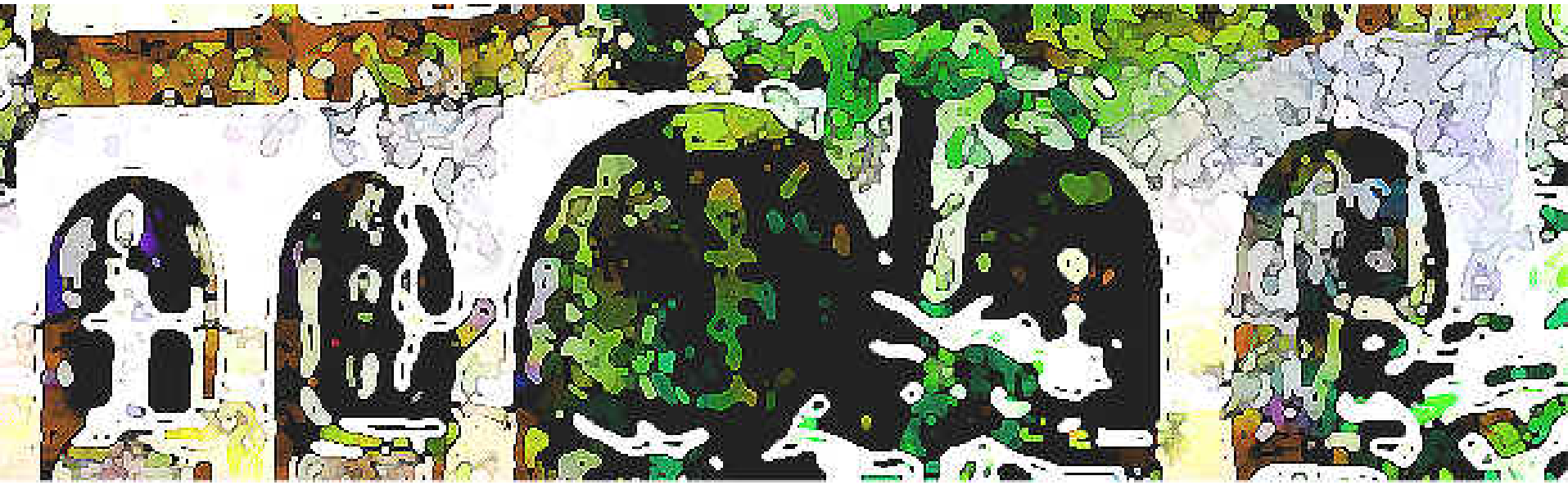
Proteomics and Antibody Bank

蛋白質體與抗體庫

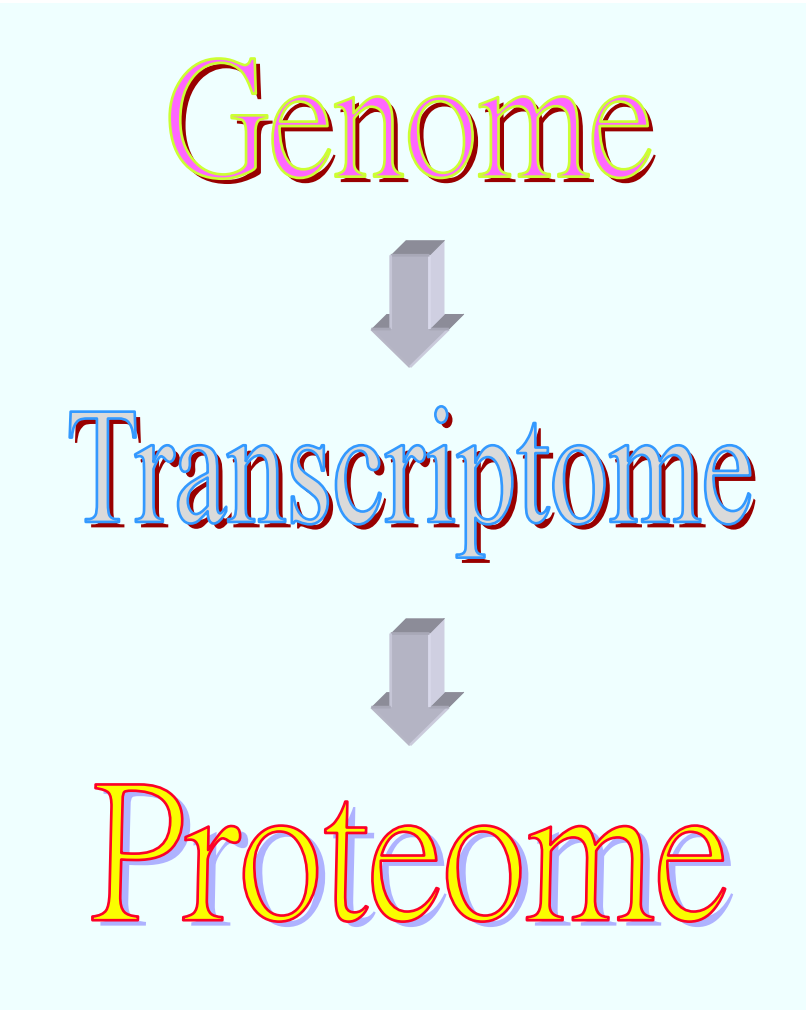


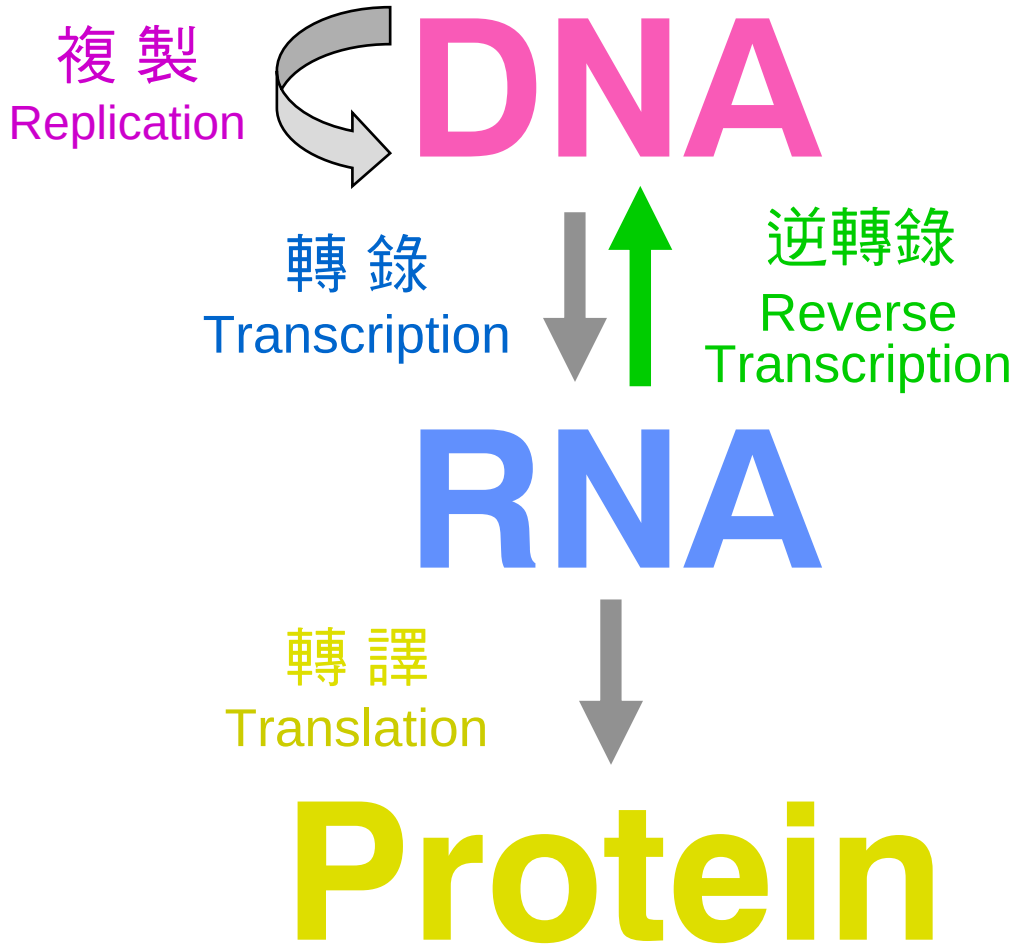
國立台灣大學 生化科技學系

莊 榮 輝

Central Dogma – 所有生物學的基本教條

蛋白質的胺基酸排列是由其基因的核苷酸序列所決定





Juang RH (2005) Proteomics

Genome

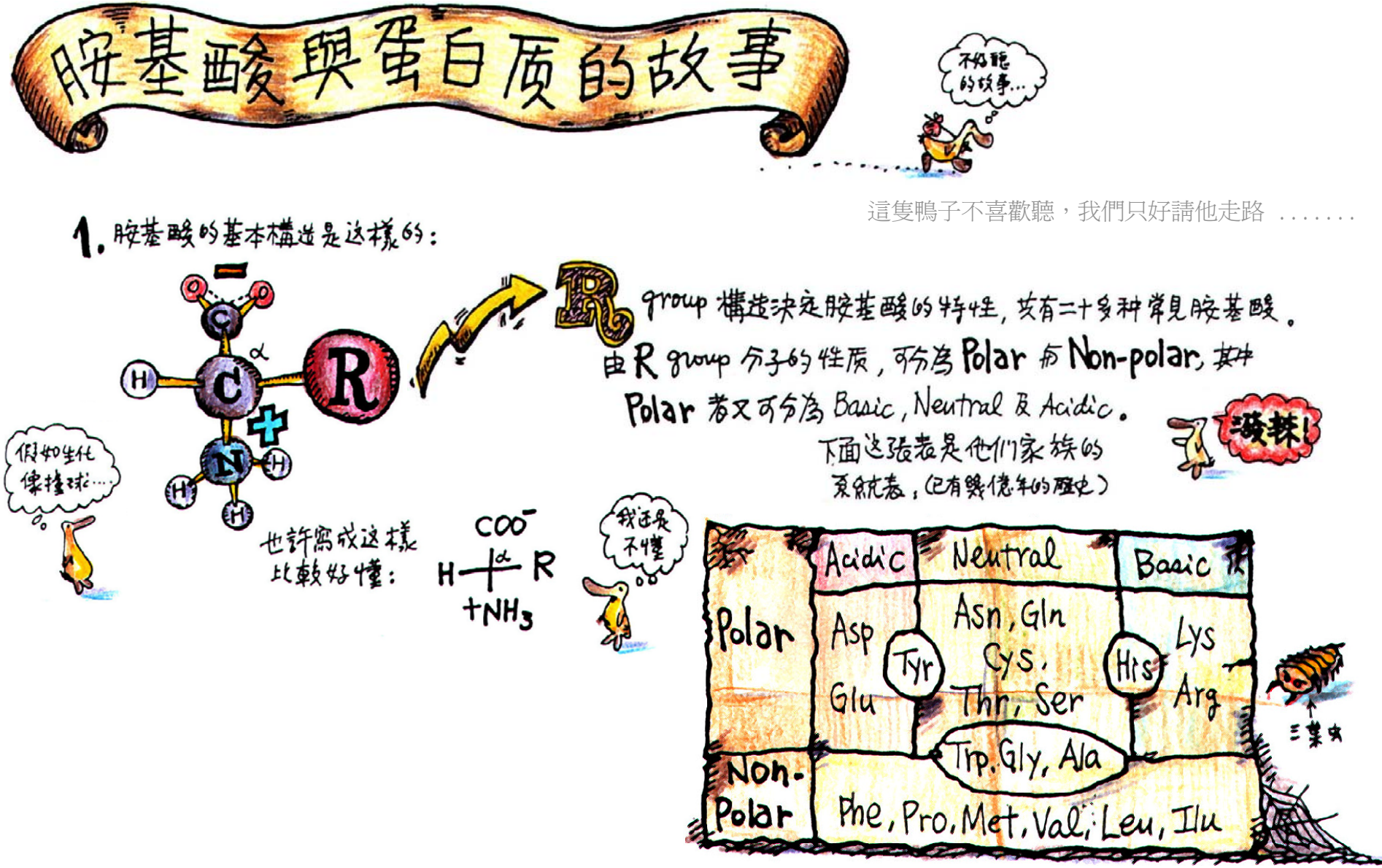
基因表現不一定完全反映在蛋白質

由基因體較難預測蛋白質的修飾及調控

也無法預測蛋白質間的交互作用

Proteome

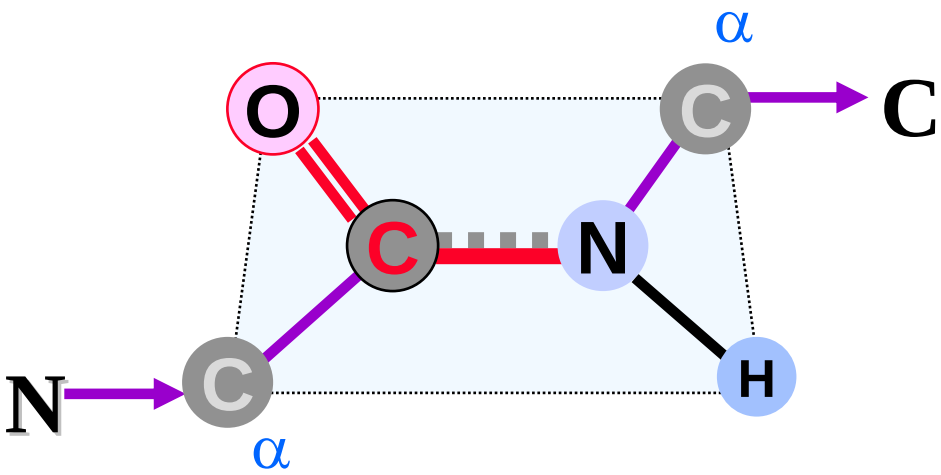
Juang RH (2005) Proteomics

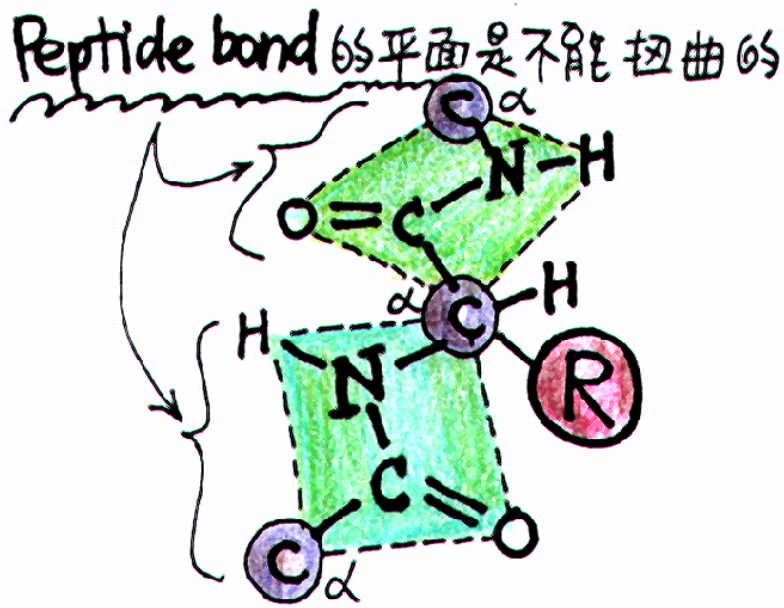


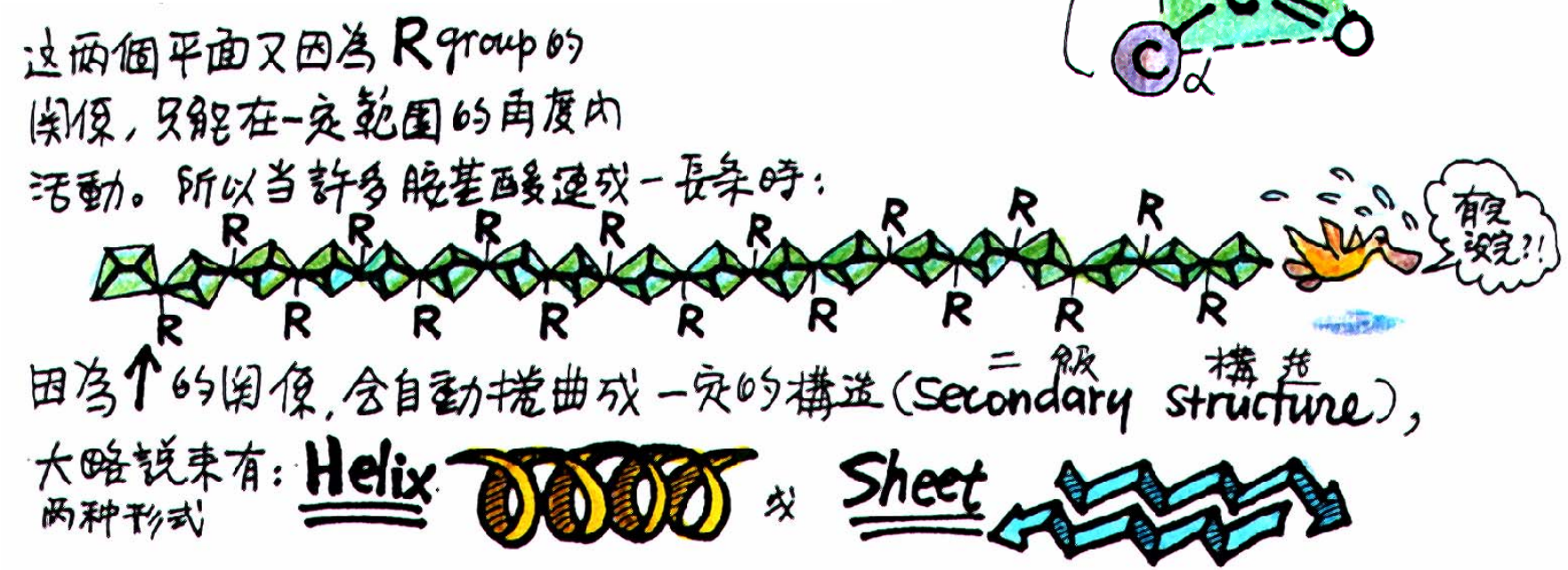
胺基酸側鏈基團 R 的化學性質不同

Juang RH (2005) Proteomics

胜鍵是組成蛋白質構形的最基本單位



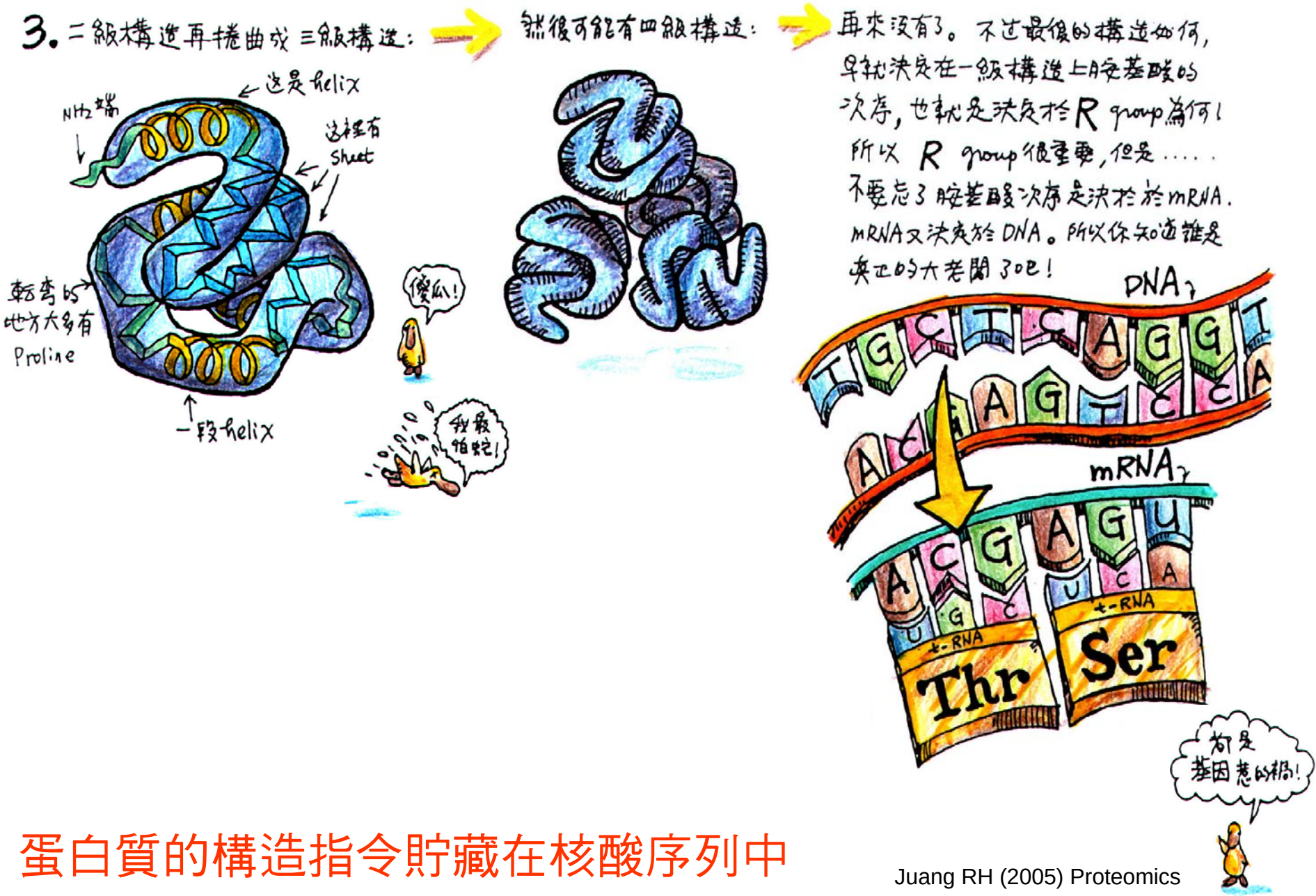




二級構造主要有兩種形式

Juang RH (2005) Proteomics

三級構造已經有特定構形與功能



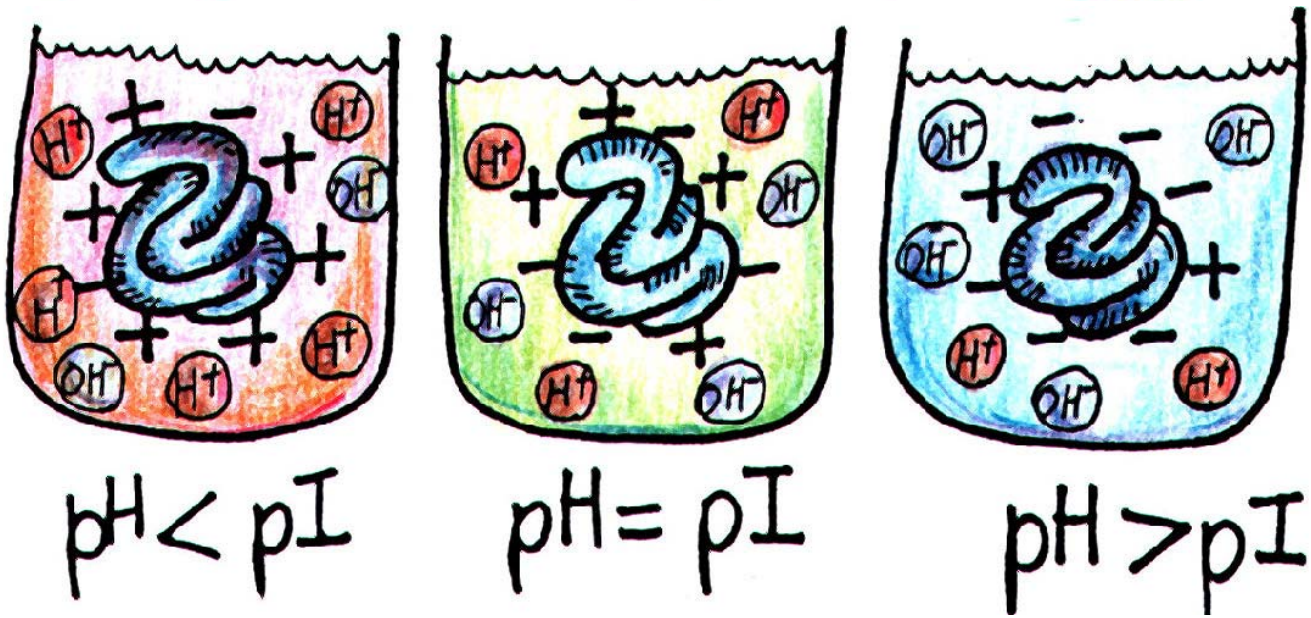
蛋白質的構造指令貯藏在核酸序列中

Juang RH (2005) Proteomics

蛋白質的帶電性質會影響其功能

4. 有一個很重要的概念，就是胺基酸或蛋白質的淨电荷是正是负，完全決定於环境的 pH！

若环境的 pH > pI，則其淨电荷為負。
若环境的 pH = pI，則其淨电荷為零。
若环境的 pH < pI，則其淨电荷為正。

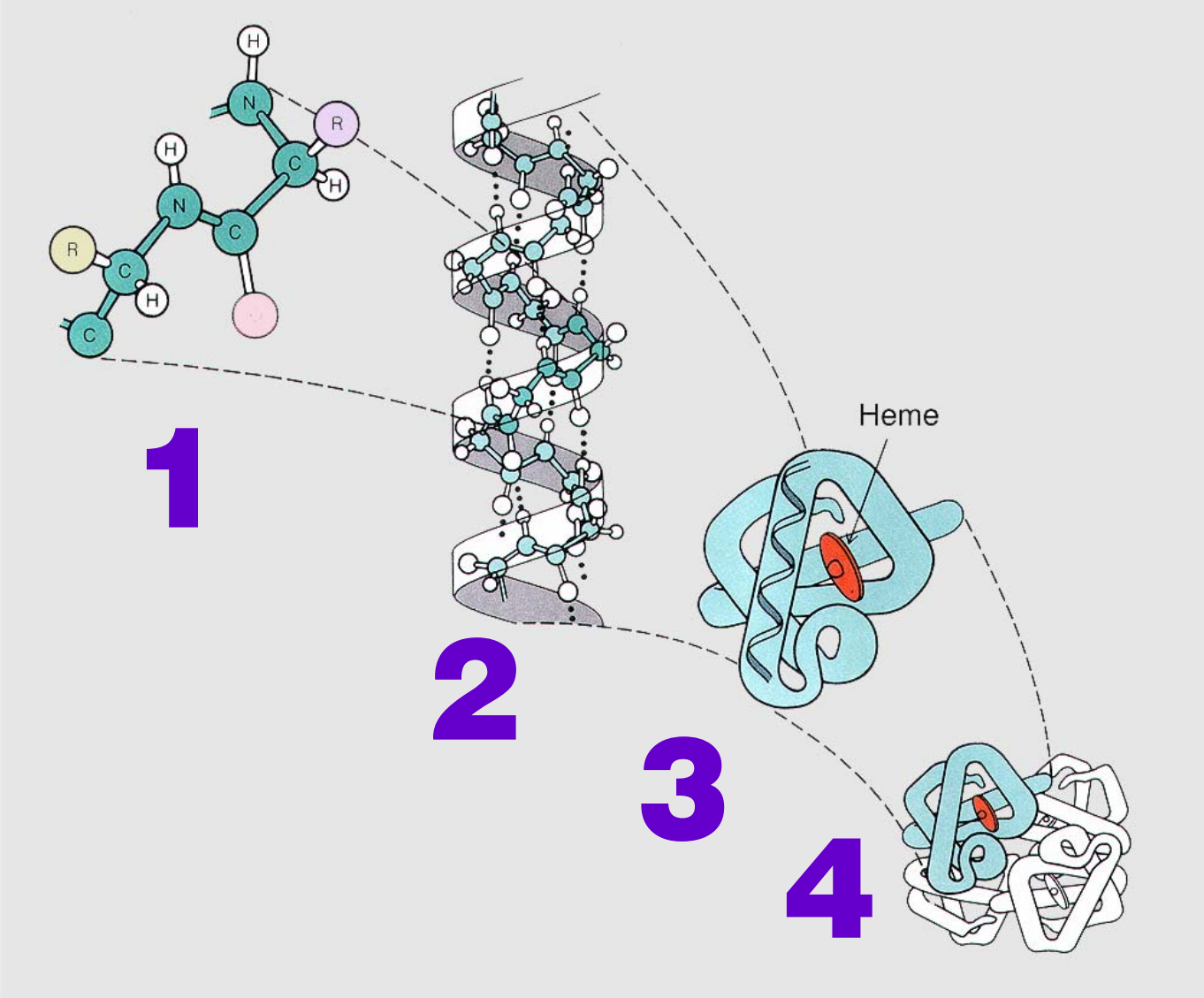


環境的酸鹼度影響蛋白質的電荷性質

Juang RH (2005) Proteomics

由化學物質組成具有活性的巨分子

蛋白質分子構形的組成有四個層次



序列

↓

構形

↓

活性

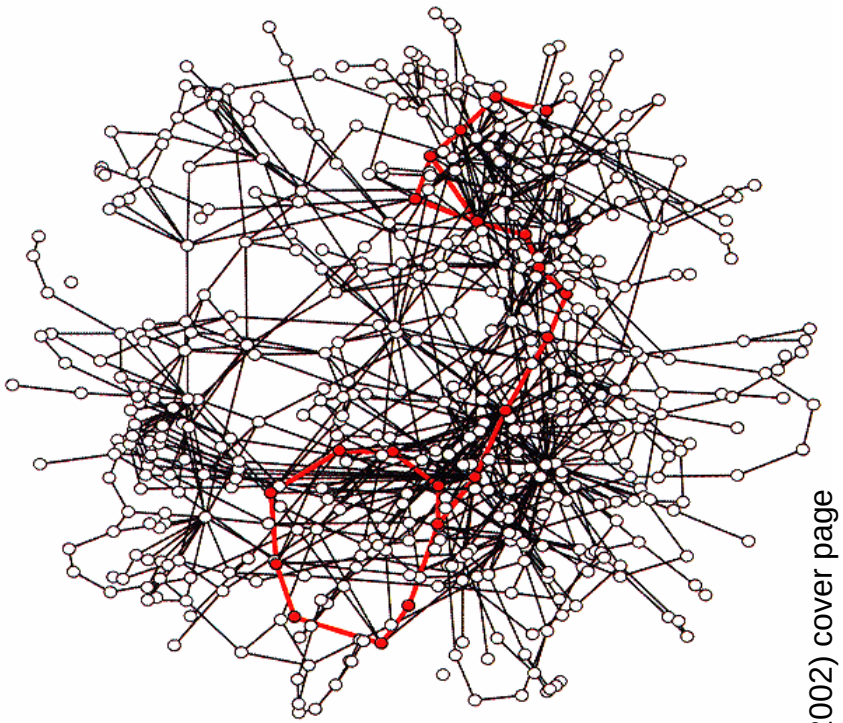
↓

調節

Mathews et al (2000) Biochemistry (3e) p.195

由蛋白質體學延伸到系統生物學

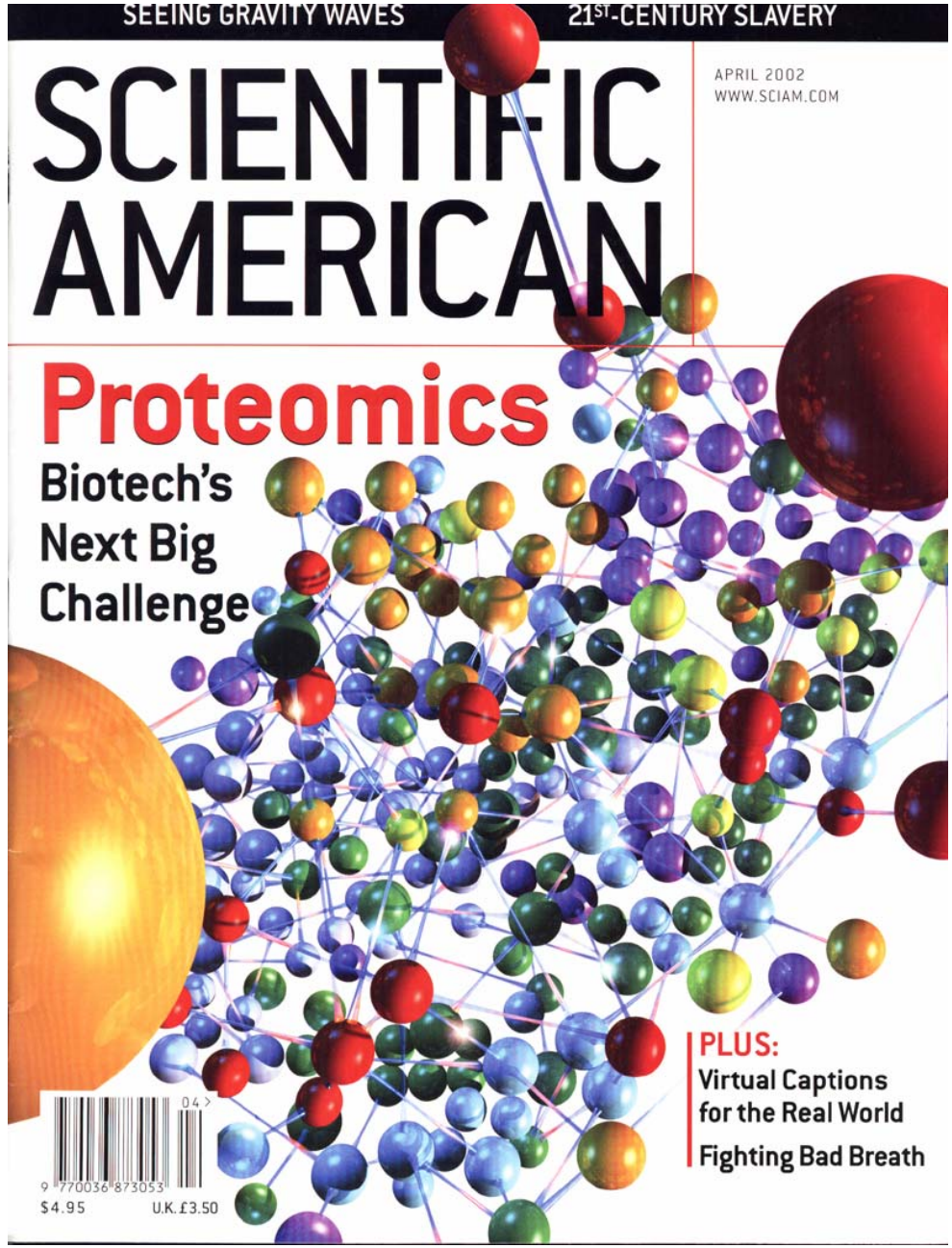
代謝路徑立體圖



Alberts et al (2002) Molecular Biology of the Cell (4e) p.107

Systems Biology

整體性的生物學觀念與工具



Scientific American (2002) cover page

基因表現不一定完全反應在蛋白質圖譜

One Gene, One Protein?

細胞中

(1) 細胞內的每個基因不一定都在表現
(2) 各器官、組織的基因表現都不相同
(3) 基因表現隨著生長時期而有改變
(4) 蛋白質表現後有進一步的修飾與調控
(5) 蛋白質在細胞內的代謝速率不同

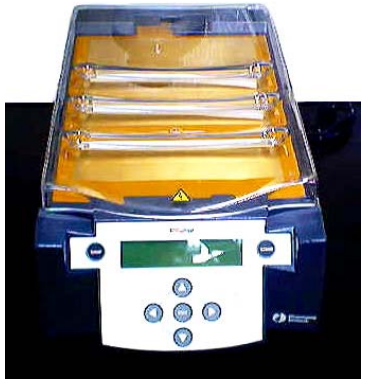
試管中

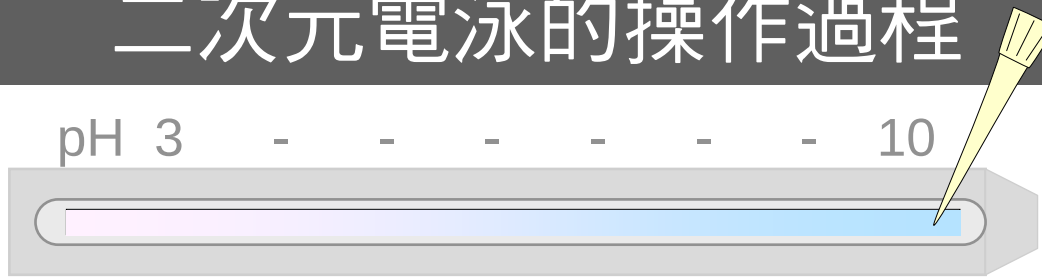
(1) 蛋白質的水溶性會影響抽取效率
(2) 蛋白質的含量差異很大
(3) 蛋白質在抽取後的安定性與半衰期不同

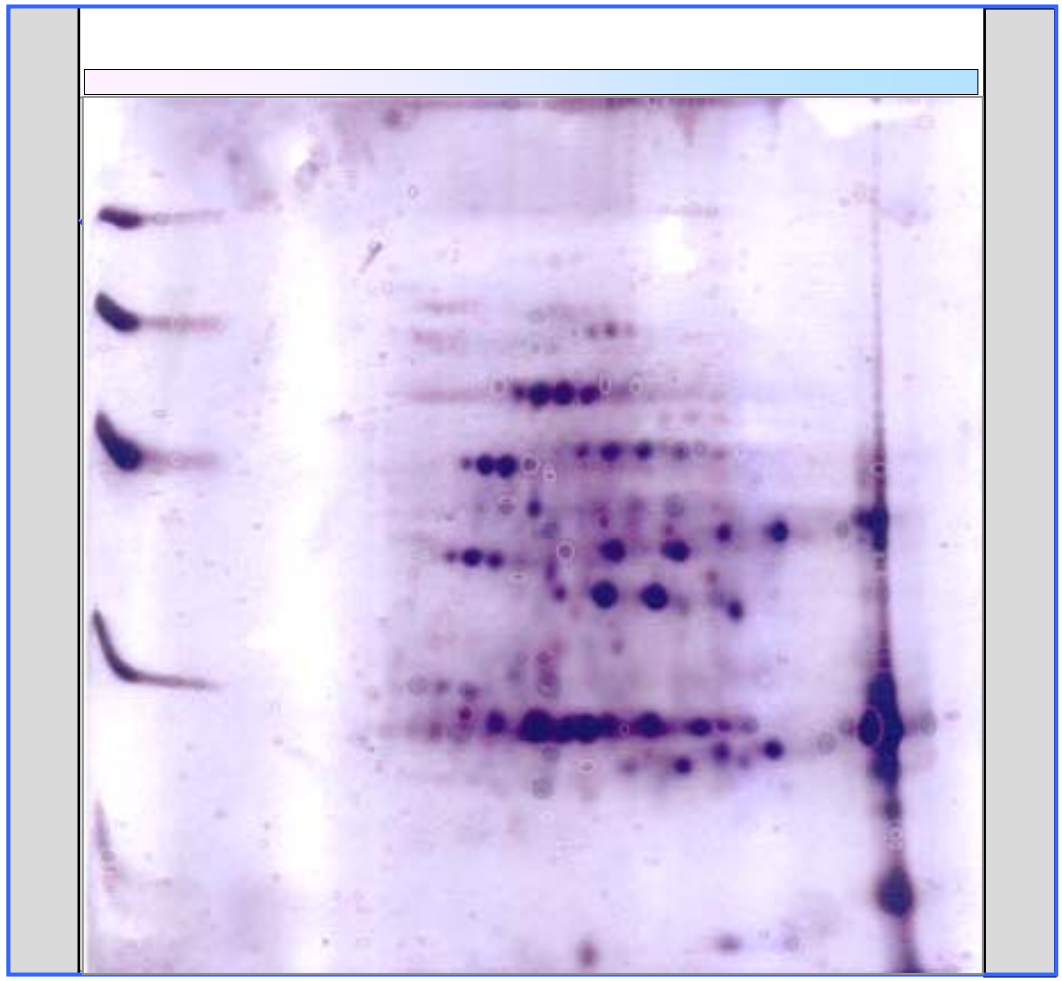
Juang RH (2005) Proteomics

二次元電泳的操作過程

(1) IEF
等電焦集電泳





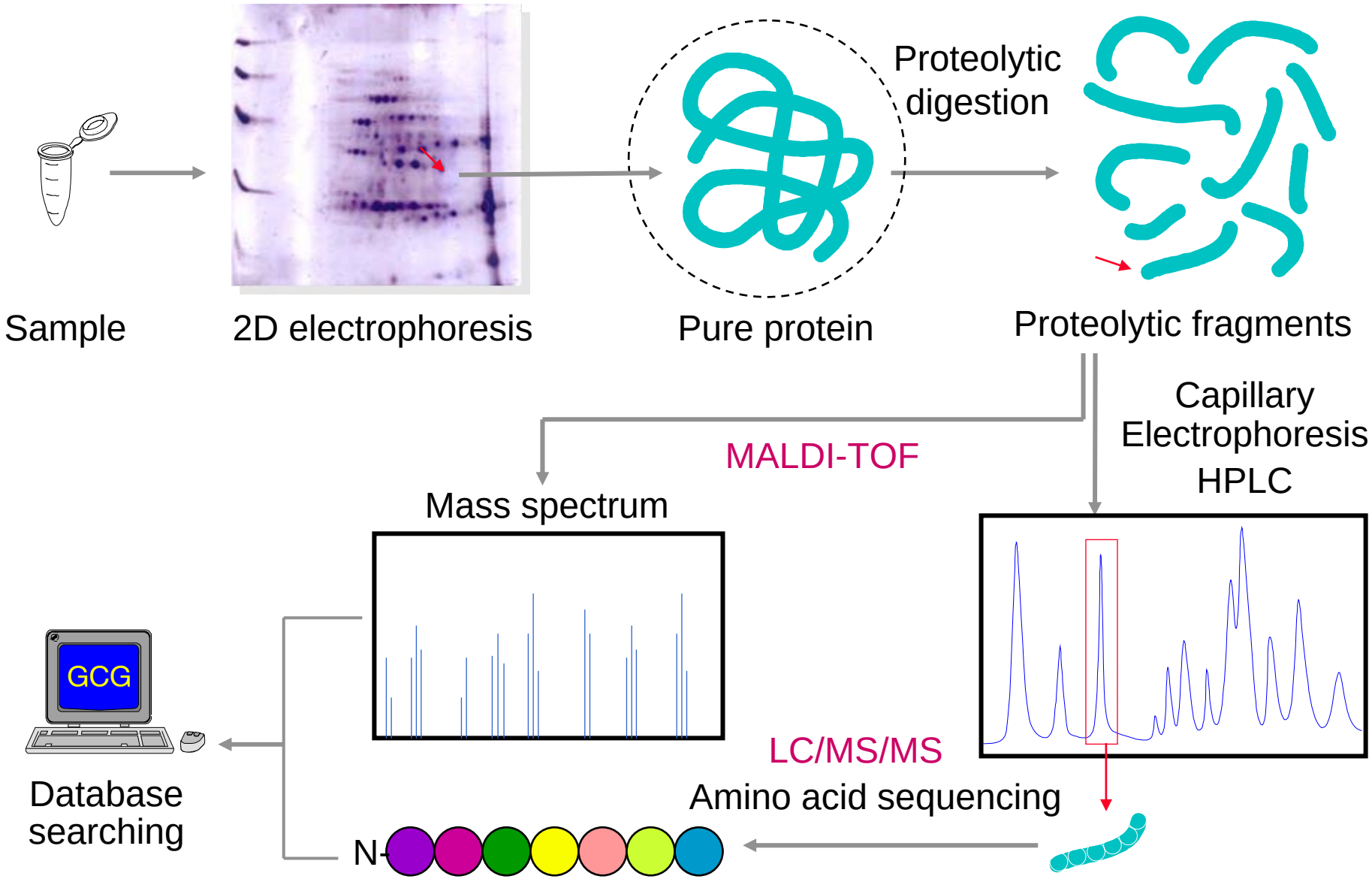


(2)
SDS-PAGE
分離膠體

(3)
染色脫色

Juang RH (2005) Proteomics

蛋白質體可綜觀蛋白質的消長與身分：



Juang RH (2005) Proteomics

蛋白質構造與組成分析

- N-端或 C-端胺基酸決定：

  通常都直接定序，C-端較爲困難

- 胺基酸組成分析
- 胺基酸定序方法：

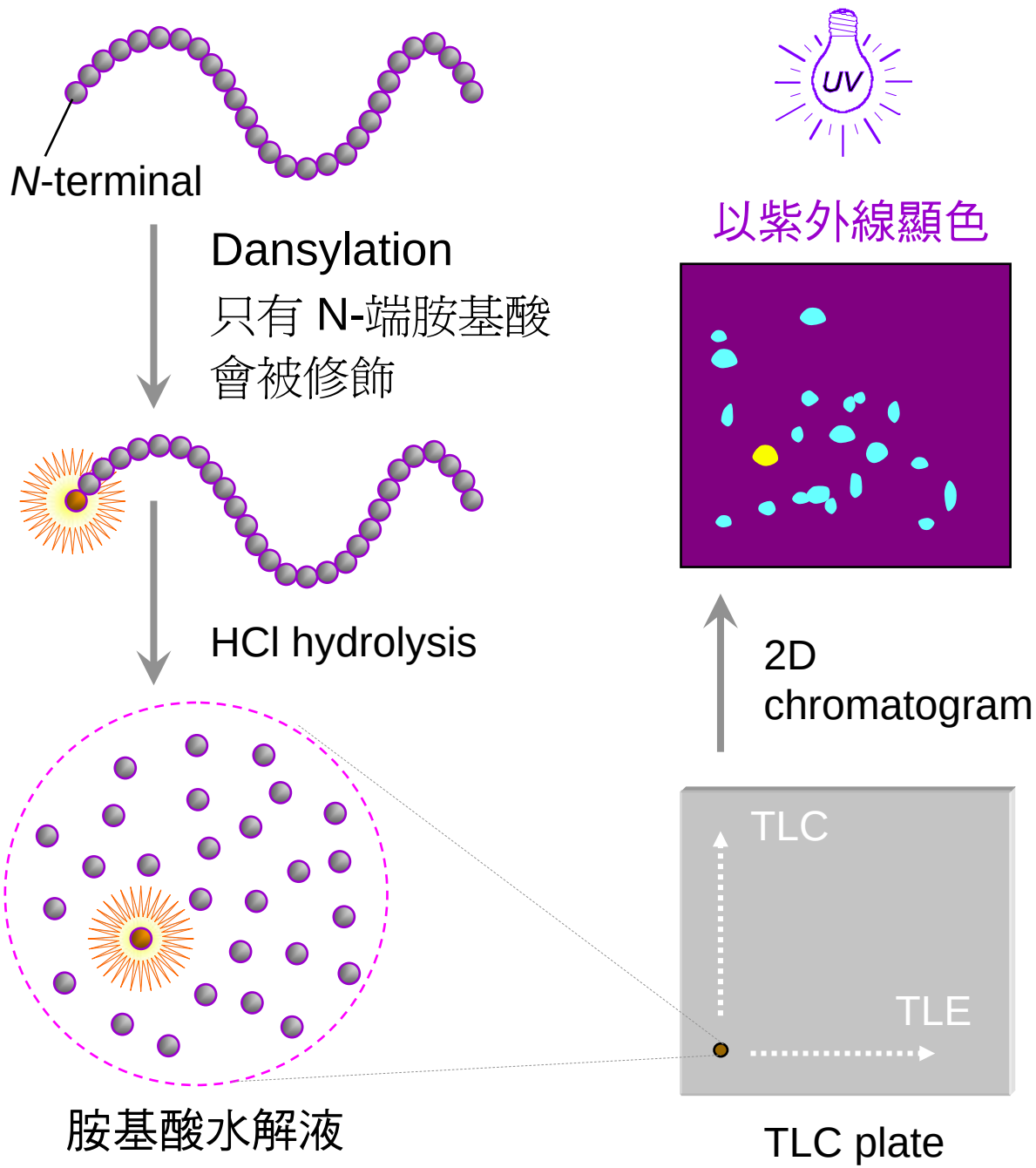
  cDNA 間推法

  Edman 直接定序法

  質譜儀定序

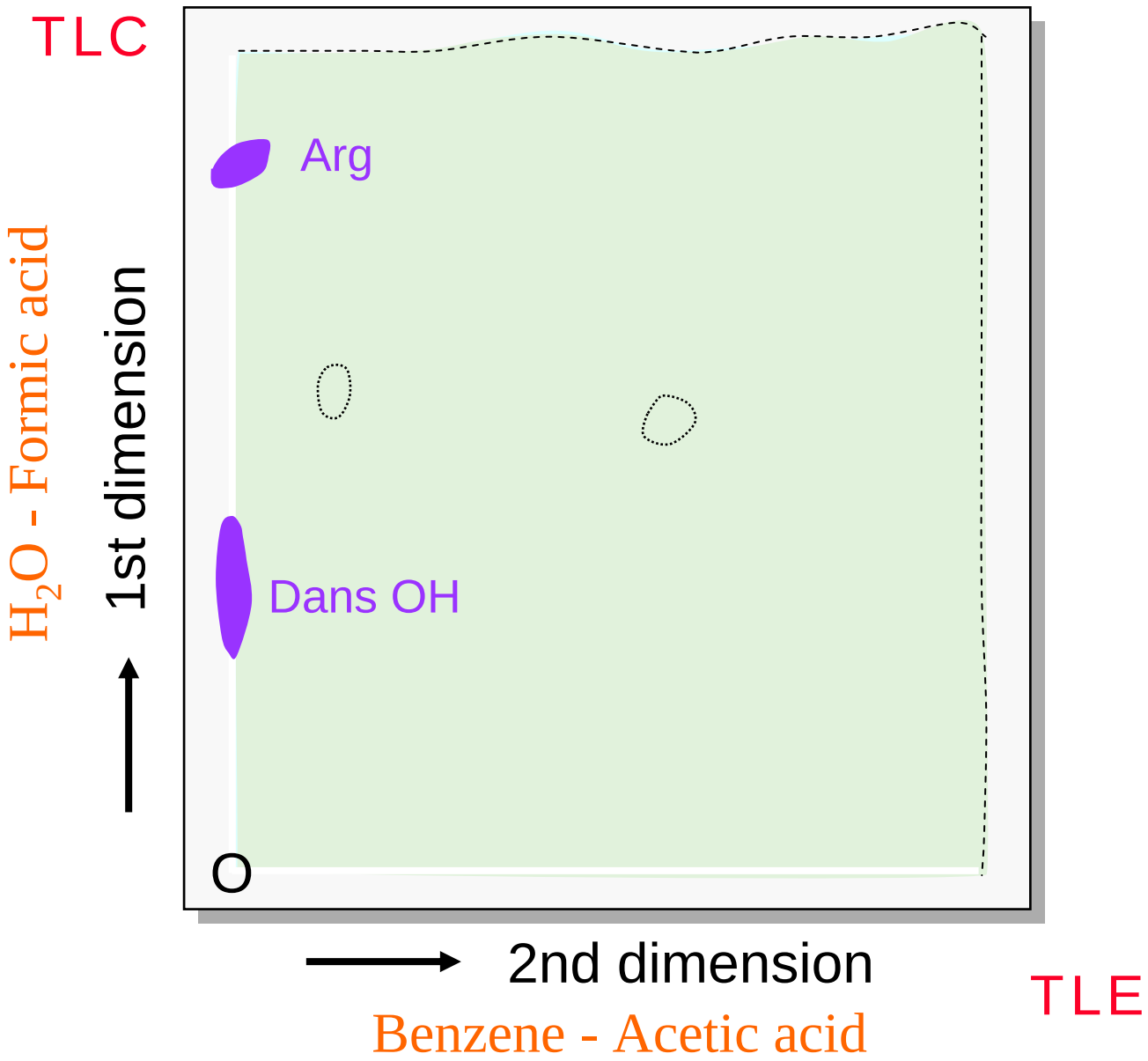
- 胜肽圖譜

決定N端胺基酸：

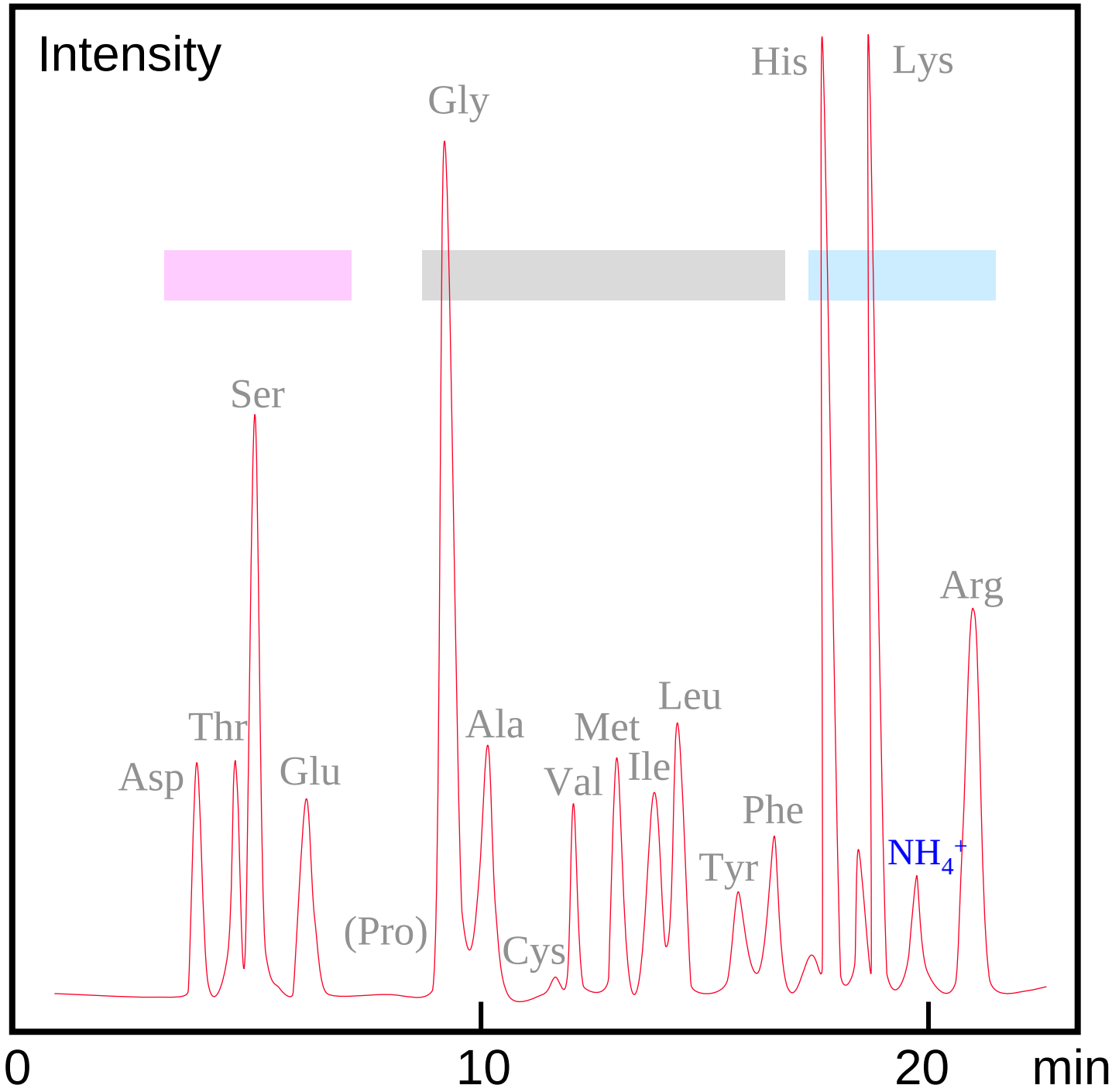

N-terminal
Dansylation
只有 N-端胺基酸
會被修飾
HCl hydrolysis
胺基酸水解液
UV
以紫外線顯色
2D
chromatogram
TLC
TLE
TLC plate

以薄層層析法鑑定胺基酸：

- 二次元薄層層析電泳可分離出二十種胺基酸

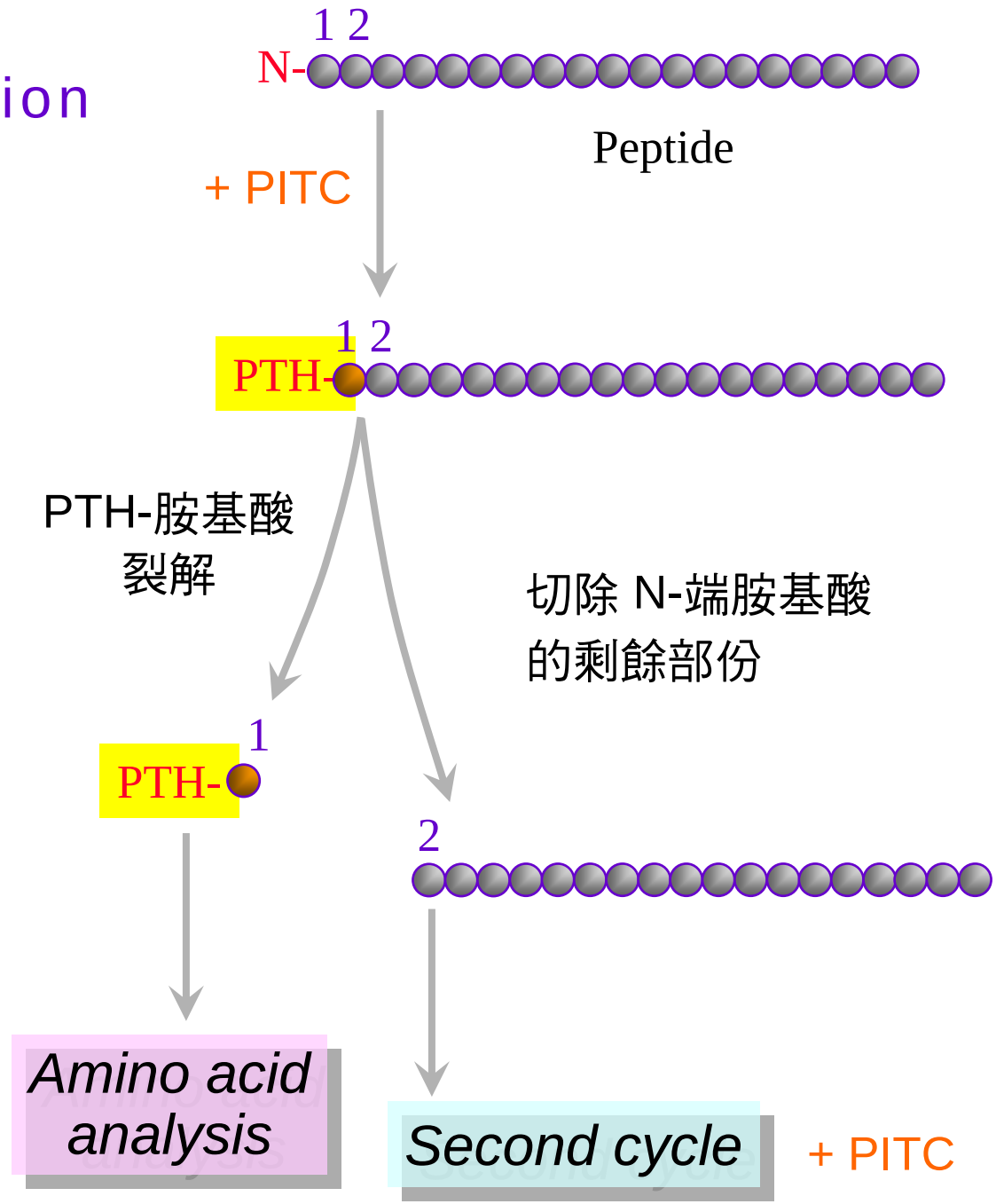


以液相層析分離鑑定胺基酸：

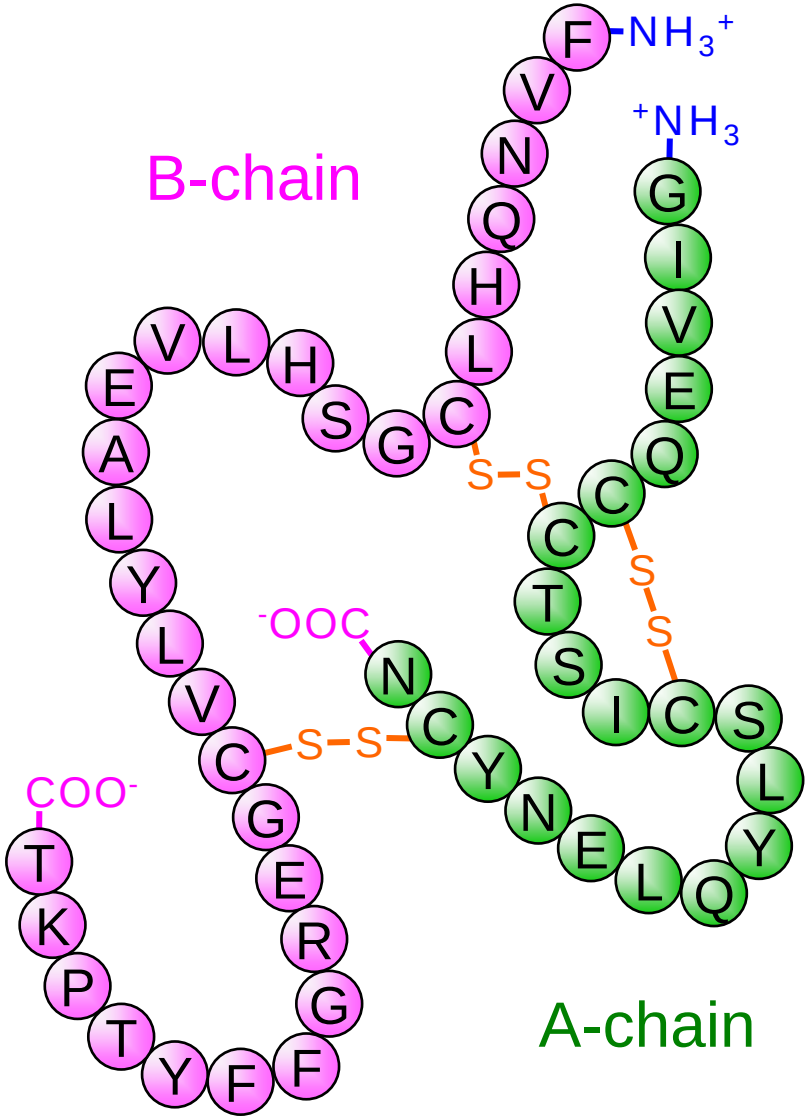


決定胺基酸序列：

Edman degradation

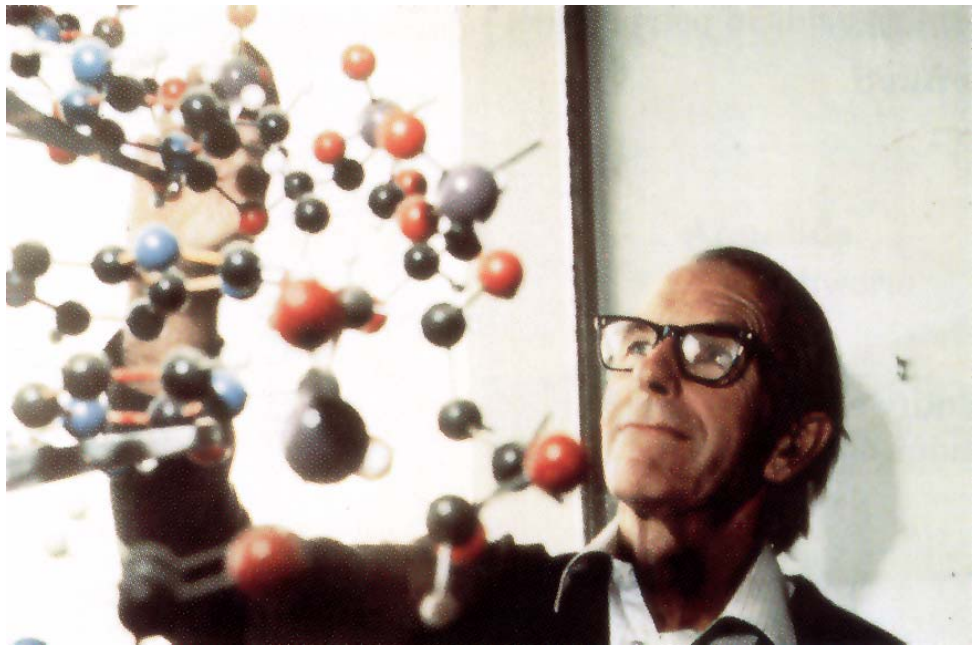


以傳統胺基酸定序法決定蛋白質序列：



F. Sanger (1980, Cambridge U)

Insulin 胰島素 (A, B chains)



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

蛋白質體分析工具建立

大型二次元電泳

蛋白質序列分析

蛋白質的專一性水解：

- 專一性內切酶：

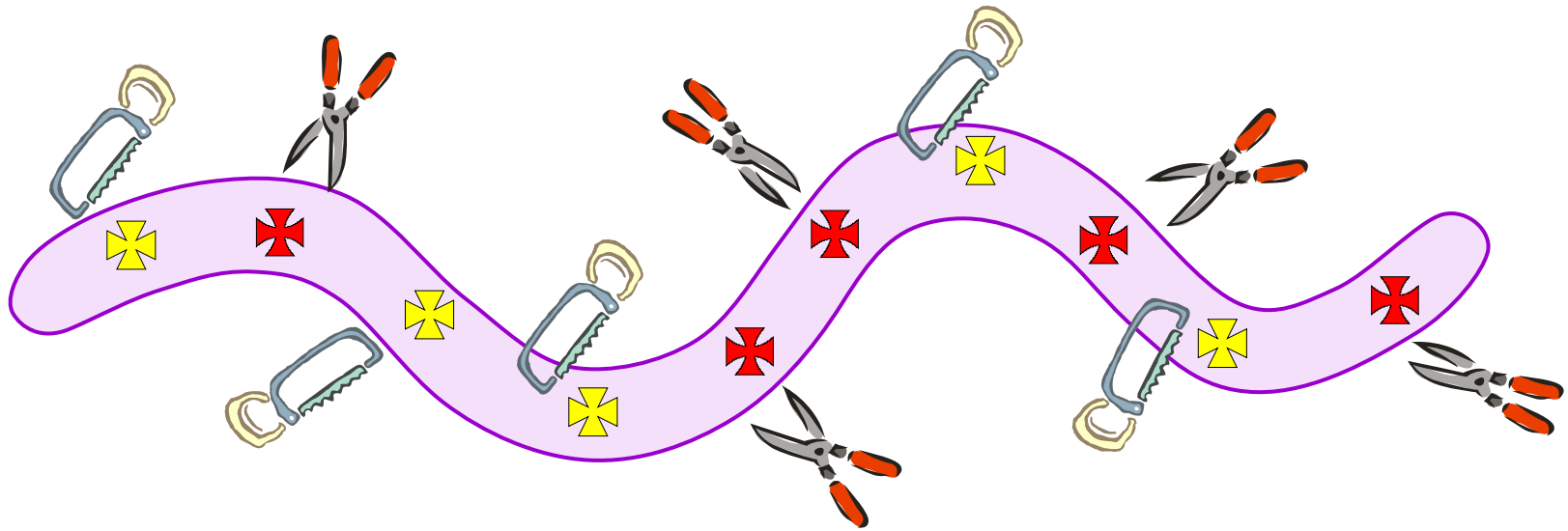
  Trypsin, Chymotrypsin

- 檢定胜肽群的方法：

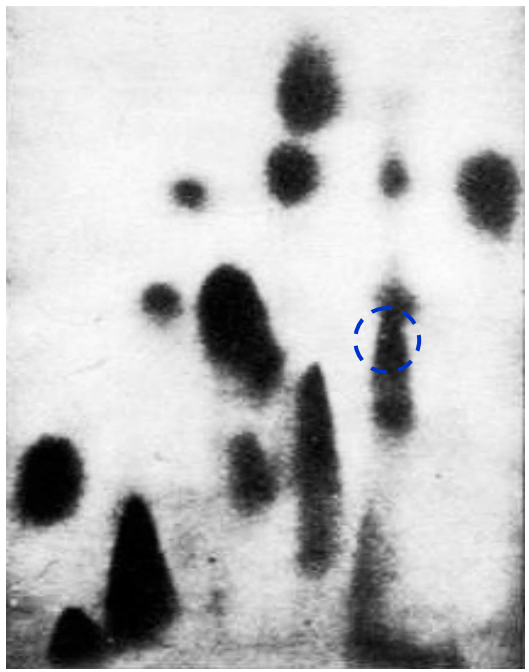
  TLE/TLC HPLC SDS-PAGE

  MALDI-TOF LC/MS/MS

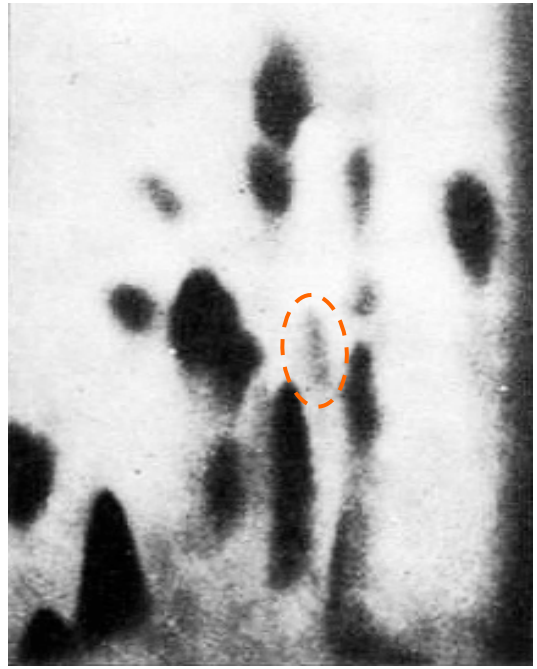
使用專一性蛋白酶



以雙向層析電泳鑑定胜肽：

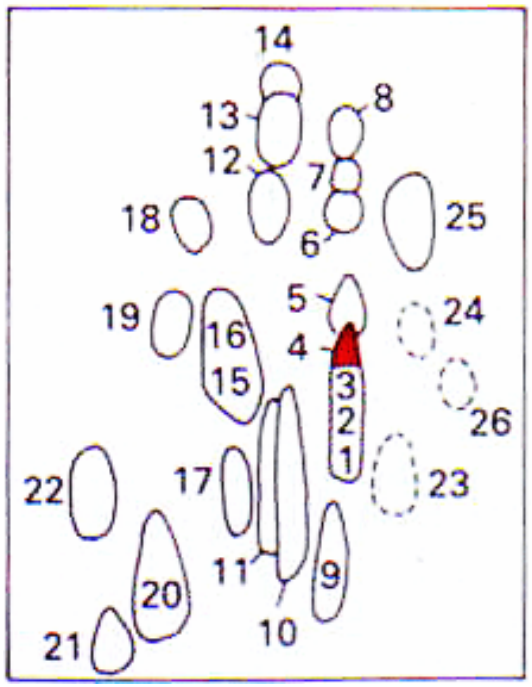


Hemoglobin A

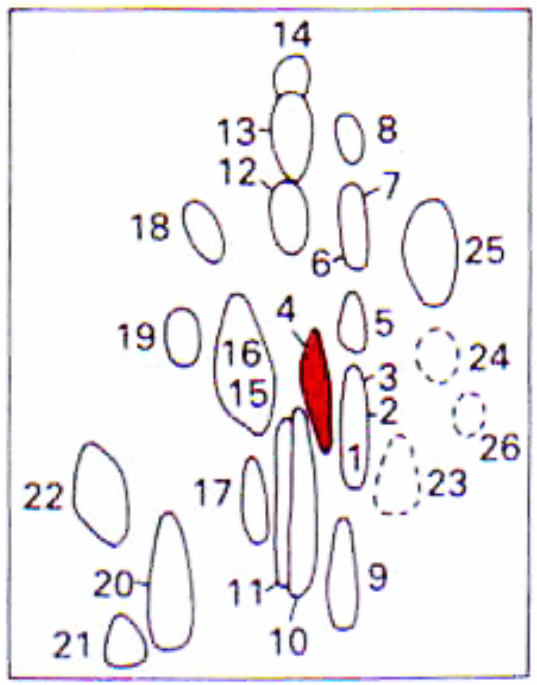


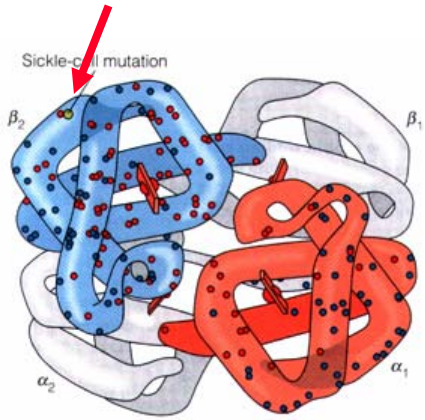
Hemoglobin S

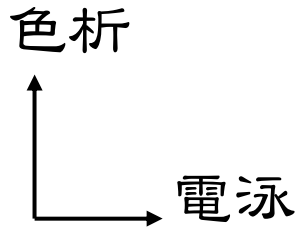
鐮型血球



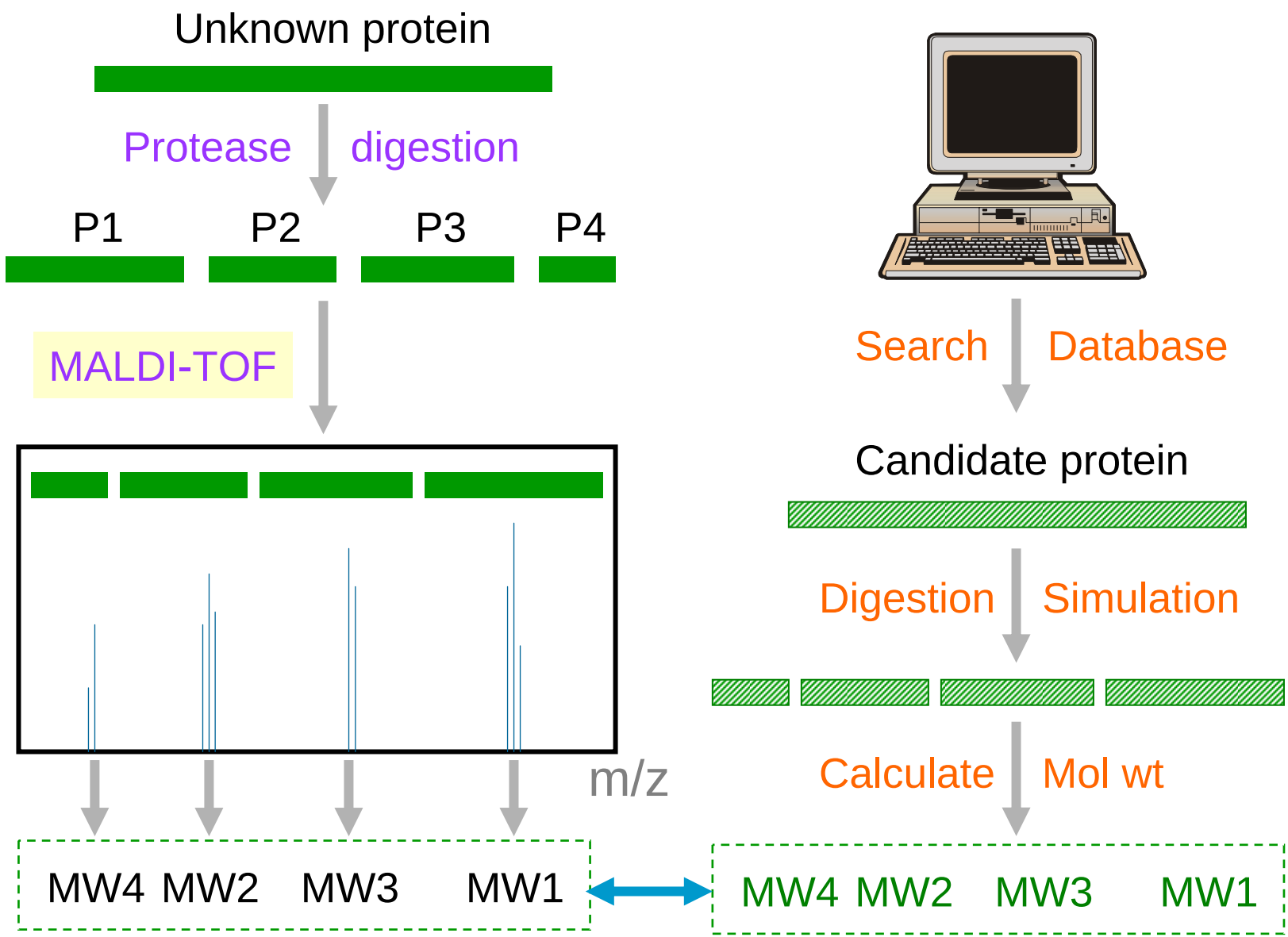
血紅蛋白四號片段







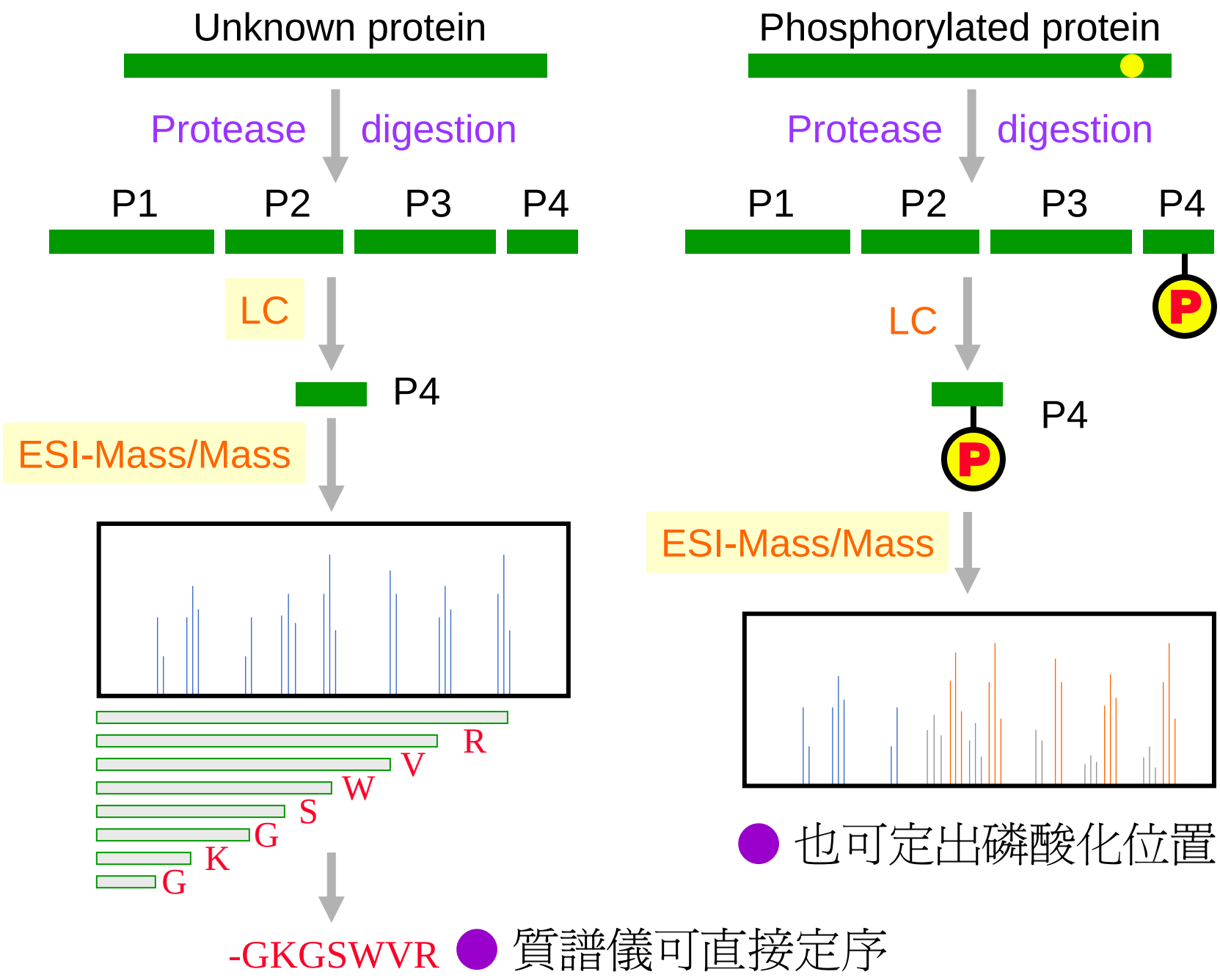
質譜儀可檢定蛋白質身分：



- 比對各片段分子量可確定該蛋白質身分

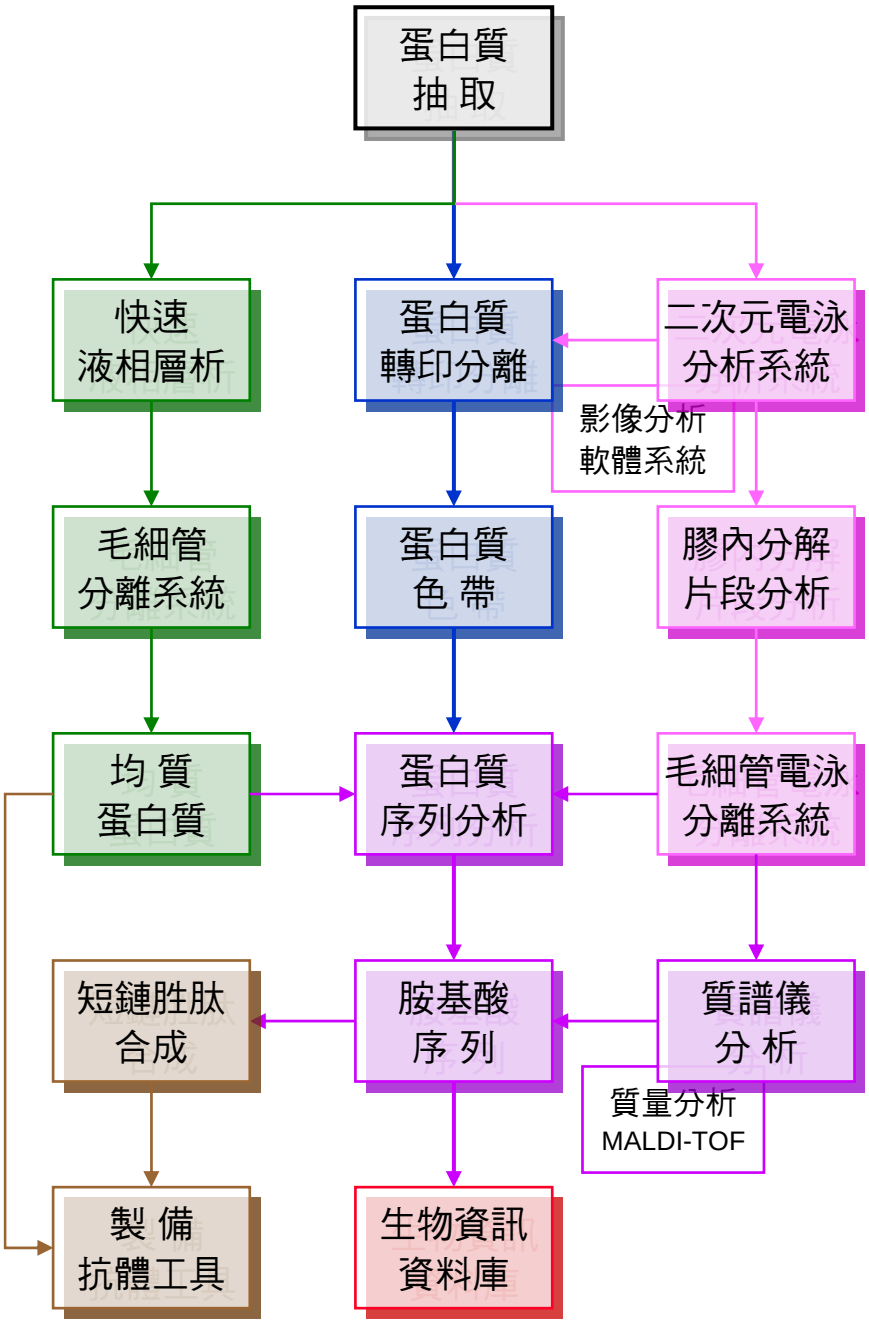
Juang RH (2005) Proteomics

質譜儀可進行蛋白質序列分析：



Juang RH (2005) Proteomics

蛋白質的微量分離及檢定：



蛋白質科技
Protein Technology

蛋白質分離分析

1 電泳及轉印

2 二次元電泳

3 膠體內水解

4 微量分離純化

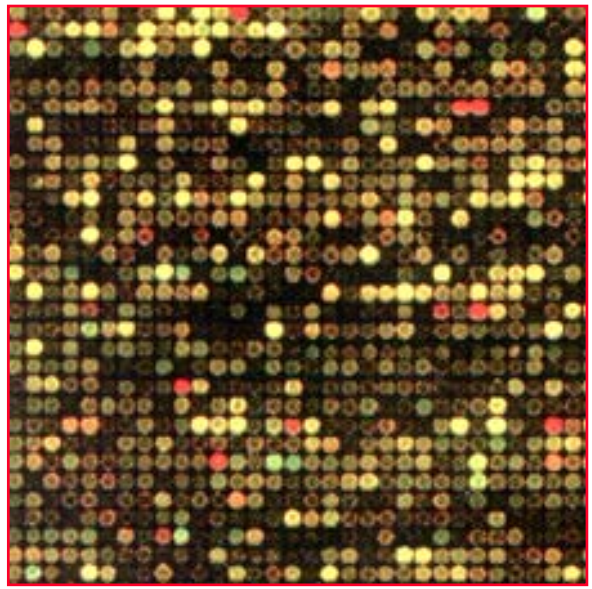
微量分析系統

抗體製備

生物資訊學

現代蛋白質科技的特點：

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



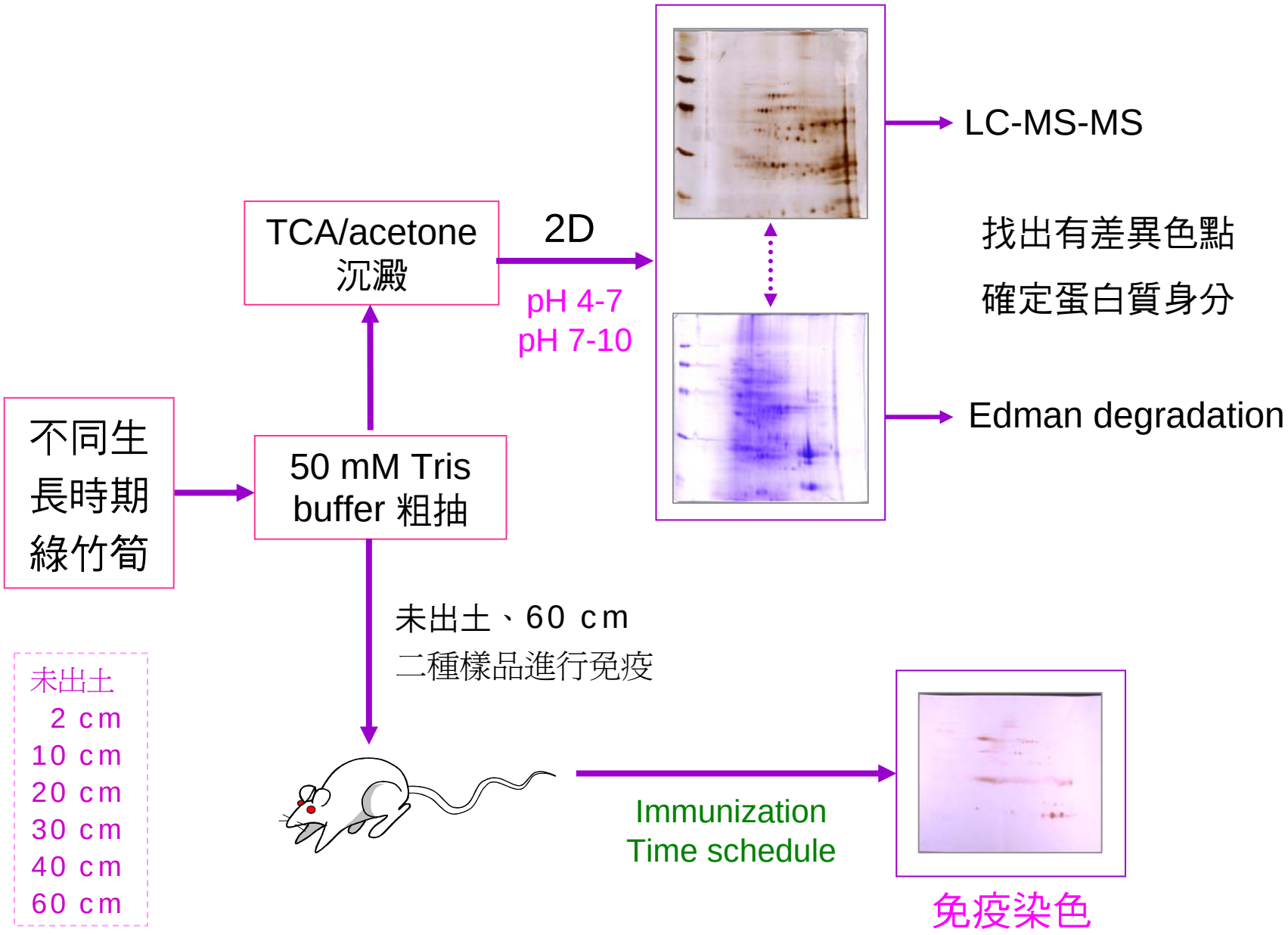
綠竹筍生長與老化機制之探討及相關功能基因之利用

2004/6/1

主持人：莊榮輝
博士班：吳裕仁
及竹筍小組

綠竹筍
(Bambusa oldhami)

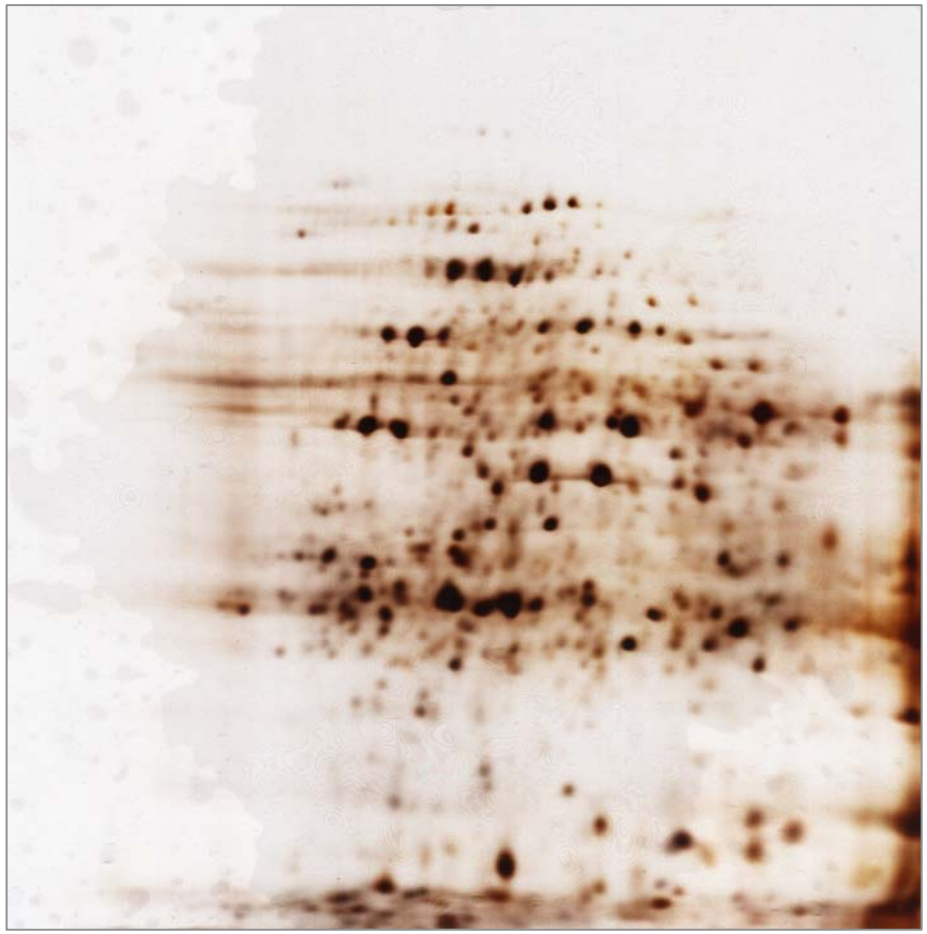
整體研究流程大綱



Juang RH (2005)

竹筍快速生長前後的蛋白質表現確有差異

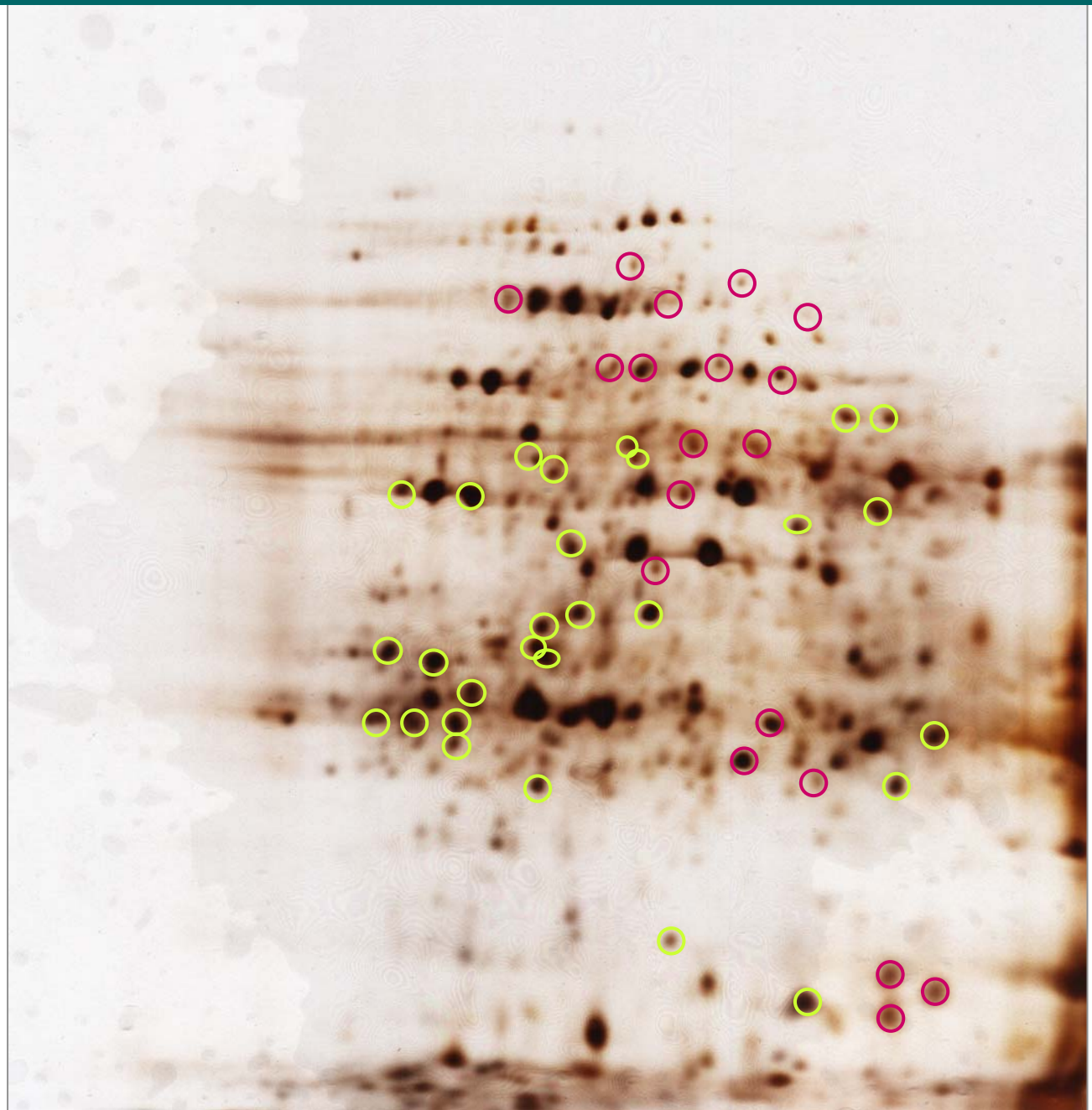
未出土



出土 60 cm

Juang RH (2005)

比對出漸增與漸減的差異



出土 60 cm

漸增

漸減

Juang RH (2005)

漸增蛋白質色點的身分

編號	Protein ID	分析方法	代謝途徑
B16	Heat-shock protein 70 Heat-shock protein 82	LC-MS/MS	Chaperone response
R26	Heat-shock protein 70	LC-MS/MS	Chaperone response
B17	20S Proteasome alpha subunit F	LC-MS/MS	Proteasome response
R25	20S Proteasome alpha subunit E	LC-MS/MS	Proteasome response
B21	Glutathione-S-transferase	N-terminal	Stress induced protein
R27	Glutathione-S-transferase	LC-MS/MS	Stress induced protein

竹筍快速生長時可能啟動抗逆境機制。

Juang RH (2005)

漸減蛋白質色點的身分

編號	Protein ID	分析方法	代謝途徑
B2	Enolase	LC-MS/MS	Glycolysis
B3	Enolase	LC-MS/MS	Glycolysis
B4	Enolase	LC-MS/MS N-terminal	Glycolysis
R4	Phosphoglycerate mutase	LC-MS/MS	Glycolysis
R5	Phosphoglycerate mutase	LC-MS/MS	Glycolysis

竹筍快速生長時醣解作用可能降低。

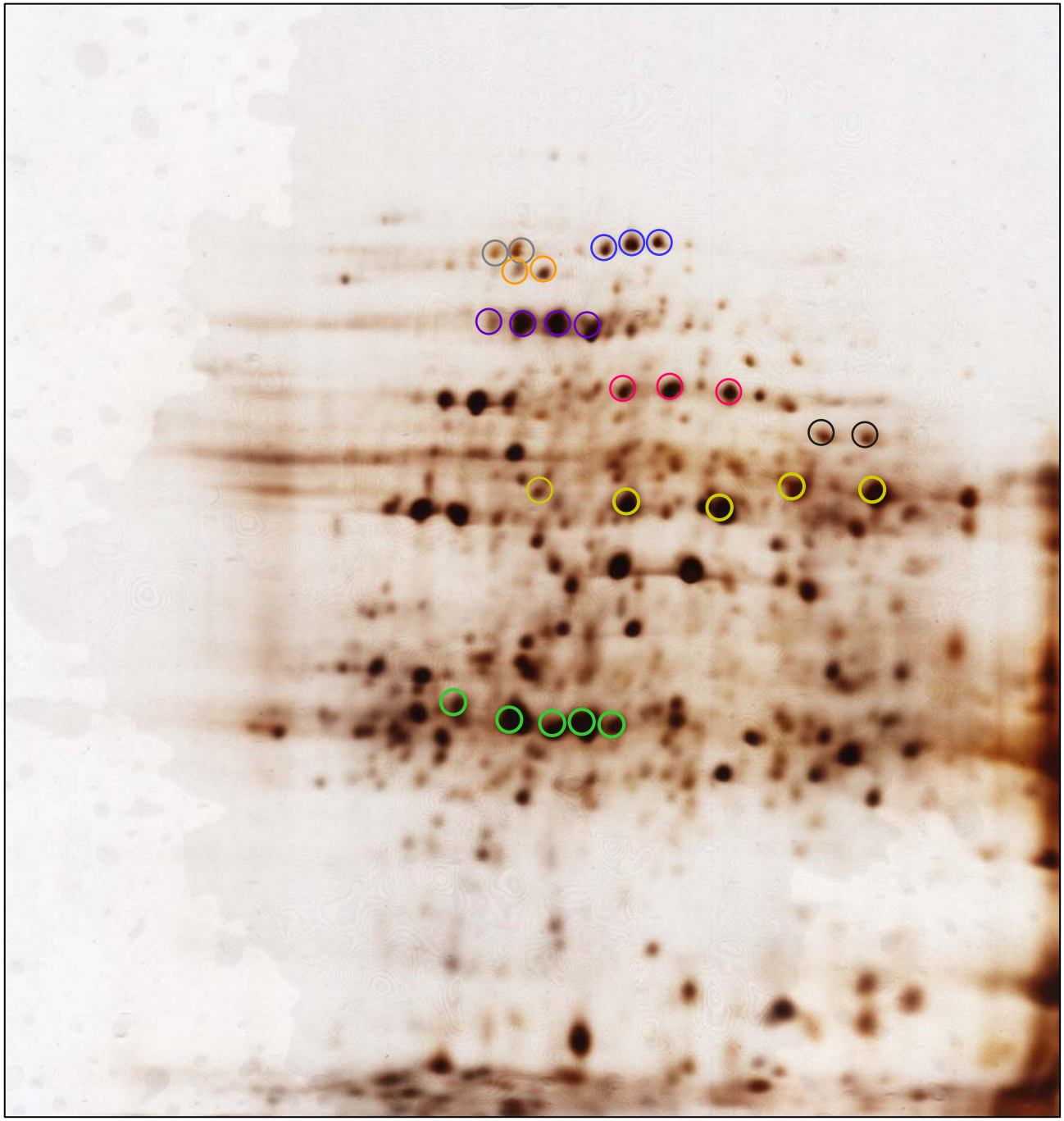
Juang RH (2005)

恆定蛋白質色點的身分

編號	Protein ID		代謝途徑
B19	Glyceraldehyde-3-phosphate dehydrogenase	LC-MS/MS N-terminal	Calvin cylce Glycolysis
B20	β 1,3-Glucanase	N-terminal	Defense protein
BI	Triosephosphate isomerase	LC-MS/MS N-terminal	Calvin cylce Glycolysis
BH	Triosephosphate isomerase	LC-MS/MS N-terminal	Calvin cylce Glycolysis

綠竹與水稻基因有相當高的同質性，
因此可以用水稻的基因體進行比對。

Juang RH (2005)

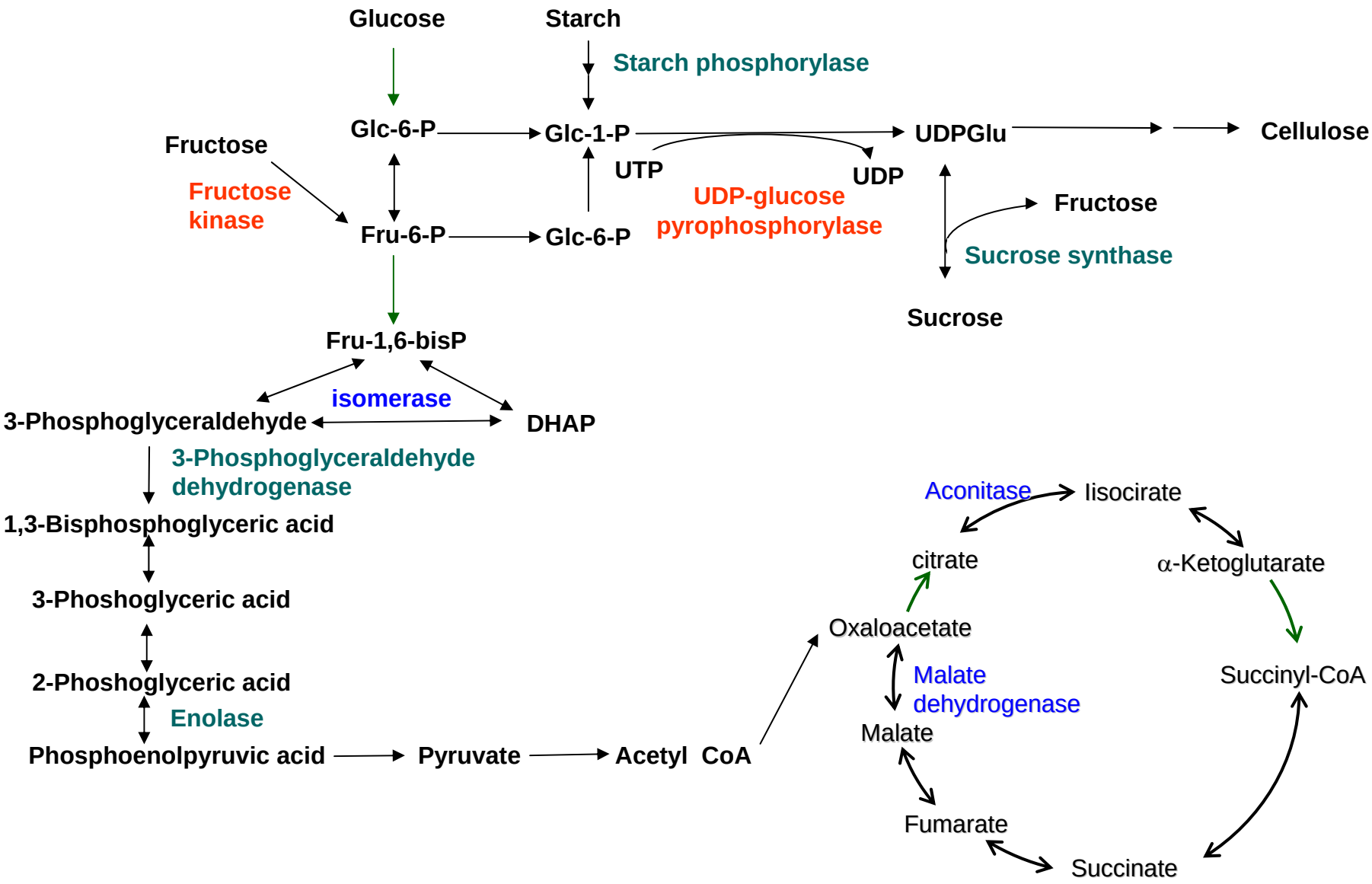


- Phosphoglycerate mutase
- Malate dehydrogenase
- Triosephosphate isomerase
- UTP-glucose-pyrophosphorylase
- Enolase
- Alcohol dehydrogenase
- Vacuolar ATP synthase catalytic subunit A
- Chaperonin (HSP60-1, mitochondrial)

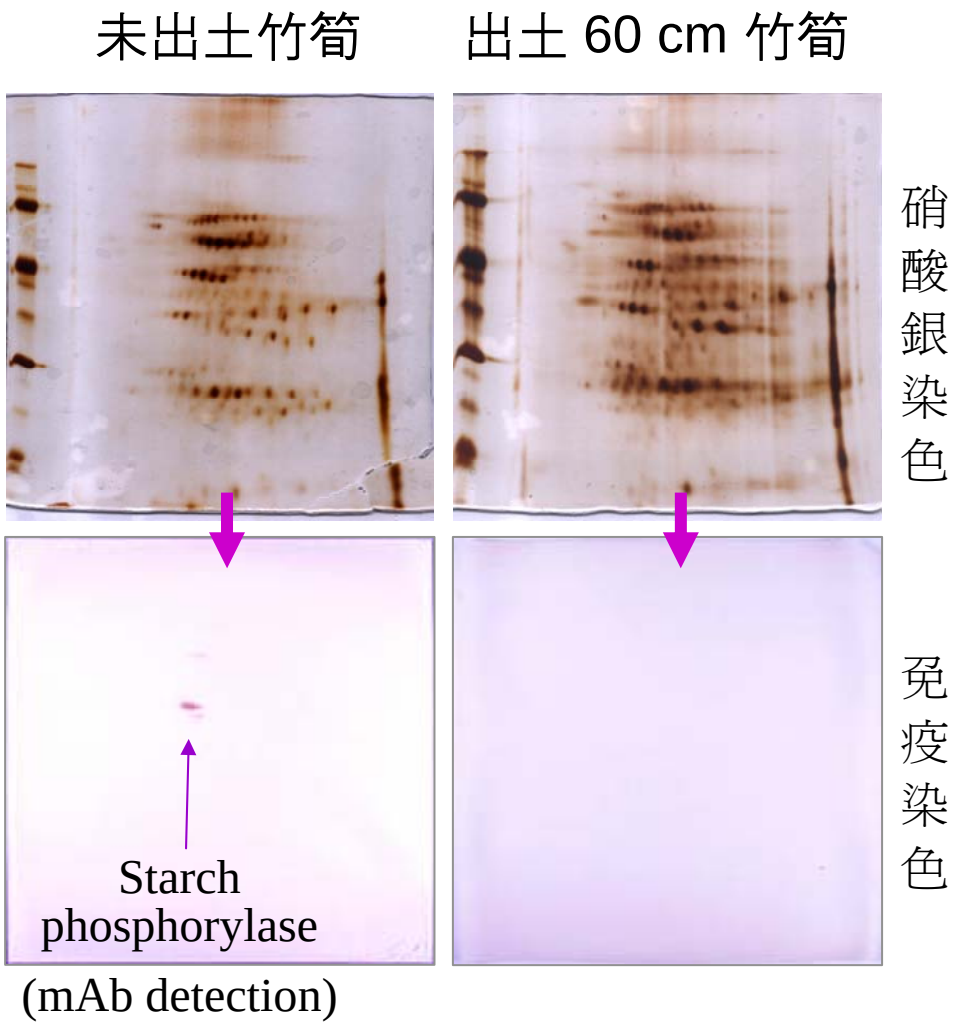
相關蛋白質顯現出水平的色點群落。

Juang RH (2005)

由蛋白質消長推測綠竹快速生長相關代謝



抗體應用在蛋白質體檢定的強大效果



首次提出

高產能抗體製備計畫構想

(1) 建立高產能抗體製備之 標準流程

(2) 大量快速 生產有用的單株抗體

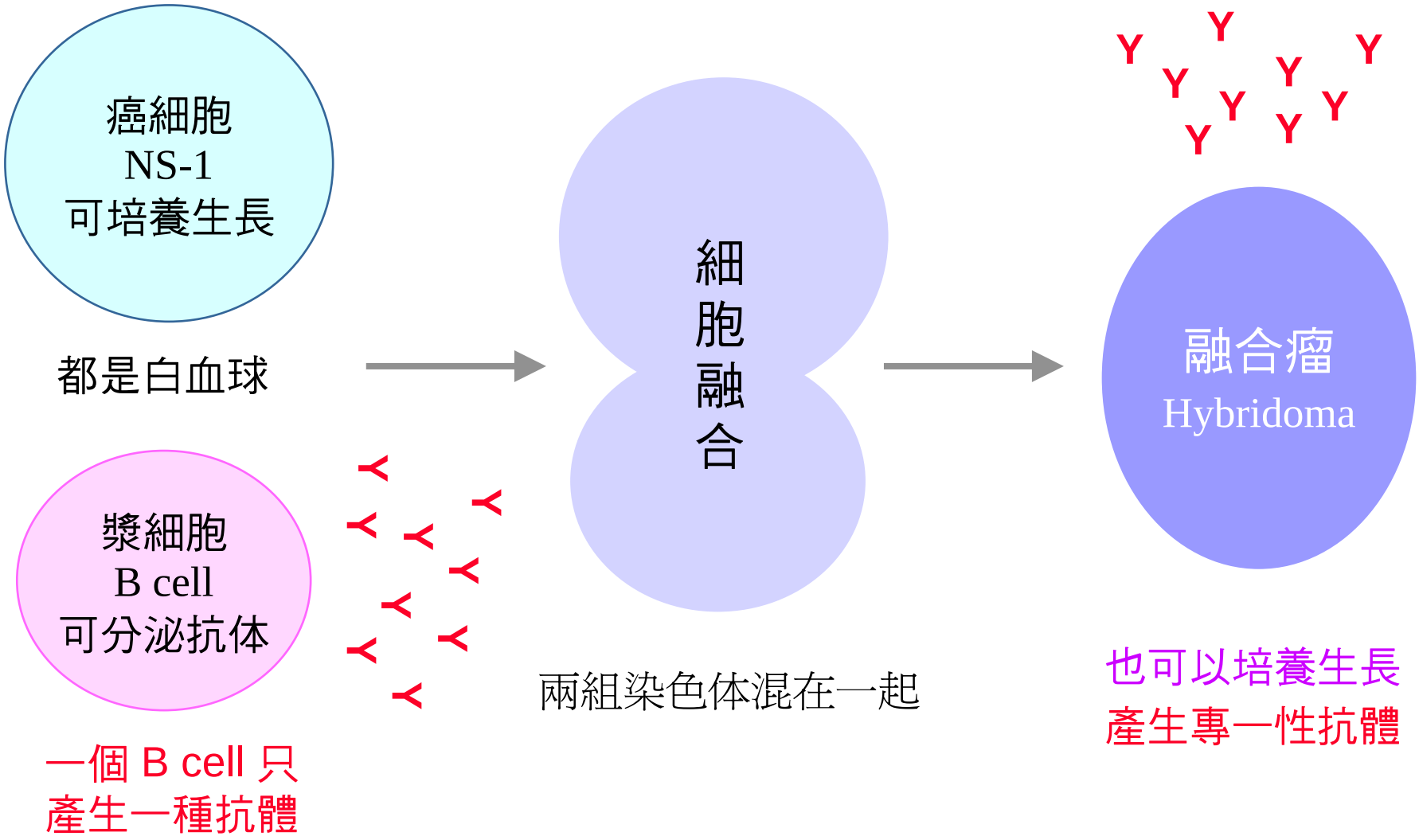
(3) 提供 抗體晶片 所需之抗體庫

均質抗原 → 單一抗體 → 整體抗原 → 全部抗體

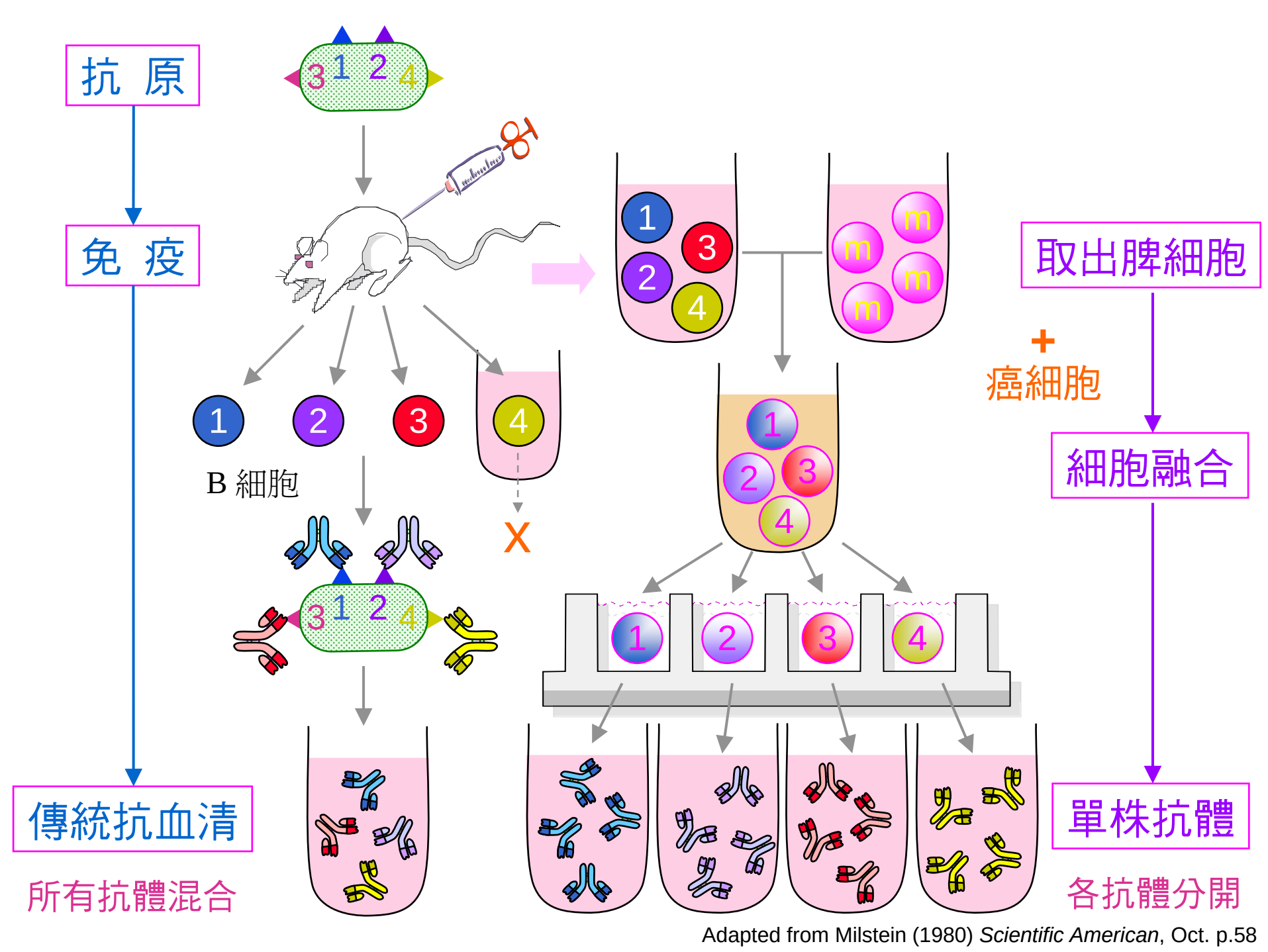
Juang RH (2005)

單株抗體

可生產有用抗體的淋巴細胞若與癌細胞融合，則形成穩定而可培養的細胞株。



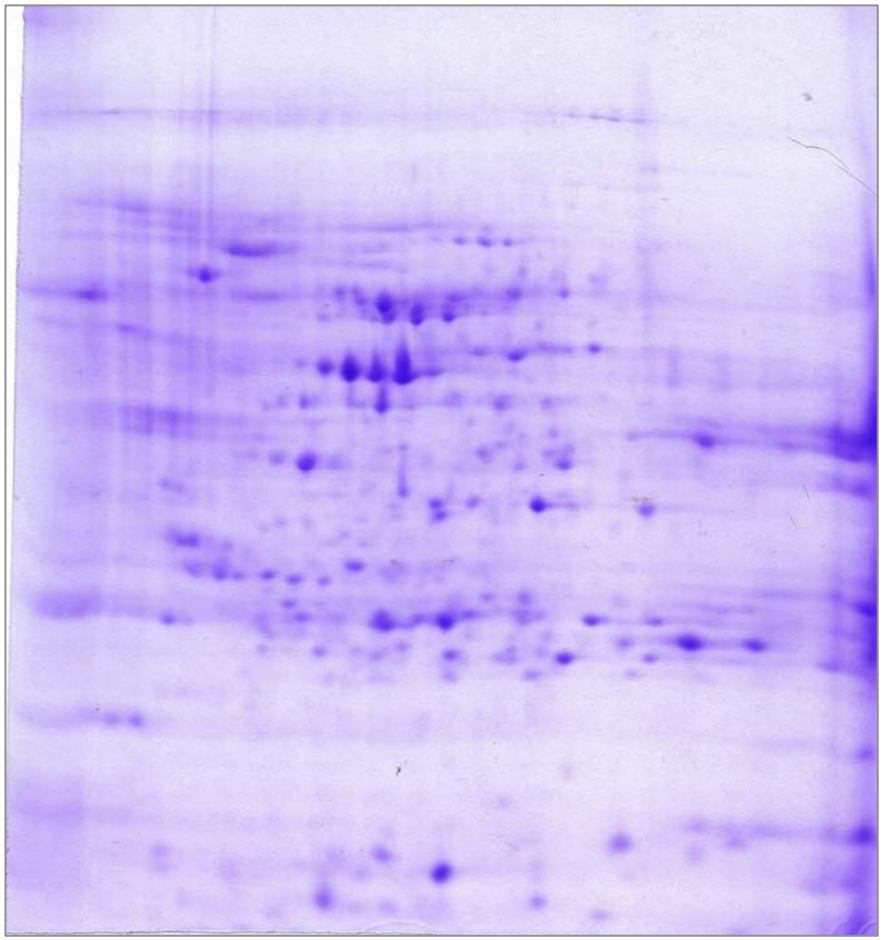
Juang RH (2005)



Adapted from Milstein (1980) *Scientific American*, Oct. p.58

竹筍蛋白質體的免疫反應

未出土竹筍樣本 CBR 染色

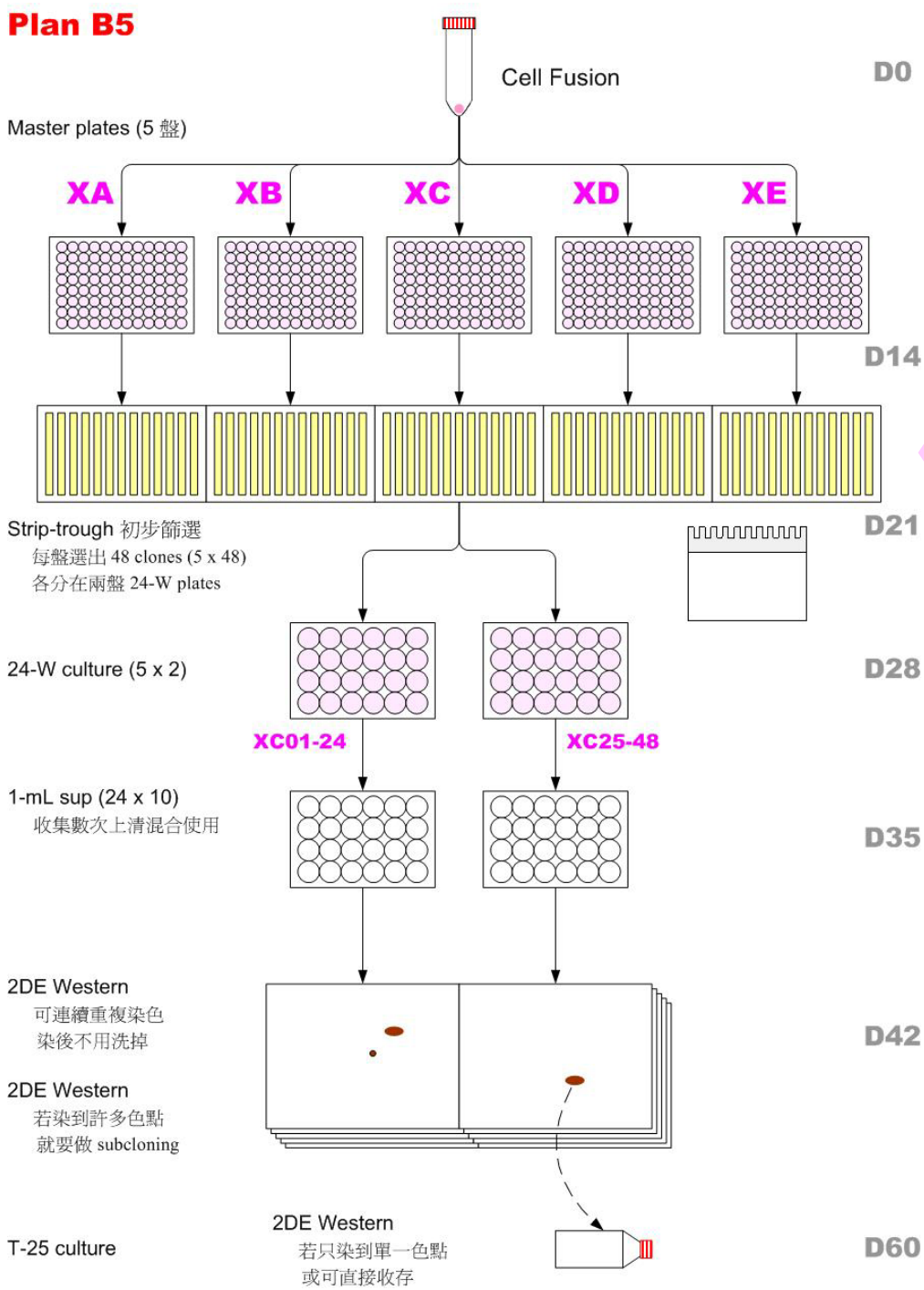


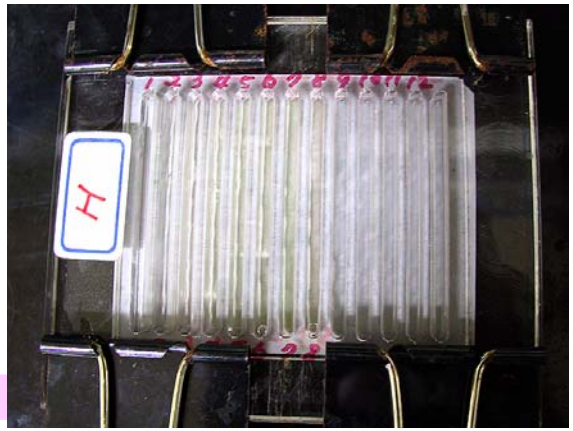
小鼠抗血清免疫染色

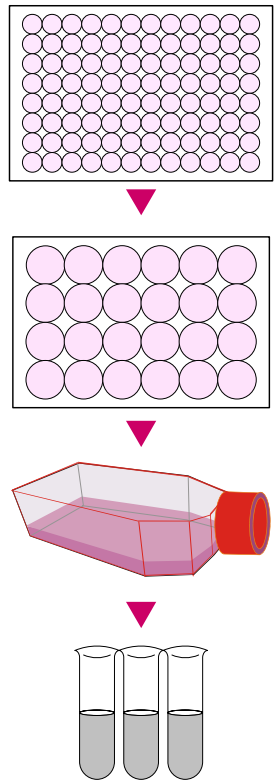
抗原性強弱不同、蛋白質含量多寡不同。

Juang RH (2005)

單株抗體高產能製備流程

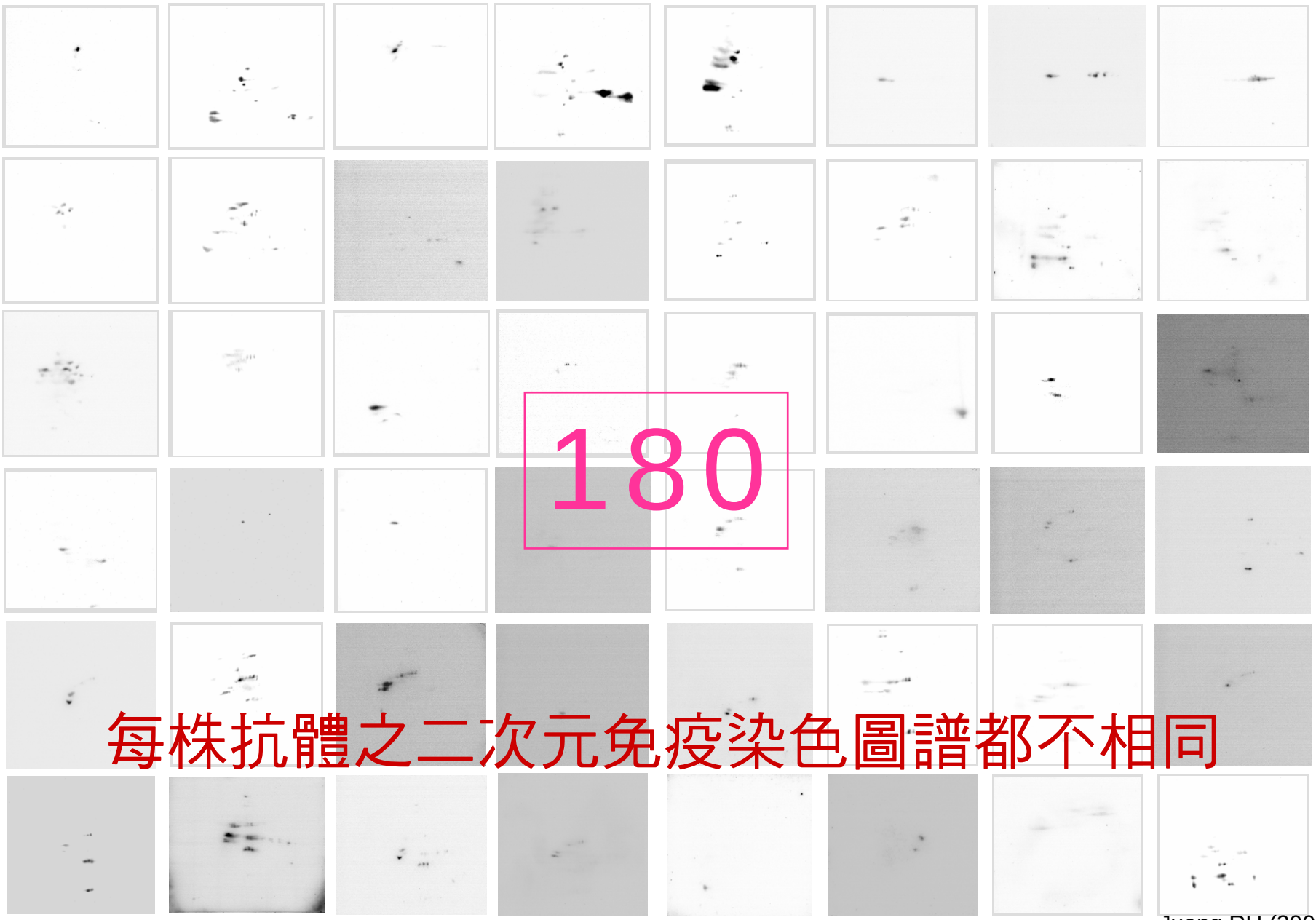






Juang RH (2005)

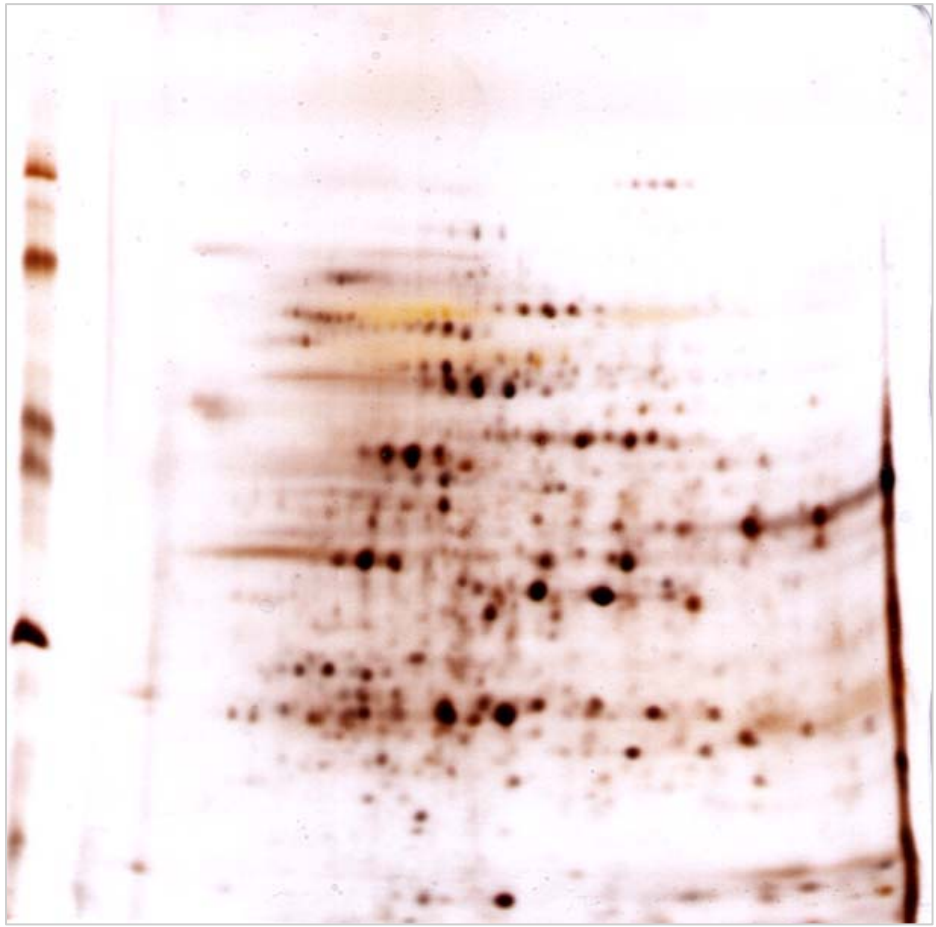
綠竹筍蛋白質體之單株抗體庫 (pH 3-10)



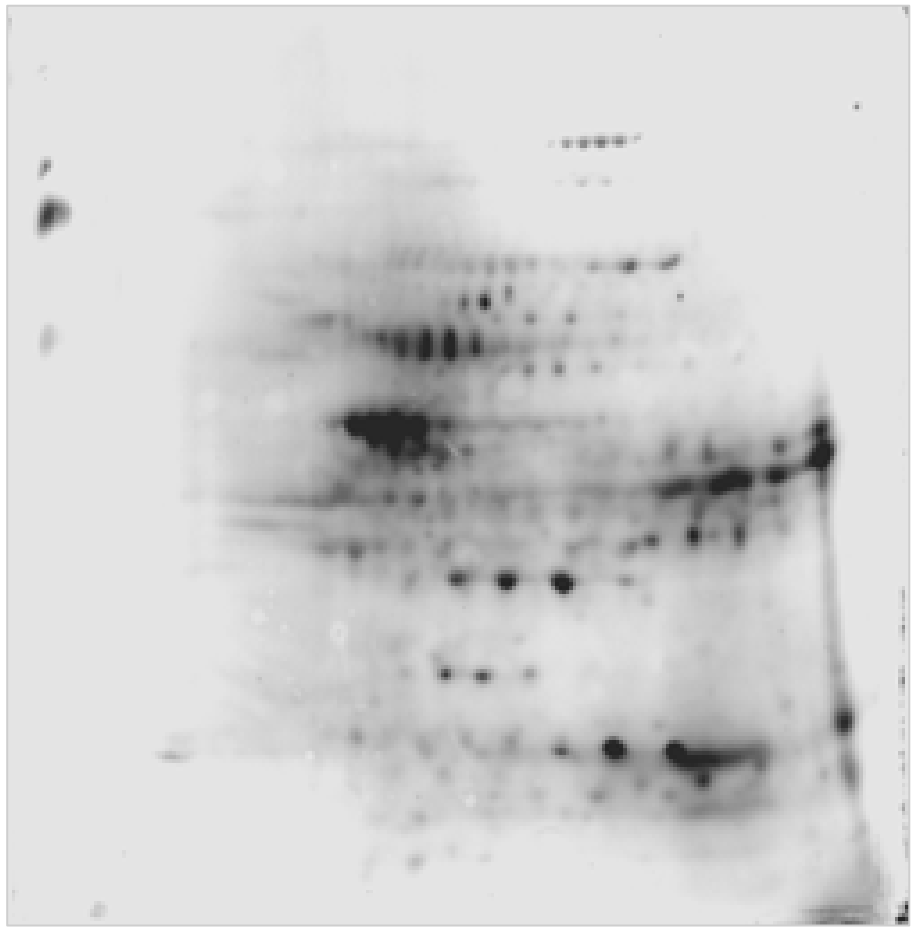
Juang RH (2005)

混合八十種單株抗體共同免疫染色

Silver staining



Immunostaining



最終目標： 蛋白質染色圖譜 = 免疫染色圖譜

11 cm strip, pH 4-7, 12.5% SDS-PAGE

Juang RH (2005)

來自中國的世界競爭

Nature (2003 Oct) 425:441

China takes centre stage for liver proteome

David Cyranoski, Changsha, China

China is set to lead a massive research project to describe all the proteins in the human liver — the liver proteome. The initiative is being coordinated by the Human Proteome Organisation (HUPO), an international group that is also overseeing plasma and brain proteome projects.

But parts of China's plan are controversial — in particular, a project to generate a range of different antibodies in one go.

Last month, China surprised the world of protein chemistry by pledging 200 million yuan ($24 million) for the three-year pilot phase of the international liver-proteome study. Two weeks later, researchers at China's annual proteomics meeting, on 18–21 September, established a Chinese branch of HUPO and elected as its director the ambitious Fuchu He, a systems-biology researcher at the Beijing Institute of Radiation Medicine. "This is a golden opportunity for China to lead an international effort," says He, who

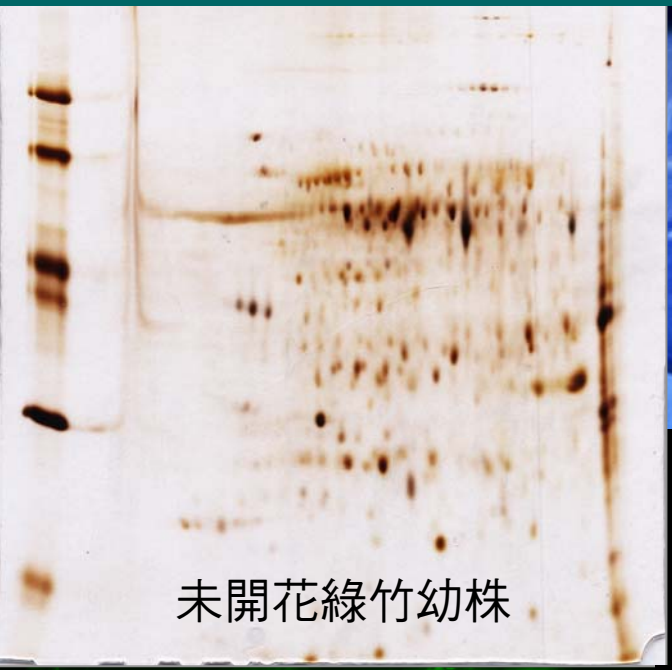
D. CYRANOSKI, LEFT & INSET

Fuchu He (left) has the task of coordinating China's project to identify liver proteins.

Conventionally, antibodies are made one at a time, but Sun's team will inject several different proteins extracted from human livers into mice and then harvest the antibodies made to each of them from the mice's blood. These antibodies will be identified individu-

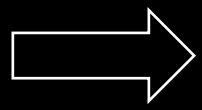
Helmut Meyer of the University of Bochum, Germany, who heads HUPO's brain-proteome project, thinks that the Chinese researchers may be biting off more than they can chew. "Sometimes it's nearly impossible to identify the protein to which an antibody binds," he says. His brain-proteome project plans to start an antibody project

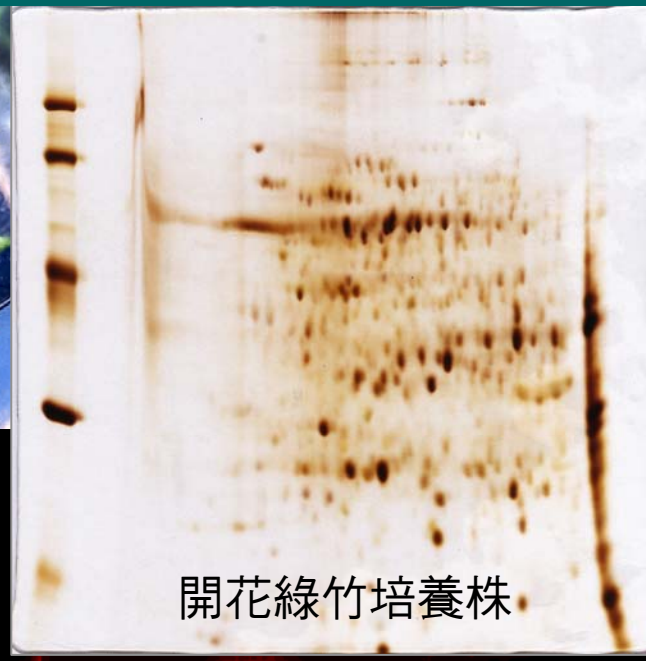
綠竹幼株開花前後的蛋白質表現差異



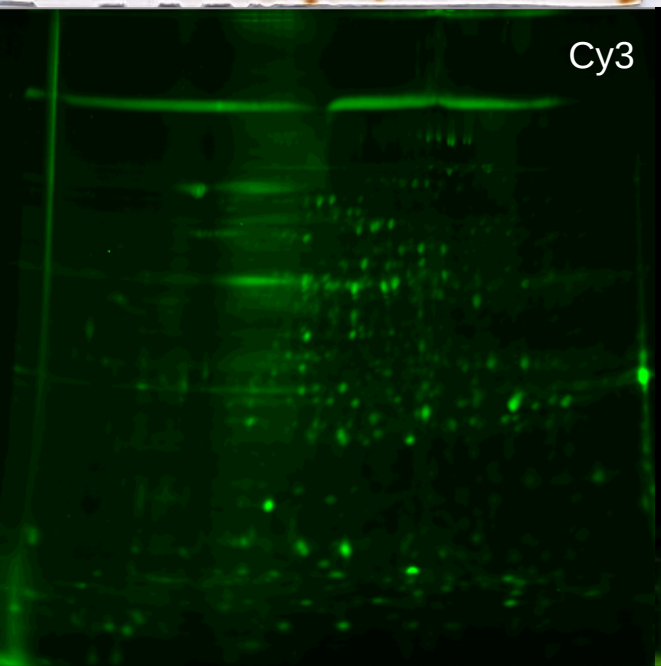
未開花綠竹幼株

中研院黃麗春教授實驗室

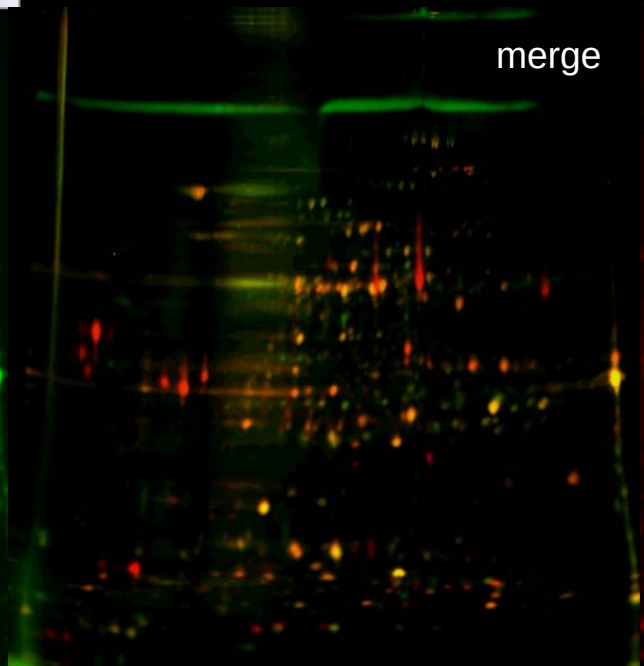




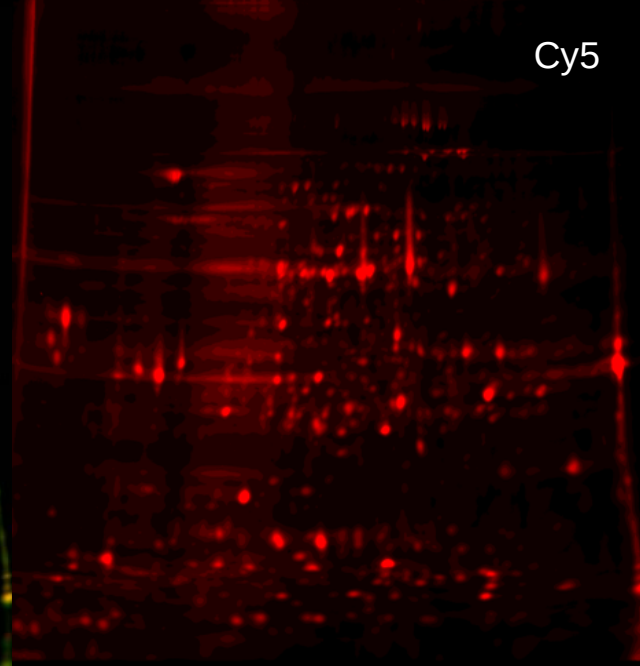
開花綠竹培養株



Cy3

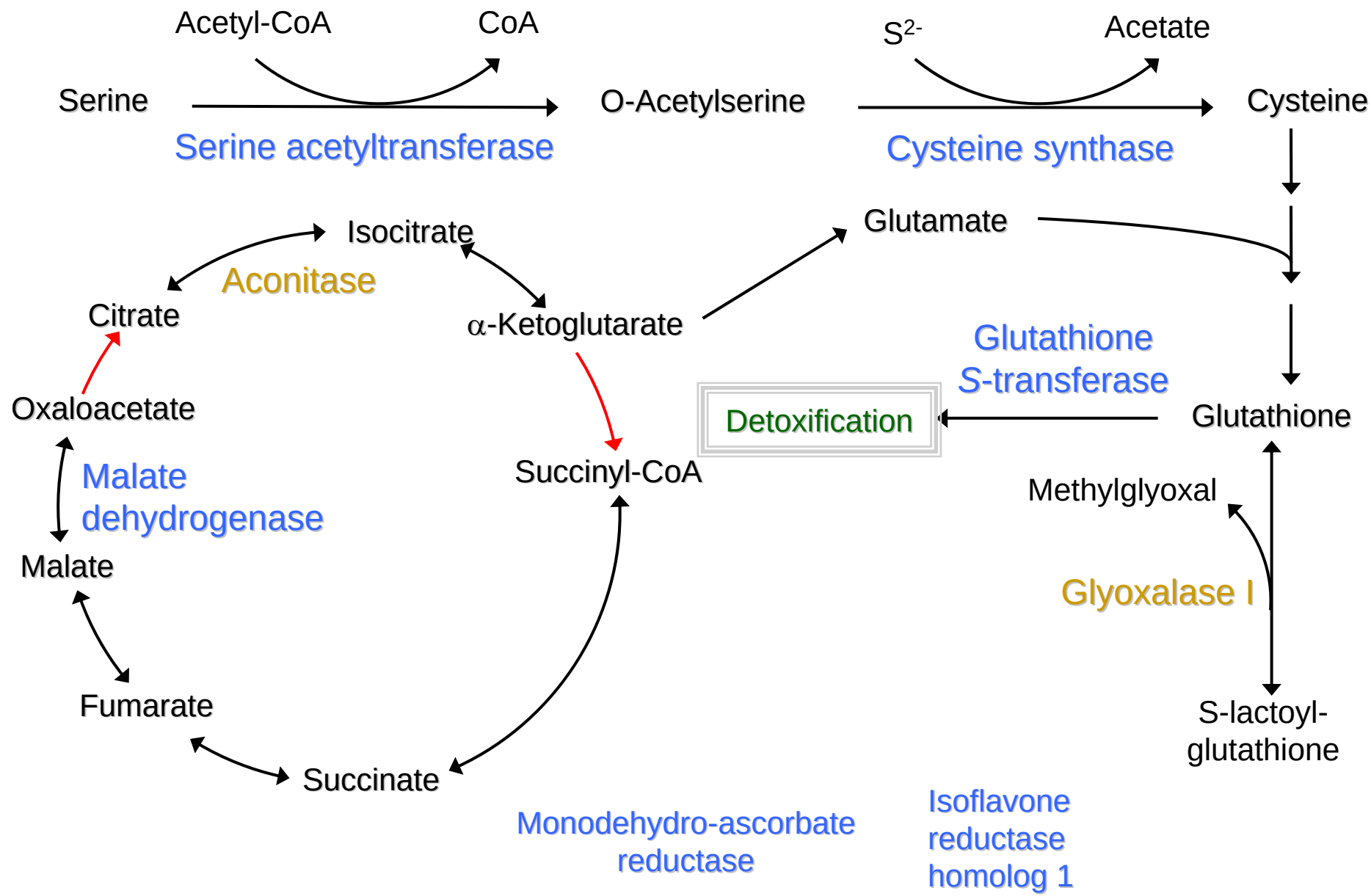


merge

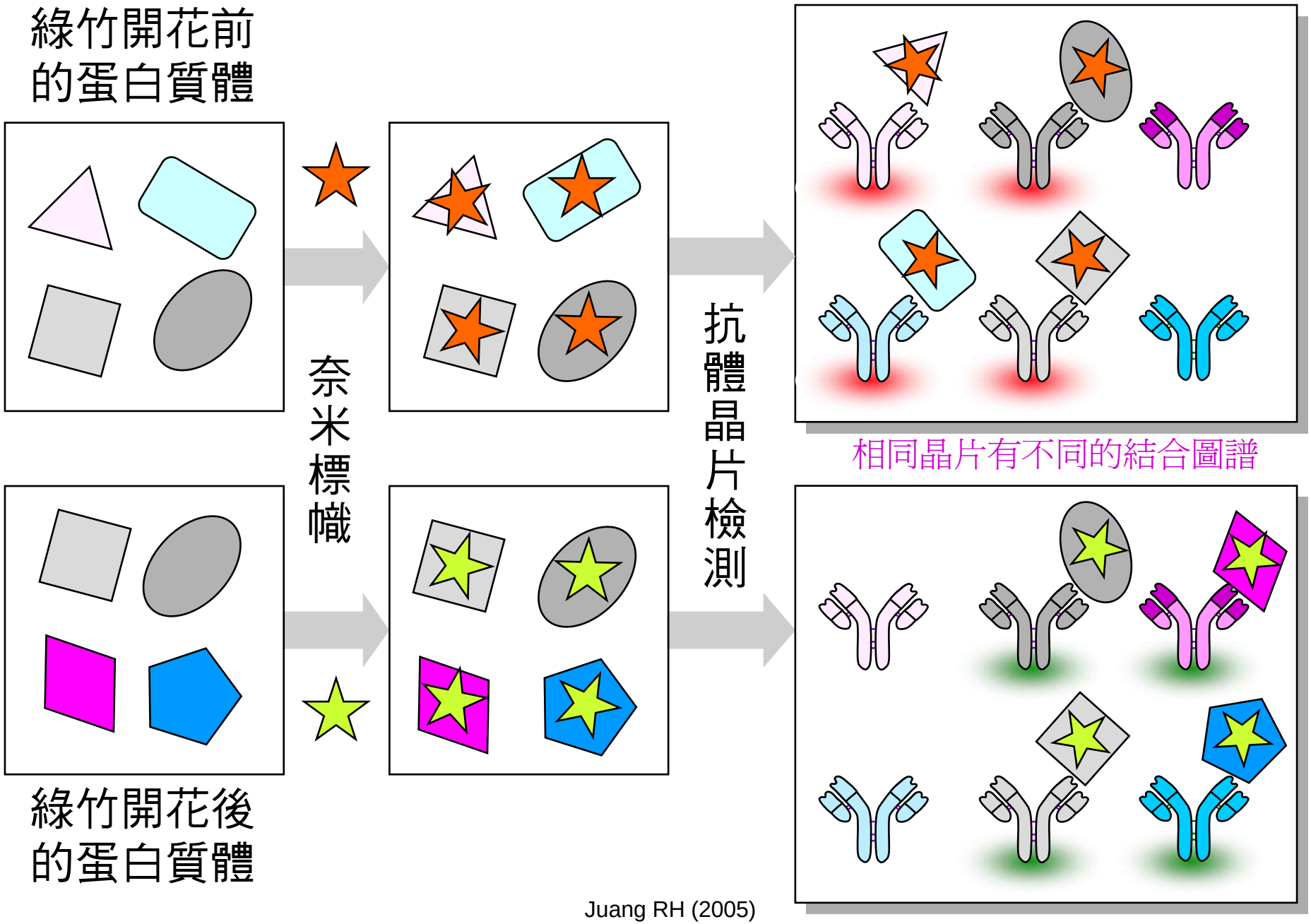


Cy5

推測與綠竹開花相關之可能代謝途徑

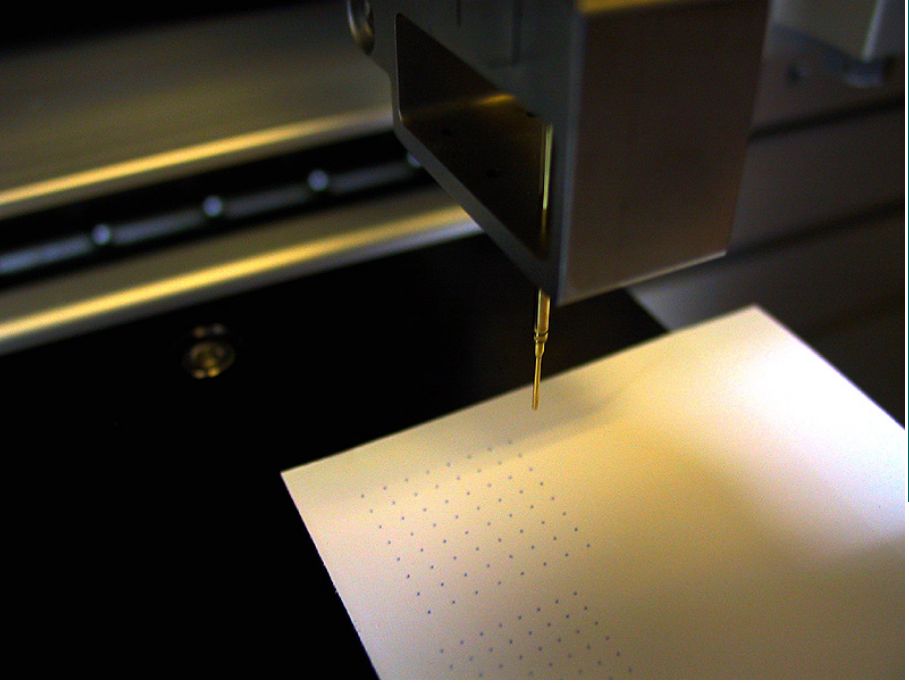


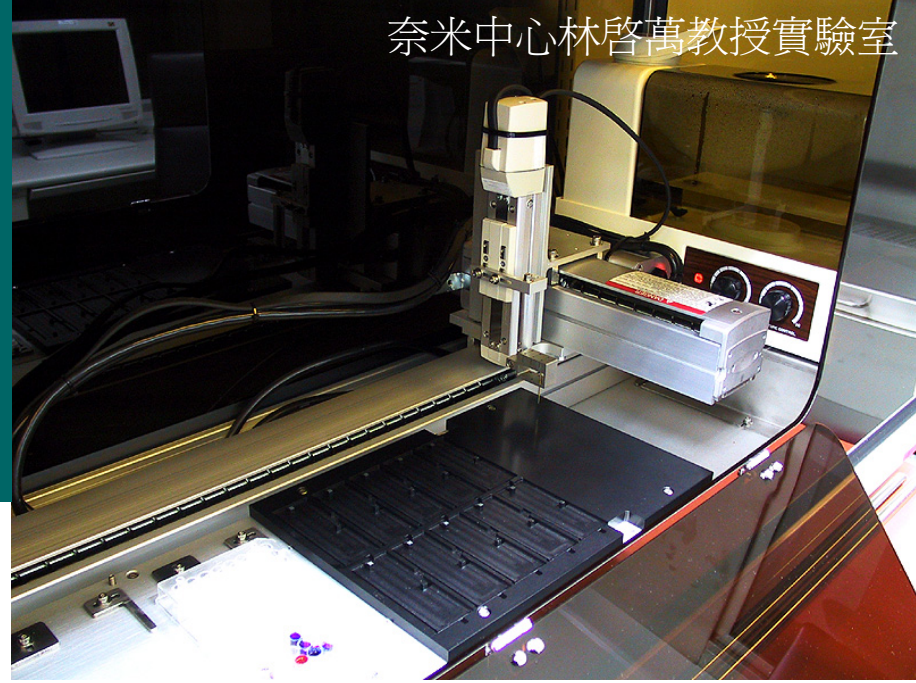
以奈米抗體晶片檢定綠竹開花前後的蛋白質體



Juang RH (2005)

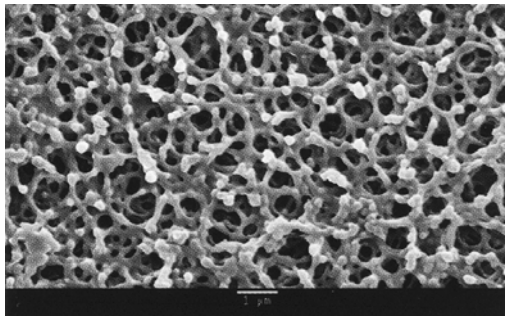
蛋白質晶片試製



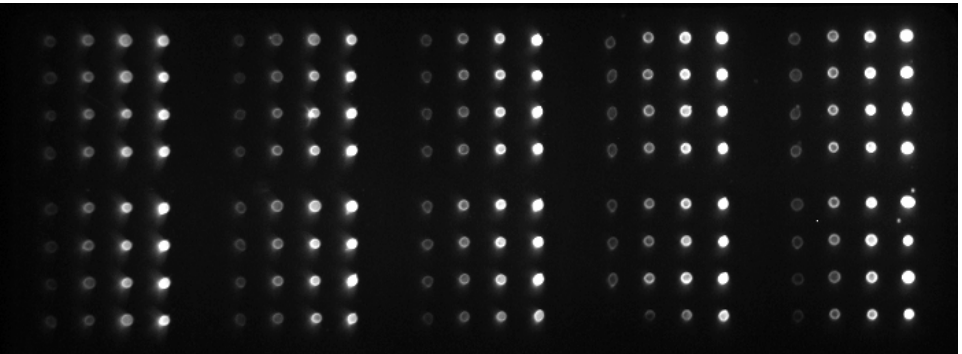


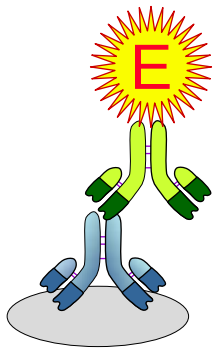
奈米中心林啓萬教授實驗室

Nitrocellulose

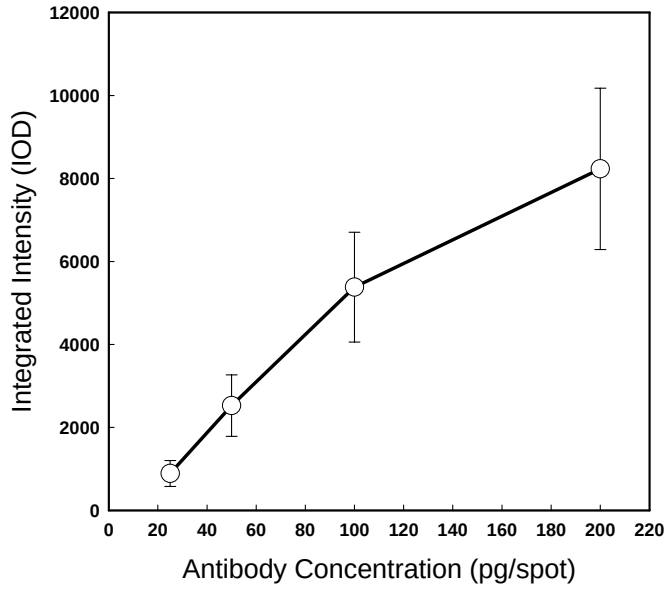


Schleicher & Schuell



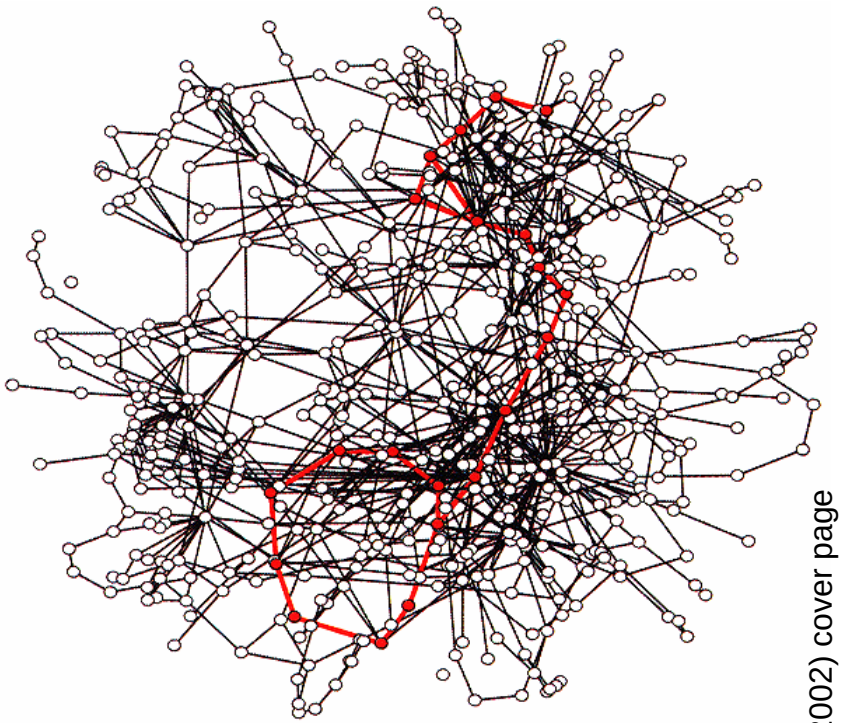


Juang RH (2005)



蛋白質分子間的交互作用 Cross-Talk

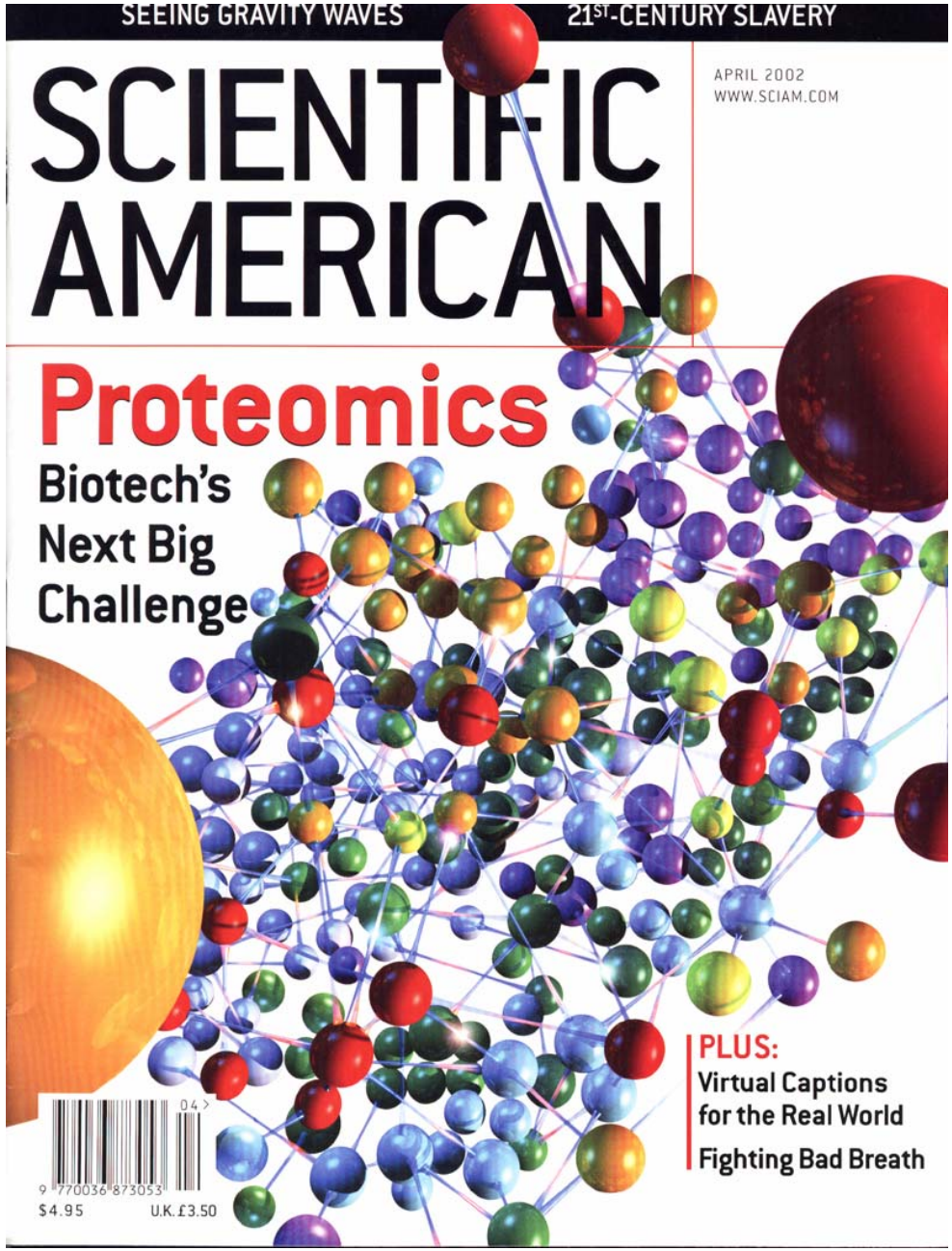
代謝路徑立體圖



Alberts et al (2002) Molecular Biology of the Cell (4e) p.107

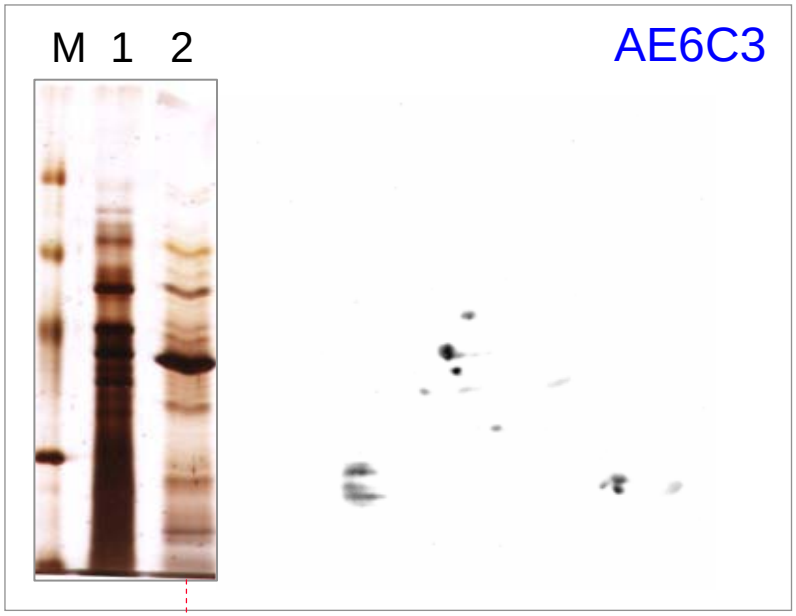
Systems Biology

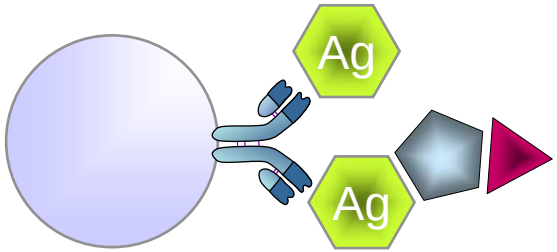
整體性的生物學觀念與工具



Scientific American (2002) cover page

抗體免疫沈澱與蛋白質交互作用

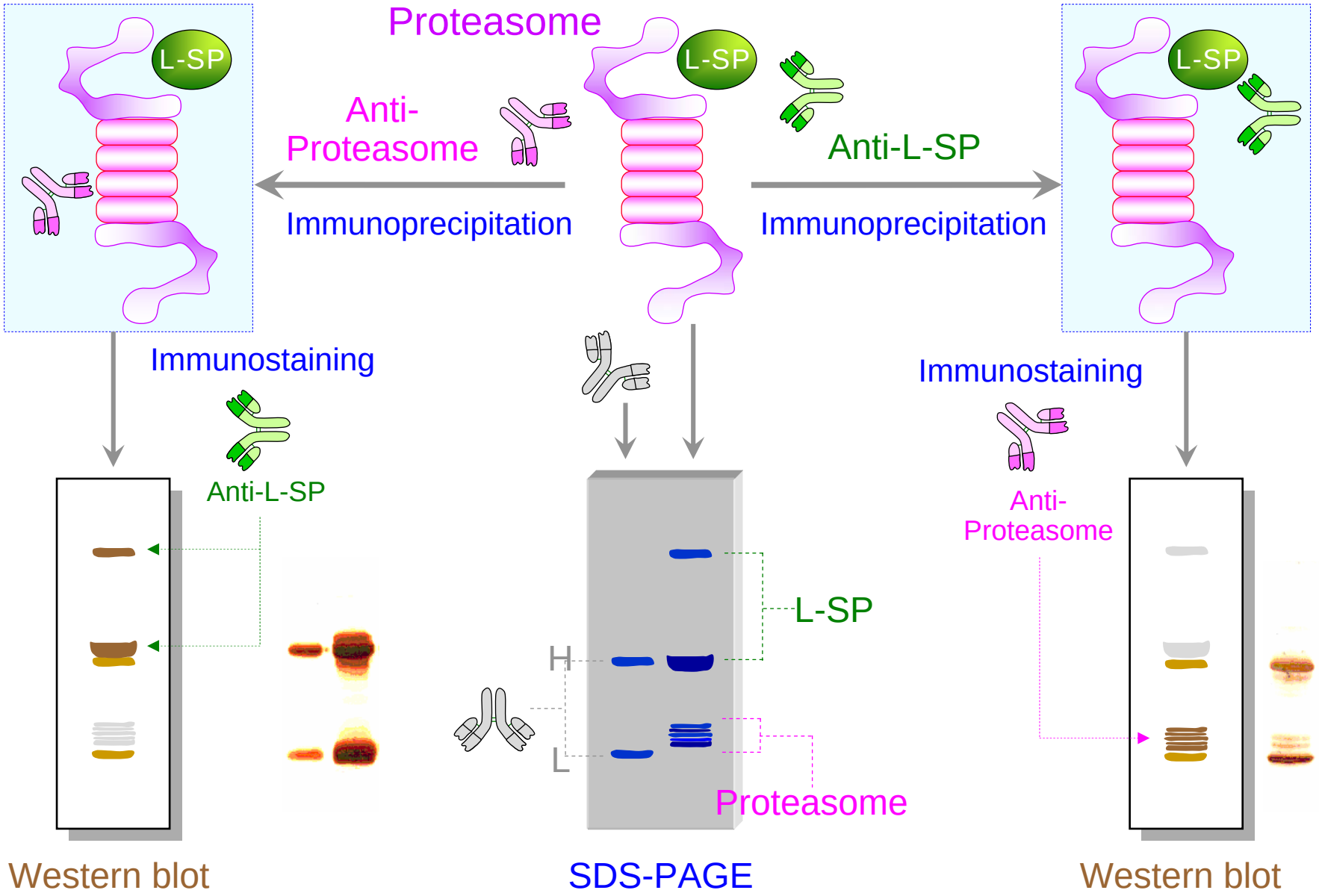




Protein ID	Match peptide
Adenosylhomocysteinase	IVLTIIR DSAAVFAWK HSLPDGLMR LVGVSEETTTGVK
Histone H4 (wheat)	IFLENVIR IDGLIYEETR TVRAMDVVYALKR
Fructose bisphophate aldolase	VTPEVIAEYTVR IGPNEPSQLAIDLNAQGLAR
Triosephosphate isomerase	TNVSPEVAESTR VIACVGETLEQR
NAD-dependent malate dehydrogenase	DDLFNINAGIVK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

Juang RH (2005)

以免疫沈澱法檢定蛋白質間的交互作用



Juang RH (2005)

BST

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