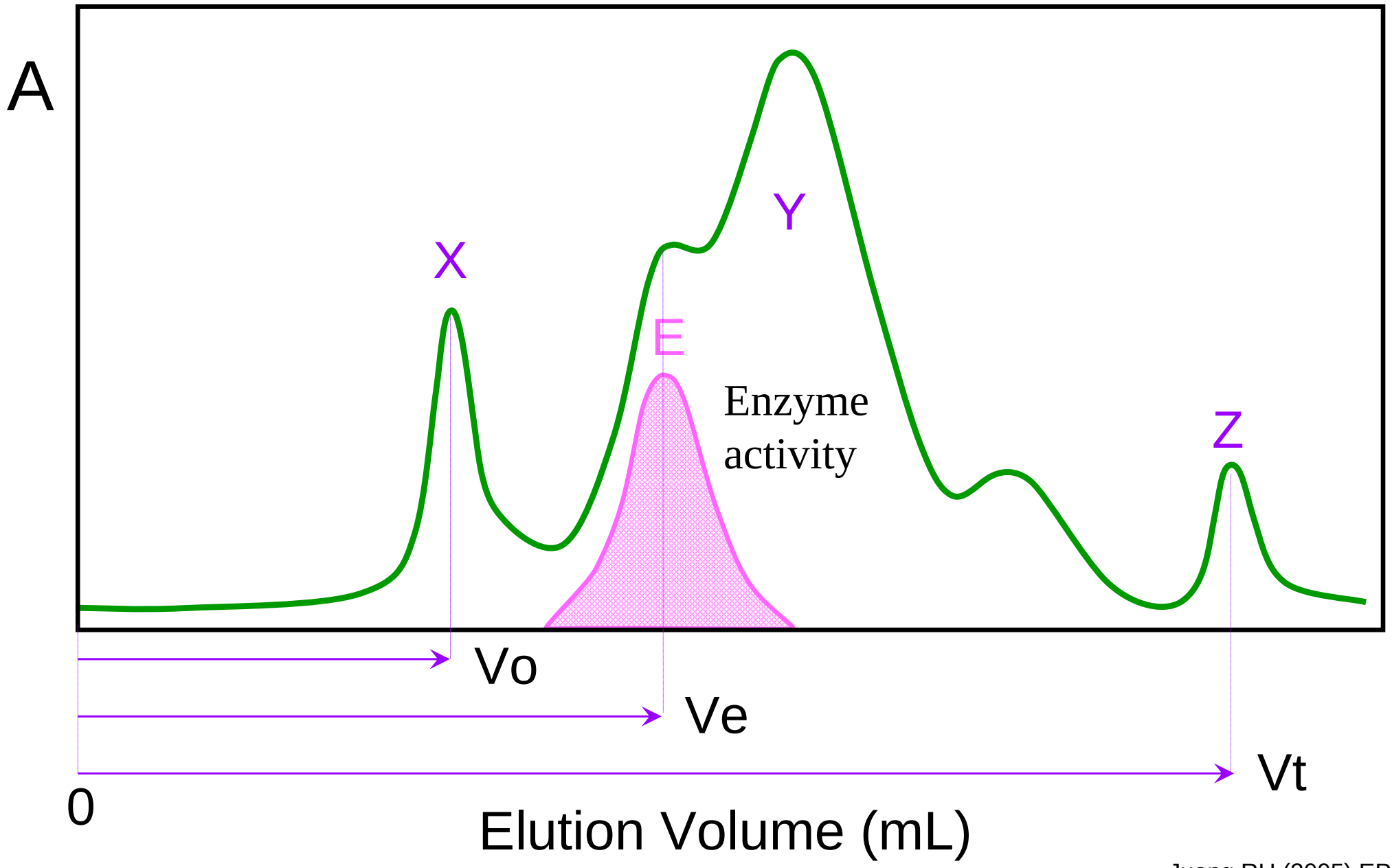
4 分子量決定法 Molecular weight determination

- 4.1 膠体過濾法 Gel filtration
  依蛋白質分子量的大小測定
- 4.2 梯度電泳法 Gradient PAGE
  可佐證分子量的測定
- 4.3 其它分子量測定方法 Other methods
  超高速離心法 Ultracentrifugation
  由胺基酸序列計算 Deduced for amino acid sequence
  質譜儀分析 Mass spectrometry

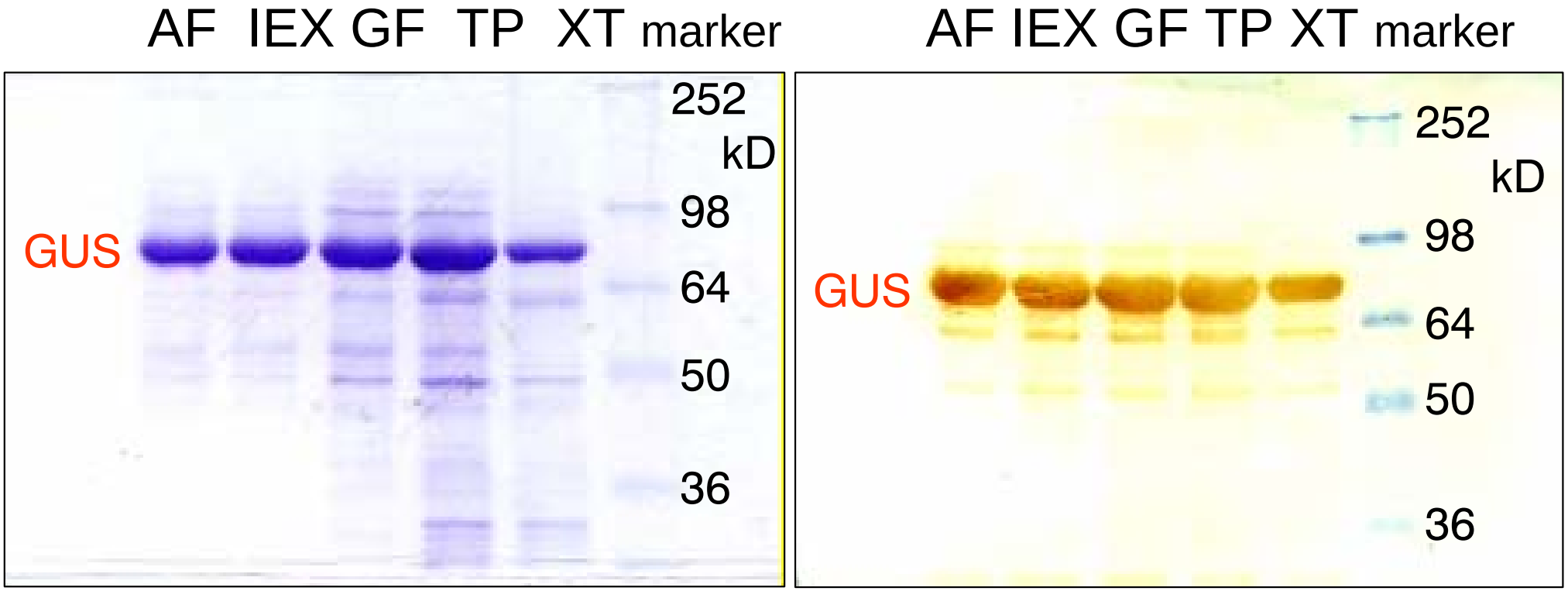
膠体過濾法依原態分子量分離 Native MW



Juang RH (2005) EPA

以電泳及抗體檢定表現蛋白質 GUS

← 純化步驟順序 Purification steps



10% SDS-PAGE

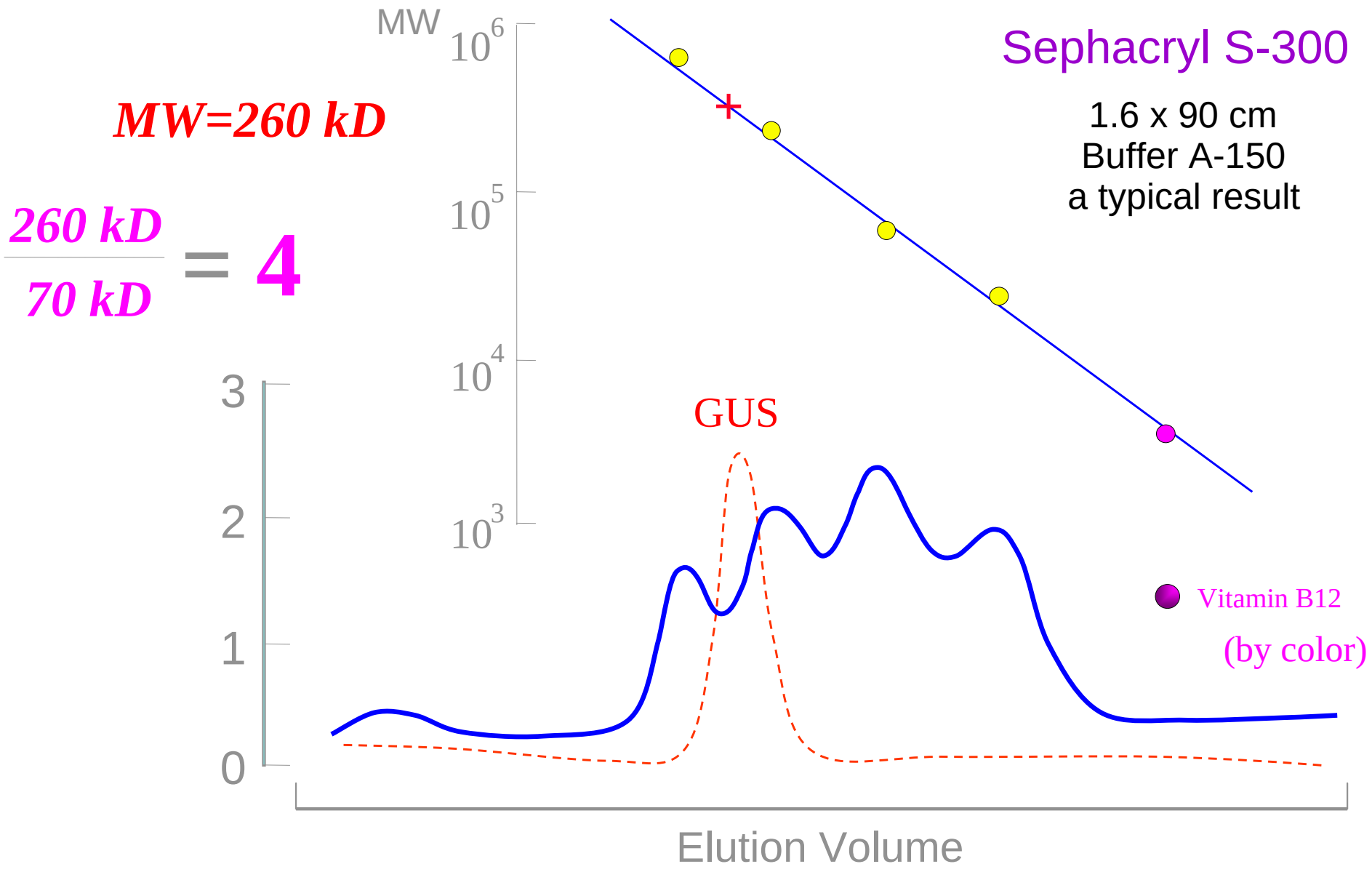
Western Transfer

MW = 70 kD

Expressed protein GUS is detected by SDS-PAGE and immunostaining

Juang RH (2005) EPA

以膠體過濾法求得 GUS 原態分子量



MW=260 kD

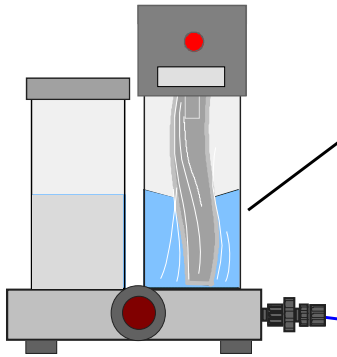
$$\frac{260\ kD}{70\ kD} = 4$$

Native molecular weight of GUS is determined by gel filtration

Juang RH (2005) EPA

梯度電泳片的製備 Prepare the gradient gel

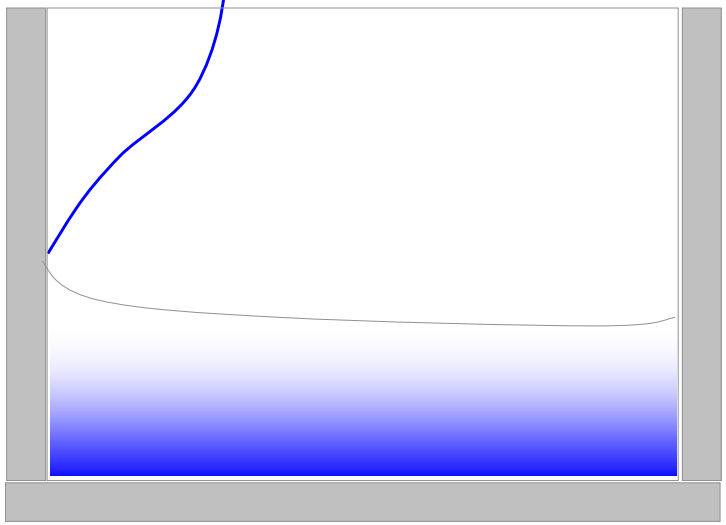
Gradient mixer



若在高限溶液中
加入少量染劑
Blue dye added in the upper limiting solution

5% 20%

Upper-limiting solution



由下方開始
注入梯度溶液
Start the gradient from the bottom

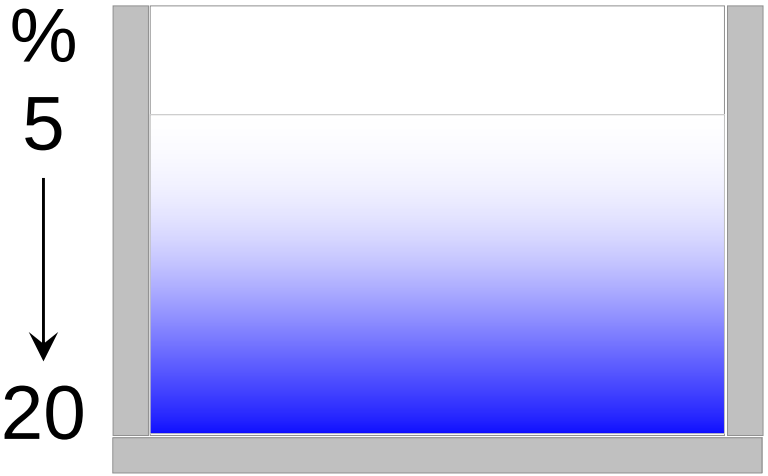
Determine MW by native-PAGE

(1) Sample protein pI < 8.0

(2) Use gradient gel

(3) Longer running time

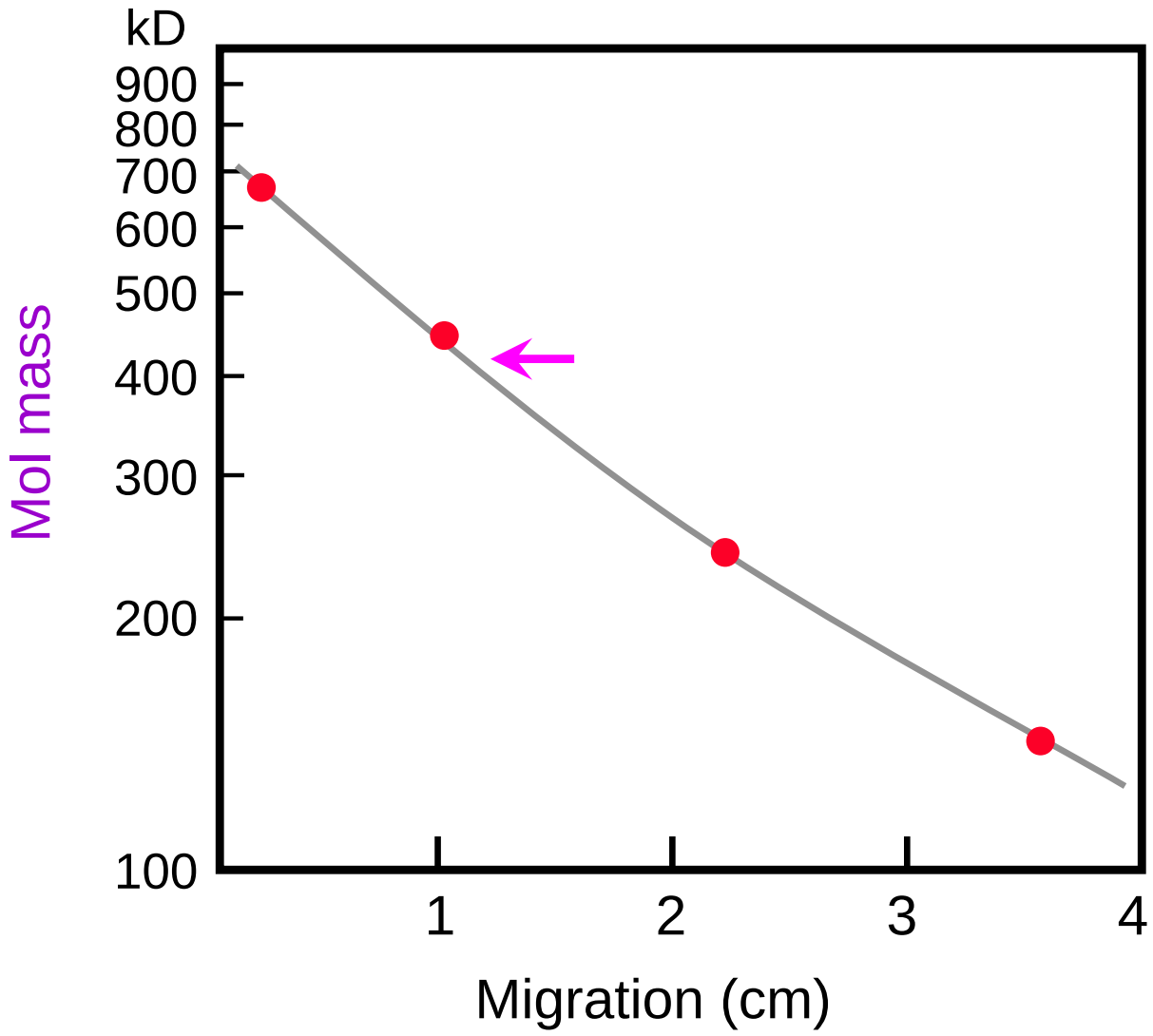
可觀察所拉梯度是否均勻
The blue color shows how the gradient formed in the gel

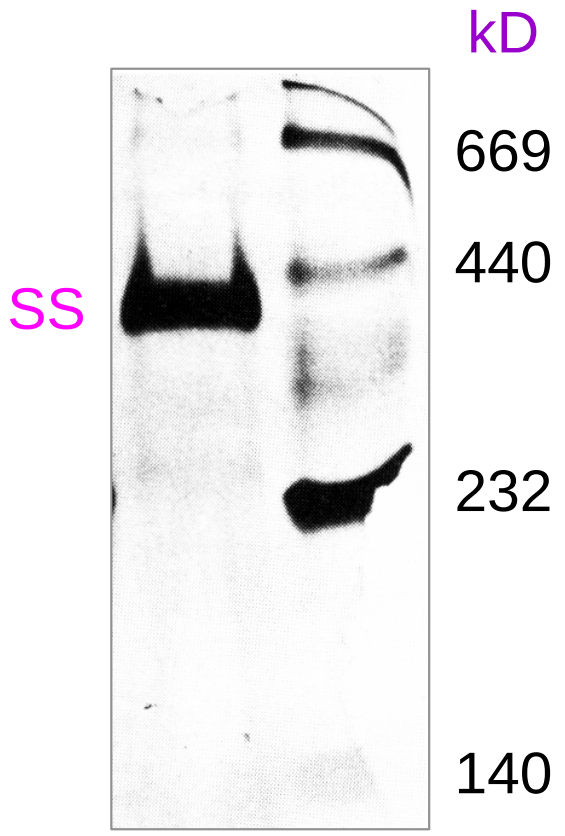


You can use commercial pre-cast gradient gel (various gel percentage)

Juang RH (2005) EPA

原態分子量 Gradient PAGE for MW determination



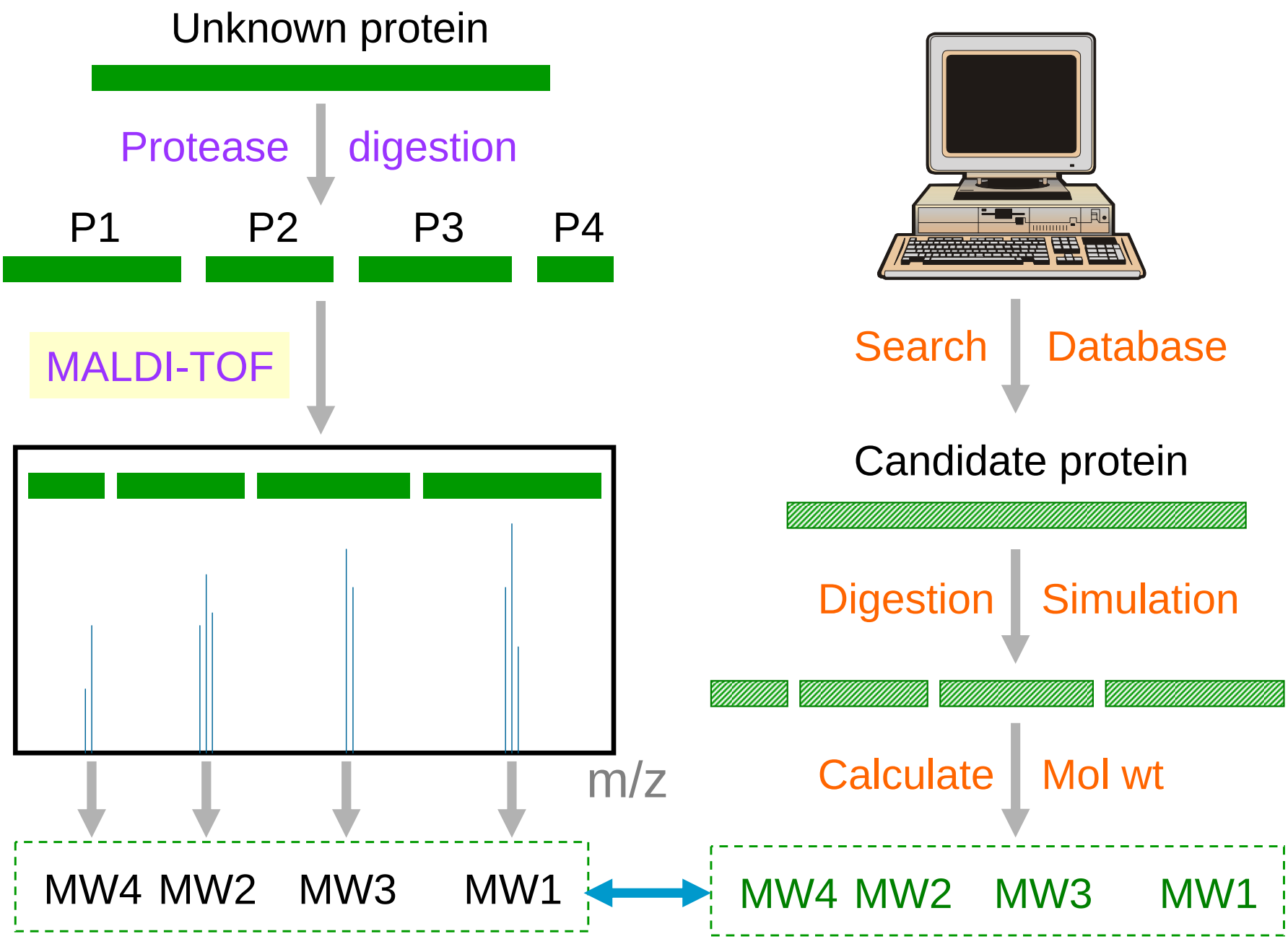


- 不能以 disc-PAGE 為唯一分子量證據

You can't take disc-PAGE as your only evidence for MW determination

Juang RH (2005) EPA

質譜儀可檢定蛋白質身分

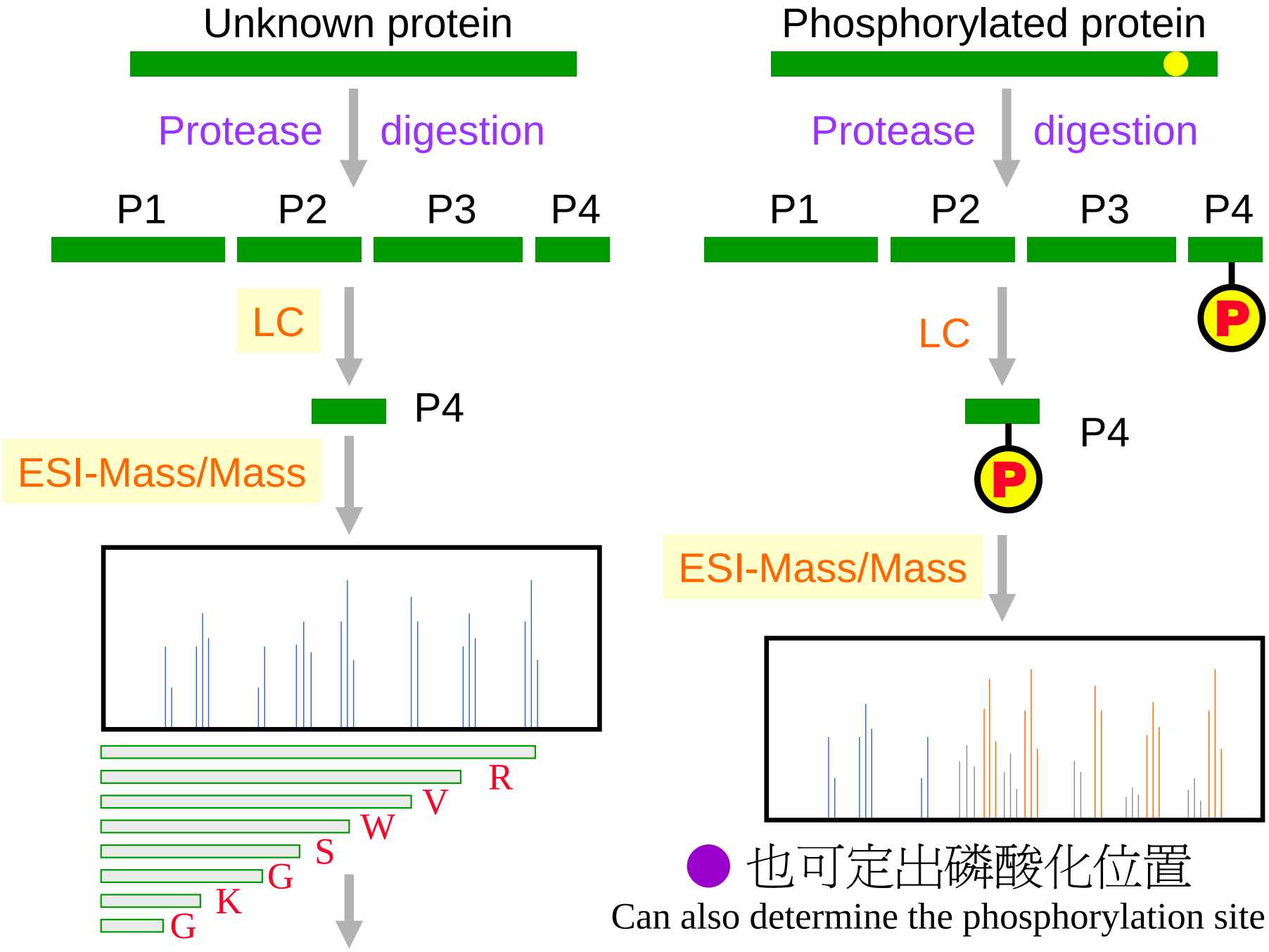


● 比對各片段分子量可確定該蛋白質身分

Tryptic fragments identified by MALDI-TOF could identify an unknown protein

Juang RH (2005) EPA

以質譜儀進行蛋白質序列分析



● 也可定出磷酸化位置

Can also determine the phosphorylation site

● 質譜儀可直接定序

Determine the amino acid sequence by LC/MS/MS

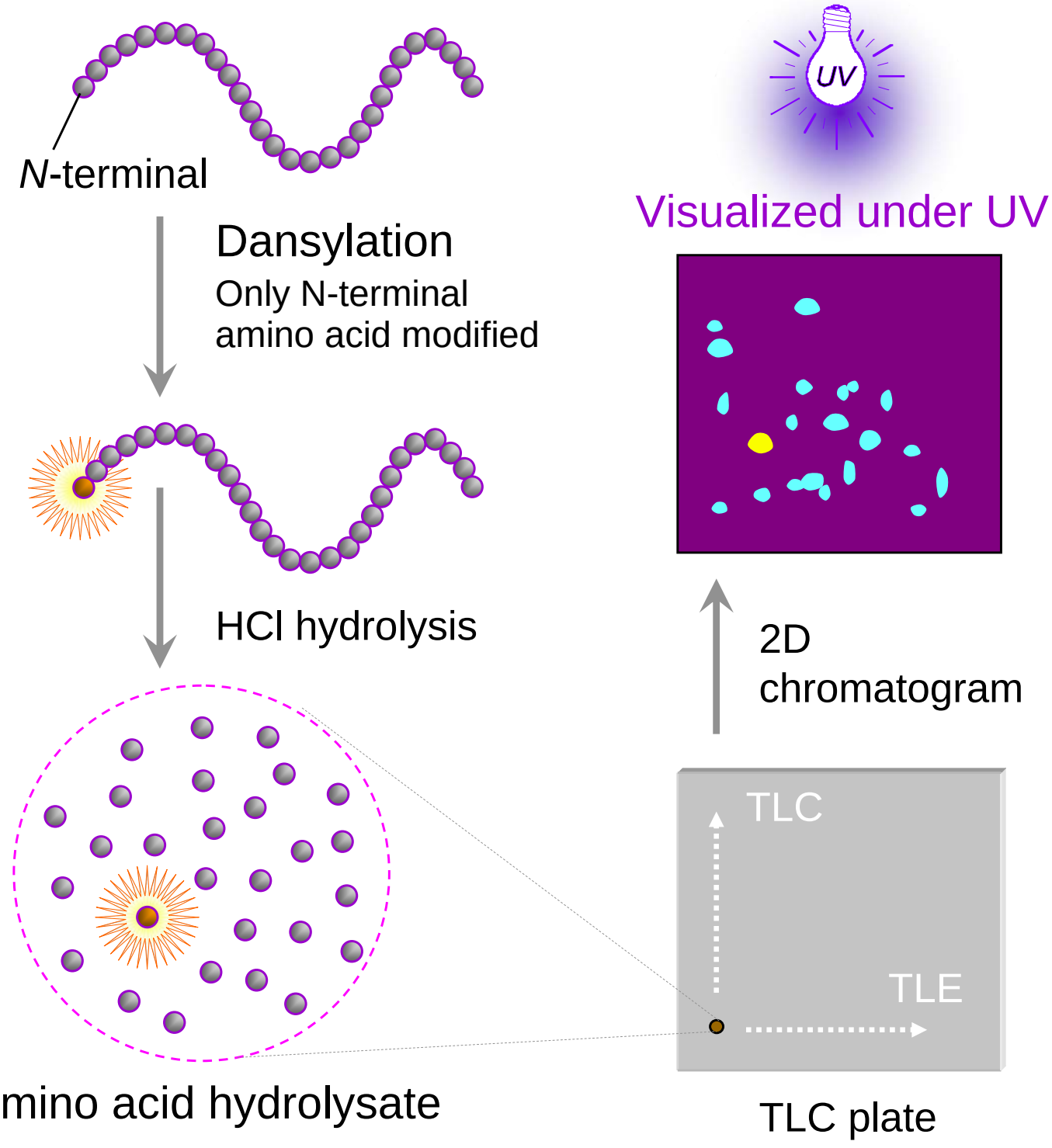
Juang RH (2005) ECX

5 蛋白質構造與組成分析 Protein structure analysis

- 5.1 N-端或 C-端胺基酸 Terminal determination
  通常都直接定序但 C-端較爲困難
- 5.2 胺基酸組成分析 Amino acid composition
- 5.3 胺基酸定序法 Amino acid sequence
  - 5.3.1 From cDNA sequence
  - 5.3.2 Edman degradation or ESI/MS/MS
- 5.4 胜肽圖譜 Peptide mapping
- 5.5 其它相關方法 Other methods

Some old techniques become very useful in modern proteomic study

決定N端胺基酸



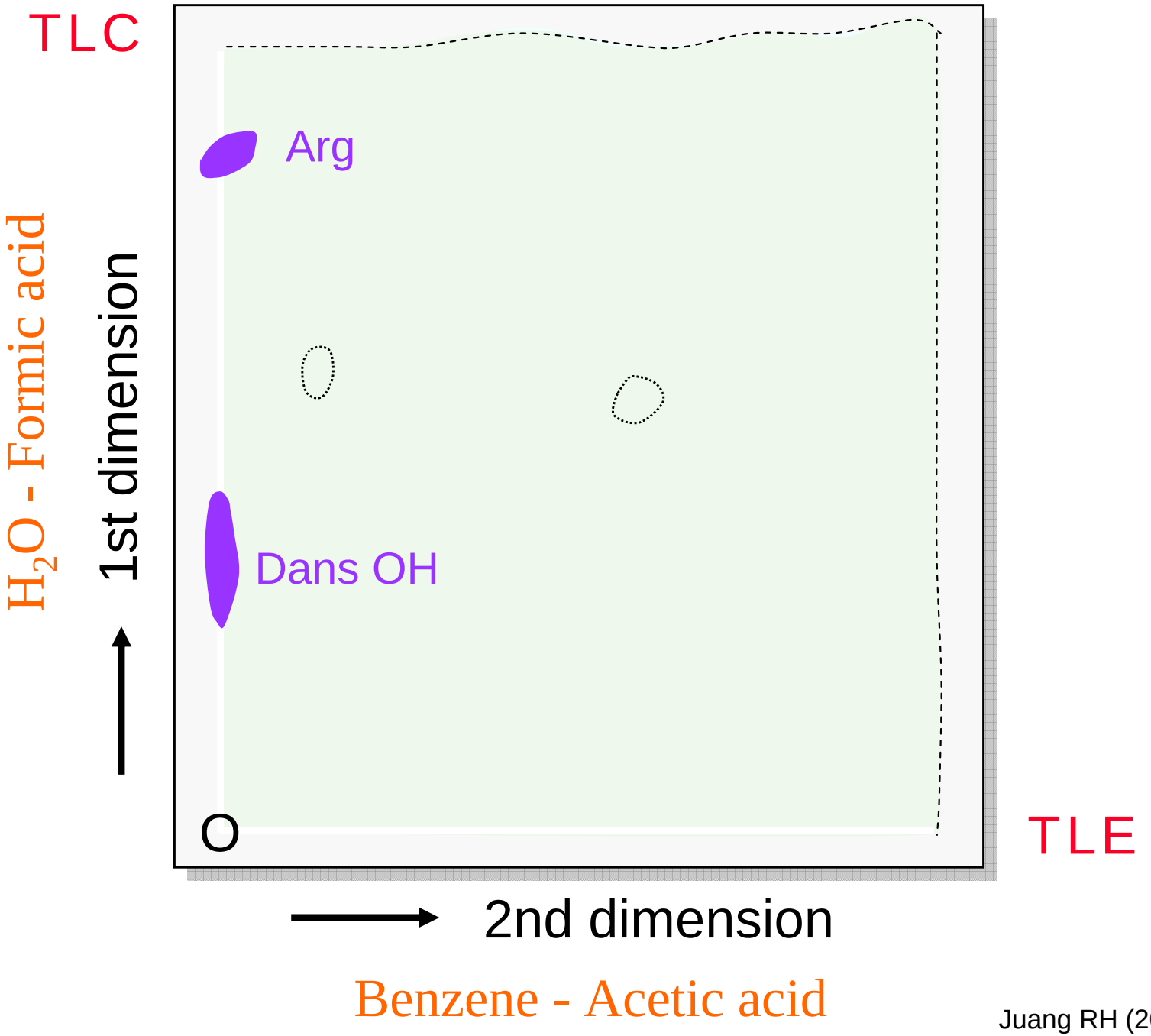
Determine the N-terminal amino acid by dansylation

Juang RH (2005) EPA

以薄層層析法鑑定胺基酸

- 二次元薄層層析電泳可分離並檢定二十種胺基酸

20 amino acids are separated and identified on 2D TLC/TLE



Juang RH (2005) EPA

蛋白質酸性水解 Total acid hydrolysis of protein

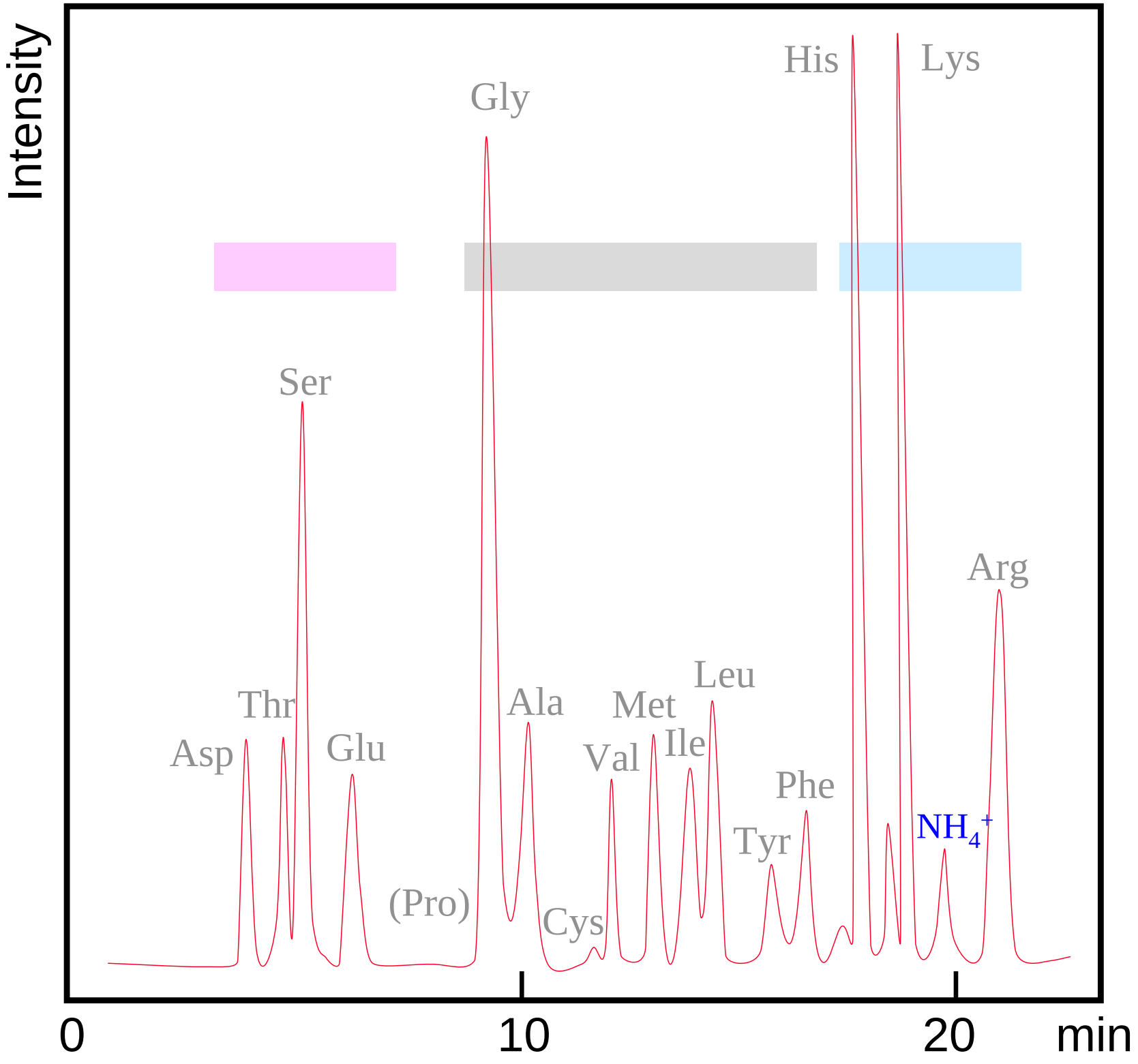
- Reagent: 6 N HCl or 4 N methanesulfonic acid
- Condition: 110C, 24 hours, under vacuum
- Detection: by HPLC (ion exchange) next slide
- Notice: Some amino acids are destroyed (Trp)

Cys-Cys broken to Cys

Gln & Asn are acidified to Glu & Asp

(Glu + Gln → Glx; Asp + Asn → Asx)

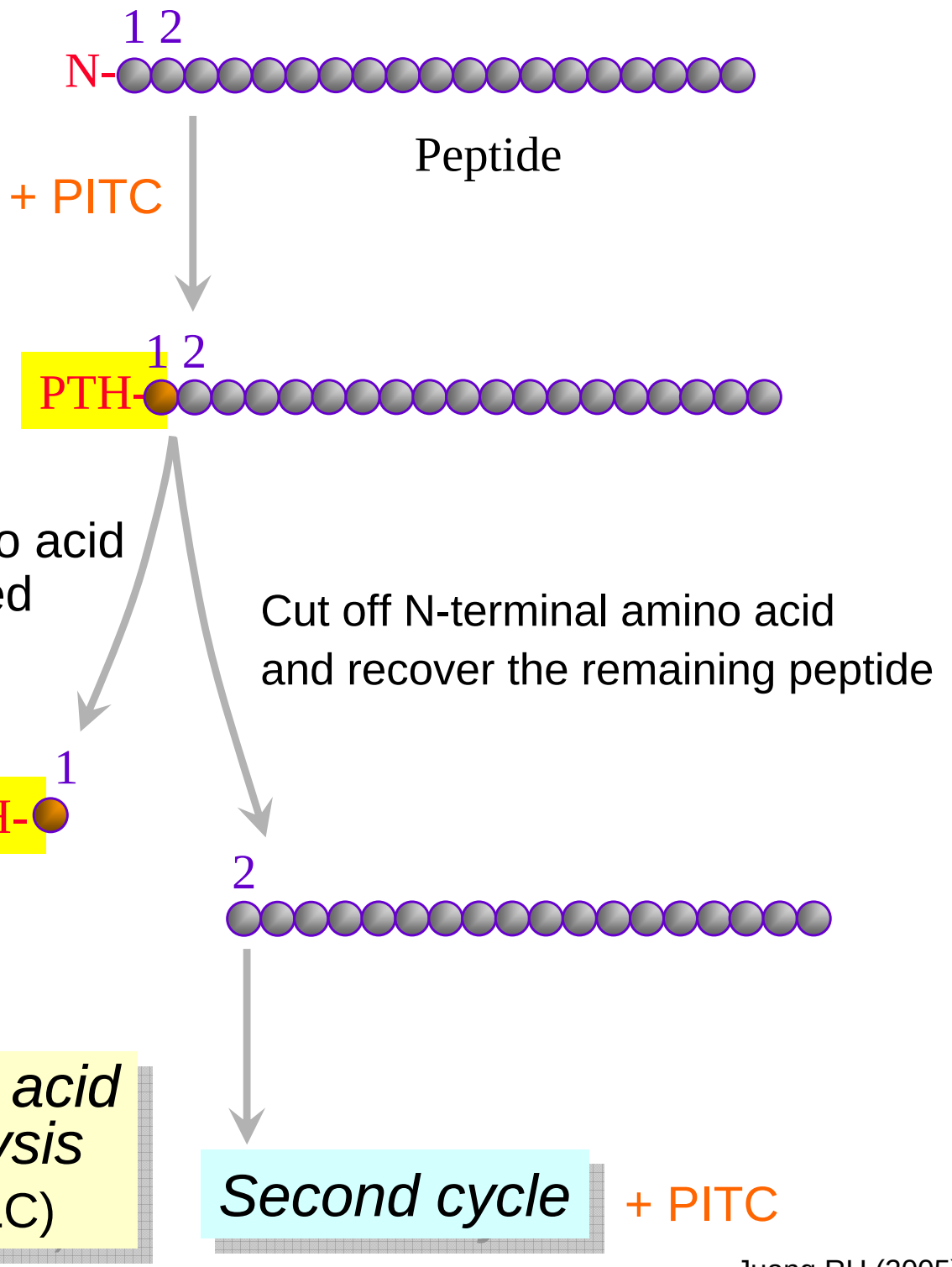
以液相層析分離鑑定胺基酸



Juang RH (2005) EPA

決定胺基酸序列

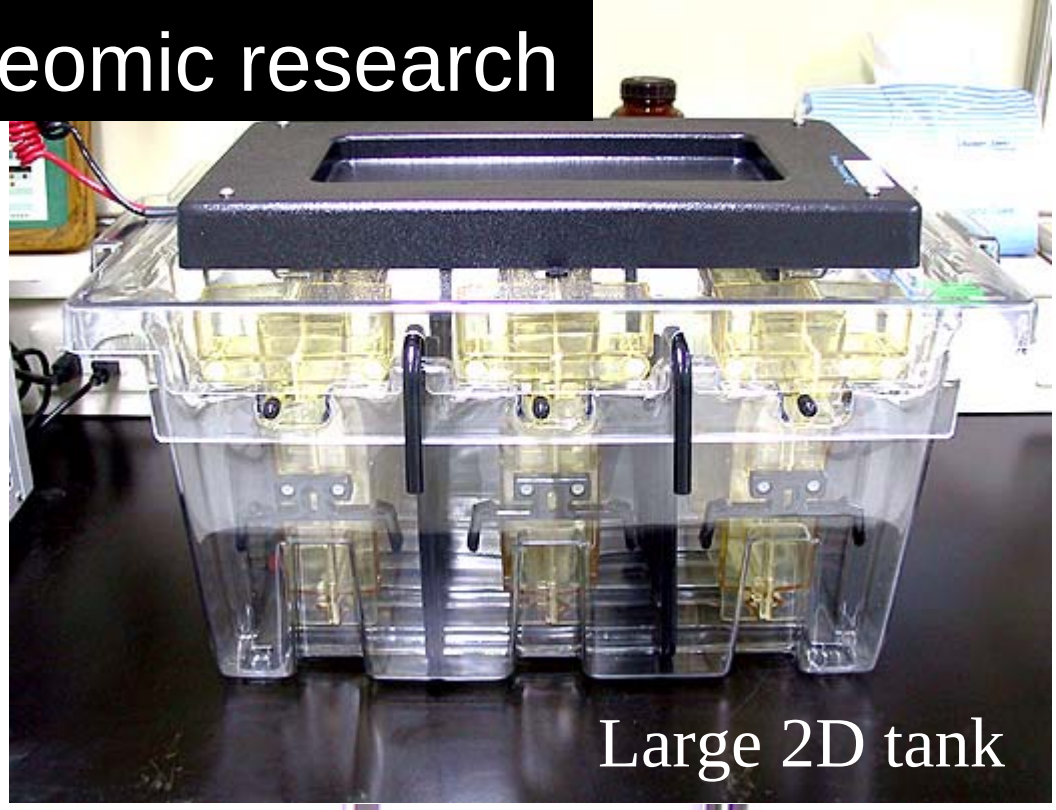
Edman Degradation
for amino acid sequencing



Juang RH (2005) EPA

Tools for proteomic research

LC/MS/MS



Large 2D tank

Amino acid sequence analyzer

Juang RH (2005) EPA

5.4 胜肽圖譜 Peptide mapping

5.4.1 蛋白質專一性水解 Specific proteolysis

- 專一性內切酶 Specific *endo*-peptidase

  Trypsin, Chymotrypsin, *Sa* protease

- 化學反應法 Chemical method

  CNBr

5.4.2 胜肽群檢定方法 Identify peptides

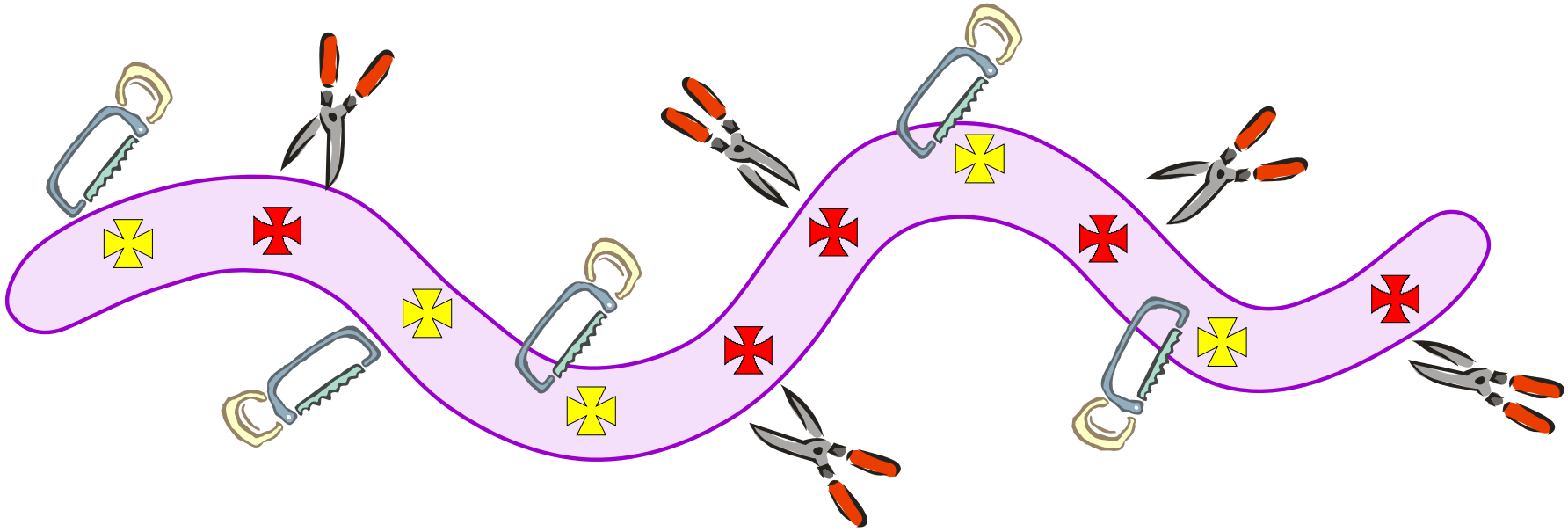
TLE/TLC HPLC SDS-PAGE

Peptide mapping has become a very important tool in proteomic study

蛋白質的專一性水解 Specific proteolysis

使用專一性蛋白酶
Use specific endo-protease

變性蛋白質
Protein denatured



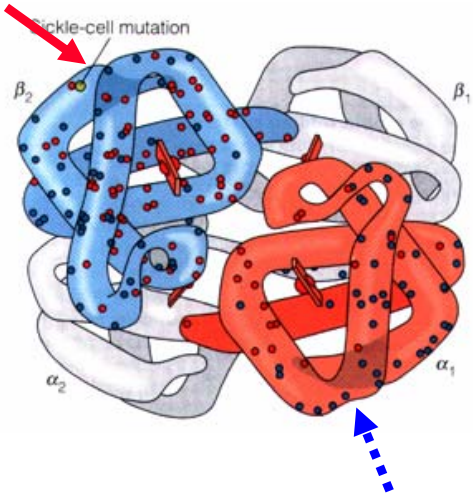
Specific protease can generate a set of fixed peptides for every protein

以雙向層析電泳鑑定胜肽

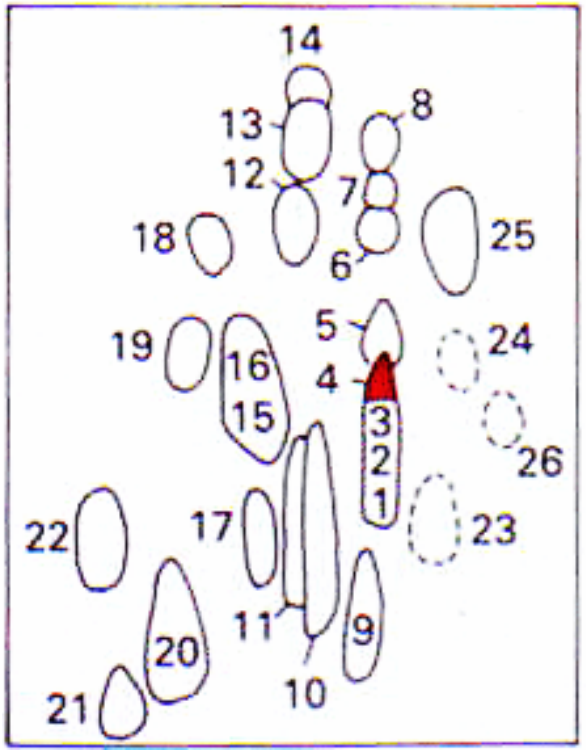
Hemoglobin A

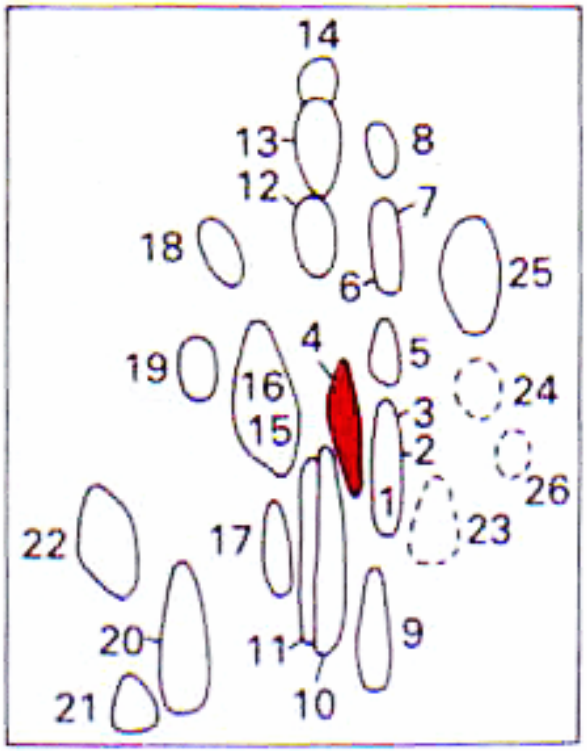
Hemoglobin S

鐮型血球
Sickle cell



血紅蛋白四號片段

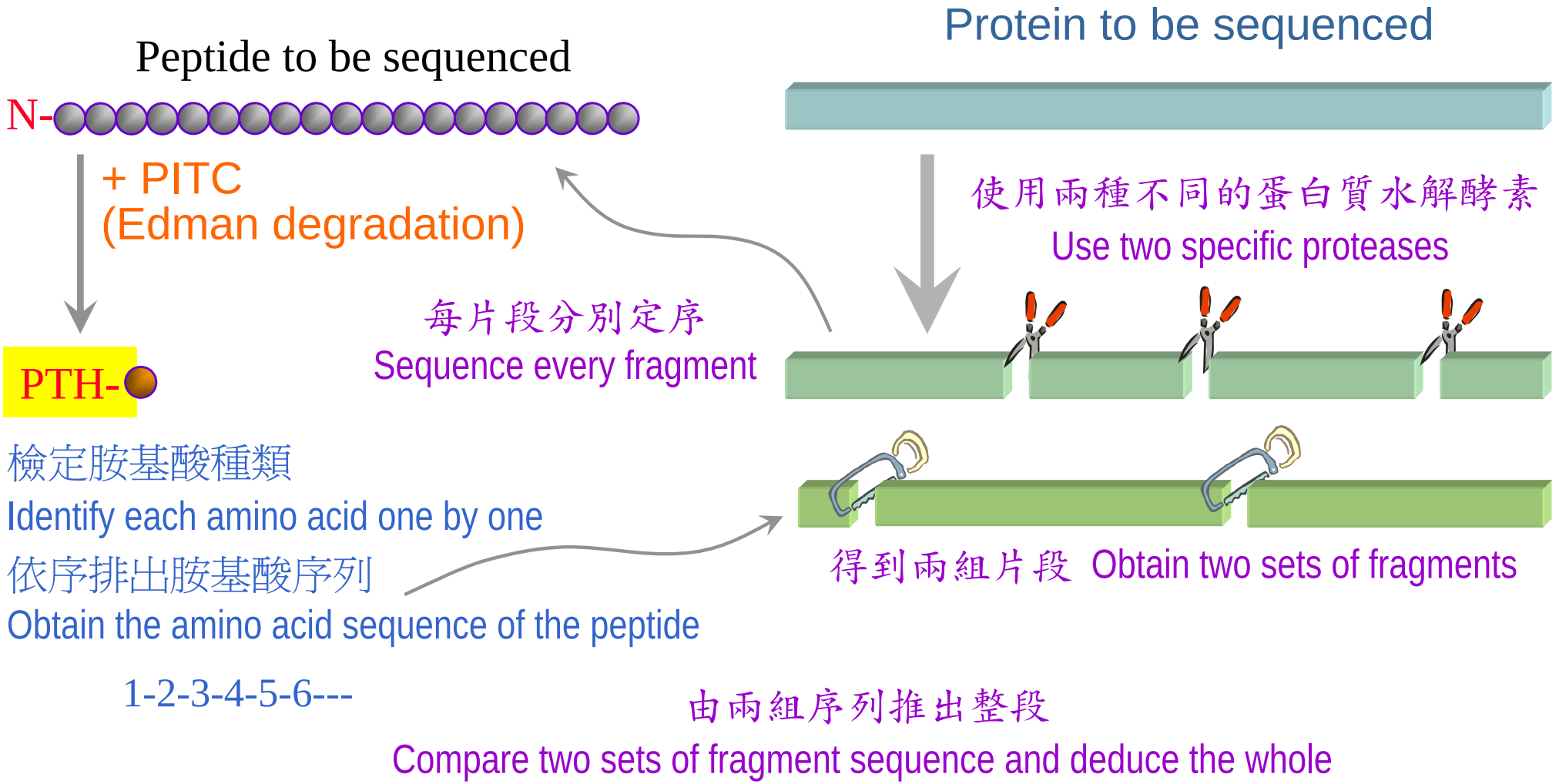




色析 TLC

電泳 TLE

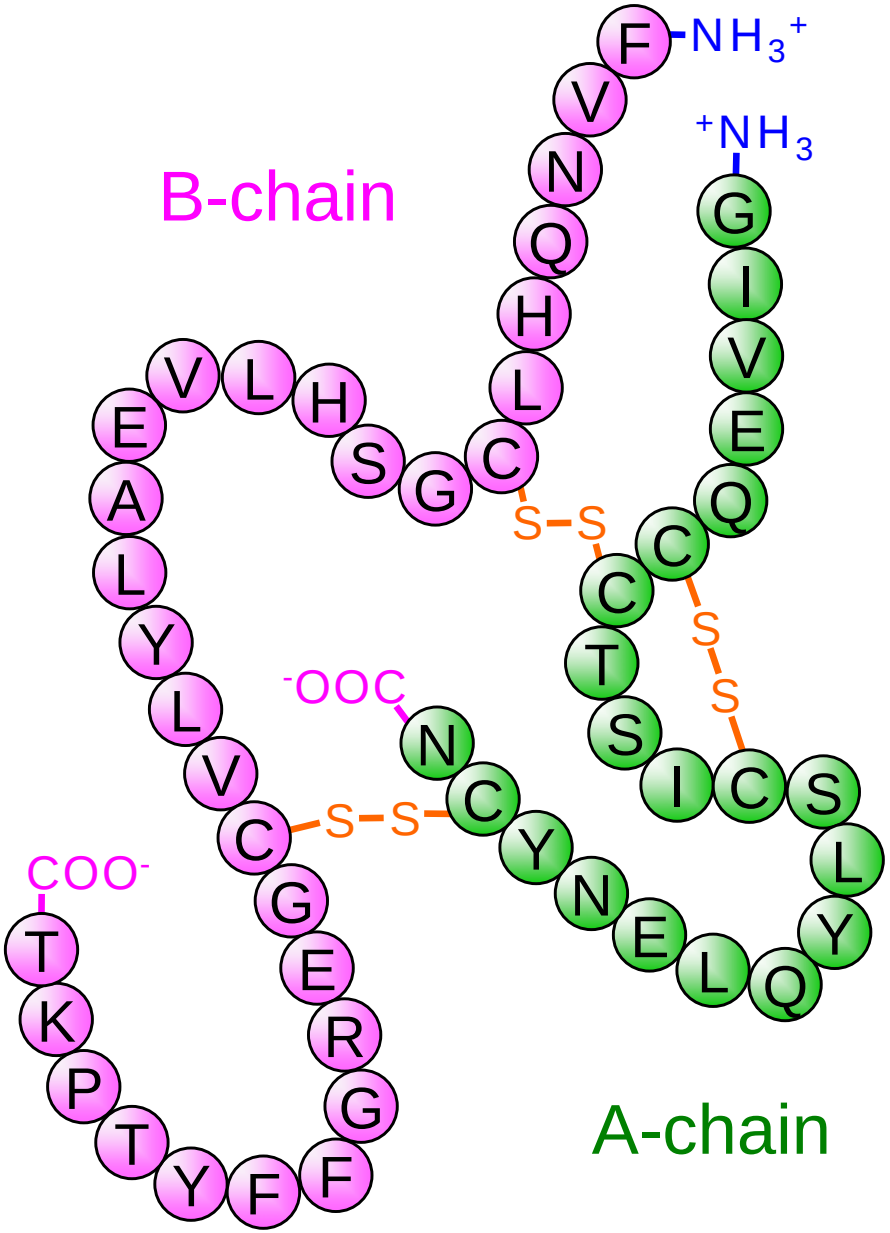
以傳統胺基酸定序法決定蛋白質序列



The traditional protein sequencing method using Edman degradation

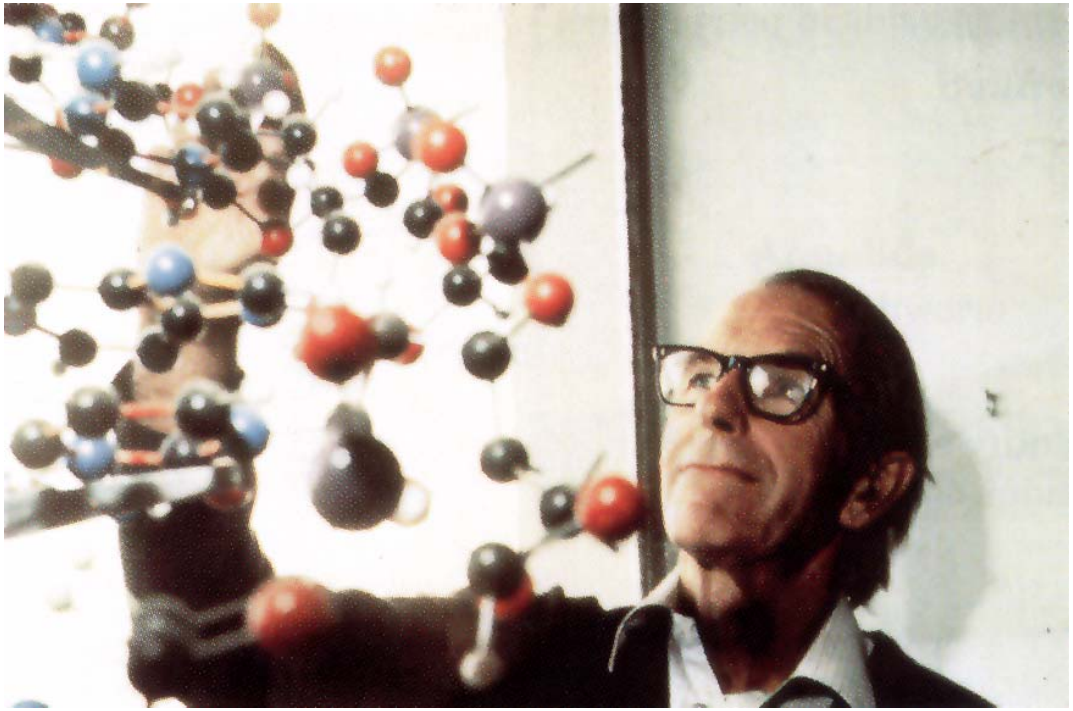
Juang RH (2005) EPA

以傳統胺基酸定序法決定蛋白質序列



F. Sanger (1958, Cambridge U)

Insulin 胰島素 (A, B chains)



Nelson & Cox (2000) Principles of Biochemistry (3e) p.142

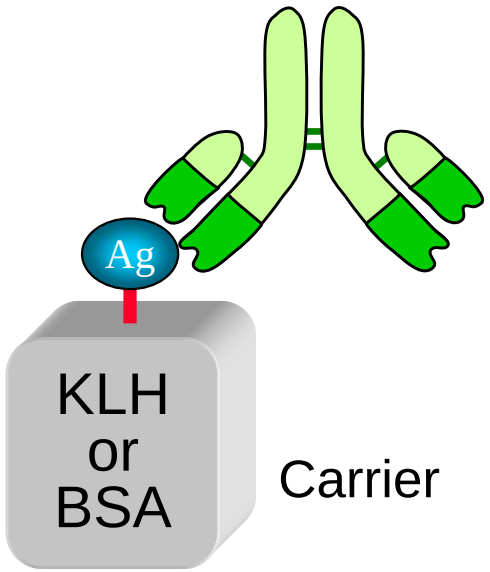
6 免疫學工具的利用 Immunological tools

- 6.1 抗原製備 Antigen preparation
- 6.2 免疫流程 Immunization protocol
- 6.3 抗體製備 Antibody preparation
- 6.4 抗體的應用 Antibody application

Juang RH (2005) EPA

抗原的種類 Antigen origin

- 巨分子抗原 Macromolecules
  - Protein, polysaccharide, nucleic acid
- 小分子抗原 Small molecules
  - Conjugated to carrier before immunization
- 半抗原 (hapten) aflatoxin, citrinin
  - Carrier is required
- 人工合成胜肽 Synthetic peptides
  - Carrier is required
  - Produce monospecific Ab

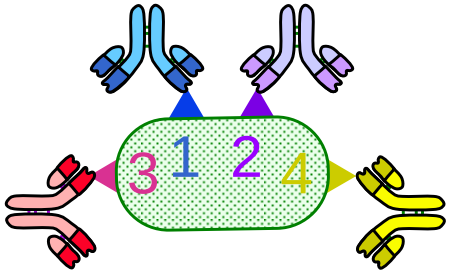


KLH: keyhole limped haemocyanin (貝類血青素蛋白); BSA: bovine serum albumin

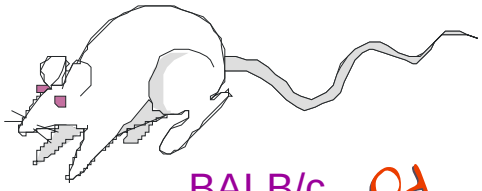
Juang RH (2005) EPA

基礎免疫學 Essential immunology

- 免疫系統： 先天及後天免疫系統
  Immune systems (innate and adaptive)
- 免疫反應： 遭遇→動員→掃蕩→休止
  Immune response (four stages)
- 抗体分子： 有兩個專一性抗原結合區
  Antibody molecule (two specific binding sites)
- 單株抗体： 只對其專一性抗原基作用
  Monoclonal antibody (very specific reagent)

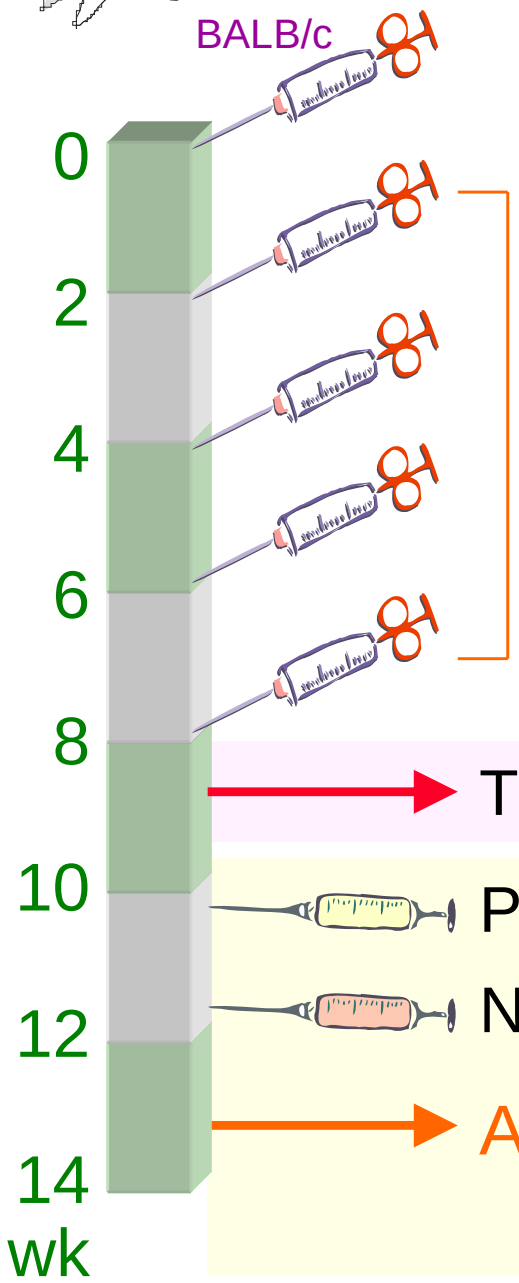


小白鼠免疫流程



BALB/c

加佐劑製成乳劑
+ adjuvant → emulsion



Antigen (50 μg/mouse)
Emulsified in 0.5 mL
Freund's Complete Adjuvant

At least three booster shots,
same dose in 0.5 mL
Freund's Incomplete Adjuvant

Booster shots might be reduced
if TiterMax is use as adjuvant

Trial Bleeding → Titer Determination

全採血

Total Bleeding, < 1 mL

Pristane (0.5 mL)

NS-1 Cell (10^6 cells)

Ascites Fluids

腹水 X mL (X = 1-10)

The immunization protocol for antiserum or ascites production

Juang RH (2005) EPA

免疫球蛋白純化流程

Ascites or serum (X mL)

↓ spin down cells (discard)

↓ + 2X mL PBS

↓ ammonium sulfate (AS) fractionation 0~40% sat.

↓ spin down pellet

Pellet

↓ resuspended in 40% AS

↓ spin down pellet

Pellet

↓ dissovled in X mL PBS

↓ dialysis in PBS three changes

↓ spin down precipitate

Supernatant

↓ + glycerol (equal volume)

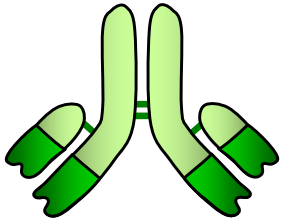
IgG (stored in freezer)

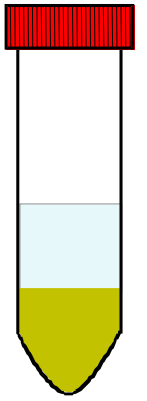
沉澱 Precipitation

清洗 Washing

透析 Dialysis

保存 Stock





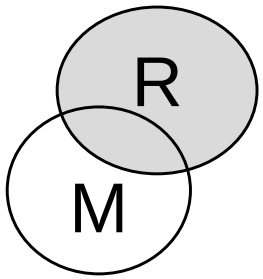
Juang RH (2005) EPA

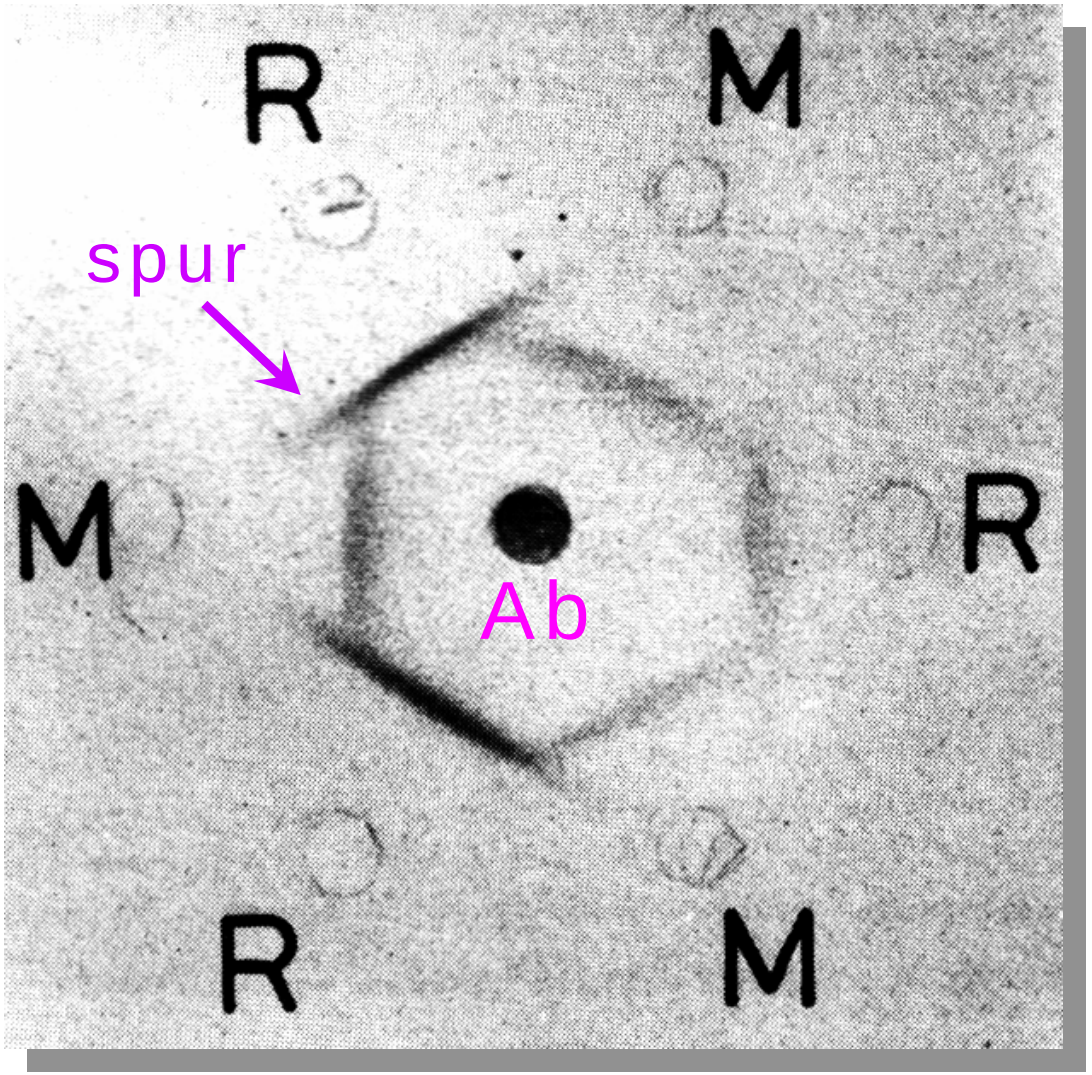
6.4 抗體的應用 Applications of Ab

- **轉印及免疫染色法** Western blot & immunostaining
  - 應用最廣 最有效率
- **免疫沉澱法** Immunoprecipitation (pull-down)
  - 另一種檢定專一性抗原的方法
- **親和層析法** Affinity chromatography
  - 最快速有效的純化方法
- **雙向免疫擴散法** Double diffusion
  - 古老但仍有其特色及應用
- **酵素免疫分析法** Enzyme immunoassay
  - 可分析大量樣本 (ELISA)
- **抗體晶片** Antibody chip
  - 專一快速地同時進行多種分析

雙向免疫擴散法

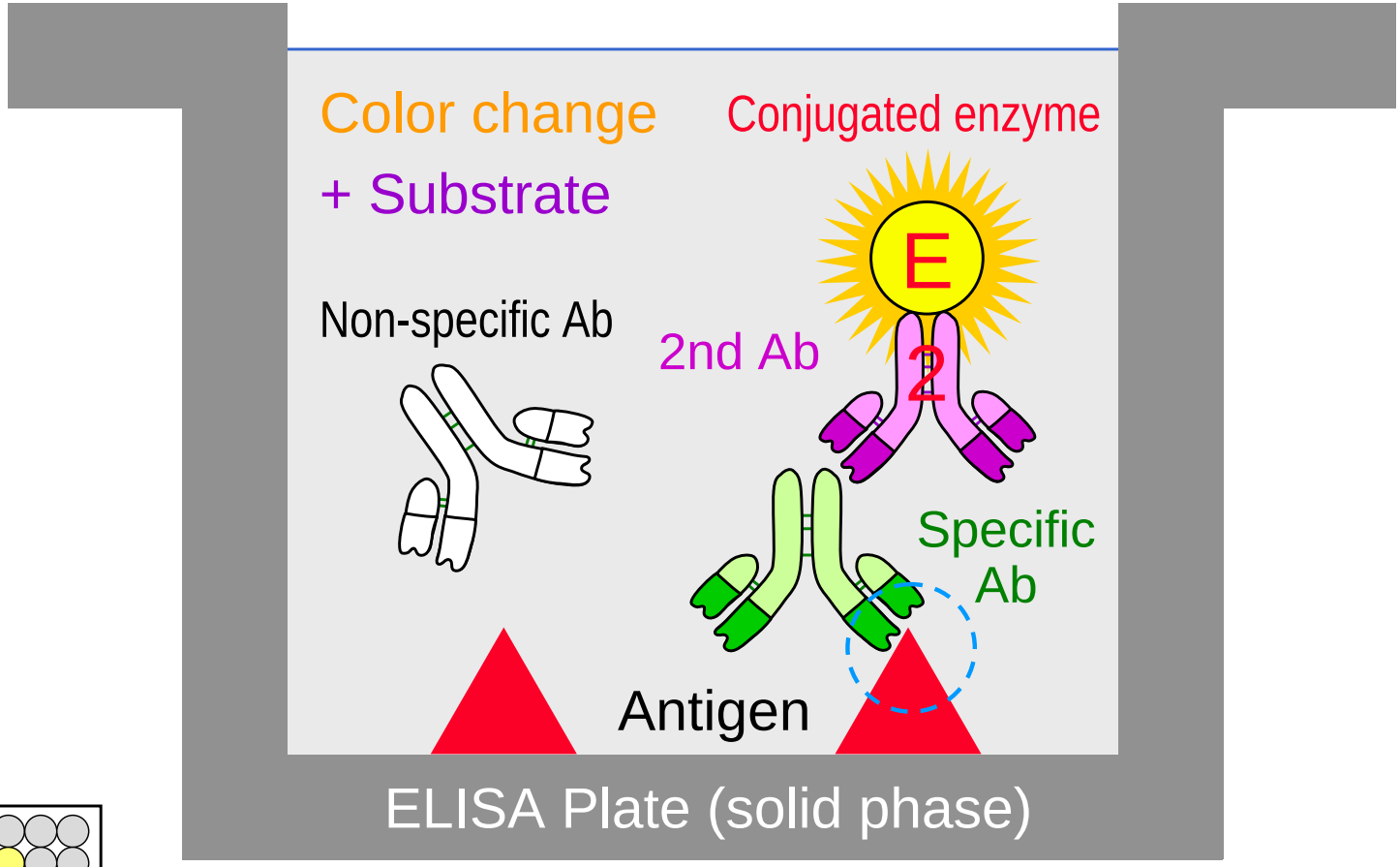
Outer wells: Rice (R) and maize (M) sucrose synthase (Ag)
Central well: Antiserum against rice sucrose synthase (Ab)

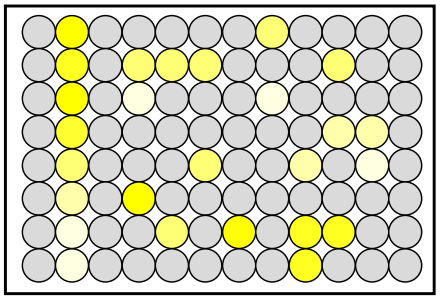




由沉澱線交叉情形得以推測抗原分子間的關係
The crossing-over of the precipitin lines reveals the structural relationship between the antigen molecules

酵素免疫分析法 ELISA

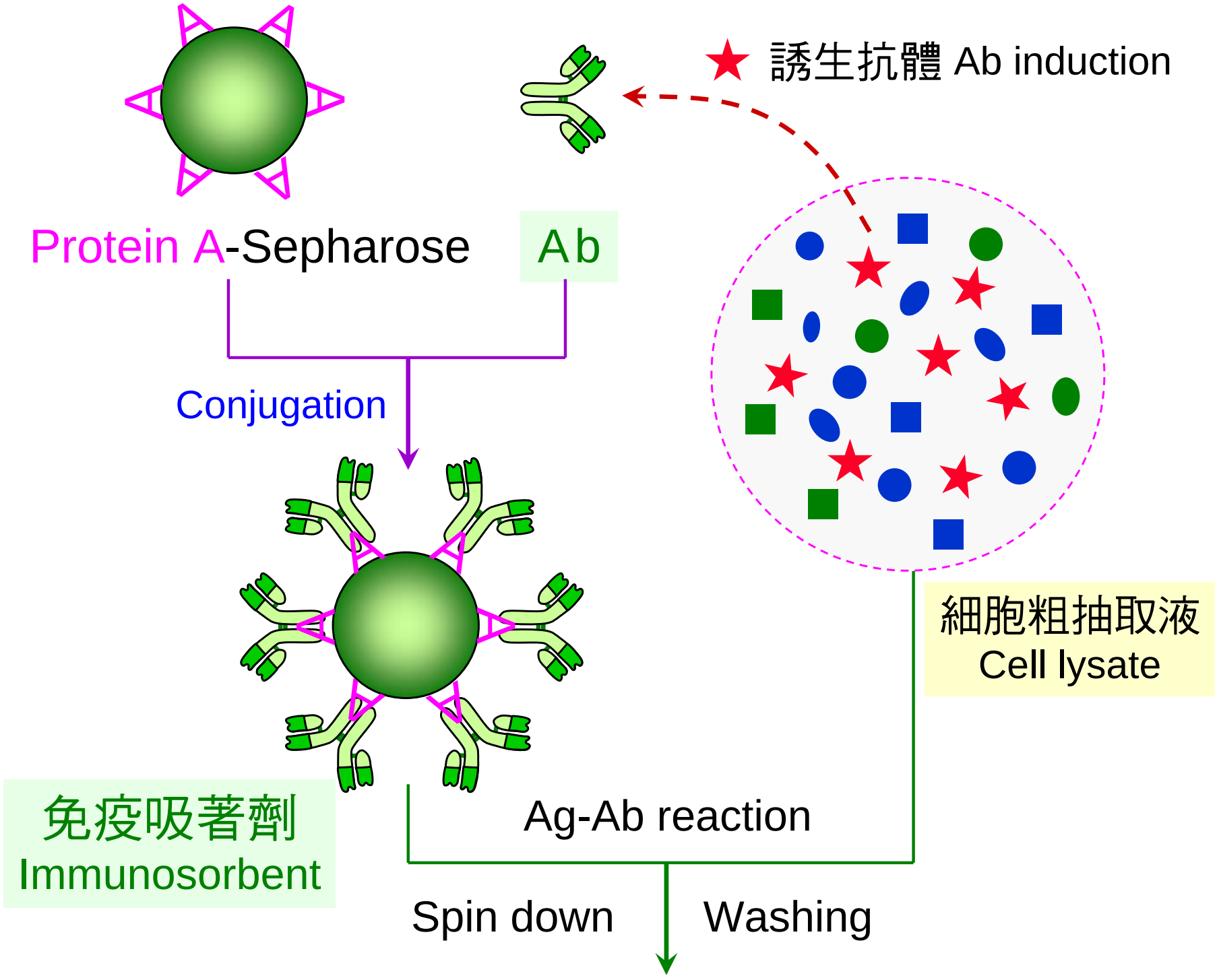




To detect the Ab in the sample

Juang RH (2005) EPA

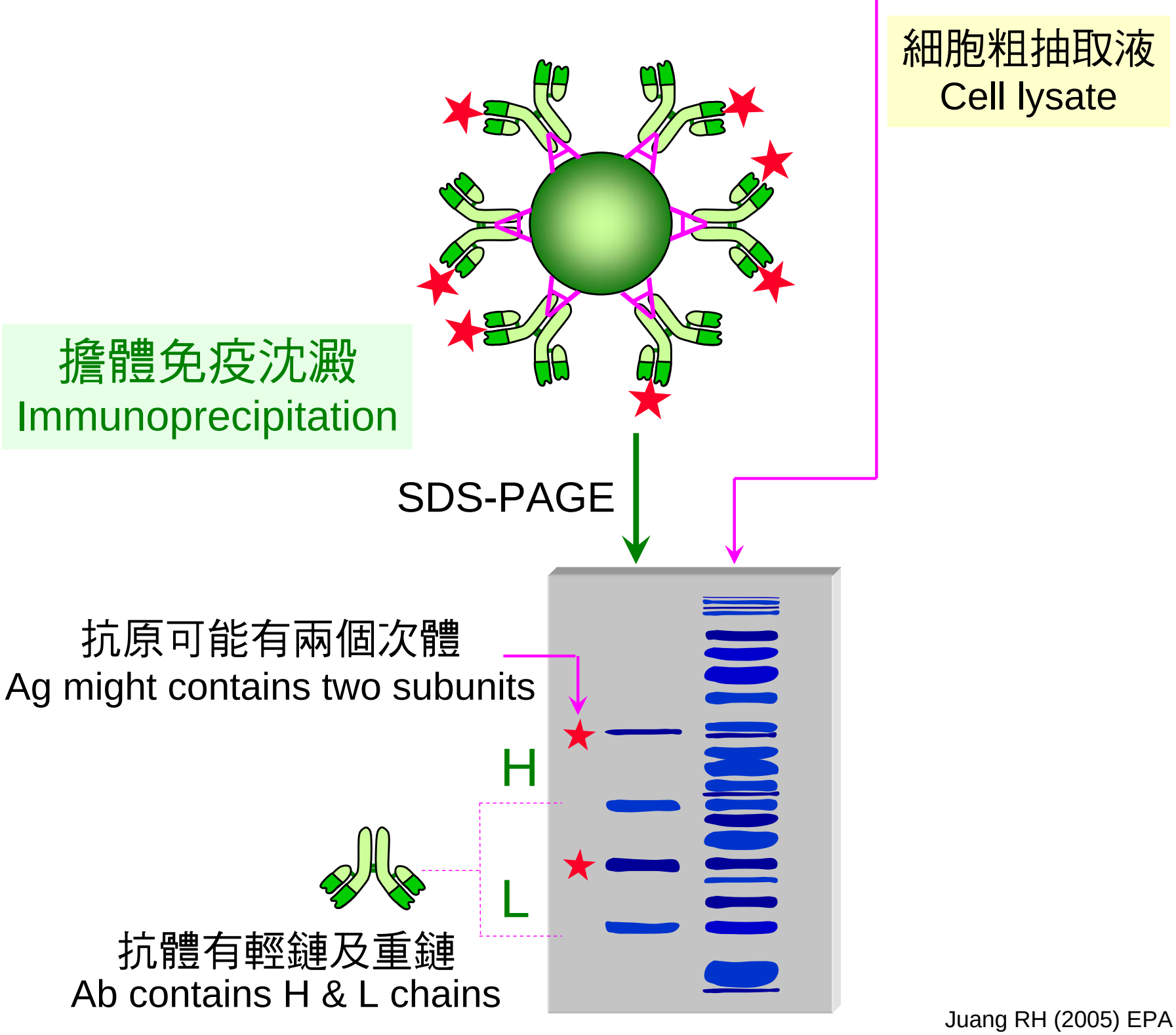
擔體免疫沈澱的原理及應用



Solid support-based immunoprecipitaion

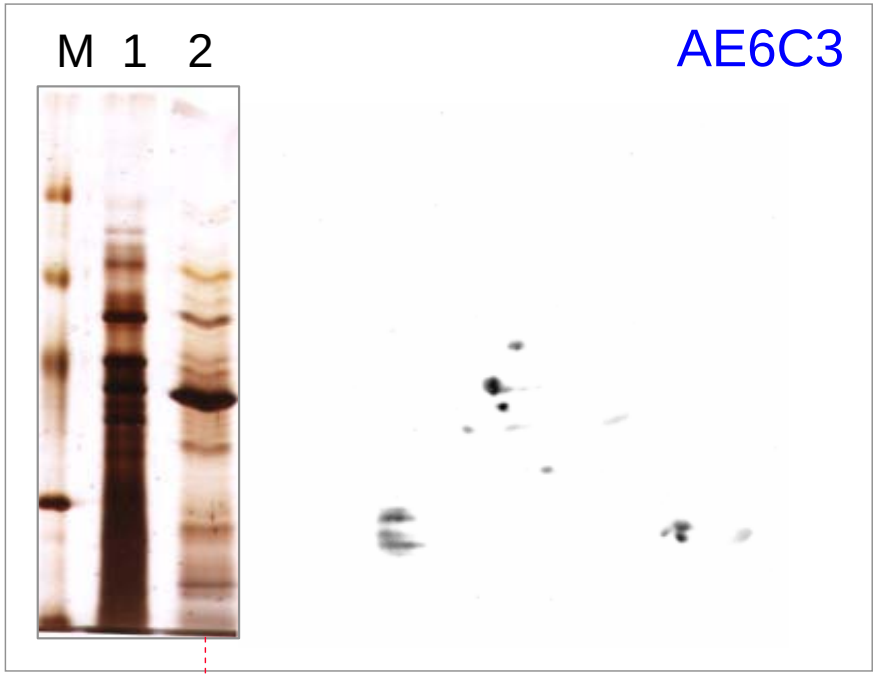
Juang RH (2005) EPA

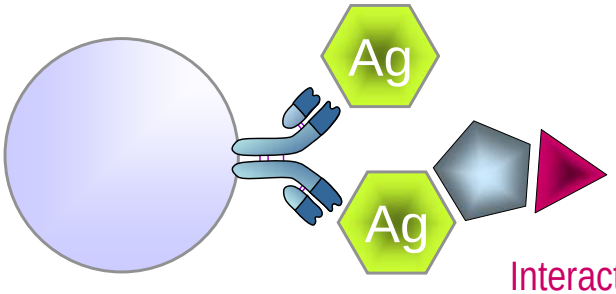
擔體免疫沈澱的原理及應用

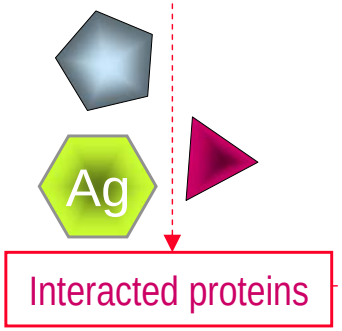


Juang RH (2005) EPA

抗體免疫沈澱與蛋白質交互作用







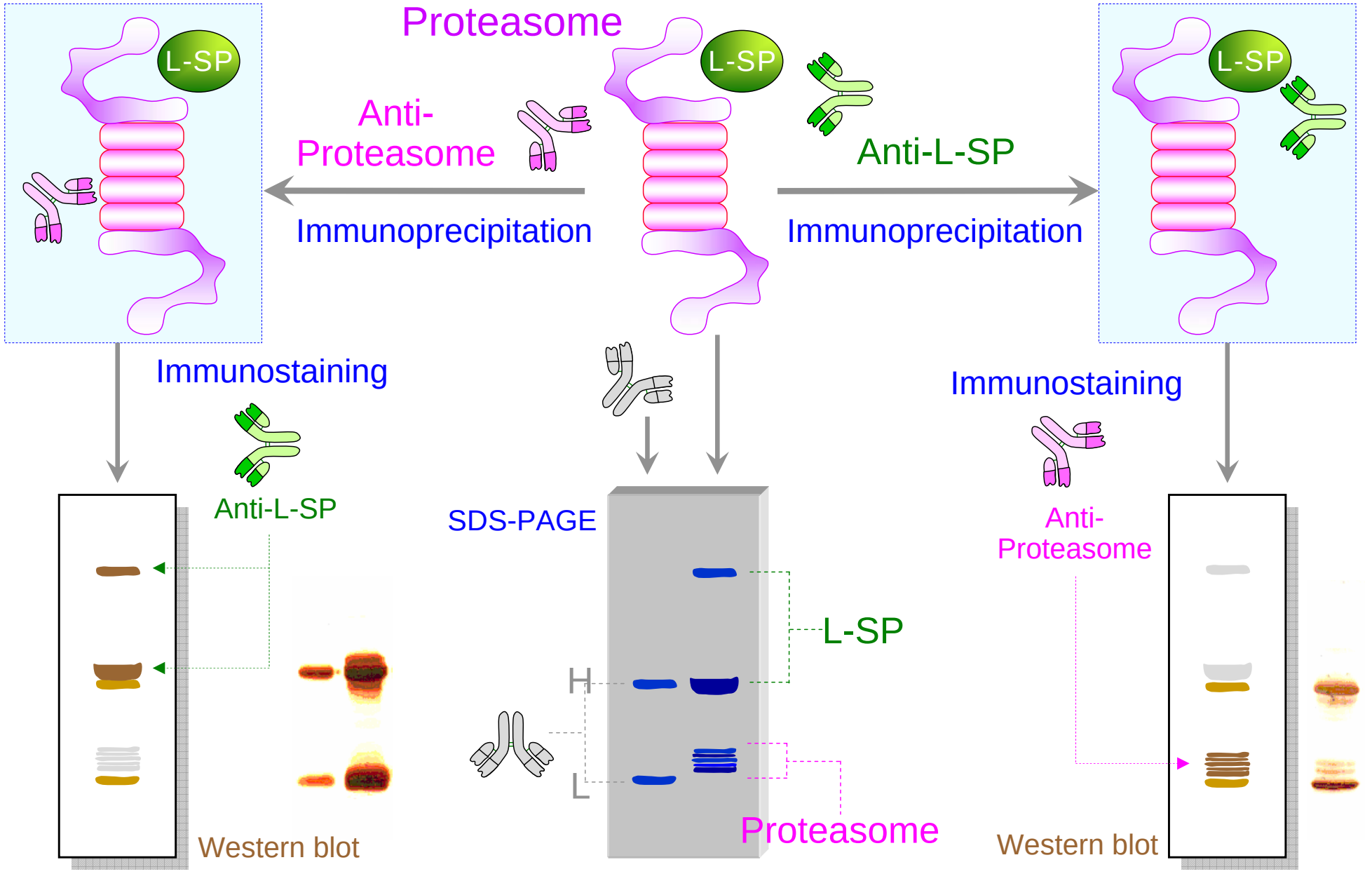
LC/MS/MS

Protein ID	Match peptide
Adenosylhomocysteinase	IVLTIIR DSAAVFAWK HSLPDGLMR LVGVSEETTTGVK
Histone H4 (wheat)	IFLENVIR IDGLIYEETR TVRAMDVVYALKR
Fructose bisphophate aldolase	VTPEVIAEYTVR IGPNEPSQLAIDLNAQGLAR
Triosephosphate isomerase	TNVSPEVAESTR VIACVGETLEQR
NAD-dependent malate dehydrogenase	DDLFNINAGIVK
Histone H3	ASAPATGGVK
Putative lipase	DQVLEEVRR

Pull down proteins interacted with Ag

Juang RH (2005) EPA

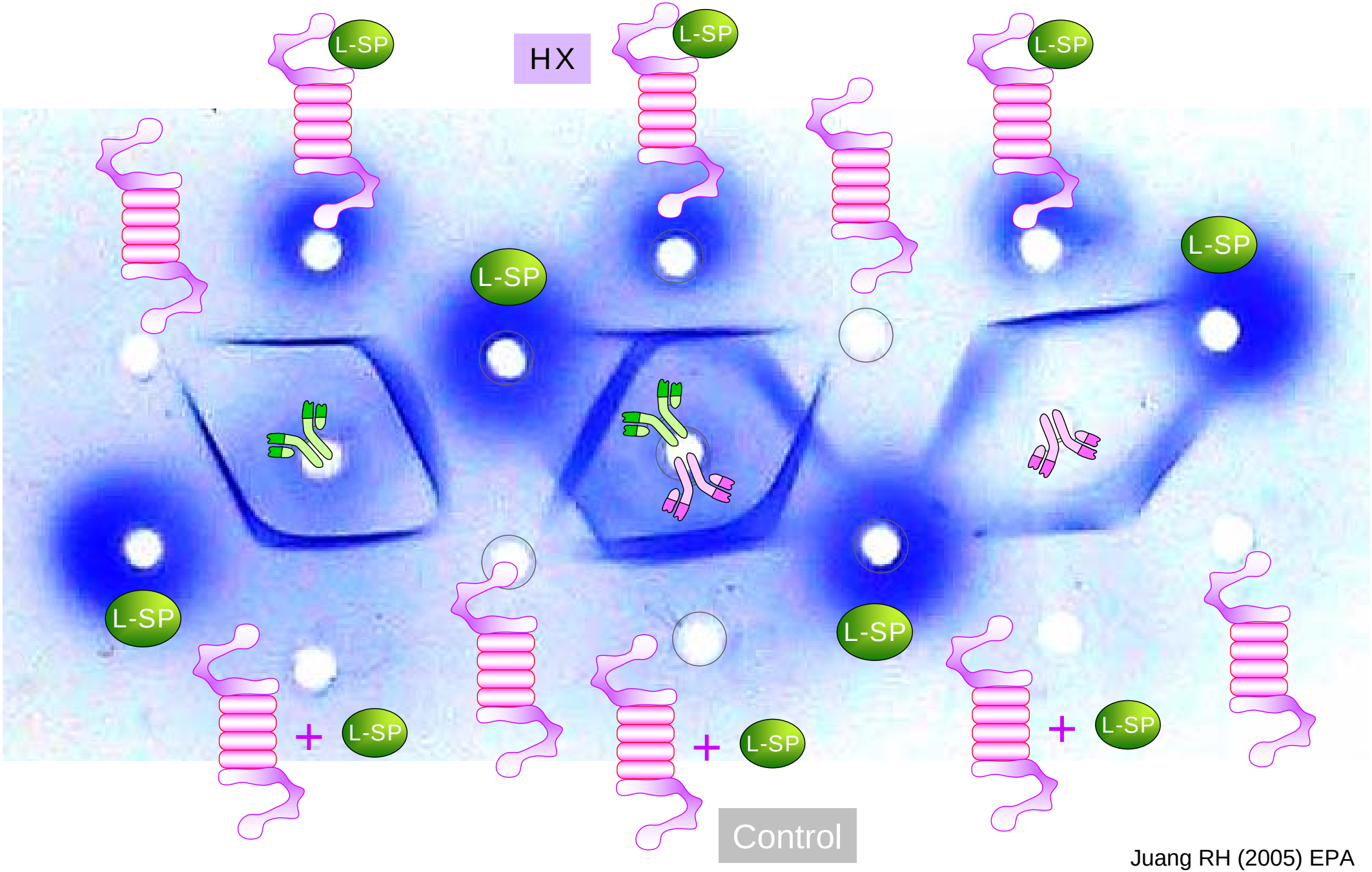
免疫沈澱證明分子間結合 Protein interactions



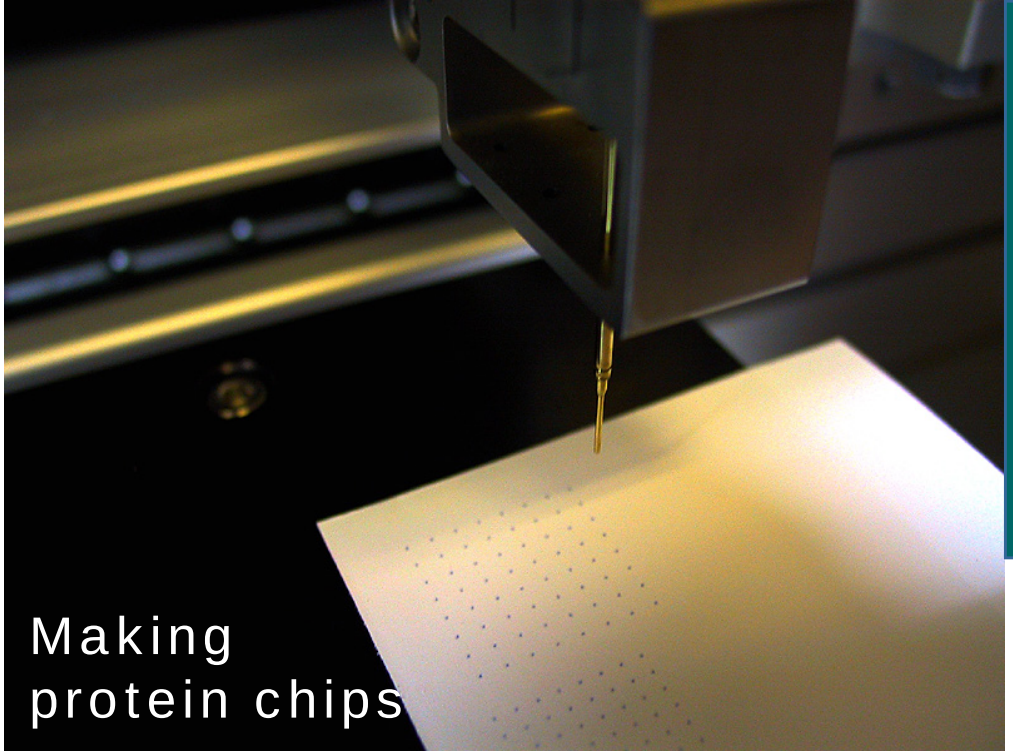
Immunoprecipitation is useful in detecting the interaction between two proteins

Juang RH (2005) EPA

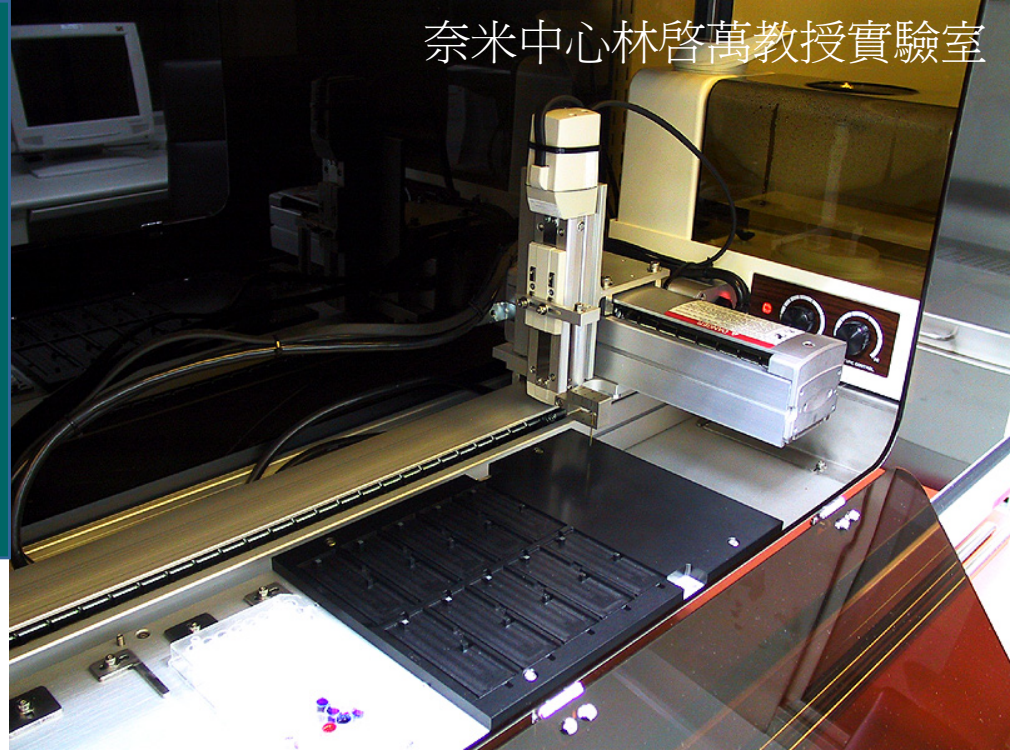
雙向免疫擴散 Double diffusion works



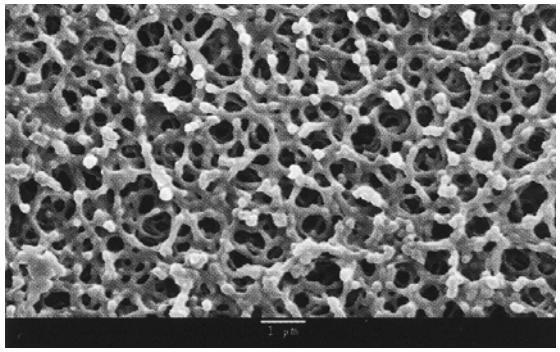
Juang RH (2005) EPA



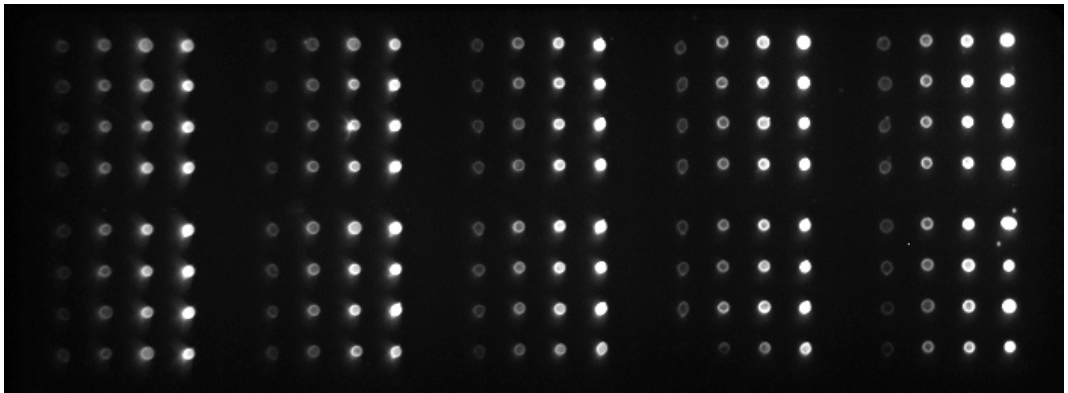
蛋白質晶片試製

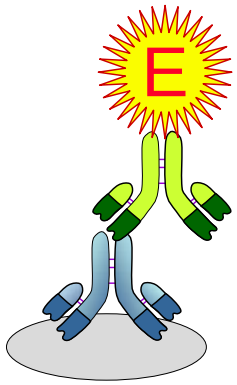


Nitrocellulose

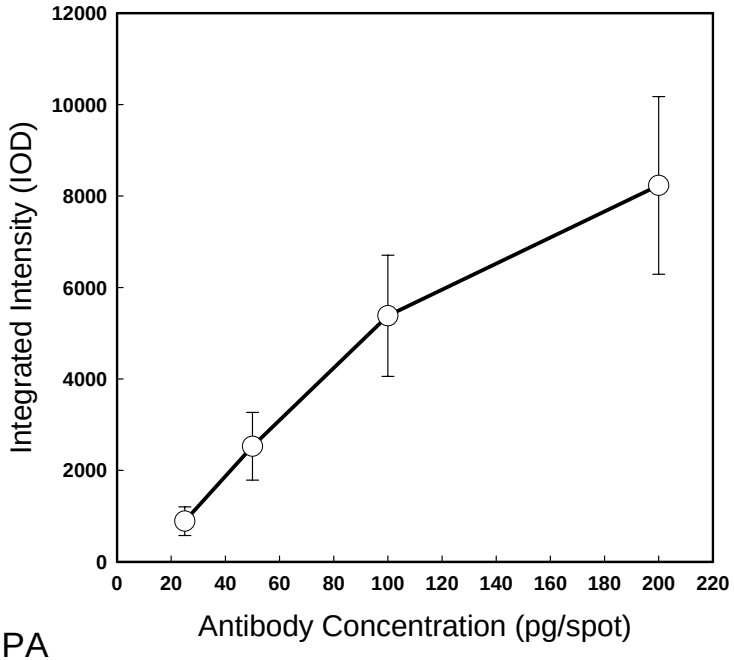


Schleicher & Schuell

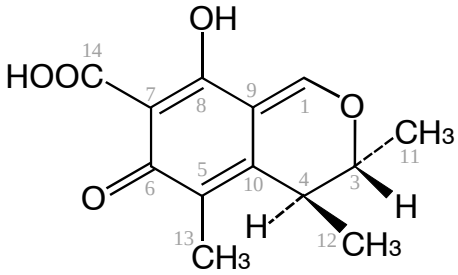




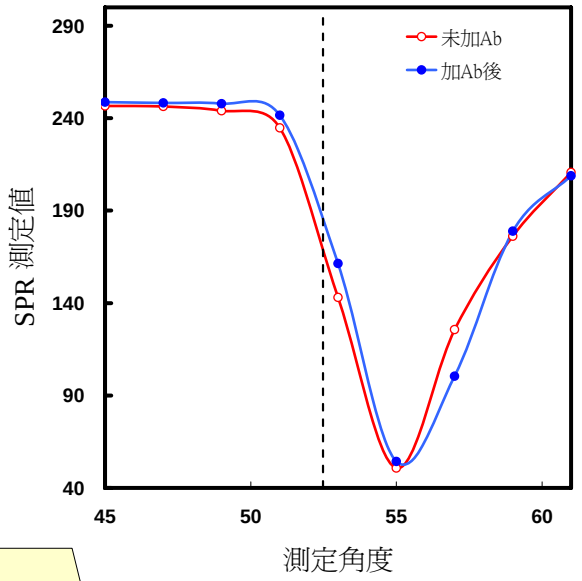
Juang RH (2005) EPA

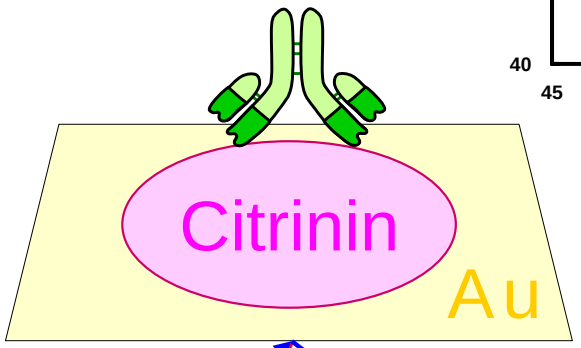


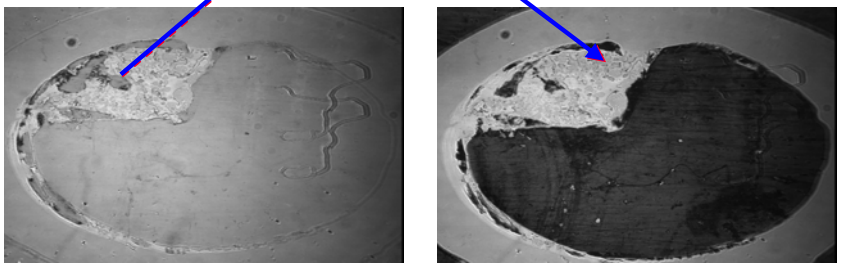
表面電漿共振 (SPR) 可檢測兩分子間的結合



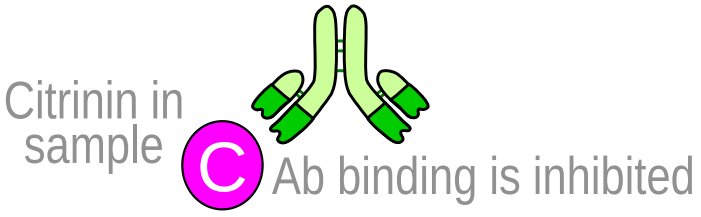
抗體抗原結合對 SPR 的影響

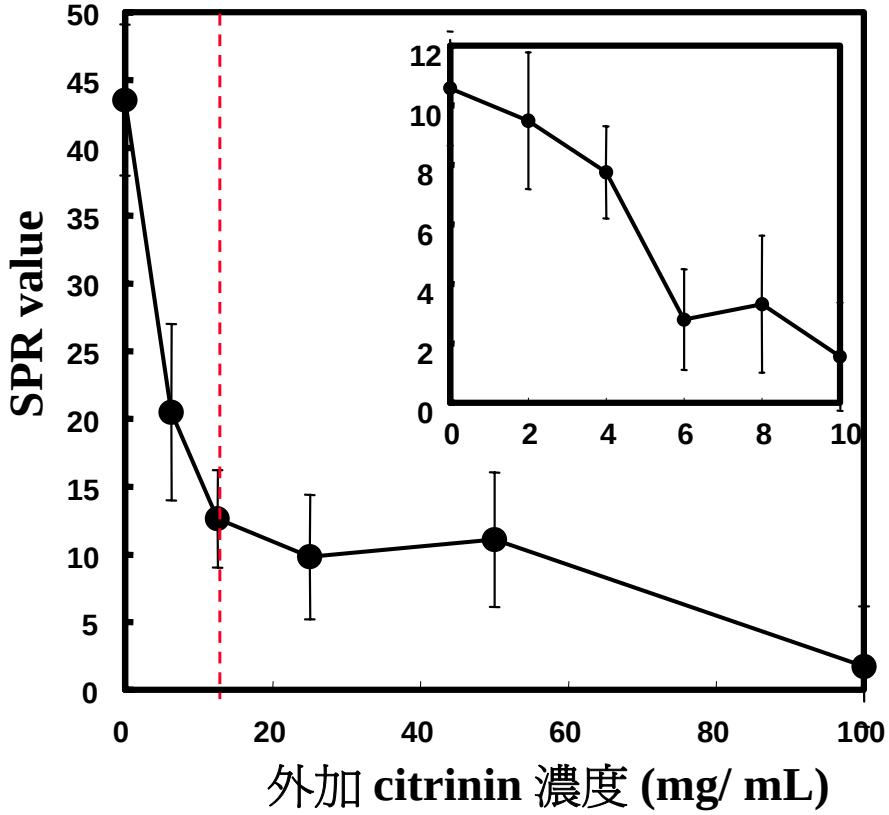






(台大醫工所林啓萬教授)





Juang RH (2005) EPA

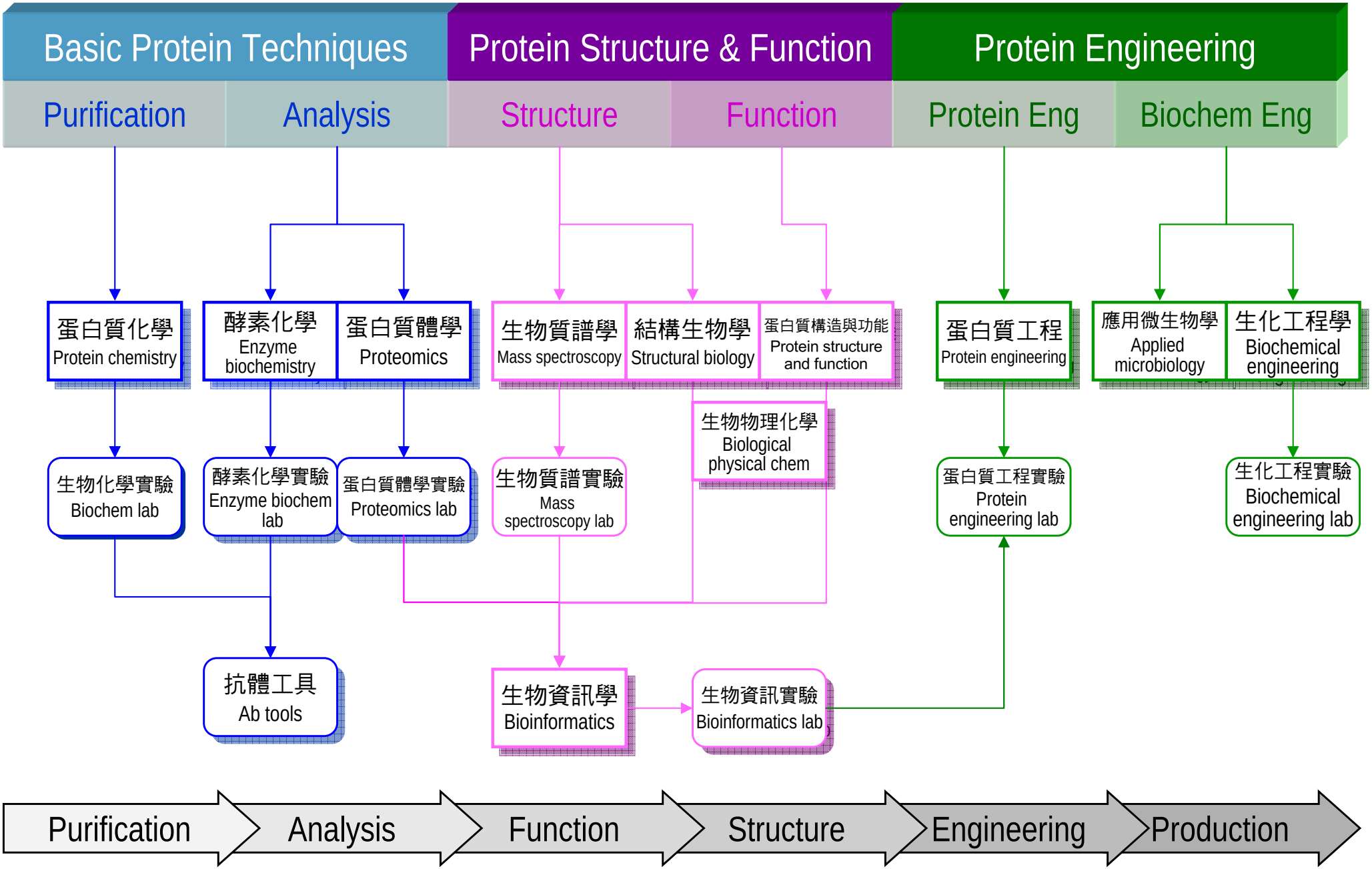
Surface plasmon resonance (SPR) detects the binding of two molecules directly

7 蛋白質科技 Protein technology

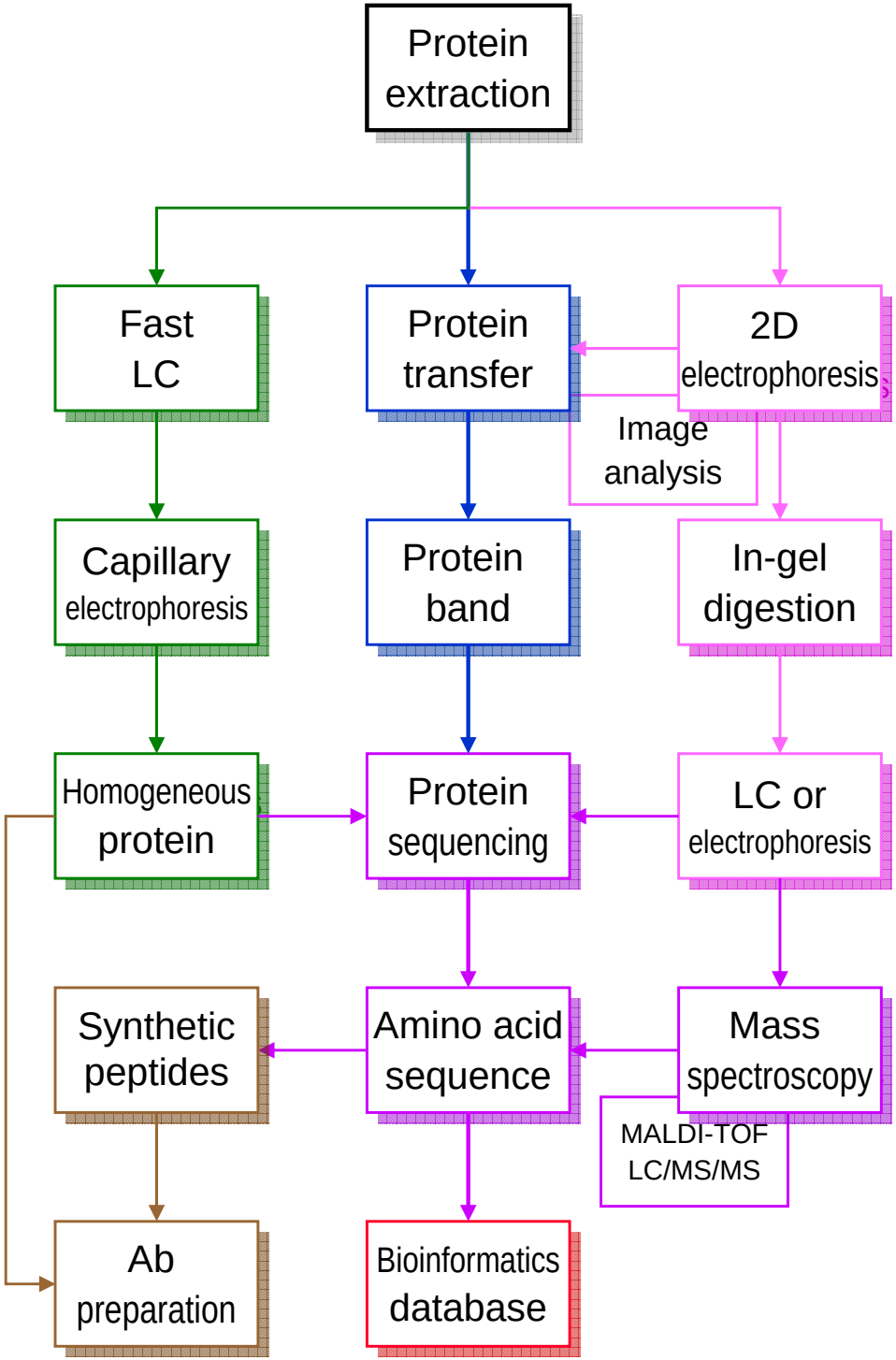
- 7.1 蛋白質科技範疇 An overview
- 7.2 蛋白質微量分析及檢定 Microanalysis
- 7.3 蛋白質體研究 Proteome research

蛋白質科技
Protein Technology

蛋白質科技相關課程與階段 Related courses



蛋白質的微量分離及檢定



蛋白質科技
Protein Technology

蛋白質純化分析新貌

A new look for EPA

1 電泳及轉印

Electrophoresis and transfer

2 二次元電泳

2D electrophoresis

3 膠體內水解

In-gel digestion

4 微量分離純化

Micropurification and analysis

微量分析系統

Microanalysis

抗體製備

Ab preparation

生物資訊學

Bioinformatics

Juang RH (2005) EPA

Genome

基因表現不一定完全反映到蛋白質

Gene expression is not totally reflected in protein level

由基因體較難預測蛋白質的修飾及調控

It is difficult to predict the protein modification and regulation from genomic level

也無法預測蛋白質間的交互作用

Nor can you predict the protein interactions

Proteome

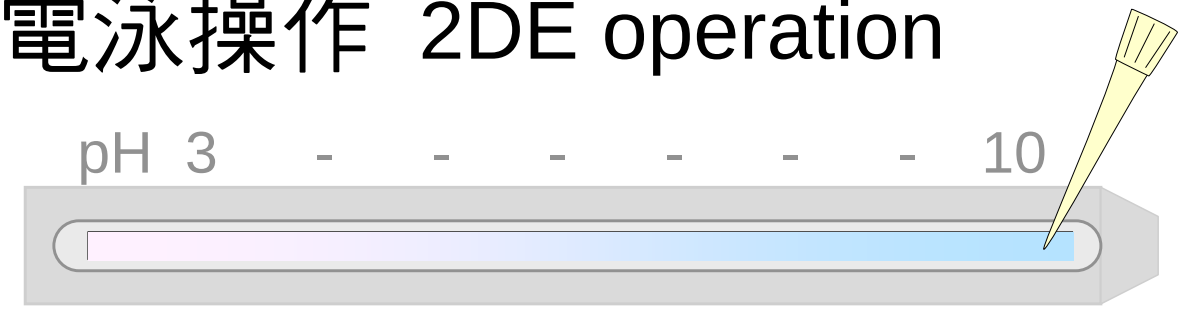
Proteome is much complex than its genome

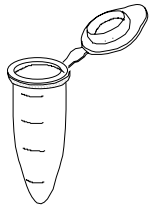
Juang RH (2005) EPA

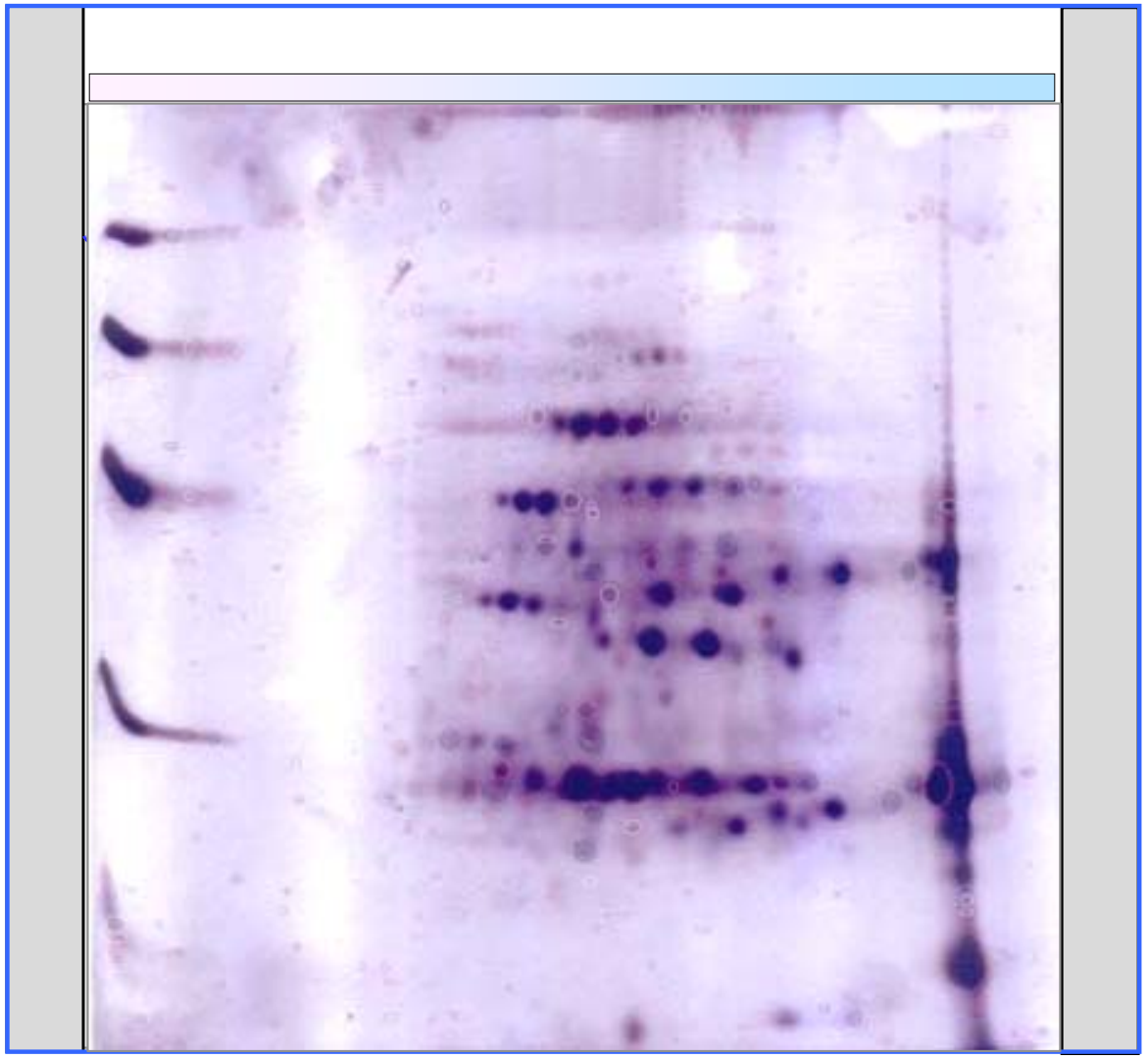
二次元電泳操作 2DE operation

Juang RH (2005) EPA

(1) IEF
等電焦集電泳



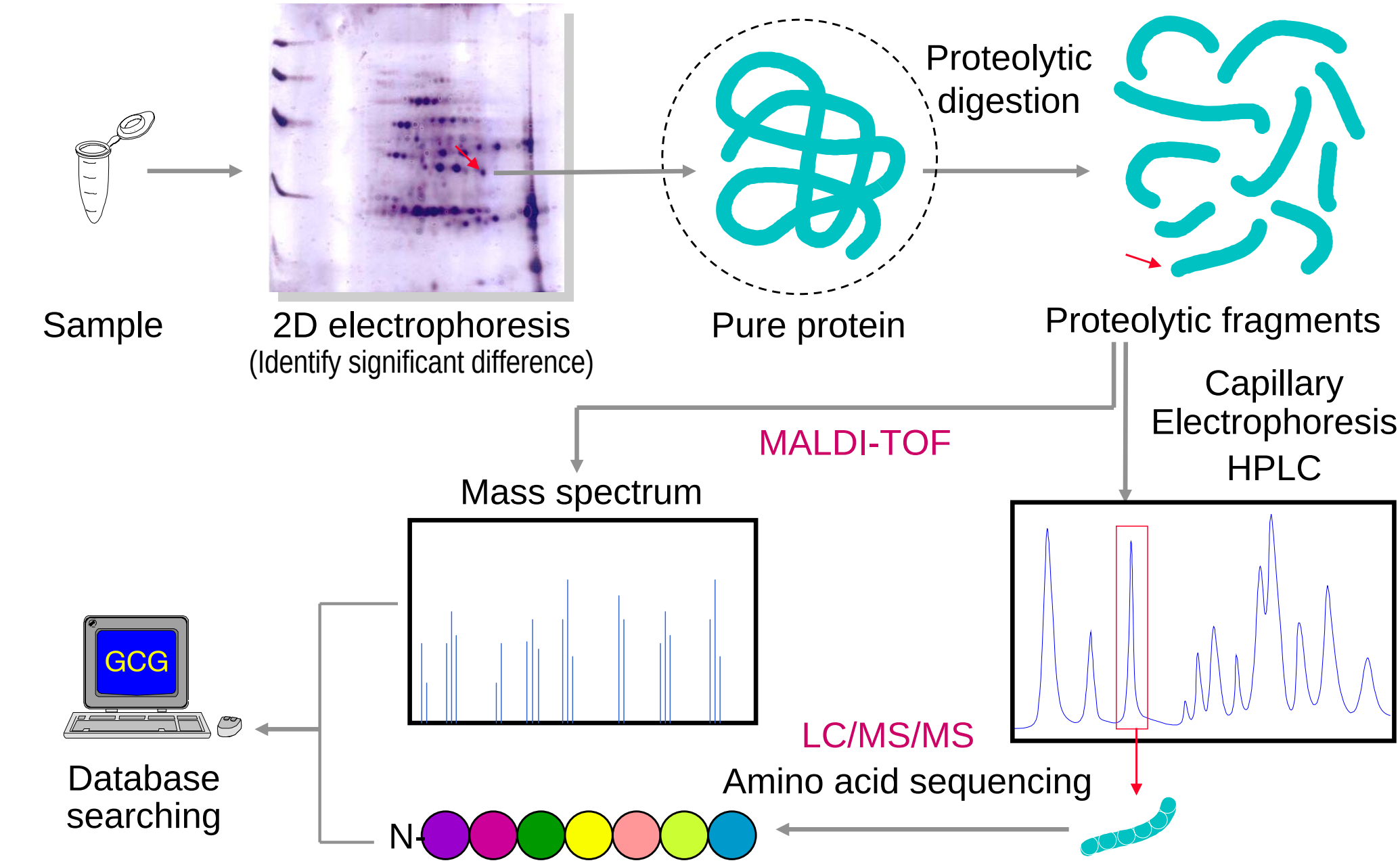




(2)
SDS-PAGE
分離膠體

(3)
Staining
染色脫色

蛋白質體可綜觀蛋白質的消長與身分



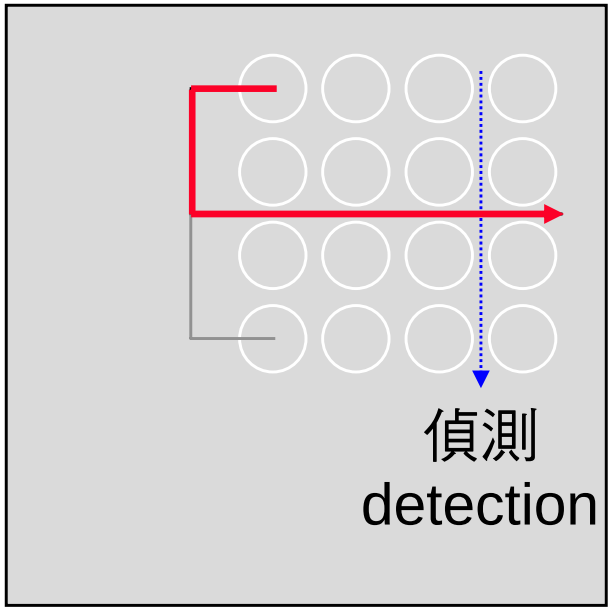
2D tool provides insight from comparing proteomic difference

Juang RH (2005) Proteomics

微流體平台 Microfluidics, Lab-on-a-chip

Agilent 所有蛋白質純化與活性分析均予微小化

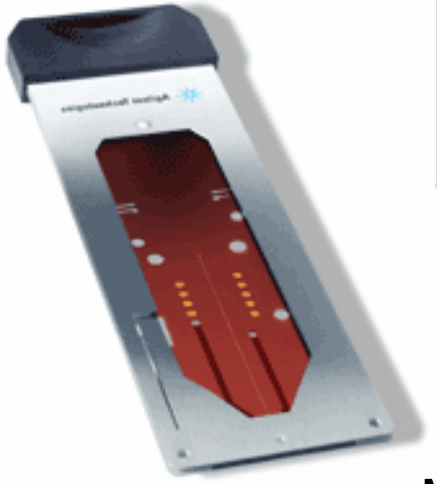
Agilent 2100 bioanalyzer

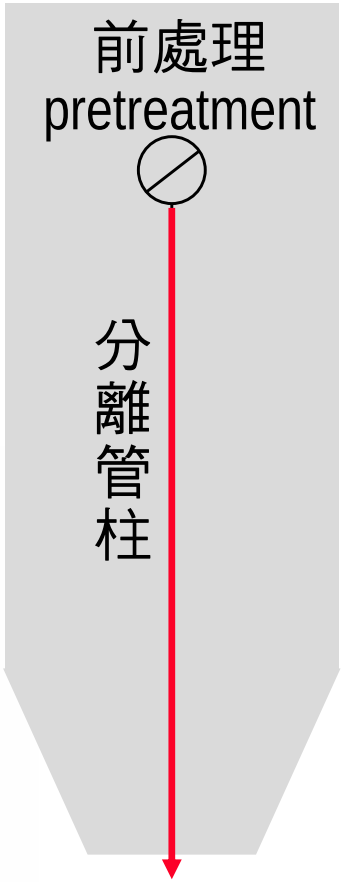


毛細管電泳
Capillary electrophoresis

http://www.chem.agilent.com/Scripts/Phome.asp

Agilent HPLC-Chip/MS



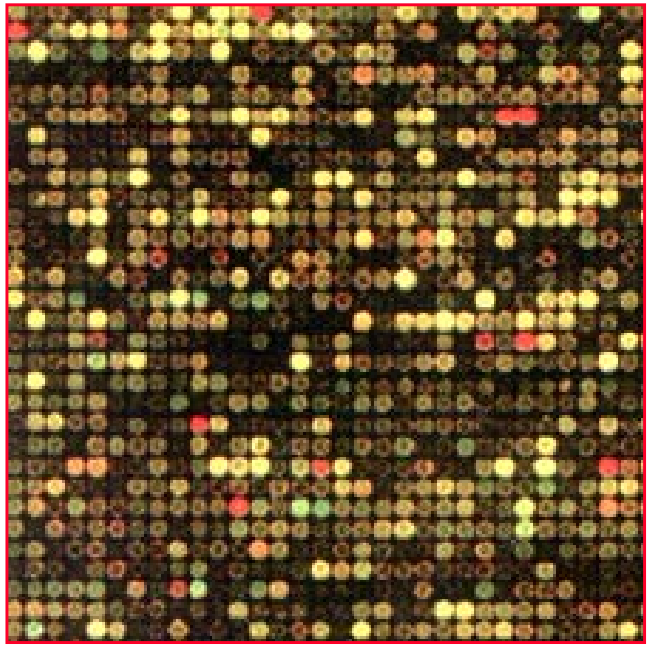


Minimize protein purification and analysis in one chip

Juang RH (2005) EPA

現代蛋白質科技特點 Modern protein technology

- 高產能 High-through put
- 快速 High-speed
- 微量 Micro-scaled



Juang RH (2005) EPA