

8.3 免疫學工具の利用 Immunological tools

8.3.1 抗原製備 Antigen preparation

8.3.2 免疫流程 Immunization protocol

8.3.3 抗体製備 Antibody preparation

8.3.4 抗体應用 Antibody application



8.3.1 抗原的種類 Antigen sources

● 巨分子抗原 Macromolecules

迴避原物種

Protein, polysaccharide, nucleic acid

● 小分子抗原 Small molecules (幾十個胺基酸以下)

Conjugated to carrier before immunization

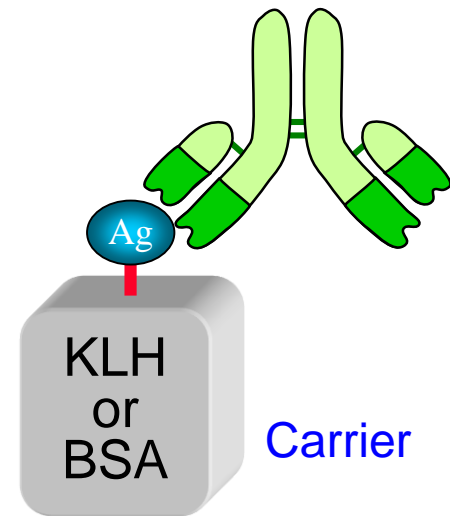
● 半抗原 (hapten) aflatoxin, citrinin

Carrier is required

● 人工合成胜肽 Synthetic peptides

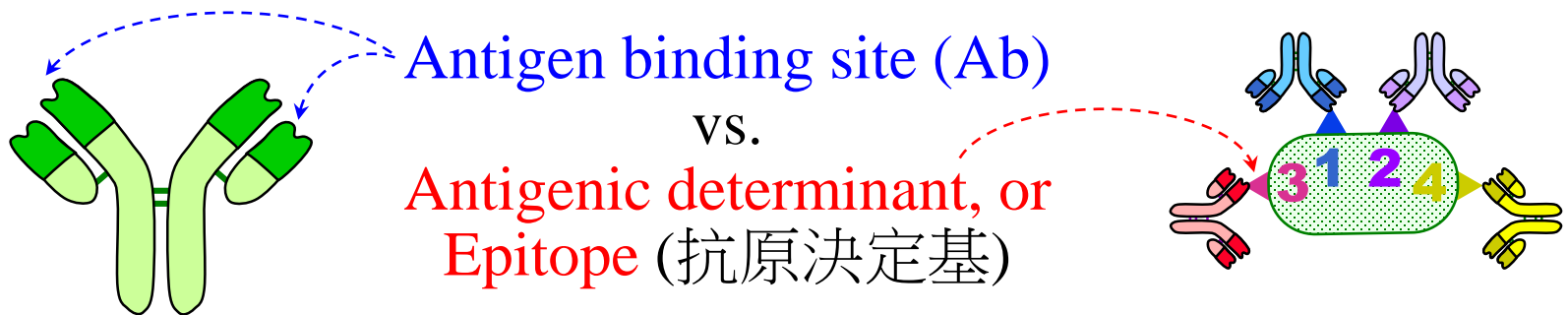
Carrier is required

Produce **monospecific Ab**

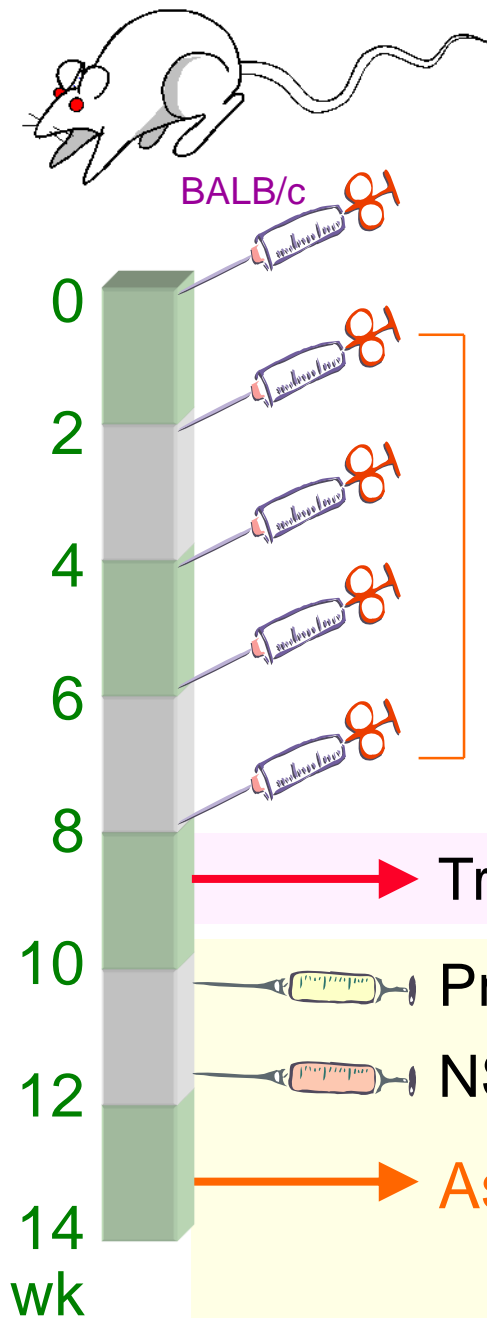


■ 基礎免疫學 Essential immunology

- **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**抗原決定基**作用
Monoclonal antibody (very specific reagent)



8.3.2 小白鼠免疫流程



BALB/c

Antigen (50 $\mu\text{g}/\text{mouse}$)

Emulsified in 0.5 mL

Freund's Complete Adjuvant

At least three booster shots,
same dose in 0.5 mLFreund's **In**complete AdjuvantBooster shots might be reduced
if **TiterMax** is use as adjuvant

Trial Bleeding

Titer Determination

全採血

Total Bleeding, < 1 mL

Ascites Fluids

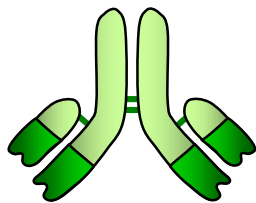
腹水

X mL (X = 1-10)

The immunization protocol for antiserum or ascites production

加佐劑製成乳劑
+ adjuvant \rightarrow emulsion

8.3.3 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

↓ dissolved 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

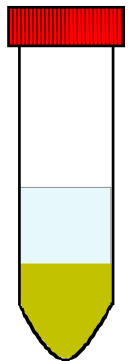
Washing

透析

Dialysis

保存

Stock



8.3.4 抗体應用 Applications of Ab

a. 轉印及免疫染色法 Western blot & immunostaining

應用最廣且最有效率 (包含細胞組織切片免疫染色)

b. 免疫沉澱法 Immunoprecipitation (pull-down)

另一種檢定專一性抗原的方法

c. 親和層析法 Affinity chromatography

最快速有效的純化方法

d. 雙向免疫擴散法 Double diffusion

古老但仍有其特色及應用

e. 酵素免疫分析法 Enzyme immunoassay

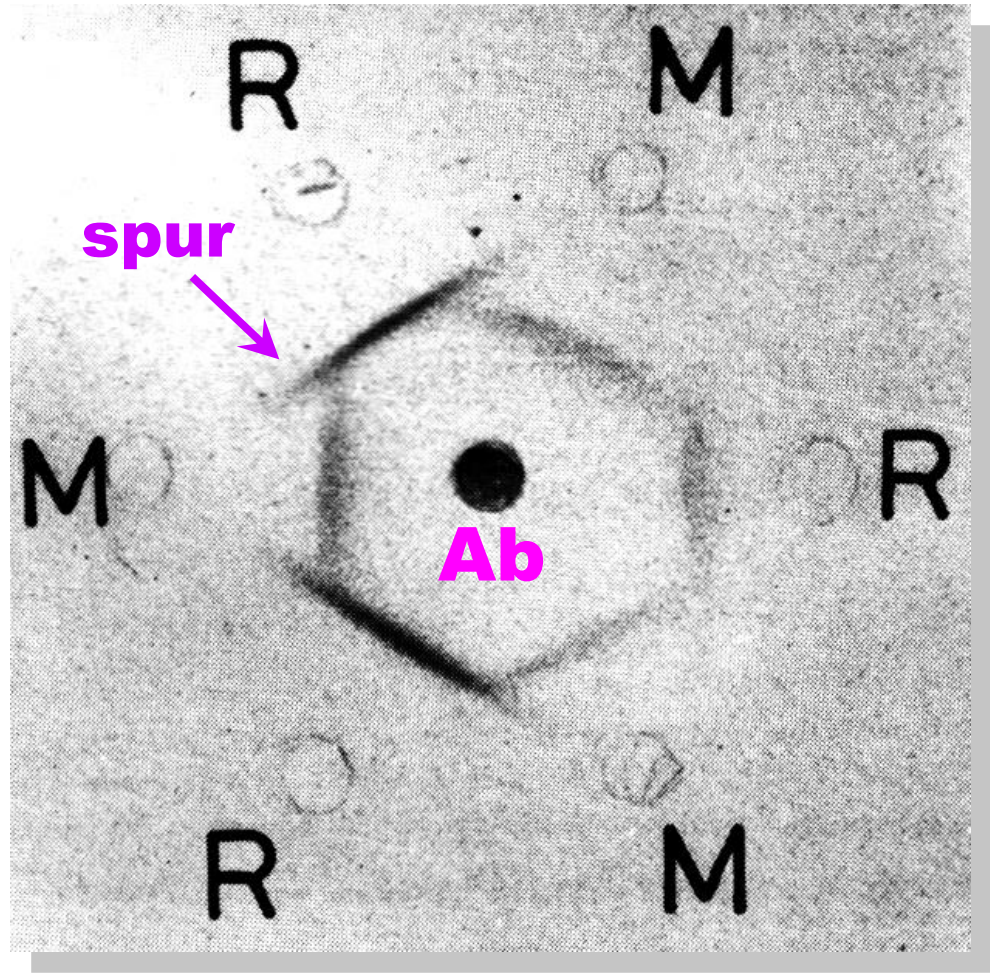
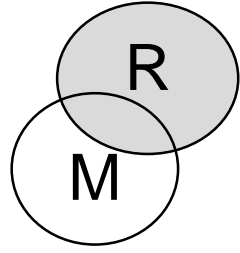
可分析大量樣本 (ELISA)

f. 抗体晶片 Antibody chip

專一快速地同時進行多種分析

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

雙向免疫擴散法

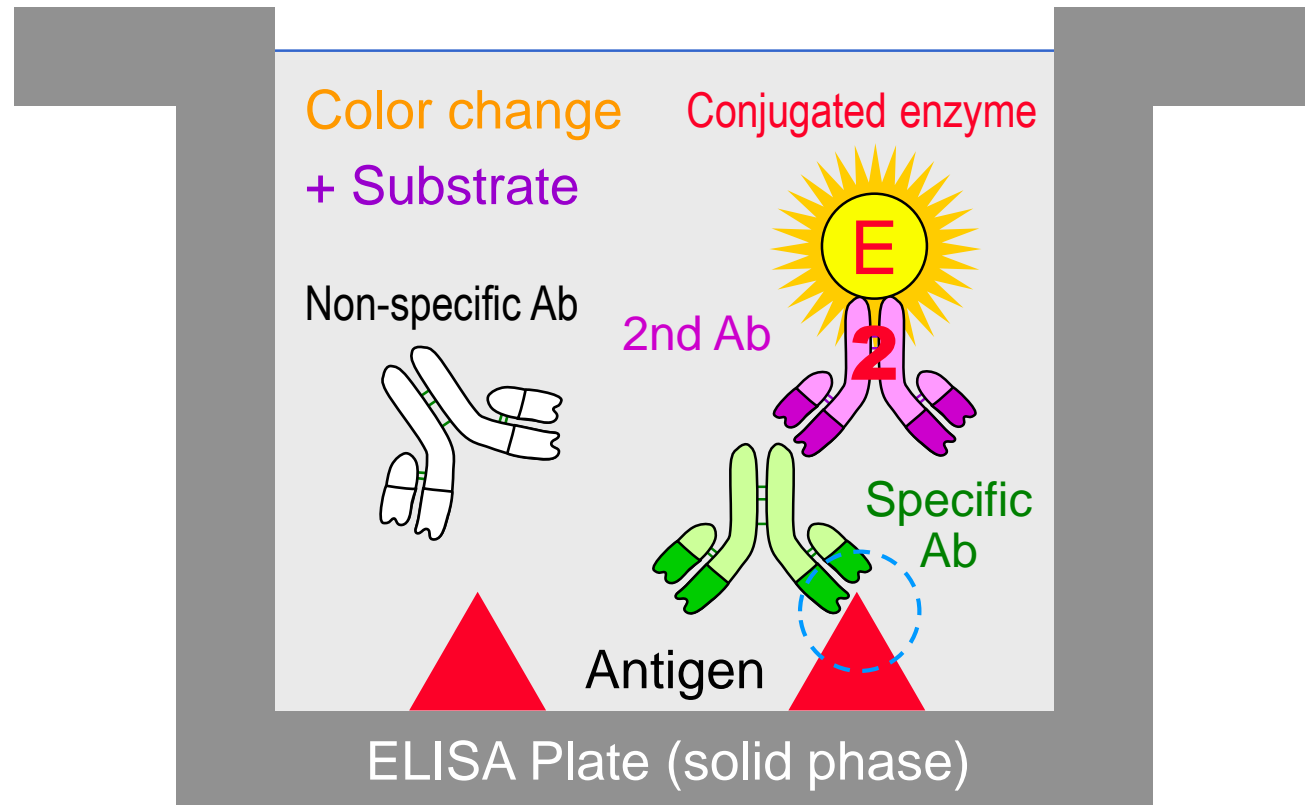
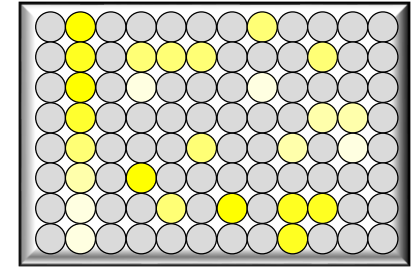


由沉澱線交叉情形可推測抗原分子間的包含關係
The crossing-over of the precipitin lines reveals the structural relationship between the antigen molecules

酵素免疫分析法 ELISA

ELISA 操作注意：

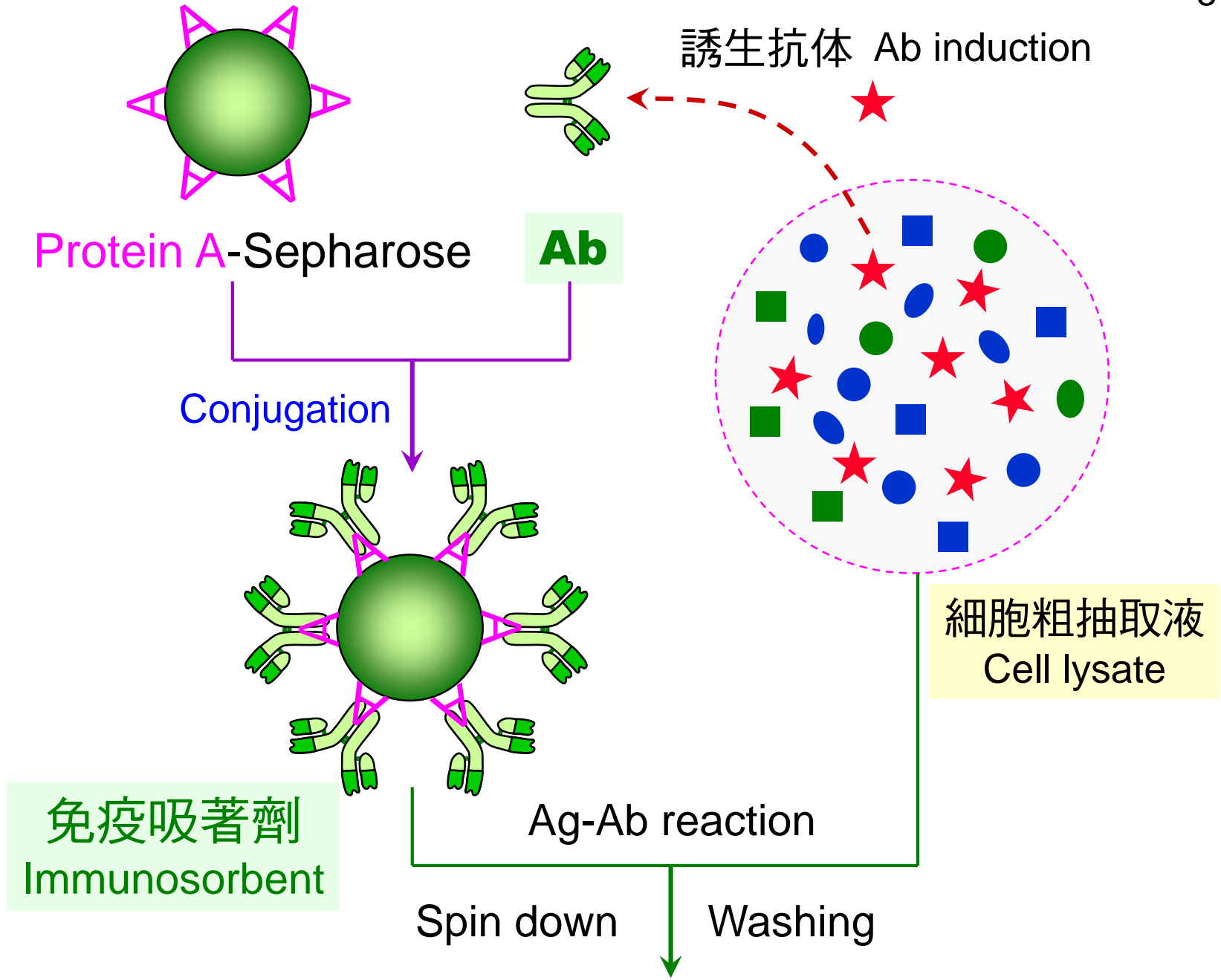
- (1) 把握 pipetting 及 timing 精準
- (2) 確定 2nd Ab conjugate 的品質
- (3) 檢查樣本中可能的干擾物質
- (4) 注意反應液在保溫箱會蒸發
- (5) 使用規格正確的 ELISA plate
- (6) 設計好關鍵的 control 對照組



To detect the Ab in the sample by ELISA

ELISA 幾乎無所不在！

擔體免疫沈澱的原理及應用

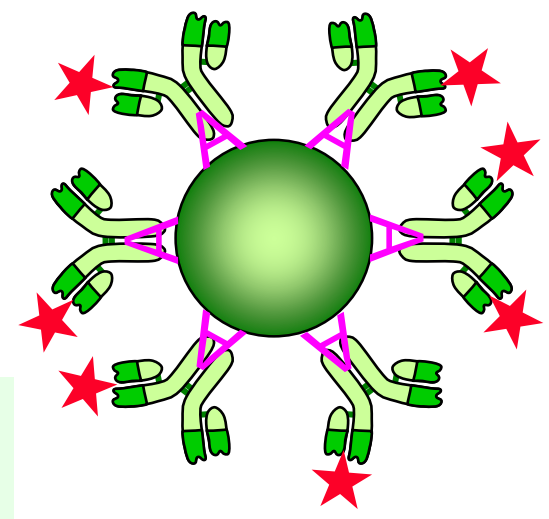


Solid support-based immunoprecipitation

圖 8.7

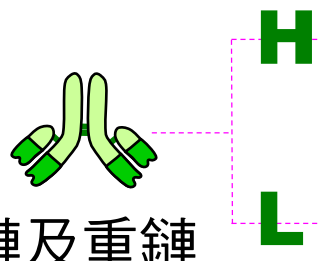
擔體免疫沈澱的原理及應用

擔體免疫沈澱 Immunoprecipitation

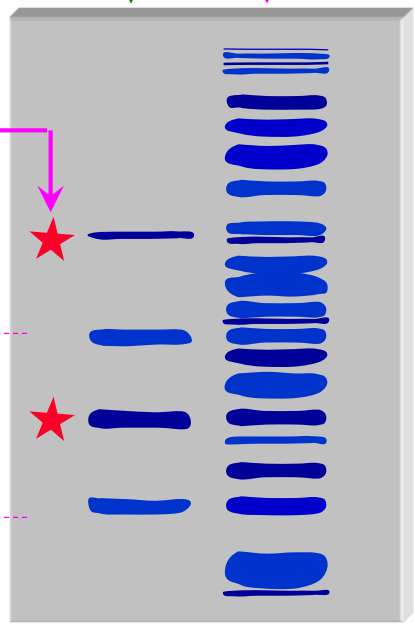


SDS-PAGE

抗原可能有兩個次體 ★
Ag might contains two subunits

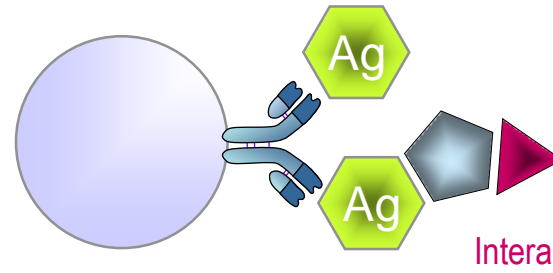
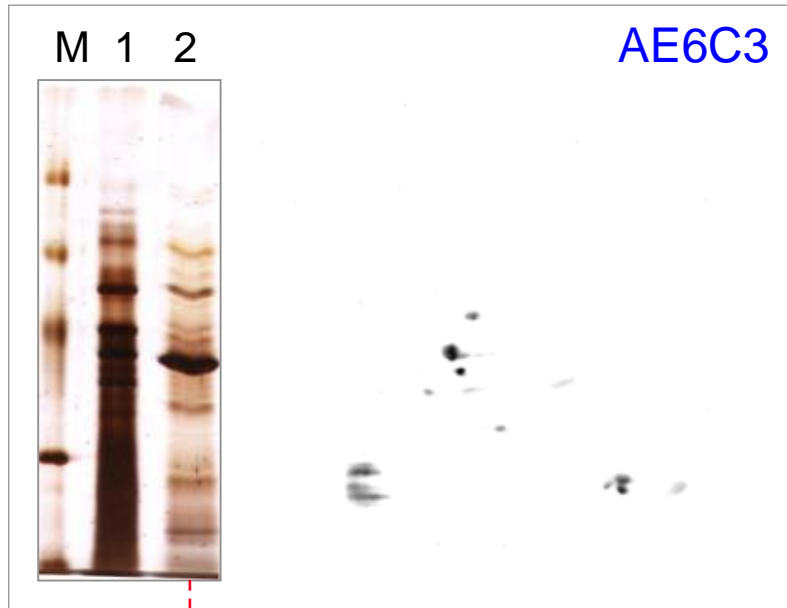


抗体有輕鏈及重鏈
Ab contains H & L chains



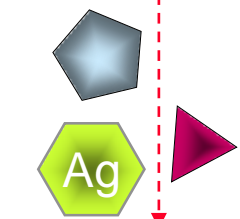
若抗原的分子量與 H 或 L 重疊怎麼辦？

■ 抗体免疫沈澱與蛋白質交互作用



要證明細胞中
也有交互作用
(How?)

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



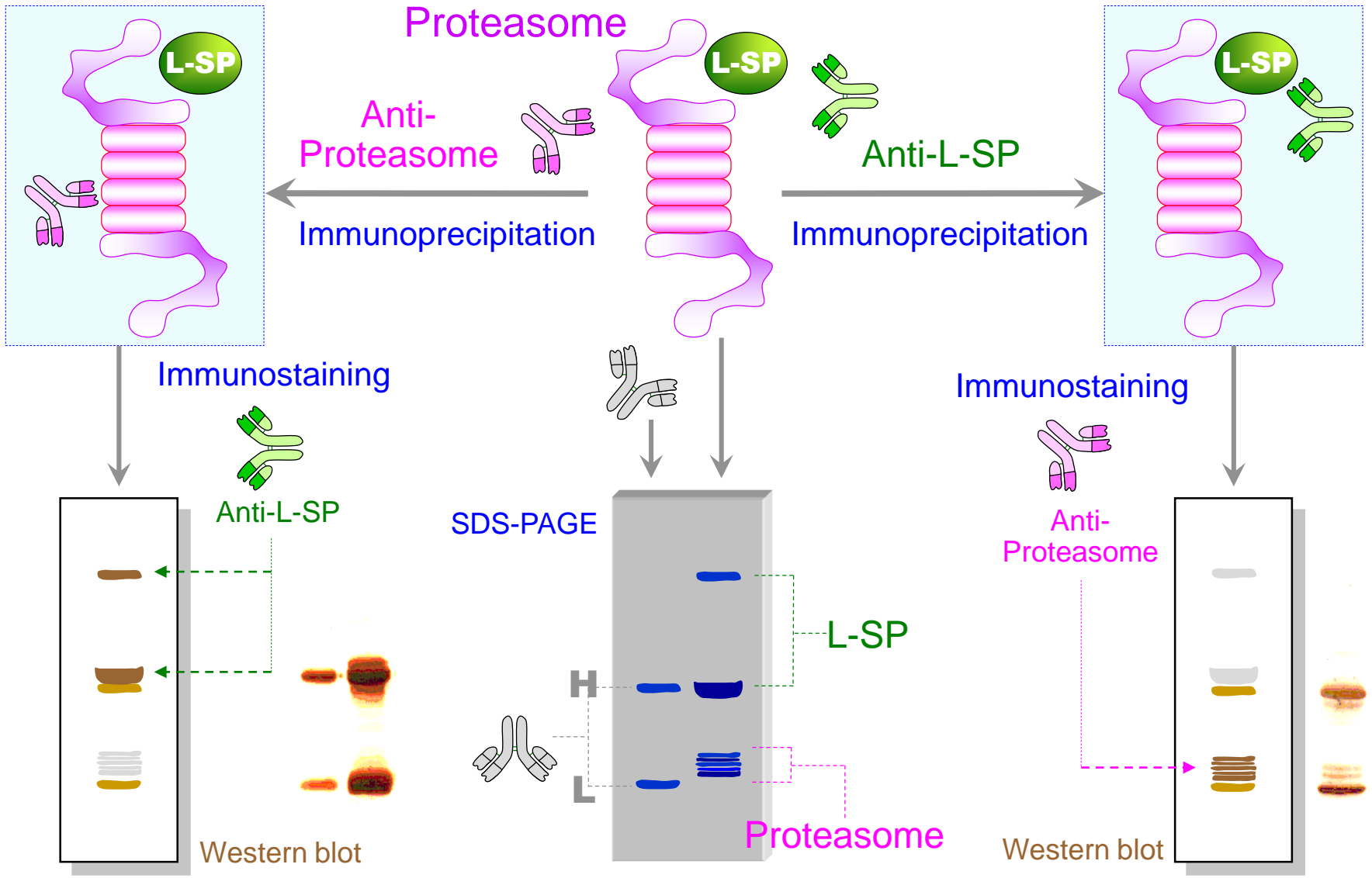
LC/MS/MS

最大的問題：

必須驗證何者是有意義的交互蛋白質
或者只是非專一性的吸附

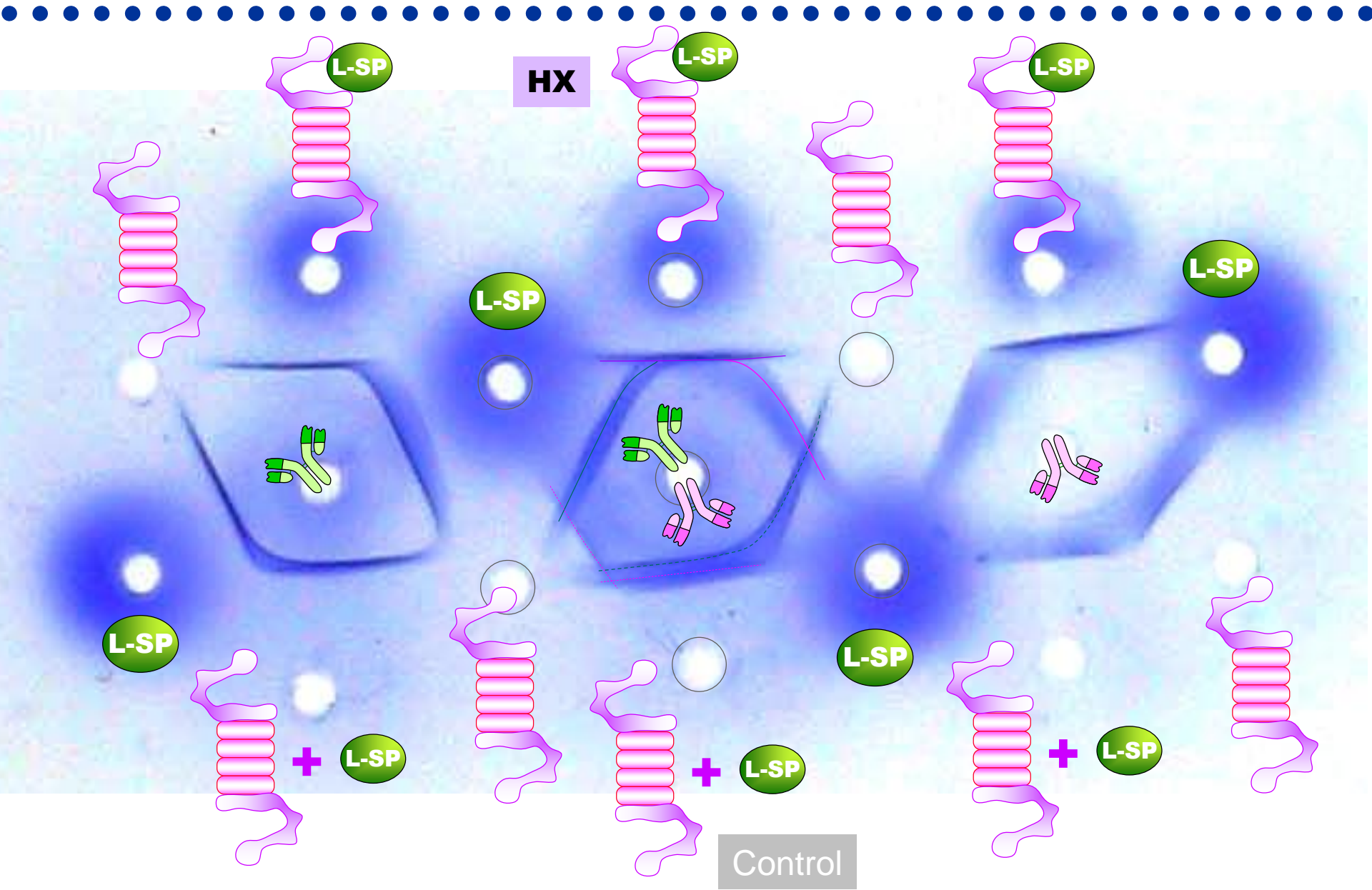
Pull down proteins interacted with Ag

免疫沈澱證明分子間結合 Protein interactions

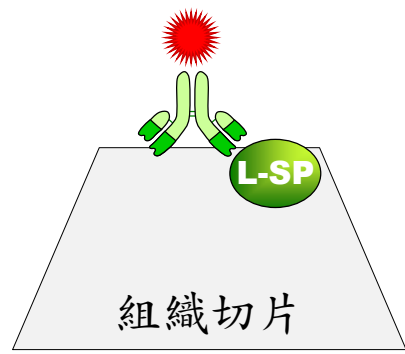


Immunoprecipitation is useful in detecting the interaction between two proteins

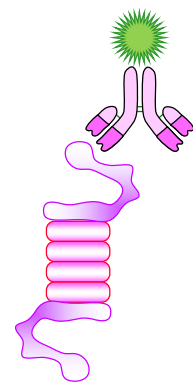
雙向免疫擴散 Double diffusion works



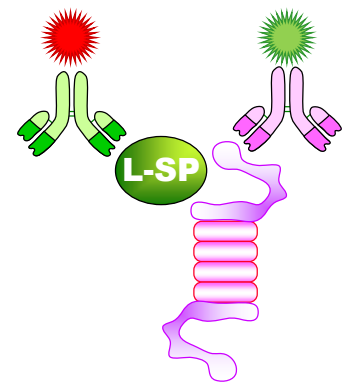
共軛焦顯微定位 Confocal microscopy



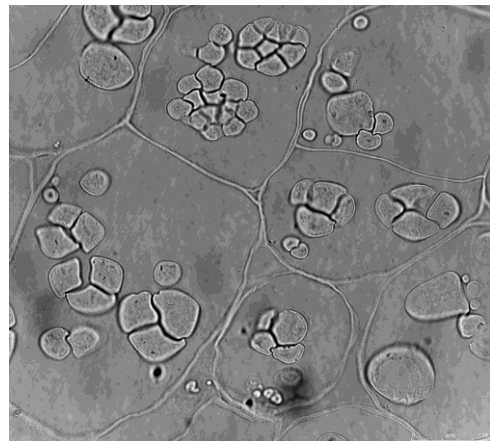
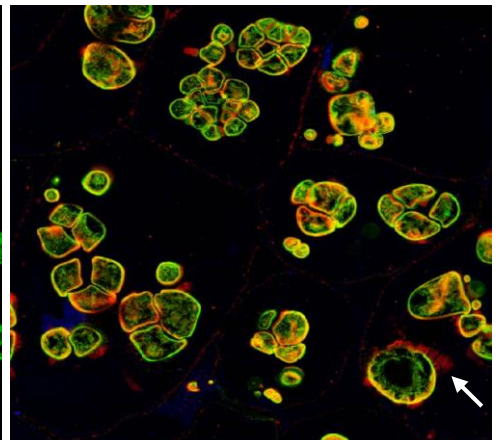
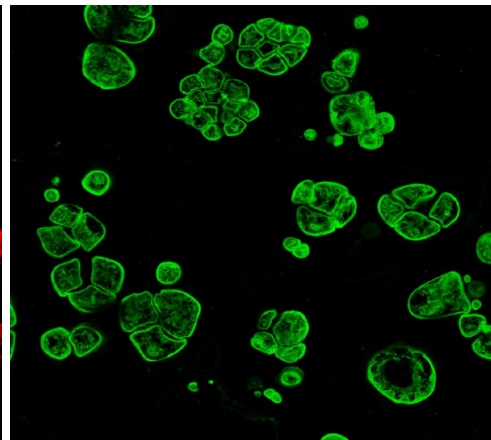
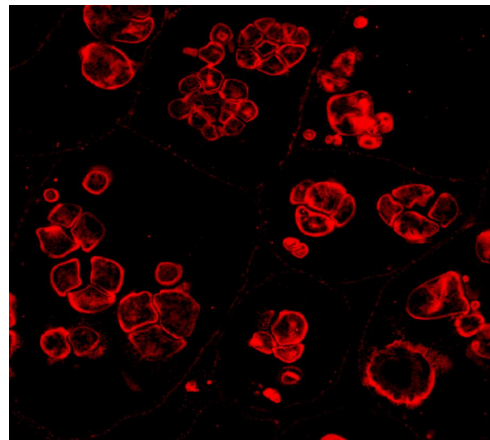
L-SP



Proteasome



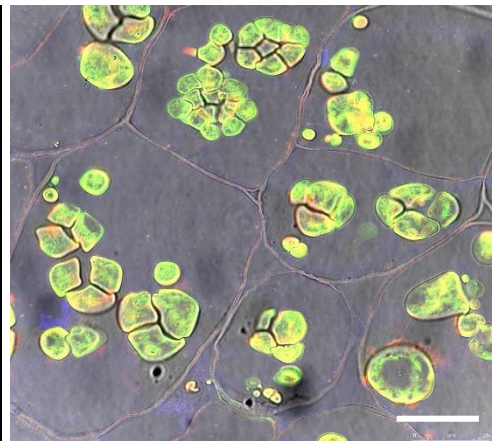
Merge (red & green)



Light

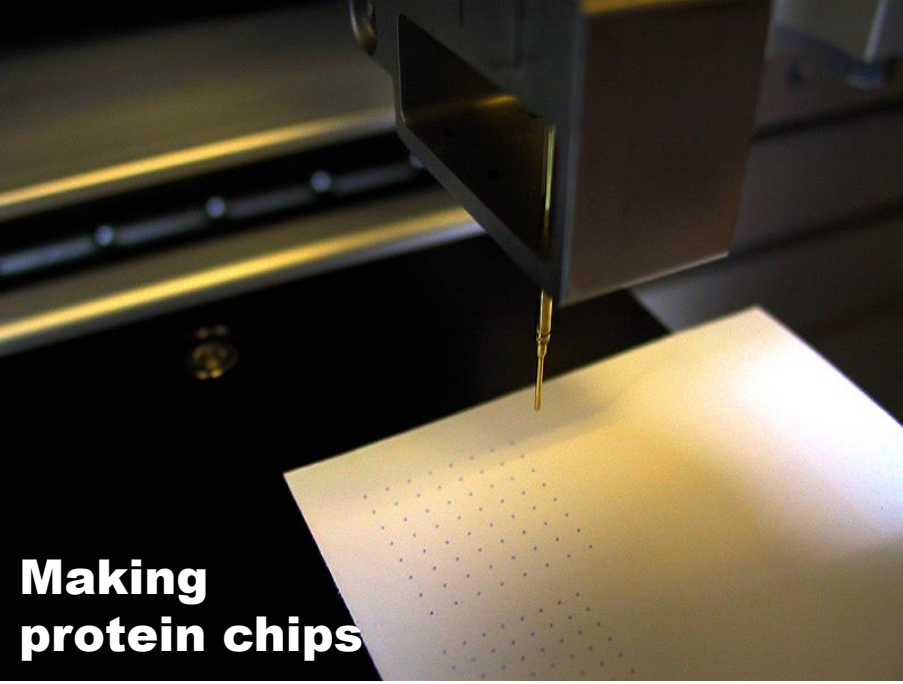


DAPI (nucleus)

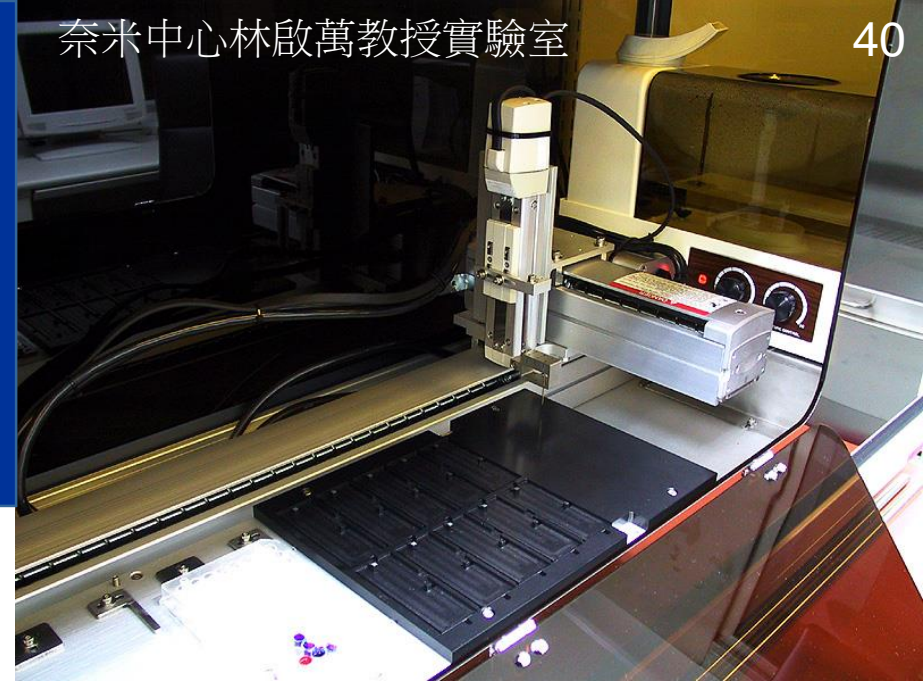


Merge (all)

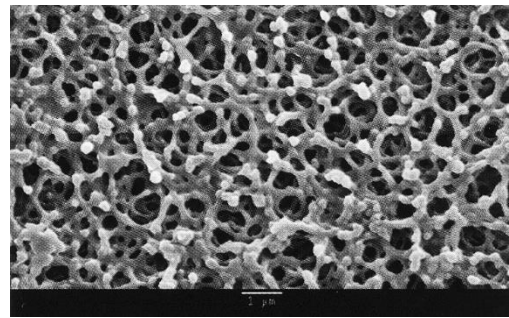
蛋白質晶片試製



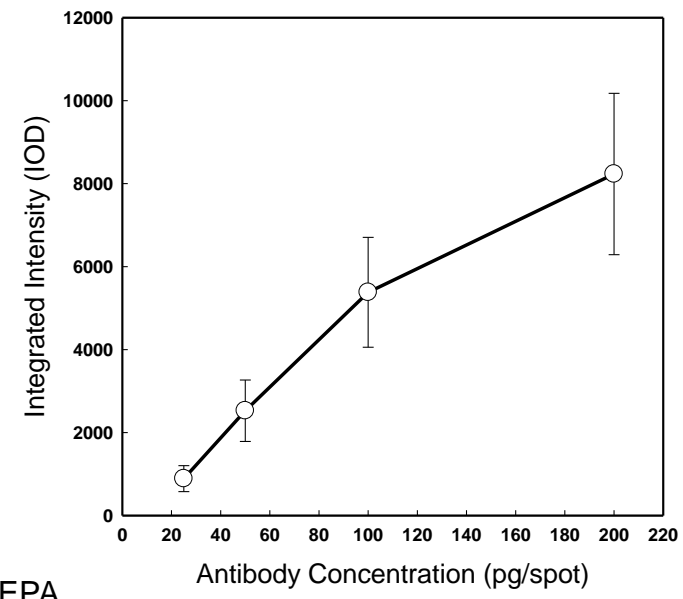
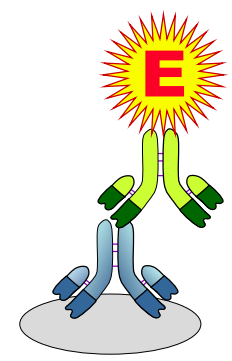
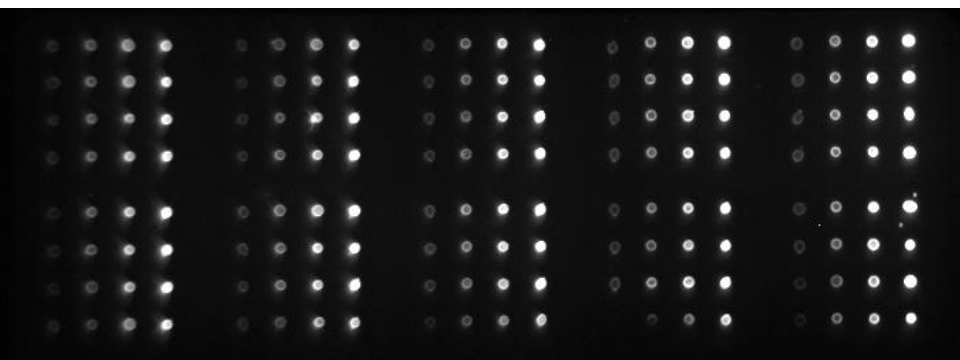
Making protein chips



Nitrocellulose



Schleicher & Schuell



8.4 蛋白質新世界 Protein New World

8.4.1 微量科技 Microscale technology

8.4.1.1 微量純化

8.4.1.2 微量分析

**Protein
Chemistry
100 years**

8.4.2 蛋白質體學 Proteomics

8.4.2.1 如何看待 Proteomics ?

8.4.2.2 Proteomics 就是蛋白質化學 ?

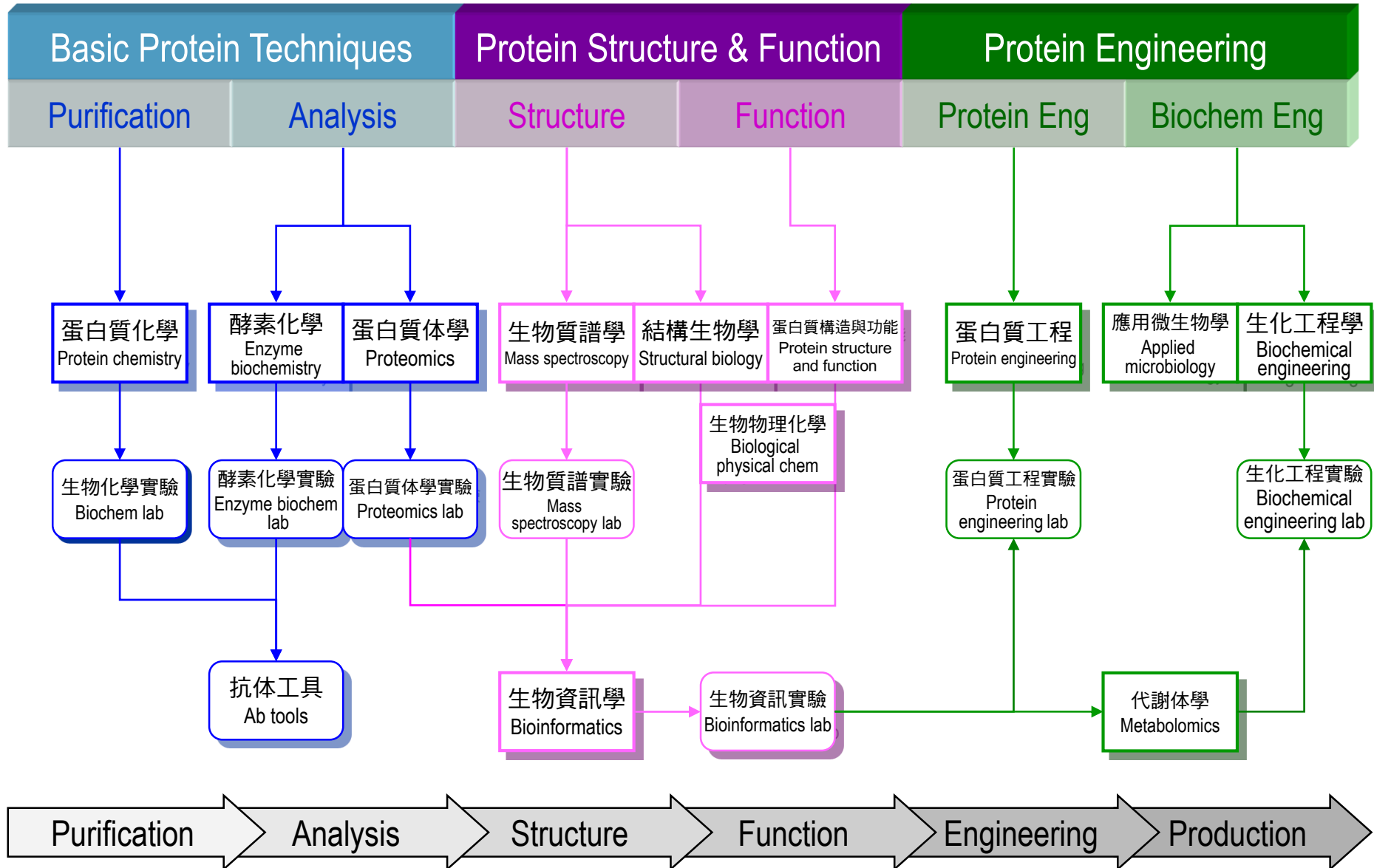
8.4.2.3 Proteomics 與代謝體

蛋白質科技

Protein Technology

- (1) 基本的蛋白質生化學沒有改變
- (2) 自動化與大數據強化分析平台
- (3) 樣本處理極微量且數目巨量化

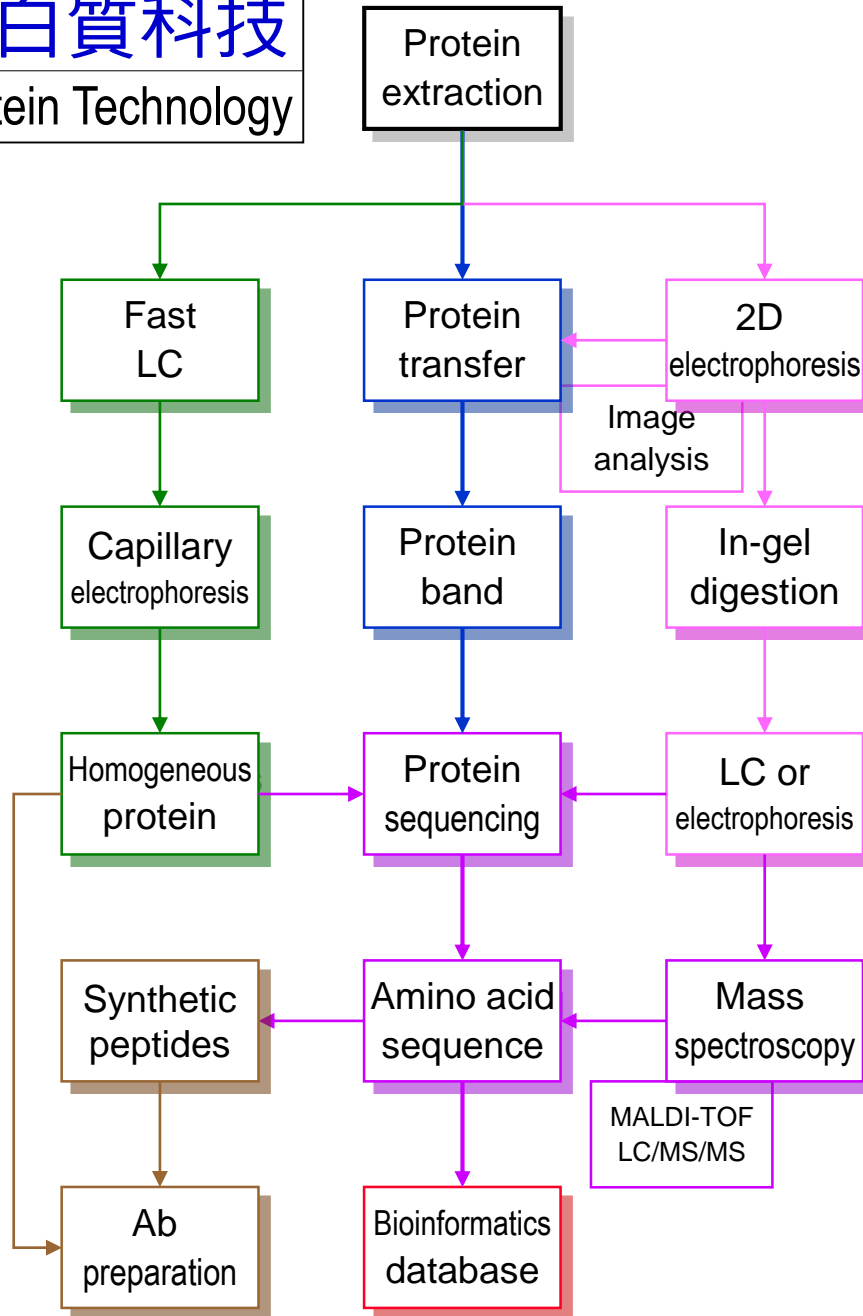
蛋白質科技相關課程與階段 Related courses



蛋白質的微量分離及檢定

蛋白質科技

Protein Technology



精準高效純化 微量快速分析

蛋白質純化分析新貌

A new look for EPA

(假如實驗課的經費無上限)

1 電泳及轉印

Electrophoresis and transfer

2 二次元電泳

2D electrophoresis

3 膠體內分析

In-gel digestion & analysis

4 微量分離純化

Micropurification and analysis

微量分析系統

Microanalysis

抗体製備

Ab preparation

生物資訊學

Bioinformatics

由基因解碼到代謝體學 Decoding the genome

細菌的基因很小，容易被解碼，也有其演化優勢

由基因密碼可解讀代謝途徑 並找出攻擊點

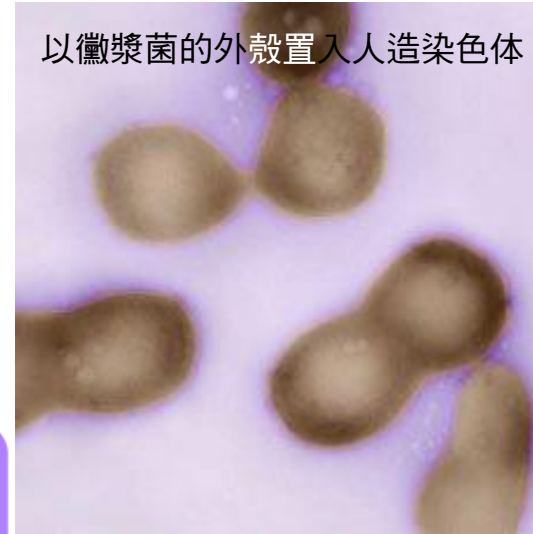
「細菌的染色体非常進，其生理功能也很直接...

是非常務實的基因」

電腦設計出來的微生物

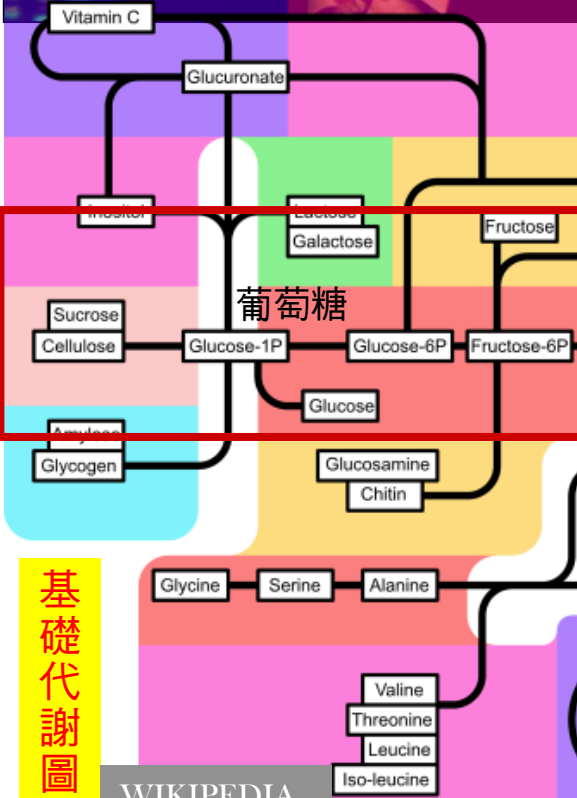
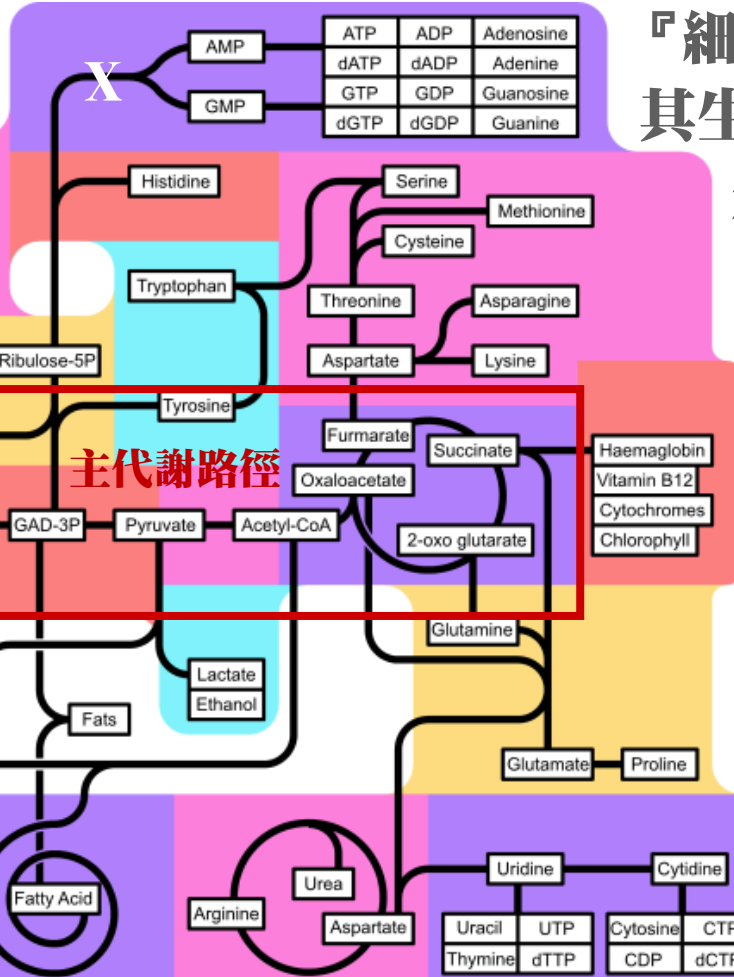
Mycoplasma laboratorium [黴漿菌]

以黴漿菌的外殼置入人造染色体



有浮水印：VENTERINSTITVTE

J. Craig Venter Institute (2010)



基礎代謝圖

WIKIPEDIA

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

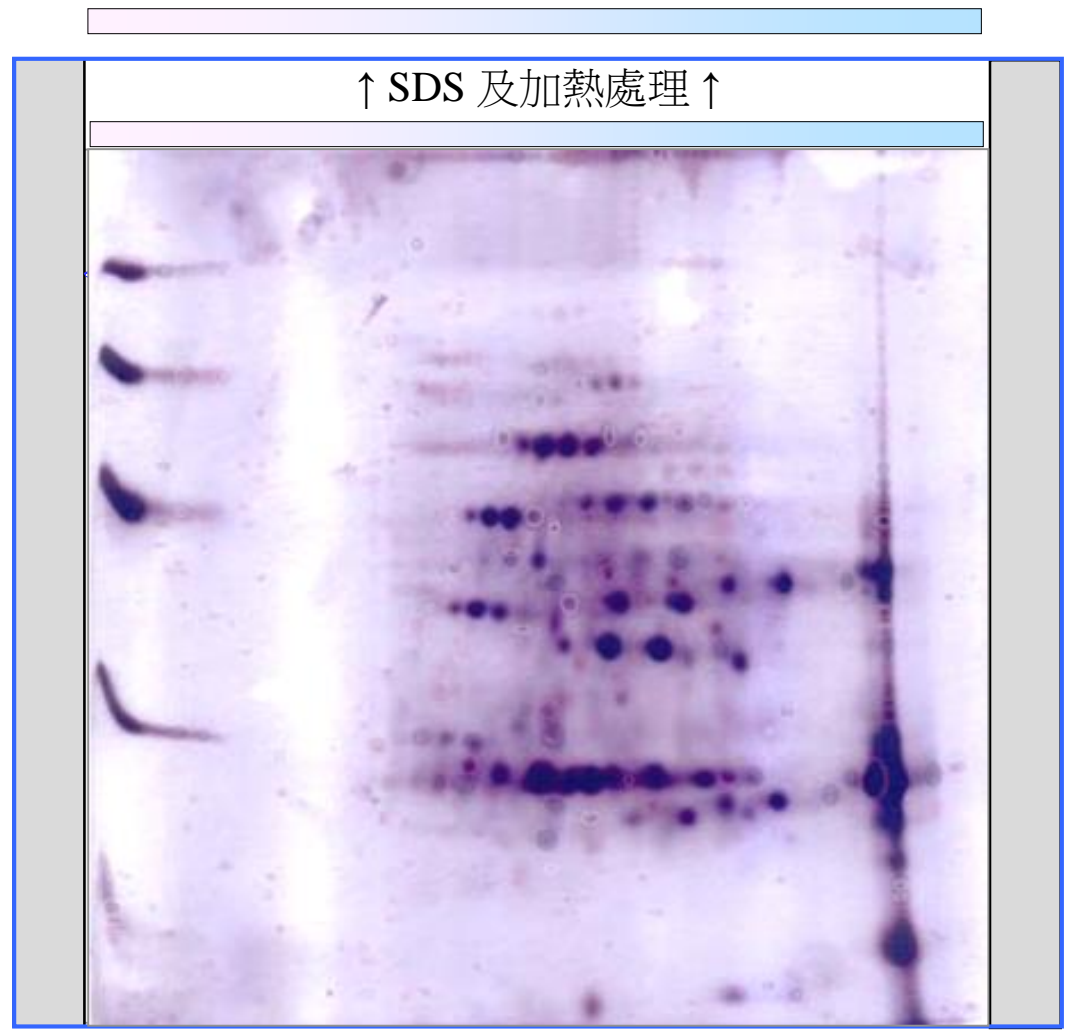
Proteome is much complex than its genome

■ 二次元電泳操作 2-DE operation

(1) IEF
等電聚焦電泳



↑ 注意 pH 梯度的產生 ↑



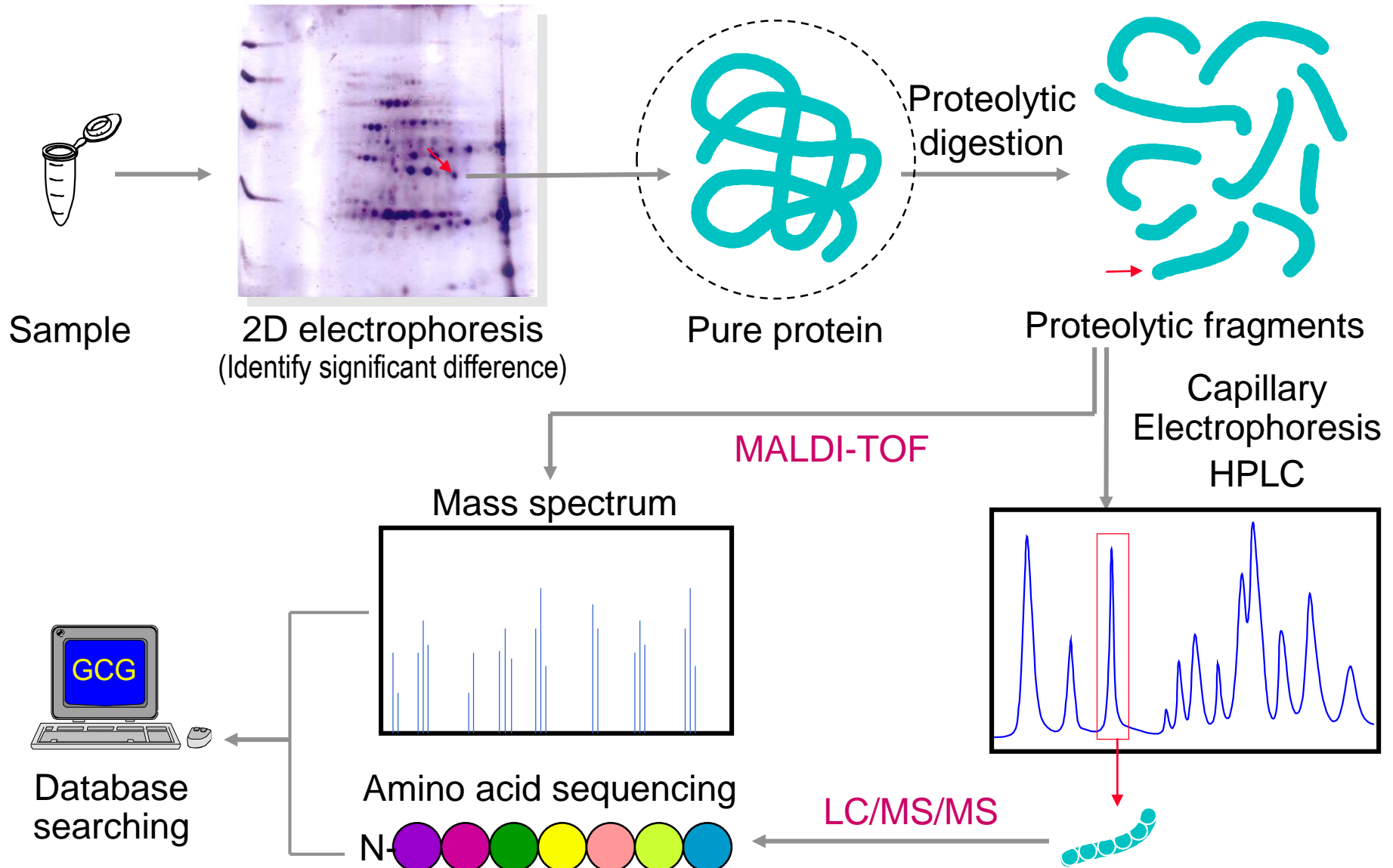
(3)
Staining
染色脫色

(2)
SDS-PAGE
分離膠體



圖 7.9

蛋白質體可綜觀蛋白質的消長與身分



2D tool provides insight from comparing proteomic difference

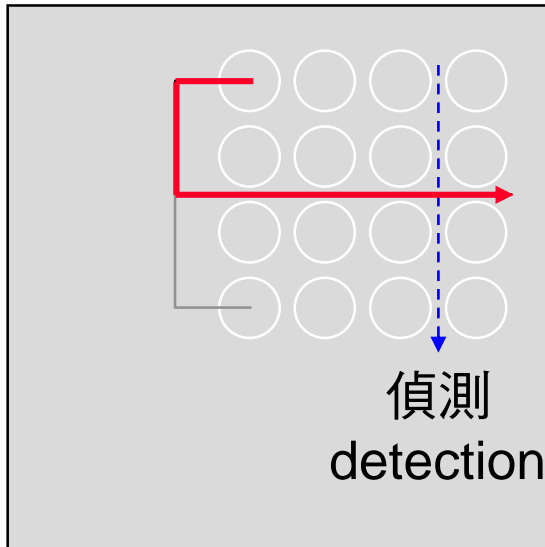
■ 微流体平台 Microfluidics, Lab-on-a-chip

Agilent 所有蛋白質純化與活性分析均微小化

Agilent HPLC-Chip/MS

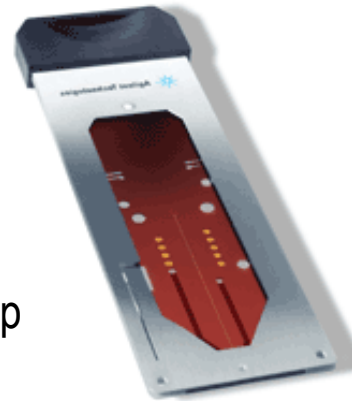
Agilent 2100 bioanalyzer

樣本 sample well



毛細管電泳
Capillary electrophoresis

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



前處理
pretreatment

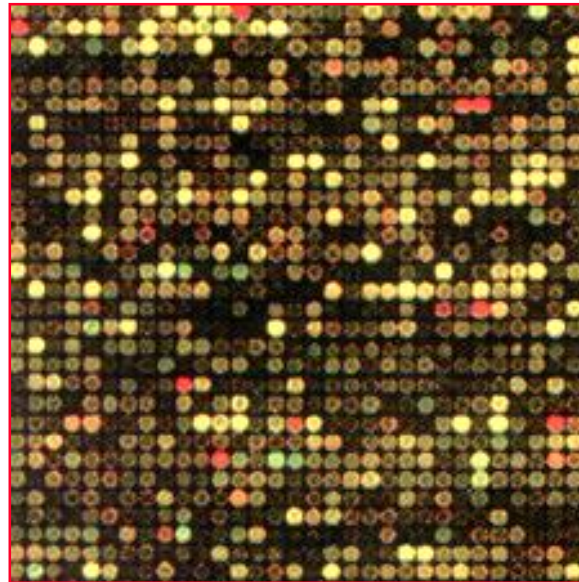
分離
管柱

質譜儀分析
Mass analysis

Minimize protein purification and analysis in one chip


■ 現代蛋白質科技特點 Modern protein technology

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



Antibody

是最**方便**取得
之**專一性**探針



Think big!

-omics

驚奇的酵素 活性之根本

