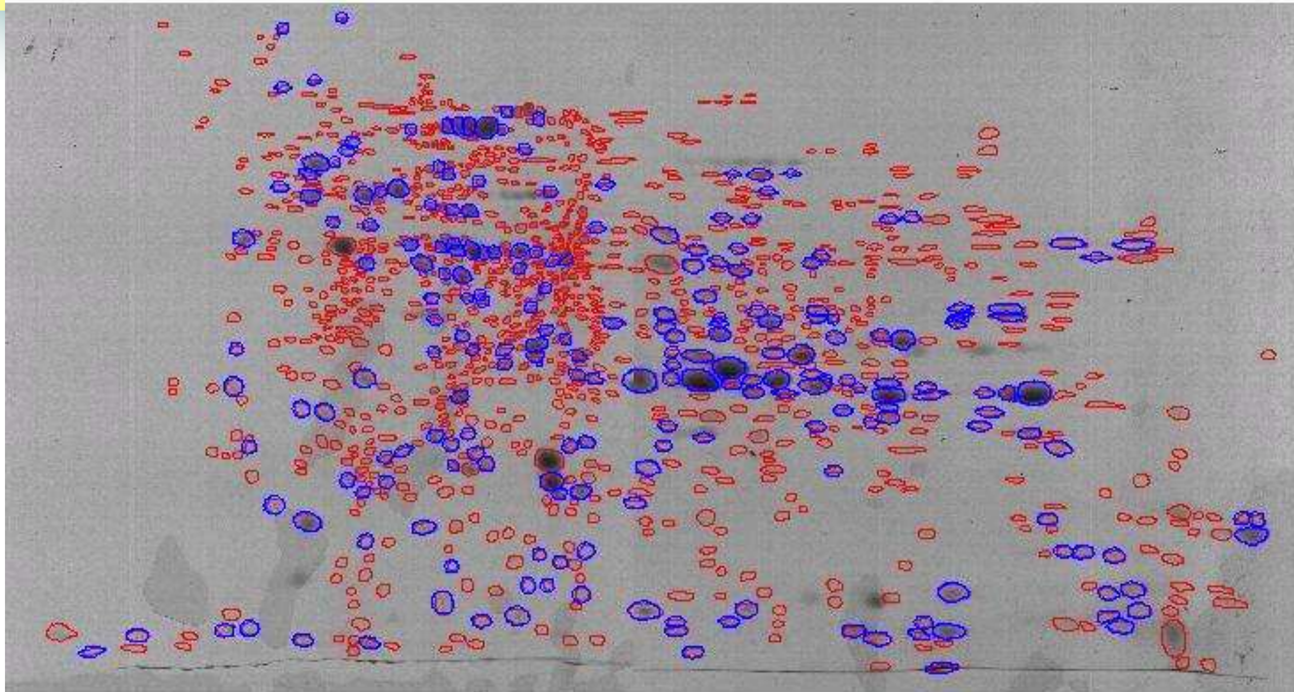
Homology search
post translational modification

Rice protein database

Preparation of antibody

cDNA cloning

Functional analysis



1 Week After Flowering
Panicle After Heading
Panicle Before Heading
Anther
Leaf Blade
Leaf Blade
Endosperm
Embryo
Leaf Sheath
Leaf Sheath
Stem
Crown
Root
Root
Callus
Seed
Seedling
Mature Plant

1)发育的蛋白质组学研究。

- 在蛋白质组学概念提出来之前，许多研究者就已采用 2-DE 技术对水稻各组织器官的蛋白质组分进行分离及比较，Komatsu 等用 2-DE 分析水稻胚、胚乳及叶的蛋白质，考马斯亮蓝染色后分别检测出约600、100、150个蛋白点，通过 N 端或内部氨基酸测序，鉴定了27种蛋白质，超过70％的蛋白质 N 端封闭。Tsugita 等对**水稻10种组织器官分离出4892个蛋白质**，对其中2.8％的蛋白质进一步分析，只有1.1％(56个)测序，由于当时基因组及蛋白质数据库有限，即使一些被测序的蛋白质也不知其功能。水稻重要的繁殖器官花药在小孢子早期发育易受环境影响，Imin 等通过 2-DE 分离、银染得到约**4000种花药蛋白**，代表了整个基因组的10％，其中53个点得到鉴定，大部分是持家蛋白，分子伴侣，富甘氨酸蛋白，以及调控翻译的肿瘤蛋白(首次在花药研究中报道)等

同时，一些与代谢密切相关的亚细胞结构也开展了蛋白质组学研究，如水稻线粒体，通过质谱分析鉴定了149个蛋白，并确定了其中85个蛋白质的功能。在制备亚细胞蛋白质样品时，如有必要可先用显微激光切割技术进行专一样品的富集后再进一步裂解。其他涉及研究的亚细胞结构还有质膜，高尔基体膜，叶绿体等。目前水稻蛋白质组数据库包含了23张参考图，有详细的采样部位、样品制备及电泳条件，鉴定方法，点击蛋白点链接相应的信息等 (http:\\gene64.dna.affrc.go.jp/rpd/)。建立高质量的水稻 2-DE ,参考图谱对水稻蛋白质组学以及水稻基因组功能的精确注释是非常重要的。

2)在环境胁迫下的蛋白质组学研究。

Salekdeh 等对干旱胁迫下以及恢复灌溉后水稻叶片蛋白质组进行分析，发现干旱胁迫下有42个蛋白点的丰度有显著变化，从而鉴定了一些干旱应答蛋白，这将有助于提高水稻抗旱育种以及改善作物品质和提高产量。Agrawal 等采用 2-DE 结合氨基酸测序及免疫杂交技术研究臭氧胁迫下水稻幼苗叶蛋白的表达，与对照相比，50多个点有差异，其中与光合作用相关的主要蛋白 RuBisCO 减少，另外诱导出与防御有关的蛋白累积，包括 OsPR5，OsPR10，SOD，APX，钙结合蛋白等，这些蛋白可以作为潜在的分子标记检测水稻及其他植物与臭氧

武漢大學
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the Human Liver Proteome Project (HLPP)

人类肝蛋白质组计划

What Is Required: A System for Organizing Knowledge from Data

Small Molecule

Screening

LIMS Experiment Tracking

Biological Samples

Expression

Gel Data

Protein

Function

mRNA/Gene Expression

Sequence

Biochemical Pathway

External Databases

Gene

Mass Spec

武汉大学
Wuhan University

Proteomics & Medicine

- 疾病的比较蛋白质组研究
- 病原微生物蛋白质组
- 药物蛋白质组学
- 蛋白质组与毒理学

武漢大學 Wuhan University

Disease comparative proteomics

pH →

Size ↓

Diseased or treated sample

2D Gel Electrophoresis

low ◄ abundance ► high

Differential protein abundance levels

Control sample

Laboratory of plant molecular cytogenetics

Study heart disease with proteomics

- DCM 比较蛋白质组分析

发现了100个DCM中发生特异性表达量变化的心脏蛋白。

分为三类：与细胞骨架和肌球蛋白相关；与线粒体和能量产生相关；与压力反应相关

- 动物模型蛋白质组分析

大鼠心肌细胞、狗的起搏诱导性心衰(pacing-induced heart failure)和牛的DCM的心脏蛋白质组

- 心脏特异性抗原研究

鉴定了一些与心脏移植中的急性或慢性排斥反应有关的心脏特异性抗原

武漢大學
Wuhan University

Disease Proteomics

- 肿瘤：

寻找特异性表达标记、新抗体与分化标记

实例：膀胱鳞状细胞癌（**squamous cell carcinoma, SCC**） 丹麦

- 神经系统疾病：

脑损伤和感染性蛋白质疾病

实例: 老年痴呆症（**Alzheimer's disease**）

克雅氏病**Creutzfeld-Jakob disease (CJD)**

- 心血管疾病：

相关器官与细胞的蛋白质组分析与数据库构建

实例：扩张型心肌病(**dilated cardiomyopathy ,DCM)**

病原微生物蛋白质组

- 确定致病菌毒力（virulence）
- 寻找新的诊断标记物（diagnostic marker）
- 为疫苗的研制寻找新的候选抗原（candidate antigen）
- 阐明药物抗菌机理，为新的抗生素的研制寻找靶点

武漢大學
Wuhan University

Proteomics & Drug development

- 靶点的探测

  疾病相关蛋白、信号传递通路中的关键性蛋白、病原微生物相关蛋白

- 靶点的确认

  分析“候选靶点”对药物的敏感性，判断开发价值

- 阐明分子药理机制,提供更为有效、合理的药理筛选模型
- 临床前安全性评价与毒害预测

Journal of Integrative Plant Biology 2006, 48 (8): 965–970

Proteomic Alterations of Antarctic Ice Microalga *Chlamydomonas* sp. Under Low-Temperature Stress

Guang-Feng Kan[1*], Jin-Lai Miao[2], Cui-Juan Shi[1] and Guang-You Li[2]

(1. *School of the Ocean, Harbin Institute of Technology in Weihai*, Weihai 264209, China;
2. *Key Laboratory of Marine Bio-active Substances, State Oceanic Administration*, Qingdao 266061, China)

Abstract

Antarctic ice microalga can survive and thrive in cold channels or pores in the Antarctic ice layer. In order to understand the adaptive mechanisms to low temperature, in the present study we compared two-dimensional polyacrylamide gel electrophoresis (2-DE) profiles of normal and low temperature-stressed Antarctic ice microalga *Chlamydomonas* sp. cells. In addition, new protein spots induced by low temperature were identified with peptide mass fingerprinting based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and database searching. Well-resolved and reproducible 2-DE patterns of both normal and low temperature-stressed cells were acquired. A total of 626 spots was detected in control cells and 652 spots were detected in the corresponding low temperature-stressed cells. A total of 598 spots was matched between normal and stressed cells. Two newly synthesized proteins (a and b) in low temperature-stressed cells were characterized. Protein spot A (53 kDa, pI 6.0) was similar to isopropylmalate/homocitrate/citramalate synthases, which act in the transport and metabolism of amino acids. Protein spot b (25 kDa, pI 8.0) was related to glutathione *S*-transferase, which functions as a scavenger of active oxygen, free radicals, and noxious metabolites. The present study is valuable for the application of ice microalgae, establishing an ice microalga *Chlamydomonas* sp. proteome database, and screening molecular biomarkers for further studies.

武漢大學

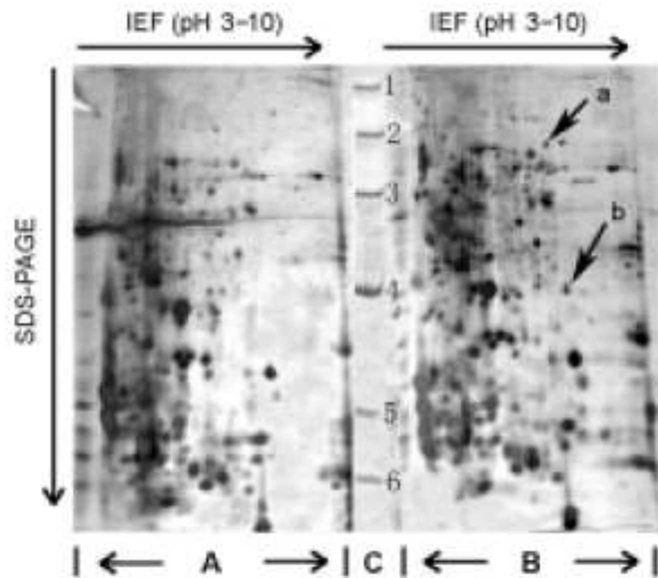

Figure 1. Two-dimensional polyacrylamide gel electrophoresis of proteins of Antarctic ice microalga *Chlamydomonas* sp. for control and **(B)** low temperature-treated cells, and **(C)** protein mark 1, 97.4 kDa; 2, 66.2 kDa; 3, 43 kDa; 4, 31 kDa; 5, 20.1 kDa; 6, kDa.

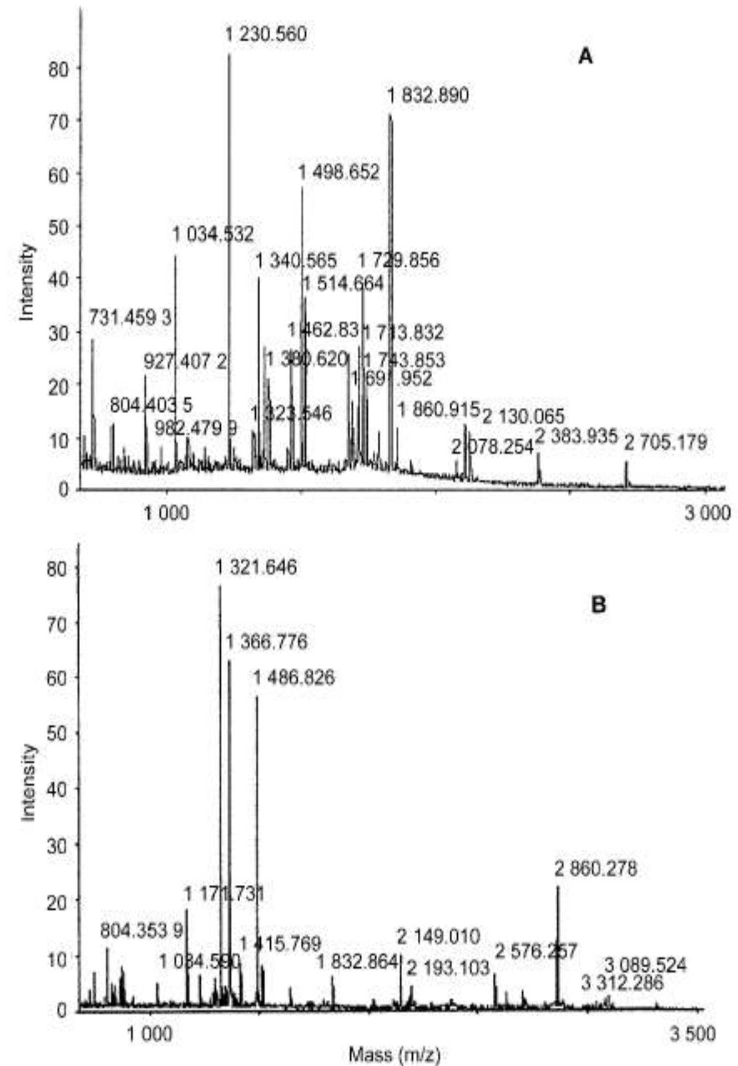

Figure 2. PMF of protein spots in Antarctic ice microalga *Chlamydomonas* sp.

Two-dimensional polyacrylamide gel electrophoresis map for **(A)** spot a and **(B)** spot b.

武漢大學

Wuhan University

Functional genomics studied by proteomics

Bent Honoré,[1]* Morten Østergaard,[1] and Henrik Vorum[2]

Summary

The human genome contains about 30,000 genes, each creating several transcripts per gene. Transcript structures and expression are studied by high-throughput transcriptomic techniques using microarrays. Generally, transcripts are not directly operating molecules, but are translated into functional proteins, post-translationally modified by proteolysis, glycosylation, phosphorylation, etc., sometimes with great functional impact. Proteins need to be analyzed by proteomic techniques, less suited for high-throughput. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), separating thousands of proteins has developed slowly over the past quarter of a century. This technique is now quite reproducible and suitable for differential proteomics, comparing normal and diseased cells/tissues revealing differentially regulated proteins. 2D-PAGE is combined with protein-identification methods, currently mass spectrometry (MS), which has been significantly improved over the last decade. Other proteomic techniques studying protein–protein interactions are now either established or still being developed, such as peptide or protein arrays, phage display, and the yeast two-hybrid system. The strengths and weaknesses of these techniques are discussed. *BioEssays* 26:901–915, 2004.
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Introduction

Completion of the draft sequence

The human genome has now been completely sequenced[1] two years after the draft sequence was published [2,3] and thirteen years since the start of The Human Genome Project in 1990. The sequence can be obtained from GenBANK at the NCBI home page (http://www.ncbi.nih.gov/Genbank/) and creates a firm foundation for the further analysis of gene

Laboratory of plant molecular cytogenetics

Table 1. Two-dimensional gel electrophoresis tools

Vendor	IPG gel strips pH range[a]	Pre-cast 2nd dimension gels[a]	Coomassie Brilliant blue/Colloidal Coomassie blue[b]	Silver staining[c]	Fluorescent labeling/staining
Amersham Biosciences	4–7; 6–9; 6–11; 7–11 NL[d]; 3–10; 3–10 NL; 3–11 NL; 3.5–4.5; 3.0–5.6 NL; 4.0–5.0; 4.5–5.5; 5.0–6.0; 5.3–6.5; 5.5–6.7; 6.2–7.5	Homogeneous 12.5%; Gradient 12–14%	Available	Available	CyDye™ DIGE Flour labels (Cy2, Cy3 and Cy5); Deep Purple™ gel stain
Bio-Rad	3–6; 4–7; 5–8; 7–10; 3–10; 3–10 NL; 3.9–5.1; 4.7–5.9; 5.5–6.7; 6.3–8.3	Homogeneous 10%, 12%; Gradient 10–20%, 8–16%	Available	Available	
Genetix	3–6; 5–8; 7–10; 3–10; 3–10 NL; 3.9–4.9; 4.7–5.7; 5.5–6.5; 6.3–7.3; 7.2–8.2; 8.0–9.0; 8.8–9.8; 9.5–10.5		Available	Available	
Invitrogen	4–7; 6–10; 3–10 NL; 4.5–5.5; 5.3–6.3; 6.1–7.1	Gradient 4–12%, 4–20%	Available	Available	
Servern Biotech	3–6; 5–8; 7–10; 3–10; 3–10 NL; 3.9–4.9; 4.7–5.7; 5.5–6.5; 6.3–7.3; 7.0–8.0; 8.8–9.8; 9.5–10.5		Available		
Sigma-Aldrich	3–5; 4–7; 5–8; 8–11; 6–11; 3–10		Available	Available	
Molecular Probes					SYPRO® Ruby gel stain; Pro-Q® Diamond Phosphoprotein gel stain; Pro-Q® Emerald 300 Glycoprotein gels stain; Pro-Q® Amber Transmembrane Protein gel stain

[a]Available in different sizes.
[b]Several reagents available. Only some vendors offers Colloidal Coomassie Blue stains.
[c]Several kits available. Some may not be compatible with MS technology.
[d]Non-linear.

 DOI 10.1002/pmic.200600094 *Proteomics* 2006, *6*, 4356–4369

RESEARCH ARTICLE

Proteomic identification of human sperm proteins

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[2] Genetics Service, Hospital Clínic i Provincial, Barcelona, Spain
[3] Unitat de Proteòmica, Faculty of Medicine, University of Barcelona, Barcelona, Spain
[4] Institut Clínic de Ginecologia, Obstetricia i Neonatología. Hospital Clínic i Provincial, Barcelona, Spain

Conventional 1-DE has in the past provided a wealth of information concerning the major sperm proteins. However, so far there are relatively few reports exploiting the potential of the present proteomic tools to identify and to study additional yet-unidentified important proteins present in human spermatozoa. In the present work, 2-DE of proteins extracted from human normozoospermic spermatozoa led to the resolution of over 1000 spots. Subsequent excision from the gels of 145 spots and MALDI-TOF MS analysis allowed the identification of 98 different proteins. The function of these proteins turned out to be energy production (23%), transcription, protein synthesis, transport, folding and turnover (23%), cell cycle, apoptosis and oxidative stress (10%), signal transduction (8%), cytoskeleton, flagella and cell movement (10%), cell recognition (7%), metabolism (6%) and unknown function (11%). As many as 23% of the proteins identified have not been previously described as being expressed in human spermatozoa. The present data provide an important clue towards determining the function of these proteins and opens up the possibility to perform additional experiments.

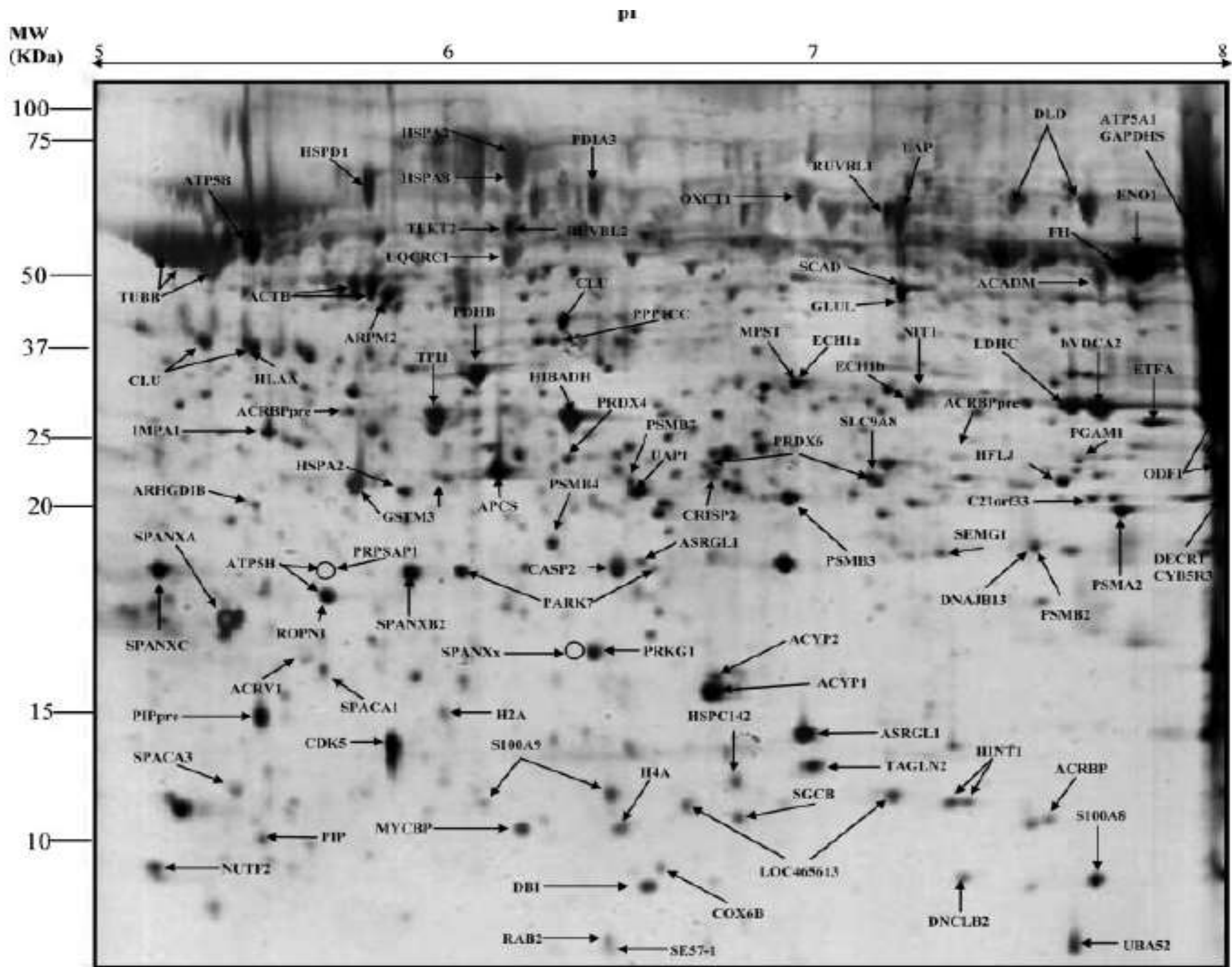

Figure 1. 2-DE map of normozoospermic human sperm proteins. The first dimension was performed by IEF on pH 5–8 IPG strips, the second dimension on 12% SDS-PAGE gels and the proteins were visualized by silver staining. The indicated spots were excised from the gel and identified by MS. Proteins are mapped and annotated by their gene name, according to HUGO database. For these abbreviations and complete information see Table 1. The two circles indicate the position of two spots absent in this particular 2-DE gel.

Table 1. Human sperm proteins identified by MS

Spot name	Protein name	Accession no.	MOWSE score	Masses match (%)	% protein covered	Mol. mass/p*I*		Function
						Determined	Expected	
Metabolism								
ACYP1	Acylphosphatase, organ-common type isozyme	P07311	249	7/187 (3)	85	16.8/6.44	11/9.52	Its physiological role is not yet clear. May be involved in phosphate metabolism
ACYP2	Acylphosphatase, muscle type isozyme	P14621	183	13/187 (6)	95	16.8/6.44	11/9.52	Same as above
GLUL	Glutamine synthetase	P15104	3.16e+003	6/60 (10)	21	44.8/6.97	42/6.43	Belongs to the glutamine synthetase family; amino acid metabolism
HIBADH	3-Hydroxy- isobutyrate dehydrogenase precursor	P31937	5.78e+003	8/18 (44)	28	31.7/6.30	35/8.38	Dehydrogenase; involved in valine metabolism
HFLJ	Hypothetical protein FLJ11342	XP_416633	1.49e+009	14/106 (13)	48	28.5/7.63	34/8.80	Predicted hydrolase involved in amino acid transport and metabolism
PRPSAP1	Phosphoribosyl pyrophosphate synthetase-associated protein 1	Q14558	1.16e+003	8/140 (5)	17	20.8/7.59	391/6.73	Negative regulatory role in PRPP synthesis and forms part of nucleobase, nucleoside, nucleotide and nucleic acid metabolism
Energy								
Glycolysis and gluconeogenesis								
ENO1	Alpha enolase	P06733	5.62e+008	13/29 (44)	36	50.2/7.84	47/7.01	Glycolysis, growth control, hypoxia tolerance, allergic responses, transcriptional repressor
GAPDHS	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific	O14556	2.05e+008	14/37 (37)	49	50.3/7.78	44/8.39	May play a role in regulating the switch between different pathways for energy production during spermiogenesis and in the spermatozoon
PGAM1	Phosphoglycerate mutase 1	P18669	3.21e+006	9/238 (3)	51	30.0/7.67	29/6.68	Interconversion of 2,3-bis-

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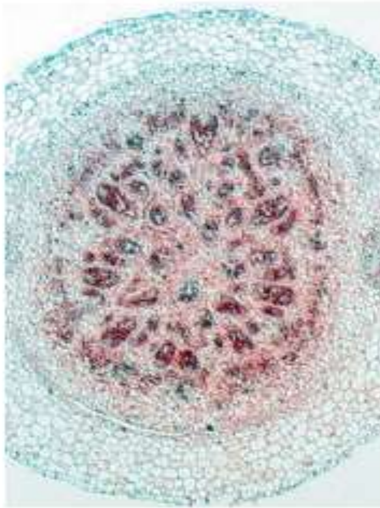
Hans-Joachim Kraus

Proteomics杂志所刊论文及研究领域



Cover Illustration

has kindly been provided by Frank Hochholdinger, ZMBP, Center for Plant Molecular Biology, Department of General Genetics, University of Tuebingen, Tuebingen, Germany.
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REGULAR

Technology

TECHNICAL BRIEF

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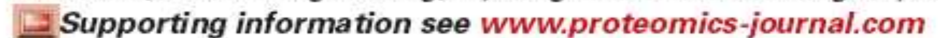
Supporting information see www.proteomics-journal.com

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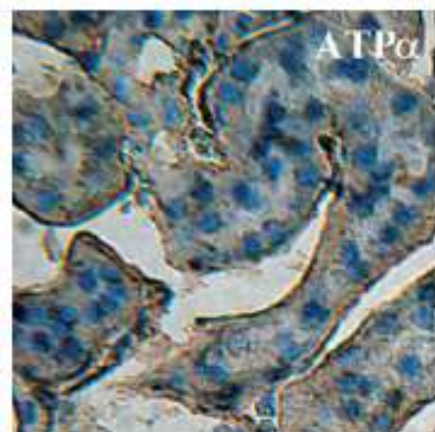
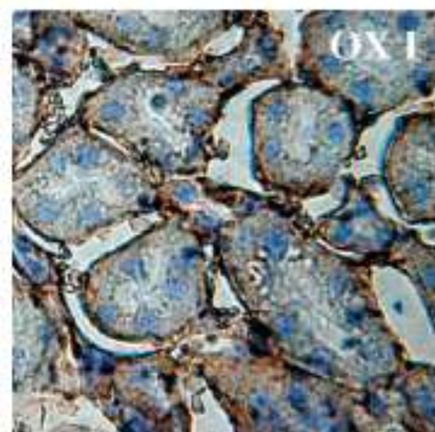
Protein Arrays

2376 **Comparative proteomic analysis reveals a function of the novel death receptor-associated protein BRE in the regulation of prohibitin and p53 expression and proliferation**
Mei Kuen Tang, Chun Mei Wang, Sze Wan Shan, Yiu Loon Chui, Arthur Kar Keung Ching, Pak Ham Chow, Lars Grotewold, John Yeuk





Cell Biology

2386 **Functional proteomics of resveratrol-induced colon cancer cell apoptosis: Caspase-6-mediated cleavage of lamin A is a major signaling loop**
Shao Chin Lee, Jason Chan, Marie-Veronique Clement and Shazib Pervaiz

2395 **Landscape of the hnRNP K protein–protein interactome**
Michał Mikula, Artur Dzwonek, Jakub Karczmarski, Tymon Rubel, Michał Dadlez, Lucjan S. Wyrwicz, Karol Bomsztyk and Jerzy Ostrowski

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2407 **Quantitative analysis of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced proteome alterations in 5L rat hepatoma cells using isotope-coded protein labels**
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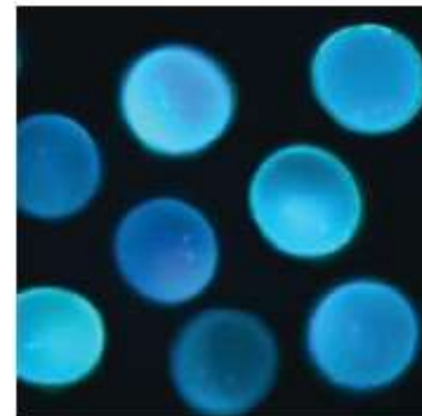
2422 **Proteomic approach to study the cytotoxicity of dioscin (saponin)**

Microbiology



2465 **Mapping and comprehensive analysis of the extracellular and cell surface proteome of the human pathogen *Corynebacterium diphtheriae***
Nicole Hansmeier, Tzu-Chiao Chao, Jörn Kalinowski, Alfred Pühler and Andreas Tauch

2477 **Shotgun identification of proteins from uredospores of the bean rust *Uromyces appendiculatus***
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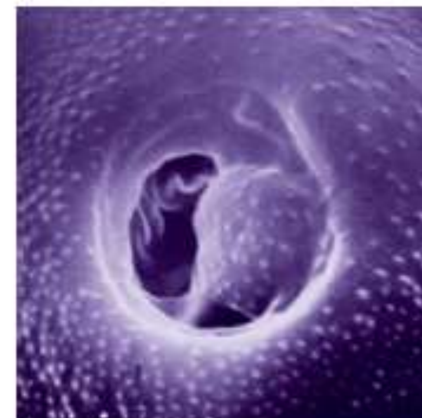
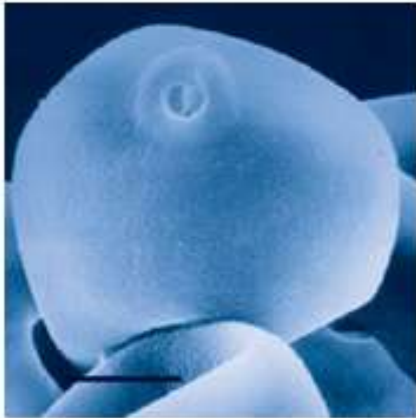
2485 **Proteins unique to intraphagosomally grown *Mycobacterium tuberculosis***
Jens Mattow, Frank Siejak, Kristine Hagens, Dörte Becher, Dirk Albrecht, Alexander Krah, Frank Schmidt, Peter R. Jungblut, Stefan H. E. Kaufmann and Ulrich E. Schaible

2495 **An important role of a "probable ATP-binding component of ABC transporter" during the process of *Pseudomonas aeruginosa* resistance to fluoroquinolone**
Jinsong Zhou, Dongyun Hao, Xudong Wang, Teimei Liu, Chengyan He, Feng Xie, Yanhong Sun and Jin Zhang





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2504 **Proteomic analyses of *Oryza sativa* mature pollen reveal novel proteins associated with pollen germination and tube growth**
Shaojun Dai, Lei Li, Taotao Chen, Kang Chong, Yongbiao Xue, Tai Wang

2530 **Proteomic analysis of shoot-borne root initiation in maize (*Zea mays* L.)**
Michaela Sauer, Andreas Jakob, Alfred Nordheim and Frank Hochholdinger

2542 **Effects of salinity levels on proteome of *Suaeda aegyptiaca* leaves**
Hossein Askari, Johan Edqvist, Mohsen Hajheidari, Mohammad Kafi and Ghasem Hosseini Salekdeh

2555 **Post-translational modifications, but not transcriptional regulation, of major chloroplast RNA-binding proteins are related to *Arabidopsis* seedling development**
Bai-Chen Wang, Hong-Xia Wang, Jian-Xun Feng, Da-Zhe Meng, Li-Jia Qu and Yu-Xian Zhu

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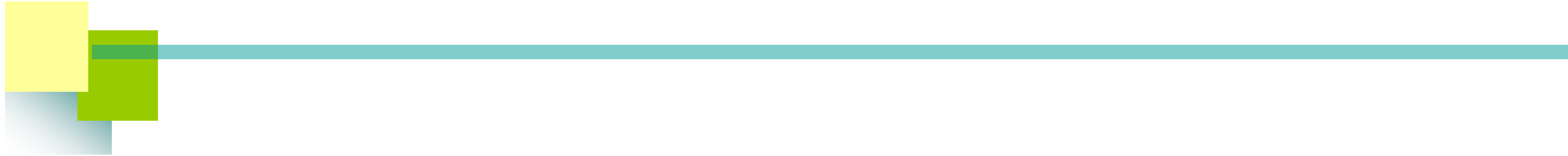

2-DE	=	Two-dimensional gel electrophoresis
ESI	=	Electrospray ionization
ICAT	=	Isotope coded affinity tag
EST	=	Expressed sequence tag
IPG	=	Immobilized pH gradient isolectric focusing gels
LC	=	Liquid chromatography
MALDI	=	Matrix-assisted laser/desorption ionization
MI	=	Myocardial infarction
MS	=	Mass spectrometry
MS/MS	=	Tandem mass spectrometry
NSCLC	=	Non-small cell lung cancer
PCR	=	Polymerase chain reaction
PMF	=	Peptide mass fingerprinting
SUMO-2	=	Small ubiquitin-related modified-2 protein