蛋白質體學及應用

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內容介紹

- 何謂蛋白質體學
- 蛋白質體學研究方法
- 蛋白質體學應用
- 實驗技術

何謂蛋白質體學

- 蛋白質體學與傳統生化學
- 傳統研究“蛋白質”的科學
  - 生物化學
  - 酵素學
  - … 等

蛋白質的構造

- 細胞的功能主要由蛋白質執行
  - 代謝
  - 生化反應
  - 繁殖…
- 蛋白質爲一長鏈的胺基酸聚合物，鏈長由數十至數千。
- 生物體約有20種胺基酸。

胺基酸構造

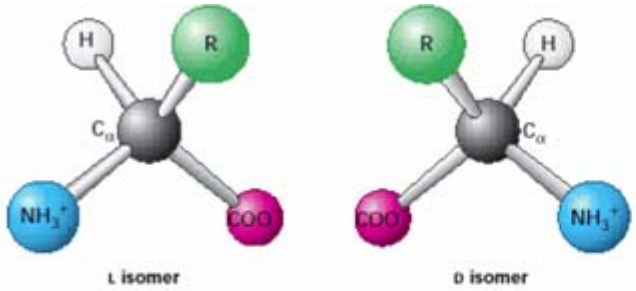


Figure 3.4. The L and D Isomers of Amino Acids. R refers to the side chain. The L and D isomers are mirror images of each other.

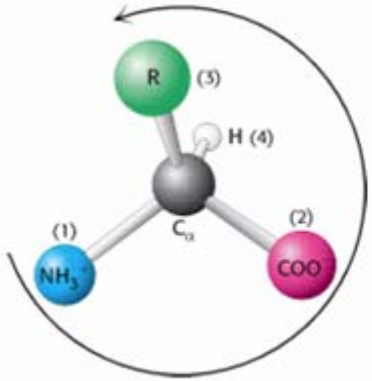


Figure 3.5. Only L Amino Acids Are Found in Proteins. Almost all L amino acids have an *S* absolute configuration

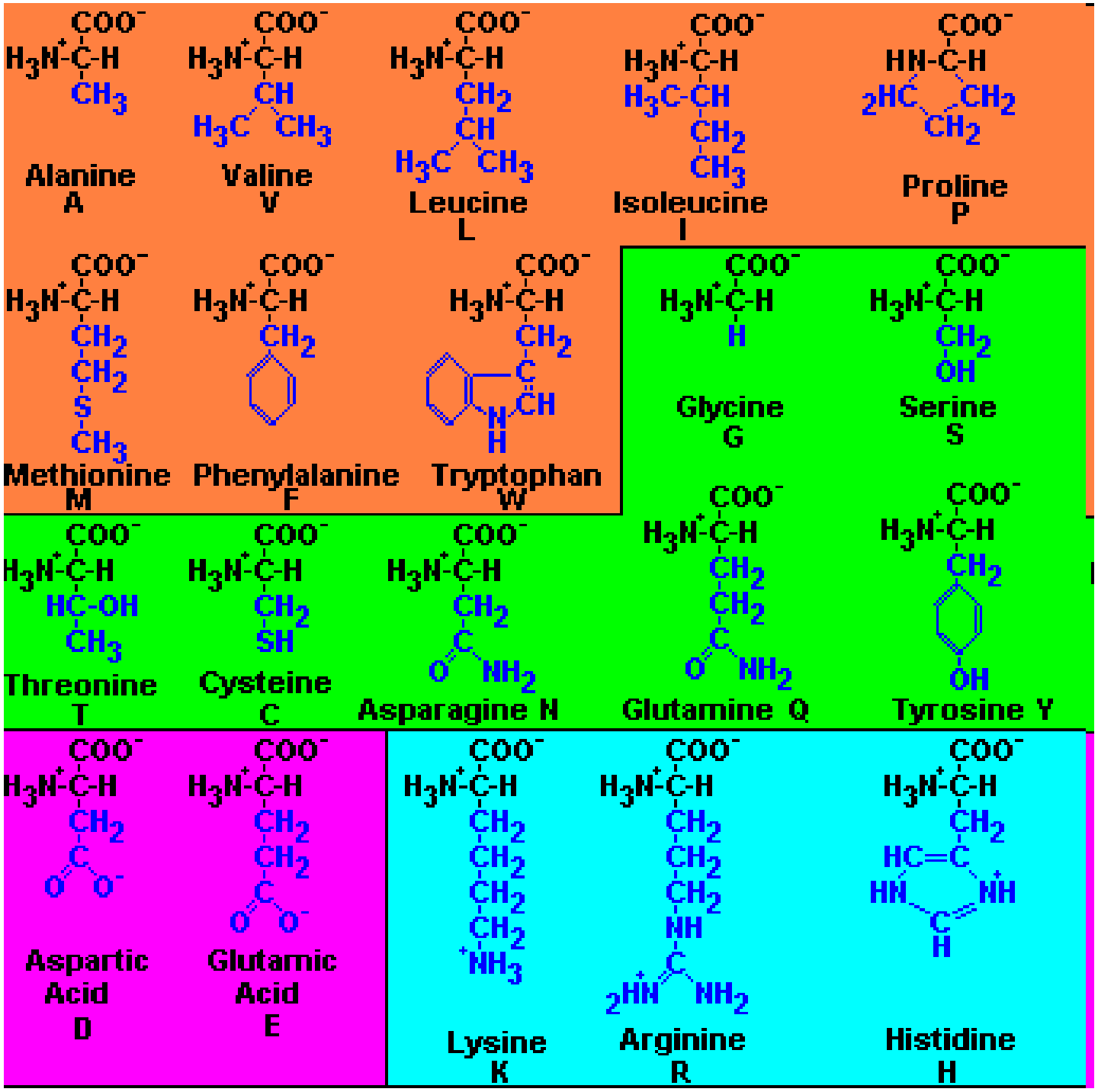
Functional group:

1.-NH_3

2.-COOH

3.-R (羧基)

4.鏡像結構



20種胺基酸:

依R group分類:

1.非極性

2.極性

3.正電

4.負電

蛋白質鏈方向性(N → C)

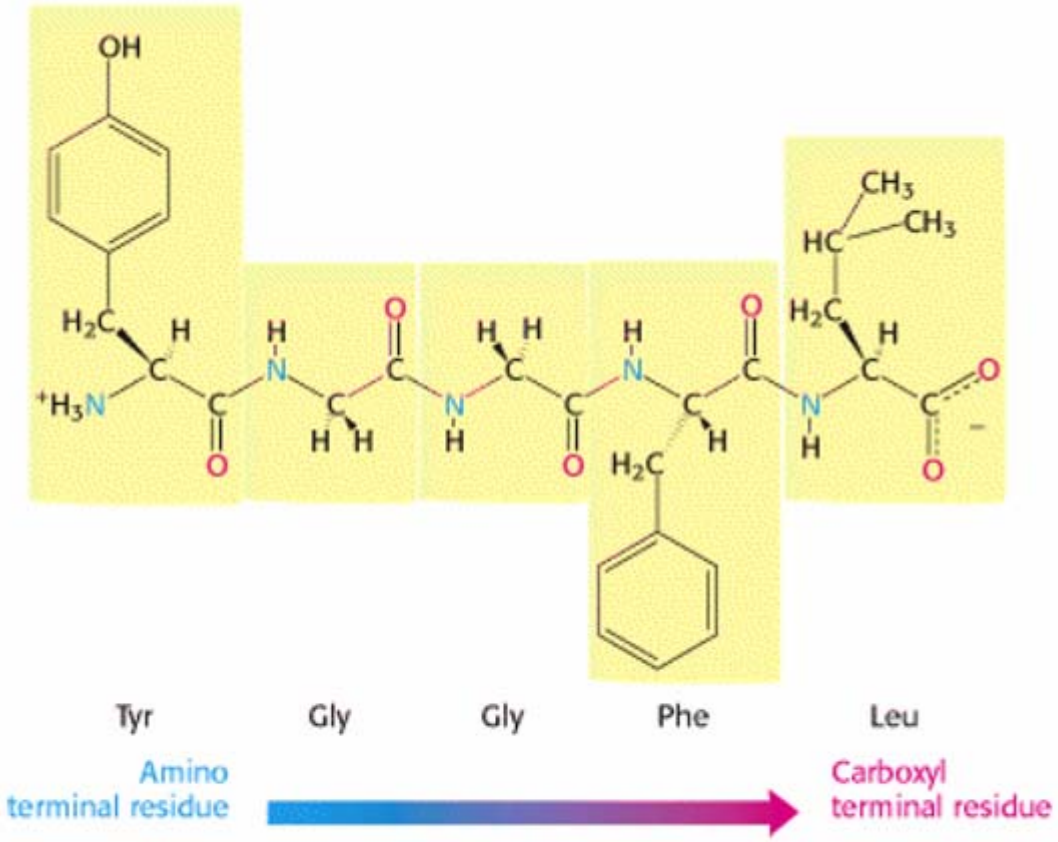
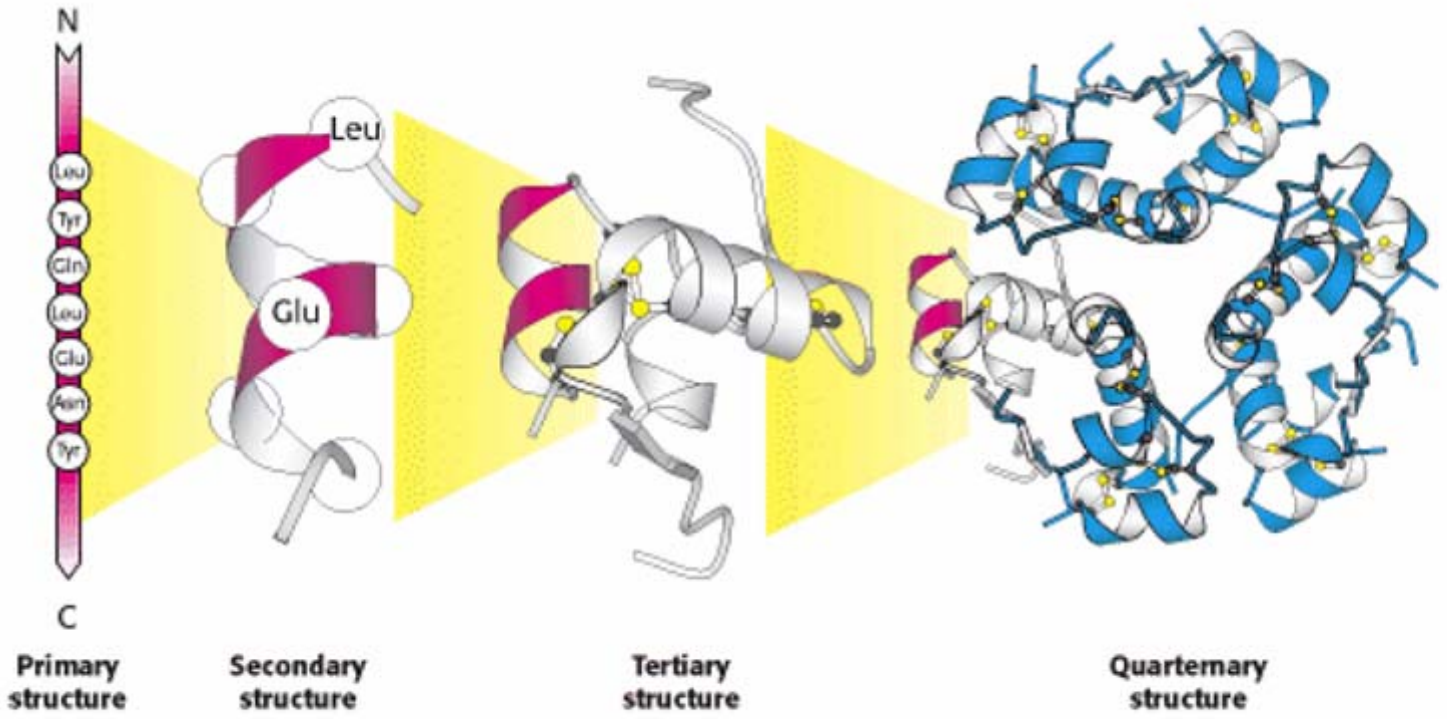


Figure 3.19. Amino Acid Sequences Have Direction. This illustration of the pentapeptide Tyr-Gly-Gly-Phe-Leu (YGGFL) shows the sequence from the amino terminus to the carboxyl terminus.

蛋白質四元結構

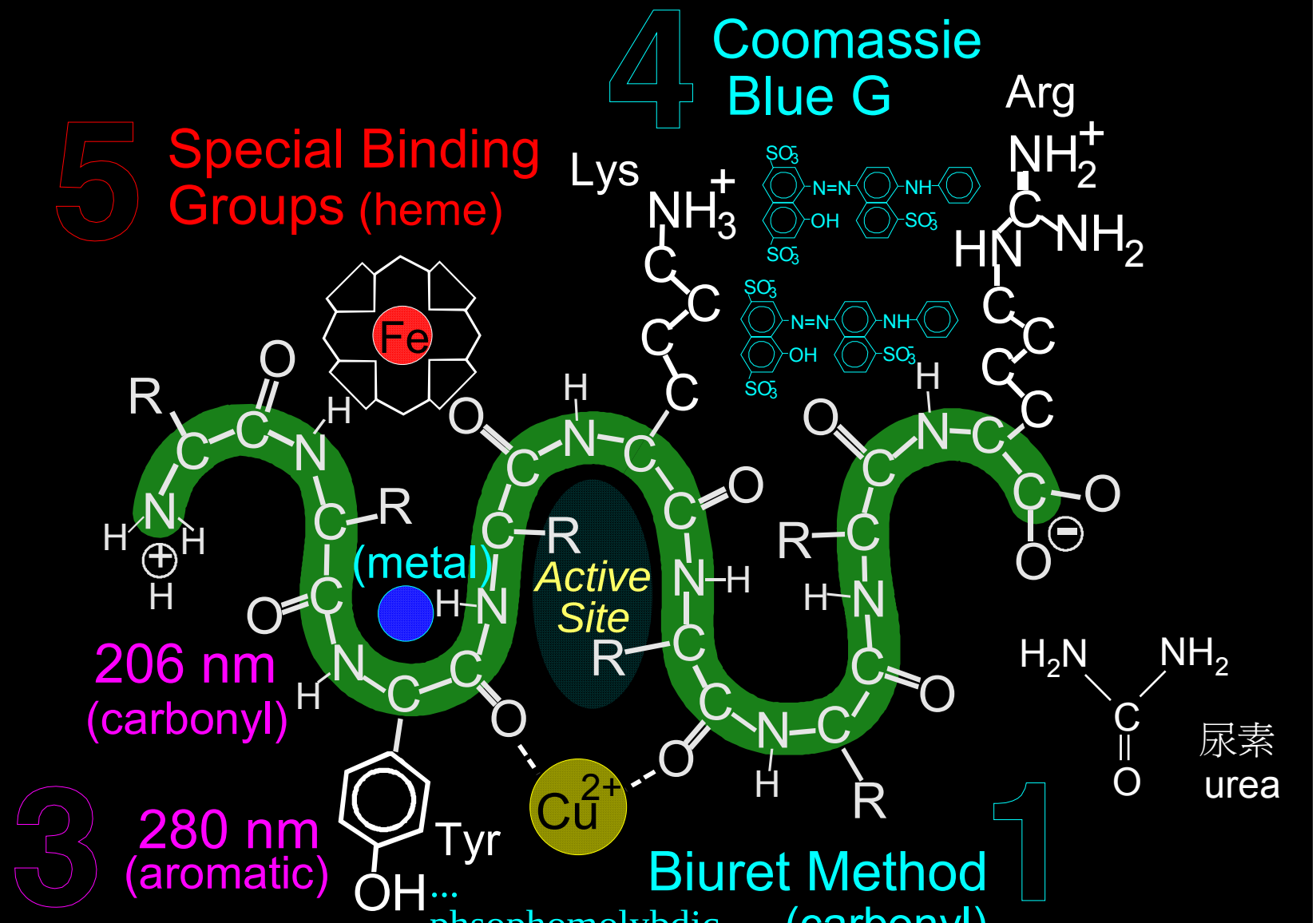


Crystals of human insulin. Insulin is a protein hormone, crucial for maintaining blood sugar at appropriate levels.

傳統研究蛋白質的方法

- 蛋白質濃度測定
- 蛋白質(酵素)反應測定
- 蛋白質分離純化
- 蛋白質定序

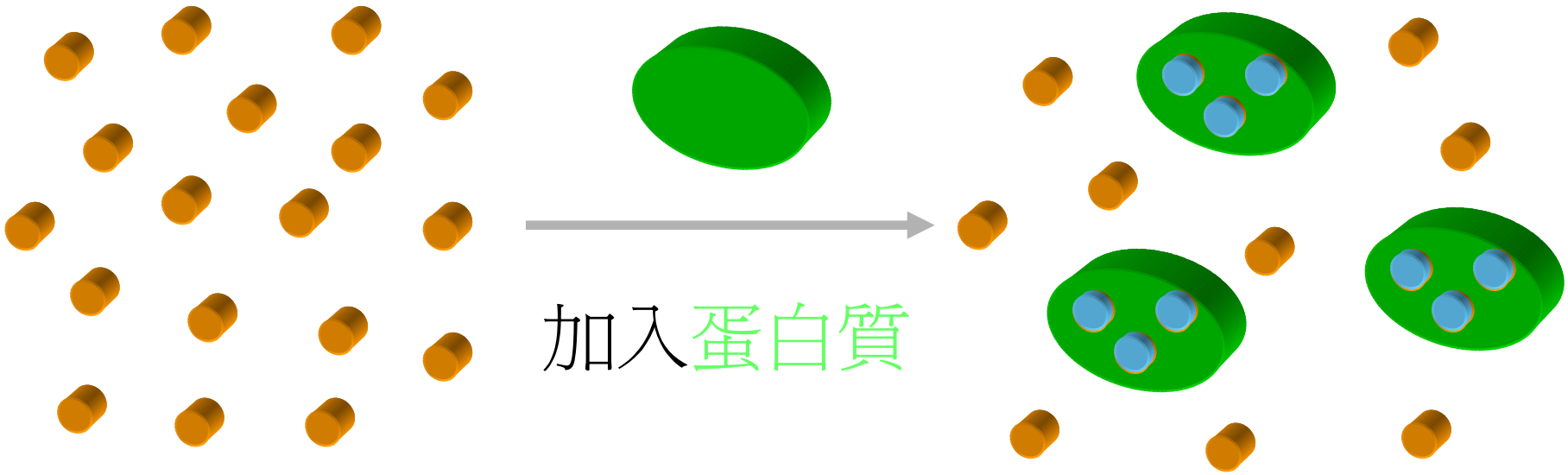
各種蛋白質定量法：


4
Coomassie
Blue G
Arg
Lys
5
Special Binding
Groups (heme)
Fe
(metal)
Active
Site
206 nm
(carbonyl)
3
280 nm
(aromatic)
UV
Absorbance
Tyr
OH...
Cu2+
phsophomolybdic-
phosphotungstate
Biuret Method
(carbonyl)
1
Lowry Method
2
尿素
urea

Bradford Method：

Coomassie Brilliant Blue G-250

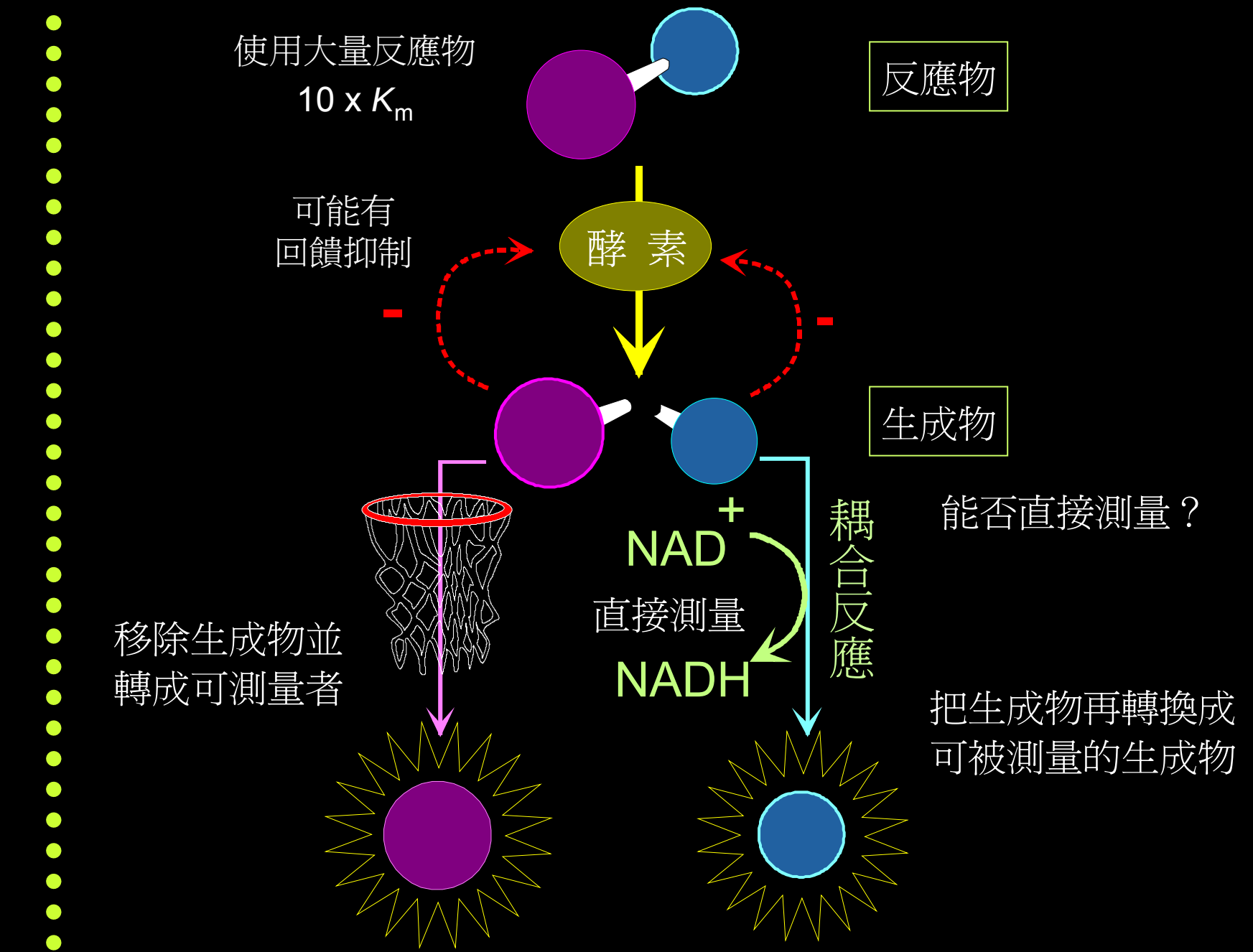
470 nm　　CBG 是一種指示劑　　595 nm



酸性環境下呈茶色

與蛋白質結合變藍色

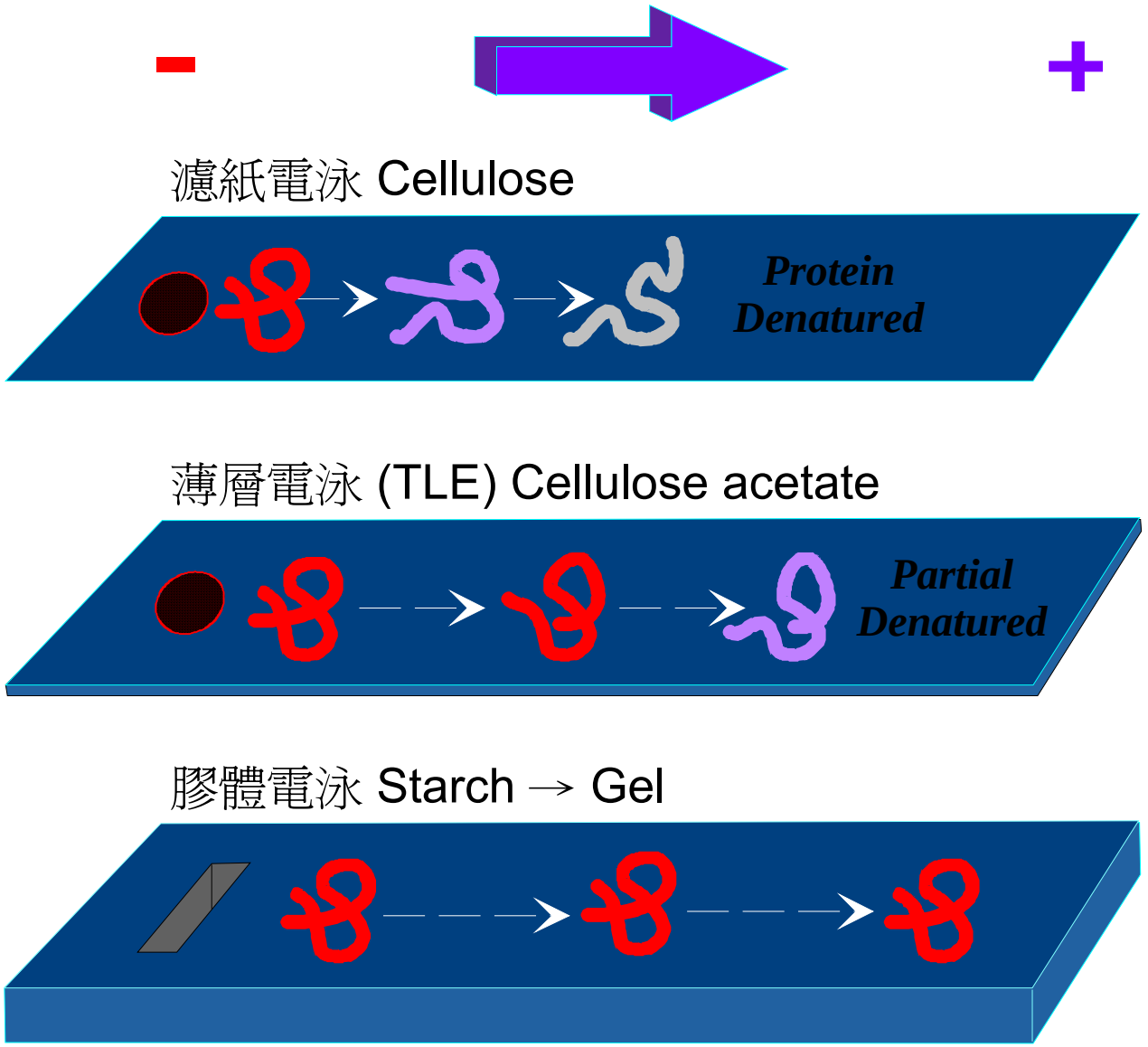
酵素反應及偵測方法：


使用大量反應物
10 x K_m
反應物
可能有
回饋抑制
酵 素
生成物
能否直接測量？
NAD+
直接測量
NADH
耦合反應
移除生成物並
轉成可測量者
把生成物再轉換成
可被測量的生成物

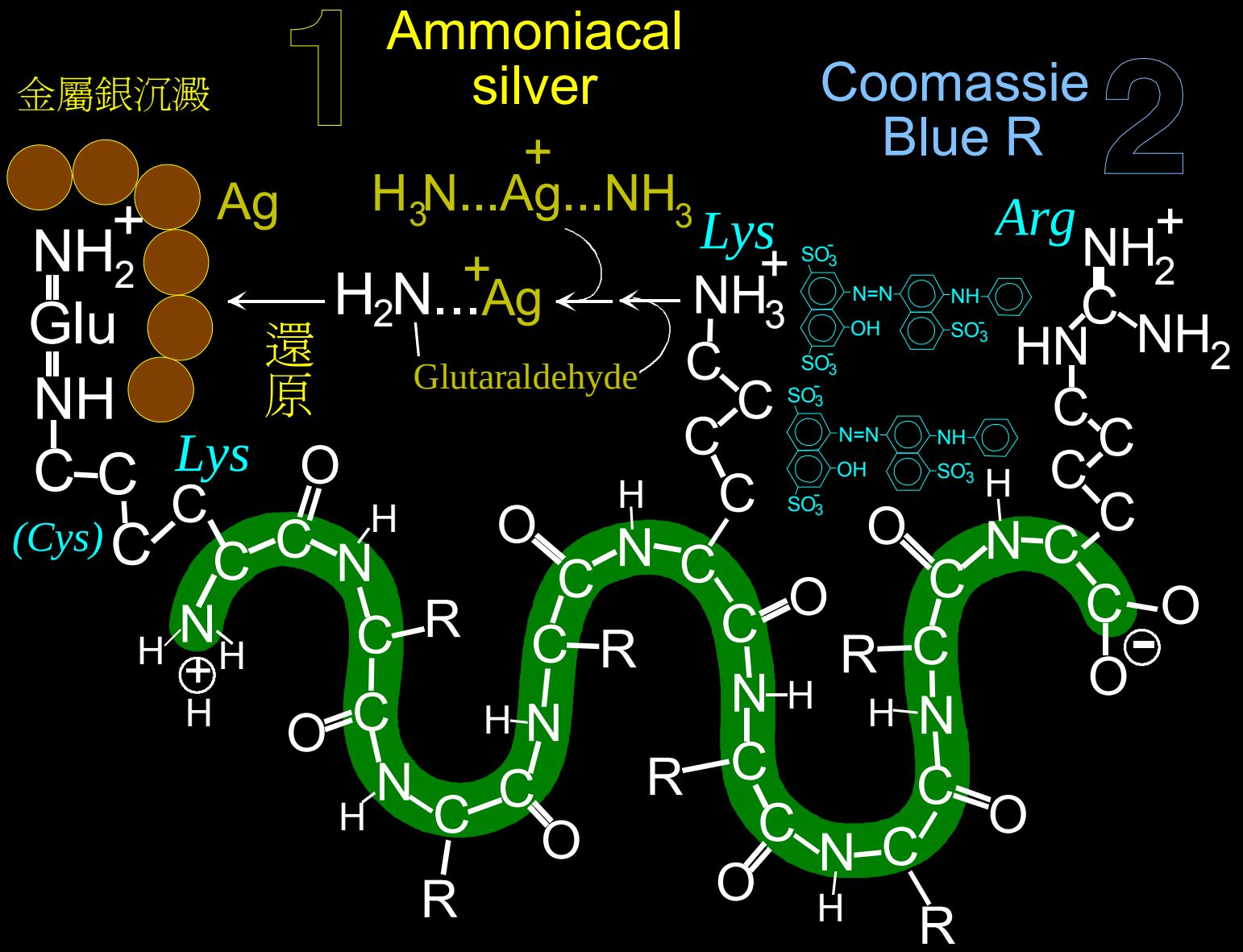
蛋白質的分離

- 電泳(electrophoresis)
- 色層分析法(column chromatography)

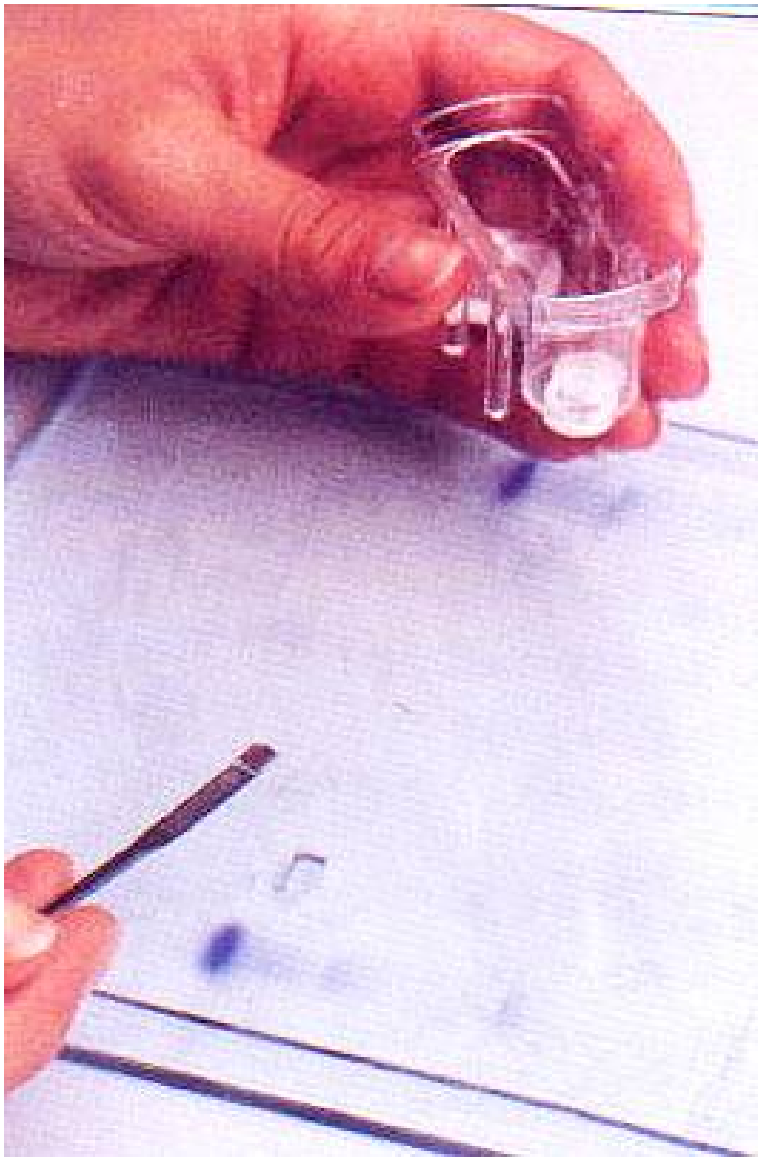
電泳形式的演進：


濾紙電泳 Cellulose
Protein
Denatured
薄層電泳 (TLE) Cellulose acetate
Partial
Denatured
膠體電泳 Starch → Gel

兩種主要蛋白質染色法：

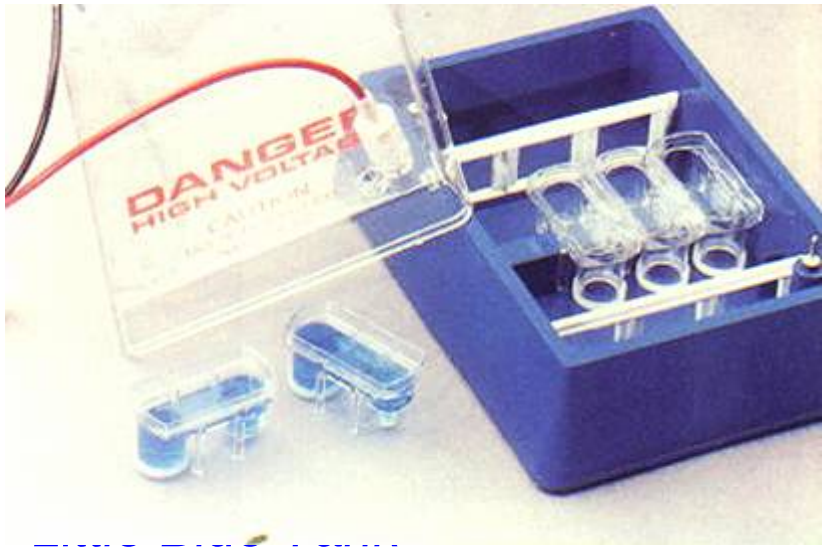

金屬銀沉澱
1
Ammoniacal silver
+
$H_3N...Ag...NH_3$
+
$H_2N...Ag$
Glutaraldehyde
還原
Ag
NH_2
Glu
NH
C–C
(Cys)
Lys
Lys
NH_3
Arg
Coomassie Blue R
2
SO_3^-
N=N
OH
NH
NH_2
HN
C
O
N
H
R

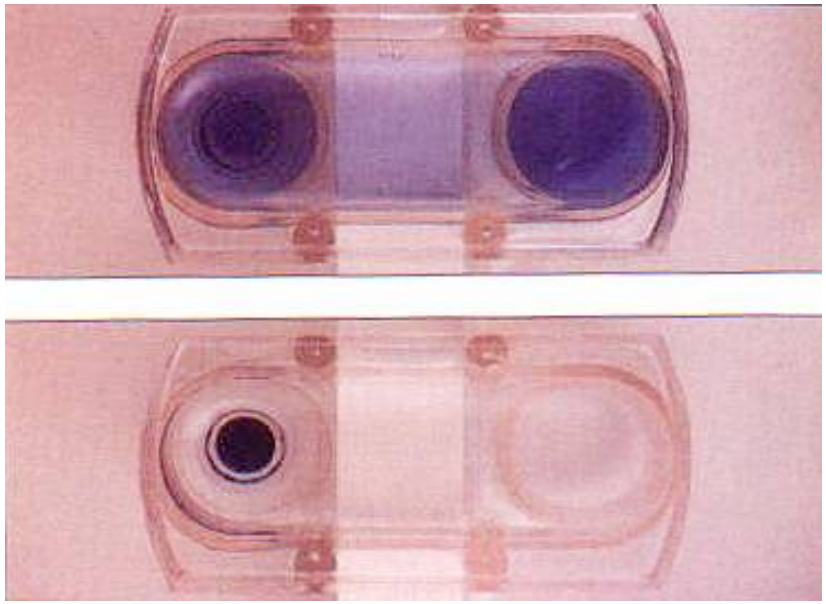
電泳膠體蛋白質的溶離：



ISCO: Little Blue Tank Concentrator

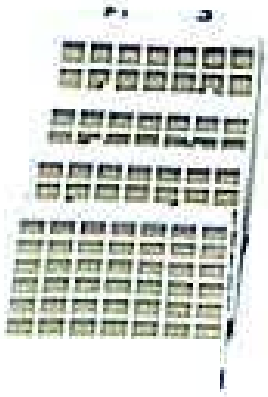
- 直接挖出膠體進行溶離或定序



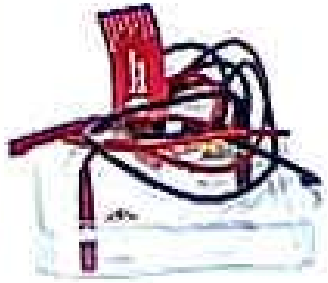


電泳槽及相關設備：

轉印三明治

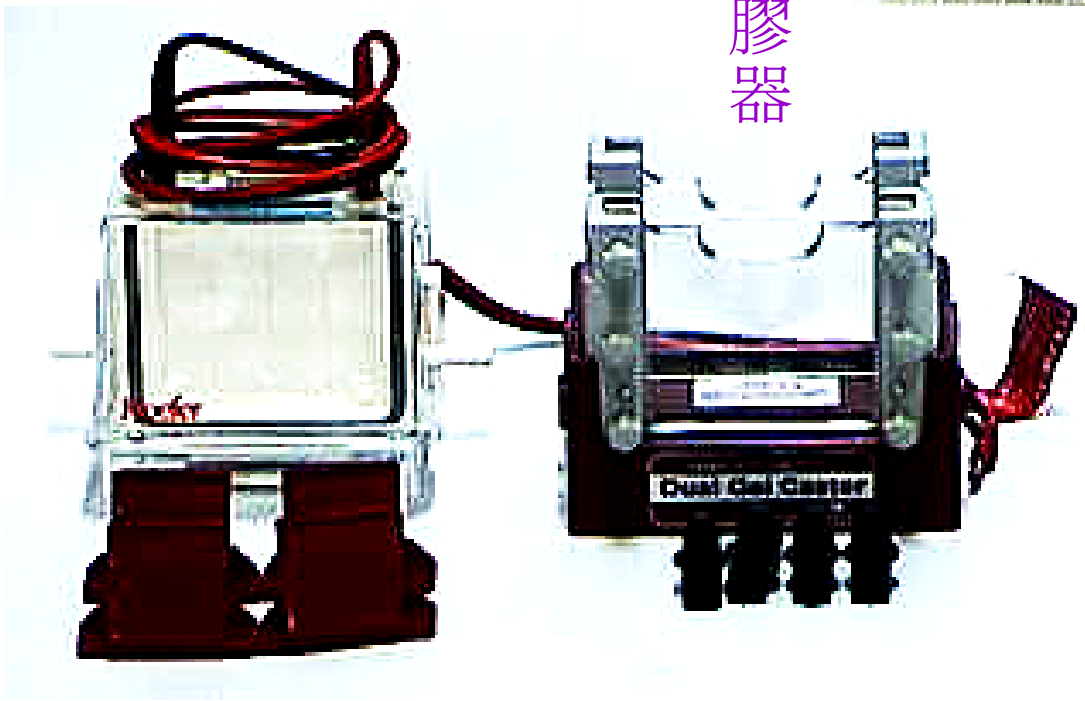


轉印槽



電泳槽

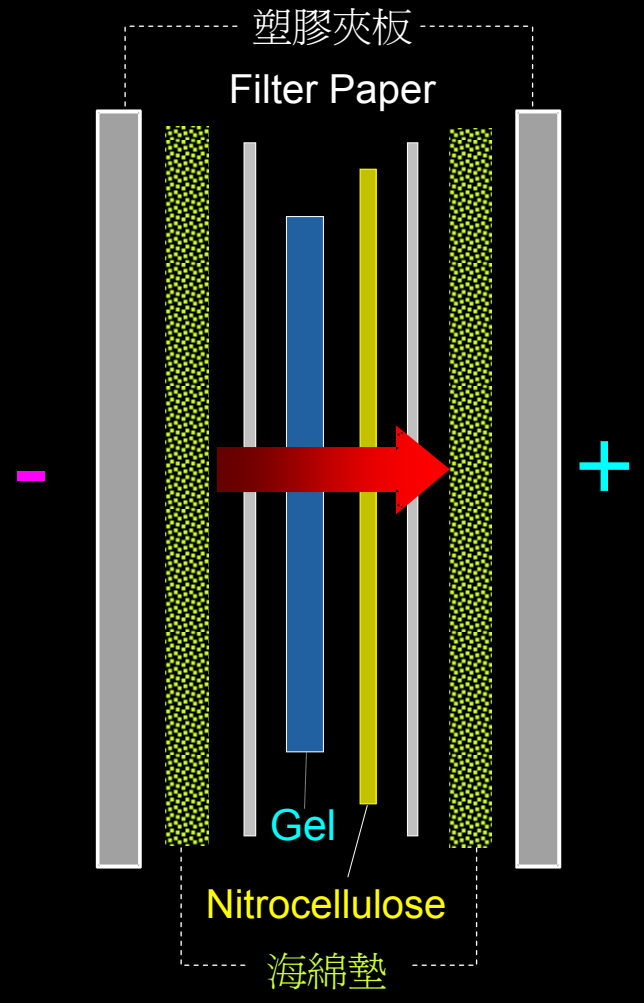
鑄膠器



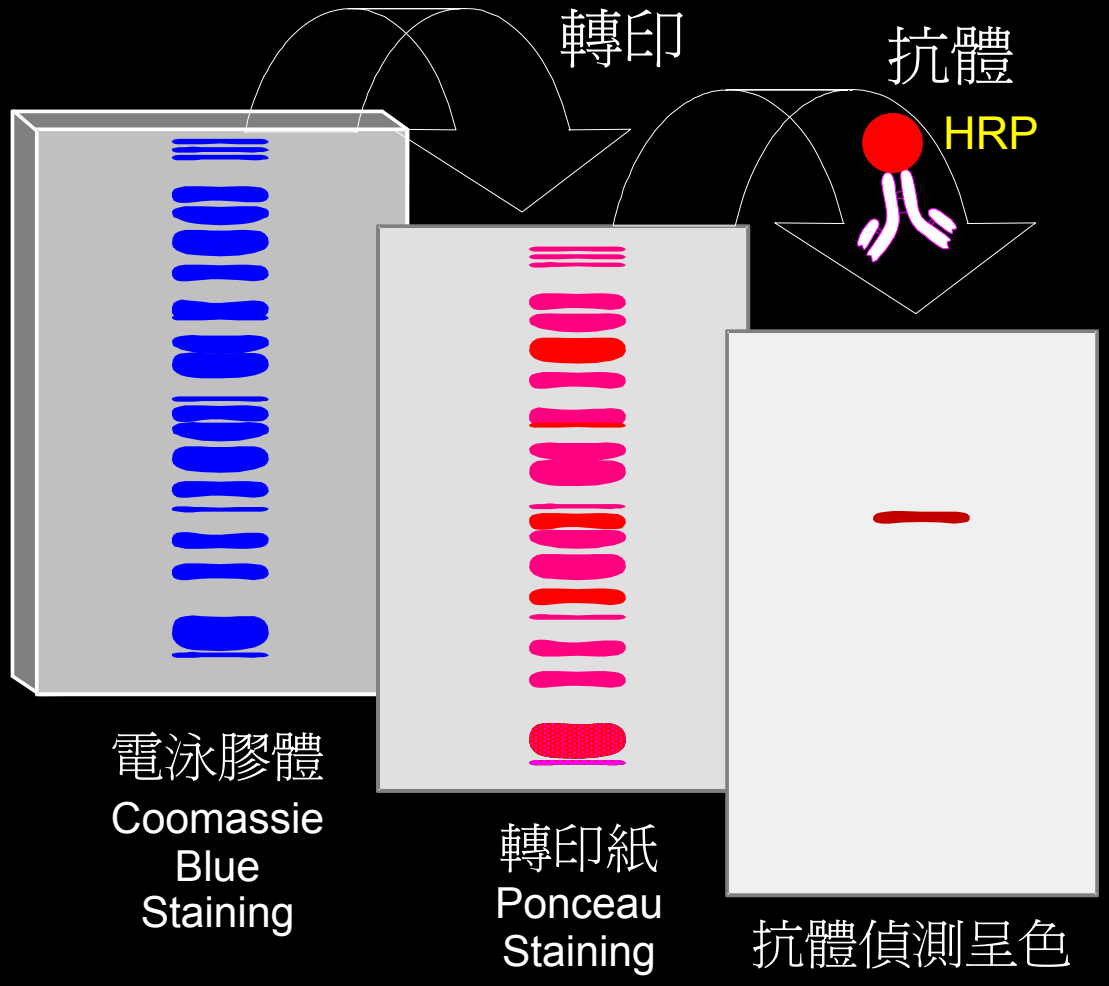
供電器

轉印及免疫染色流程：

A 轉印三明治



B 免疫染色流程及結果



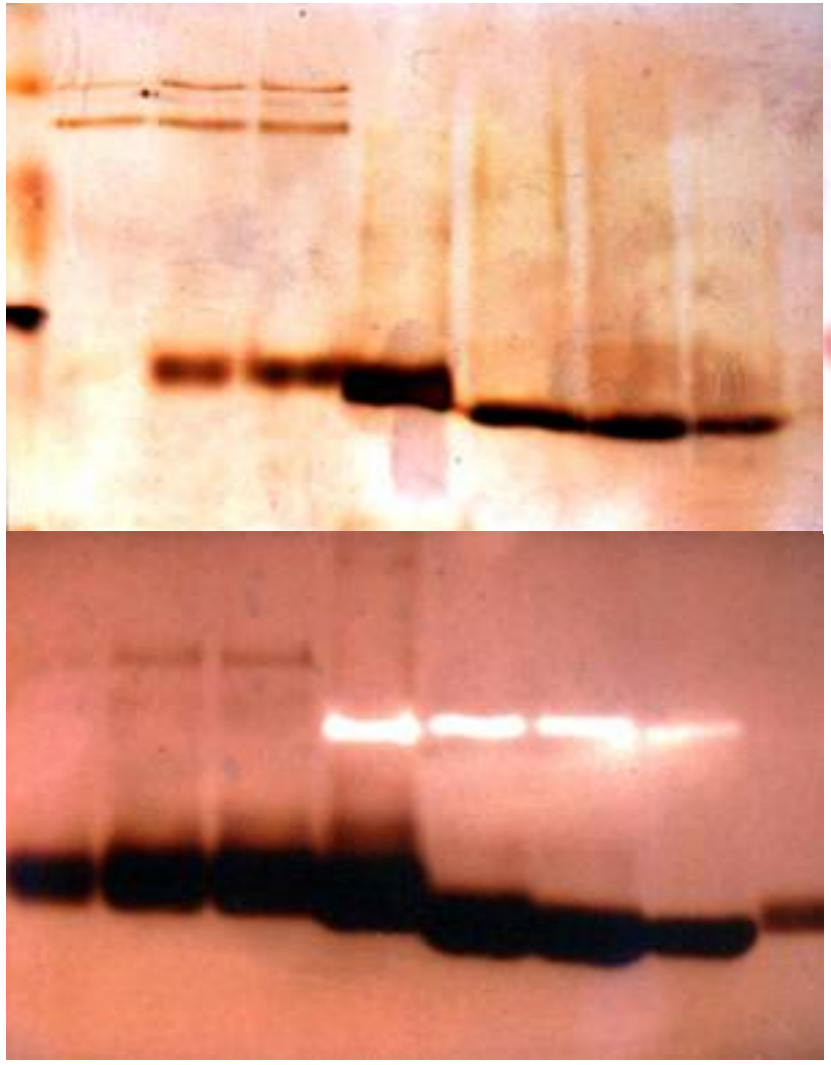
澱粉磷解　染色方法比較：

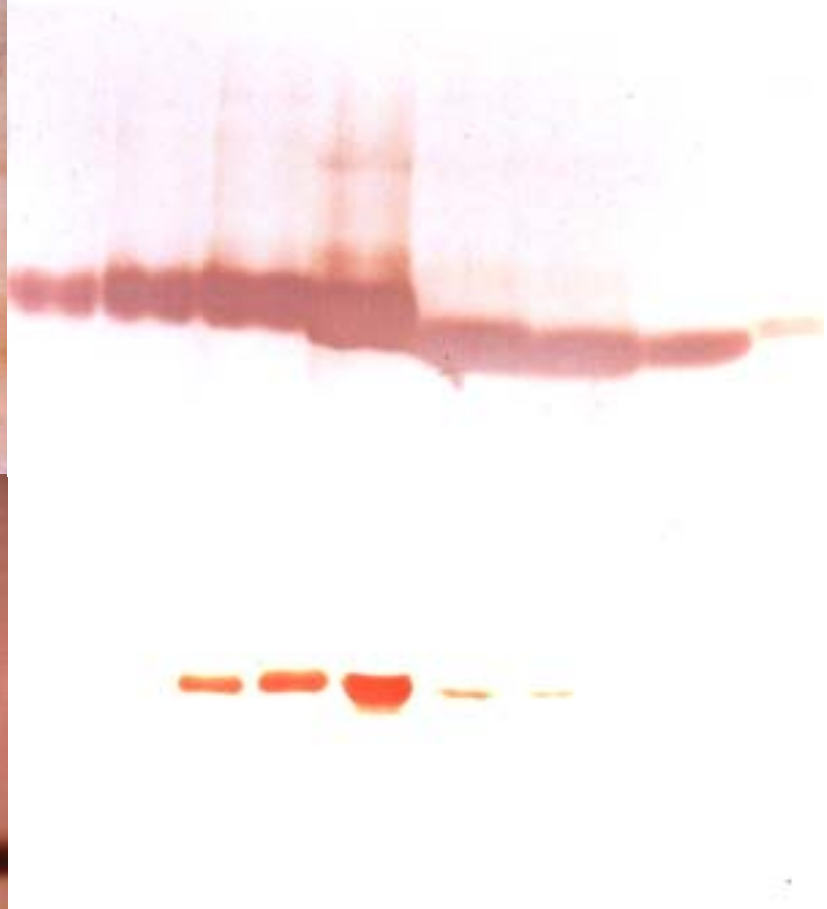
膠體過濾法各分劃

免疫轉印

硝酸銀

活性染色





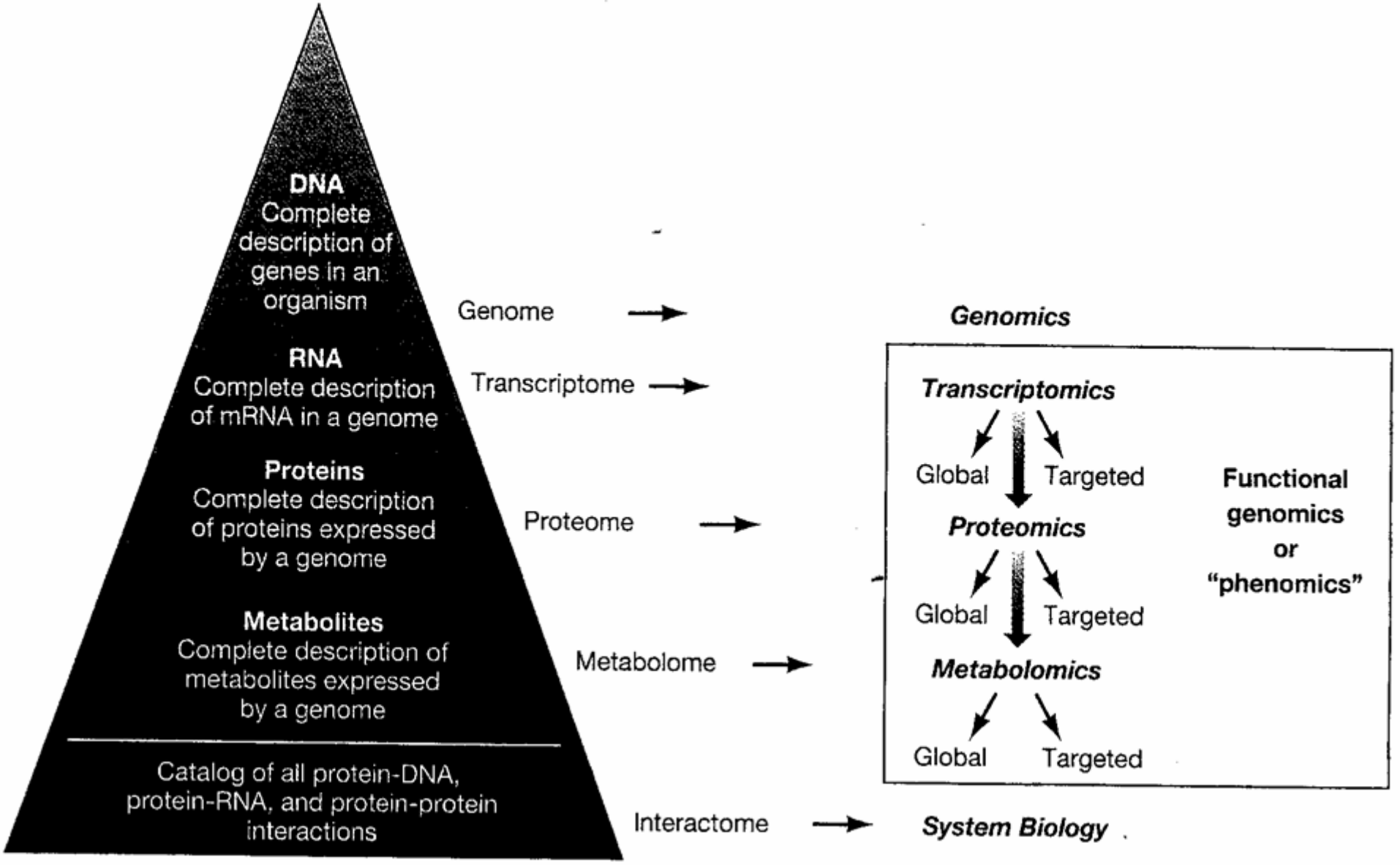
Disc-PAGE

SDS-PAGE

蛋白質體(Proteomics)

- 二維電泳(2-Dimensional protein gel electrophoresis) and HPLC were long used in protein separation.
- 近年生物質譜技術快速發展(development of mass spectrometry in recent years, ~2000 AD)
- 2002 Nobel prize:
  - John B. Fenn (Virginia Commonwealth Univ.)
  - Koichi Tanaka (Shimadzu Corporation, Japen)
  - Soft desorption ionization(軟式脫附離子化) methods for mass spectrometric analysis.
- proteins annotation(蛋白質註解).

Functional Genomics (or Phenomics)



糸統生物学

Summary of the systems

- -ome--- Compete set.
- -ics --- the utility for analyzing these domains.
- 功能基因體學 **(Functional genomics) : hierachical approaches for studying the functional analysis of novel genes**

Defining proteomics

- Functional proteomics: 利用2DE及MS研究蛋白表現
  - 二維電泳 (2DE): two dimensional gel analysis, 通常指: sample first run a 等電位電泳IEF (iso-electrofocusing) electrophoresis. 再以 SDS PAGE分離蛋白的方法.
  - 質譜(MS): mass spectrometry, basically, a method to “accurate” determine molecule weight.

比較蛋白質體學與蛋白質化學

Differences between protein chemistry and proteomics

Protein chemistry	Proteomics
Individual proteins	complex mixtures
Complete sequence analysis	partial sequence analysis
Emphasis on structure and function	emphasis on identification by database matching
Structural biology	systems biology

Conventional ideas:
Using physical biochemistry or mechanistic enzymology, study one protein or multisubunit protein complex at a time.

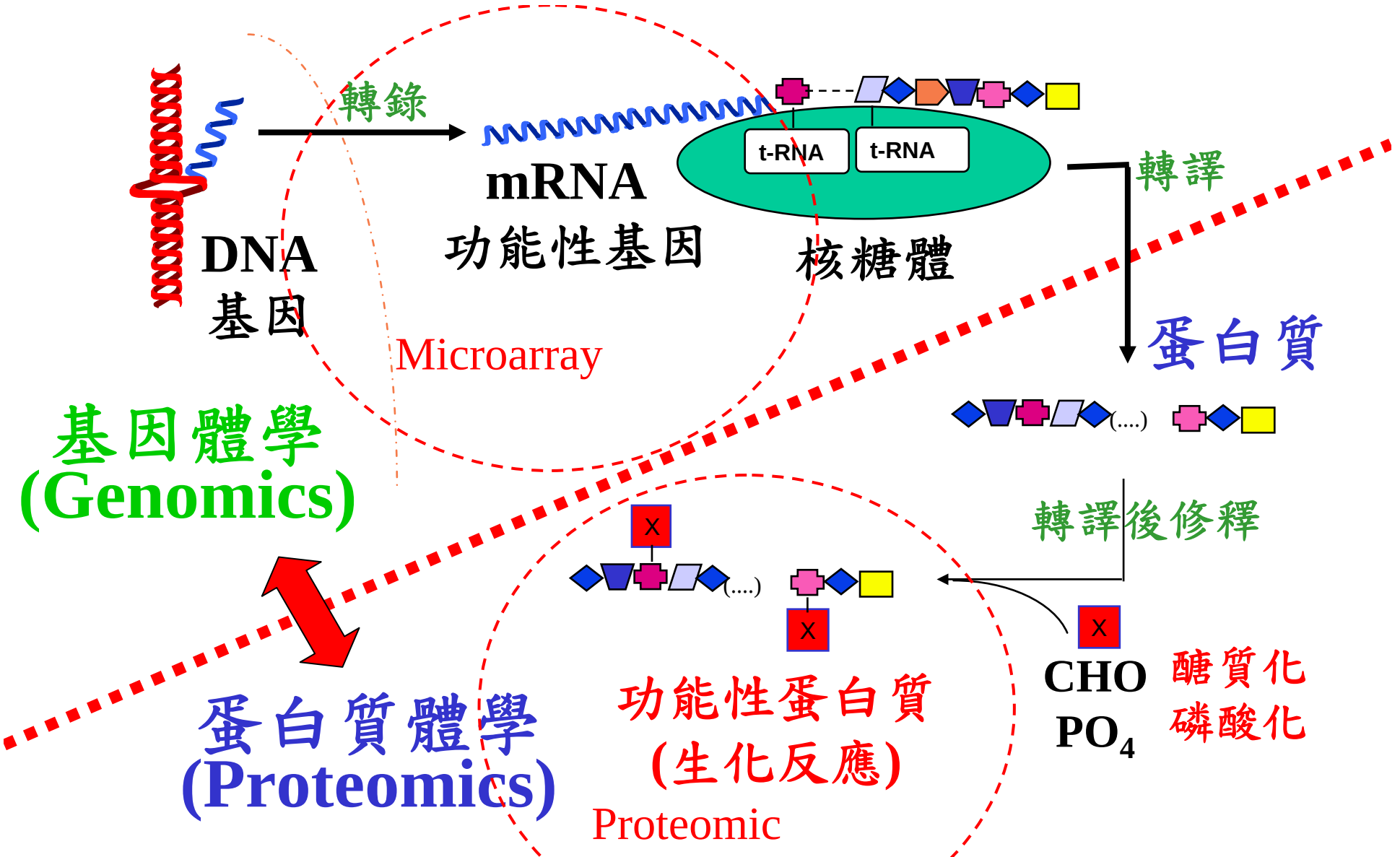
Proteomic ideas:

Study multiprotein systems, in which the focus is on the interplay of multiple, distinct proteins in their roles as part of a larger system or network.

Proteomics: to characterize the behavior of the system

- 蛋白質體學因應基因體學而來:
- Proteomics and Genomics
- Genomics
  - Genome sequencing
    - Complete DNA sequences from various organisms
  - Bioinformatics
    - Tools for sequence comparison
      - Identification of homologues
    - Tools for sequence interpretation
      - Elucidation of coding sequences / protein functions
  - Gene expression (protein) profiling
    - Transcriptomics
      - Differential expression of mRNAs (MicroArray)基因晶片

已知mRNA表現,爲何還須知道蛋白質表現?

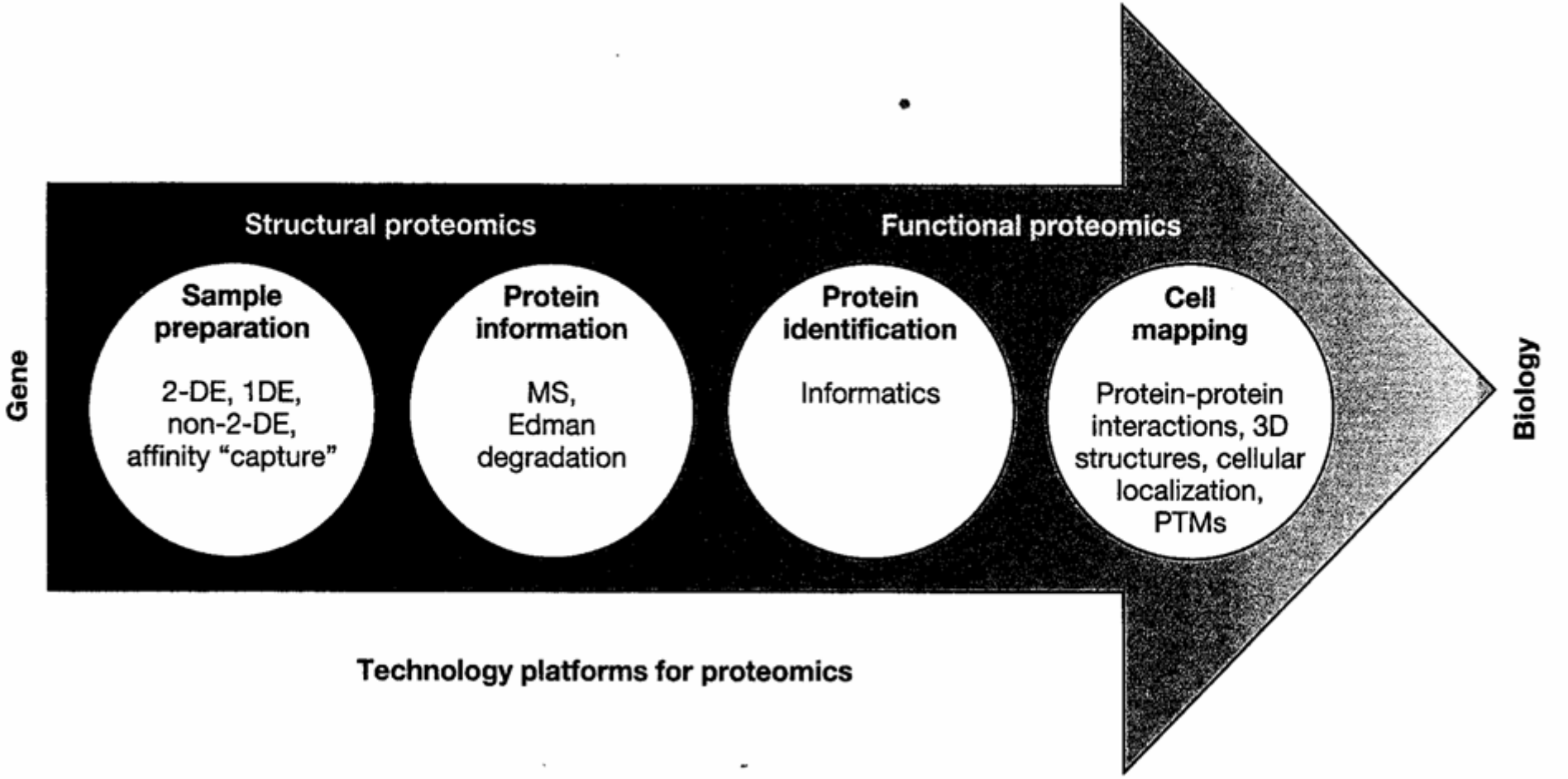


1.蛋白質體學與基因體學兩者相互互補(如表現量)但不一定相同。

2.蛋白質體學能發現在基因體學無法完成得到的結果(如醣質化及磷酸化)。

蛋白質體的四個關鍵技術:

1. Sample preparation, 樣本處理
2. Protein information,蛋白質資訊
3. Protein Identification and quantification,蛋白質註解及定量
4. Cell mapping: targeted proteomics (study of protein-protein interactions, posttranslational modifications (PTMs), cellular localization, etc.).



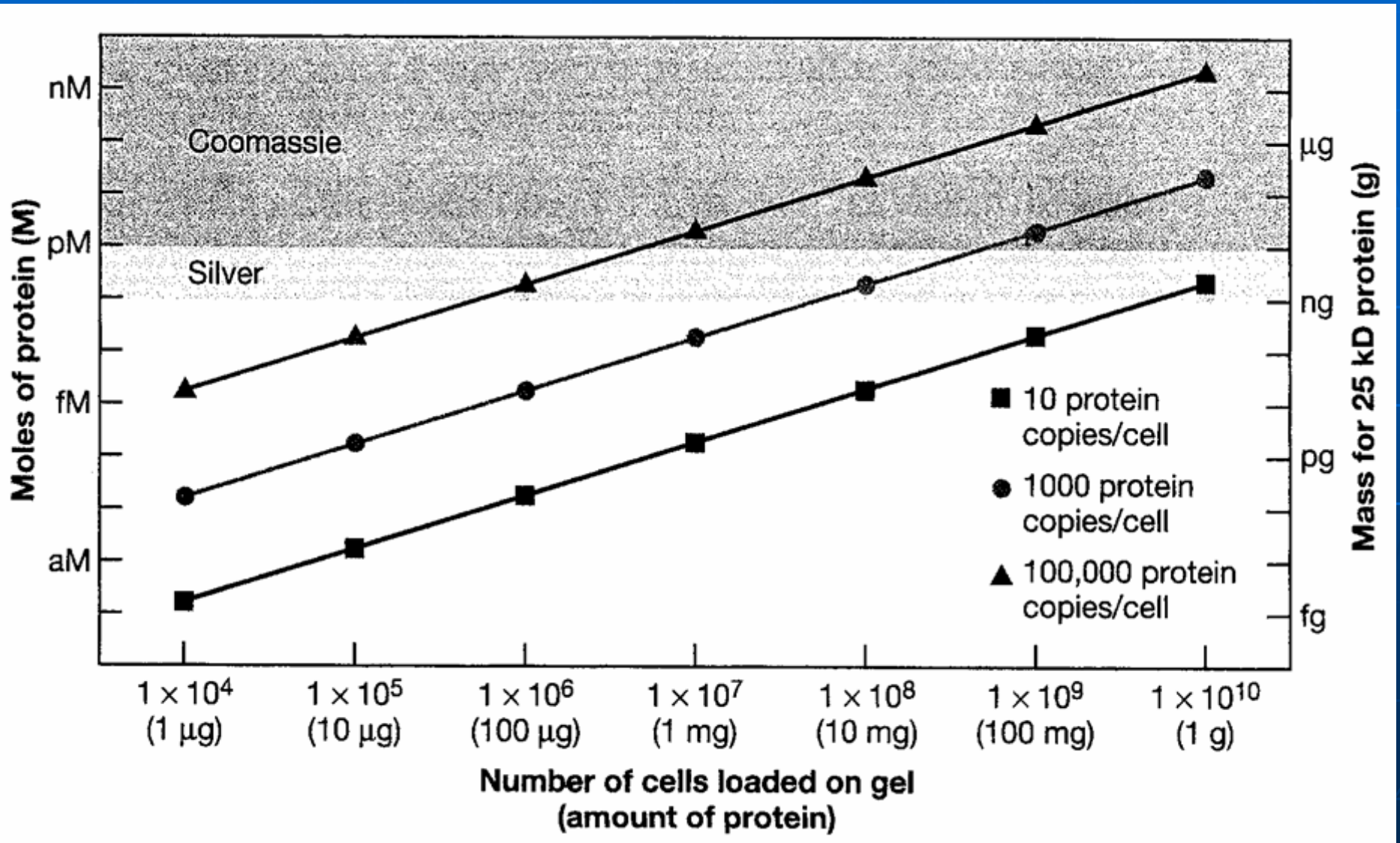
蛋白質體目前瓶頸:

Protein determination range

- 不同蛋白質表現量差異很大:~ 10^6. e.g., 10 copies/cell for transcription factor, up to 10^6 copies/cell for abundant molecules.
- 2D gels, which usually separate ~1,500 spots, present most abundant proteins if a crude protein mixture is used.

Protein Copy number and cell quantity: 無法測到低表現蛋白質

Detection in Silver stain range and coomassie stain range.



蛋白質二維電泳**2-D gel electrophoresis**

1st D - Isoelectric focusing (聚膠電泳)
- separation on basis of pI

2nd D - SDS gel electrophoresis (SDS電泳)
- separation on basis of mass

- **Isoelectric focusing**
  - separation on basis of pI
  - pH gradient
  - migrate to their pI and stabilize pH gradient

-

1st -Dimensional gel Electrophoresis

- 1st PAGE precast gels:
  - 提供高解析及高範圍生物材料分離 ("denatured" proteins, polypeptides or nucleotides) from 2,000,000 to 5,000 Da.

- **Polyacrylamide gel electrophoresis**

  Acrylamide : $CH_2 = CH - CO - NH_2$

- N,N' methylene bis acrylamide:

  $CH_2 = CH - CO - NH - CH_2 - NH - CO - CH = CH_2$

- Polymerization induced by free radicals produced from ammonium persulfate in presence of TEMED (N, N, N', N' – tetra methylethylendiamine)
- TEMED causes free radicals to form from persulfate

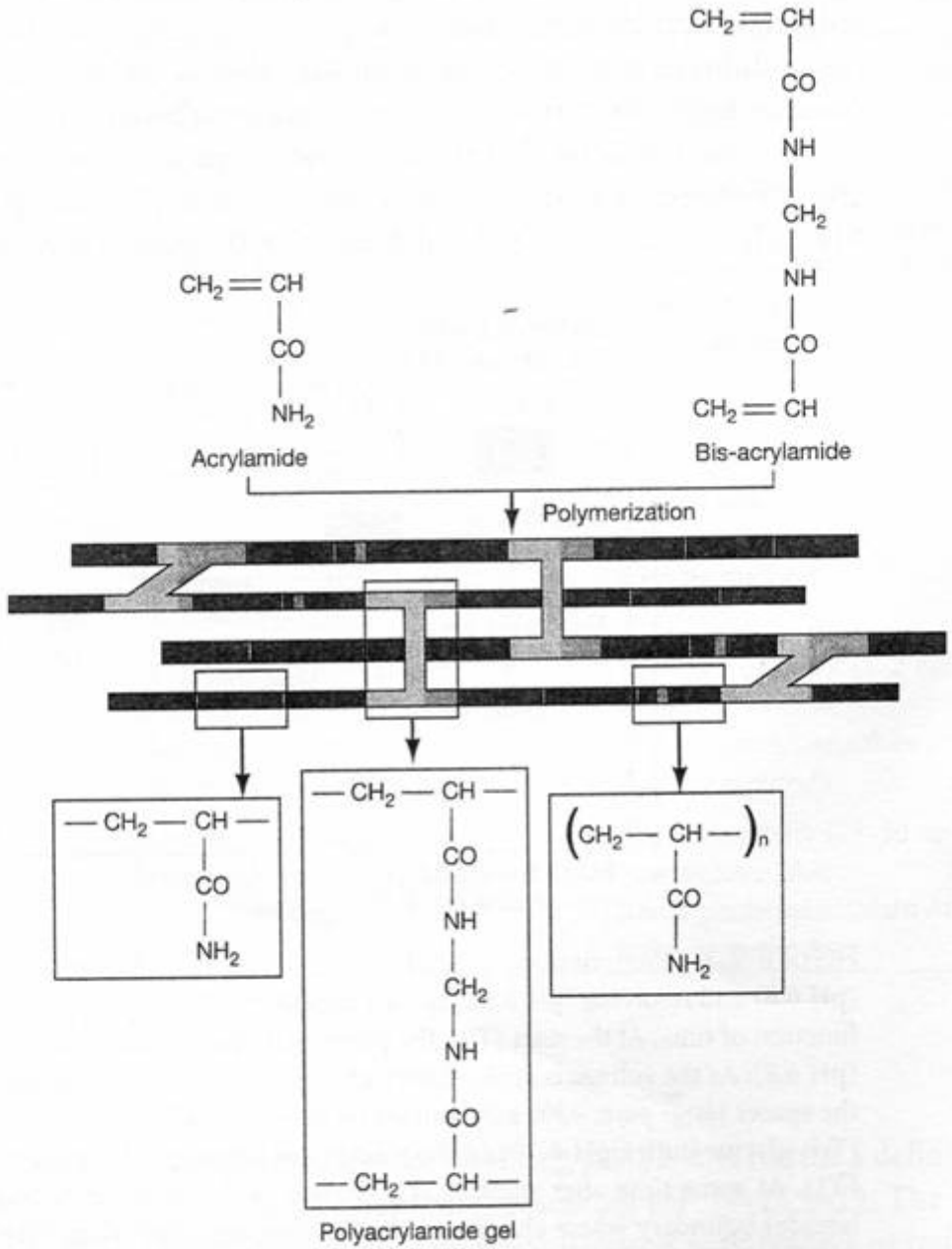


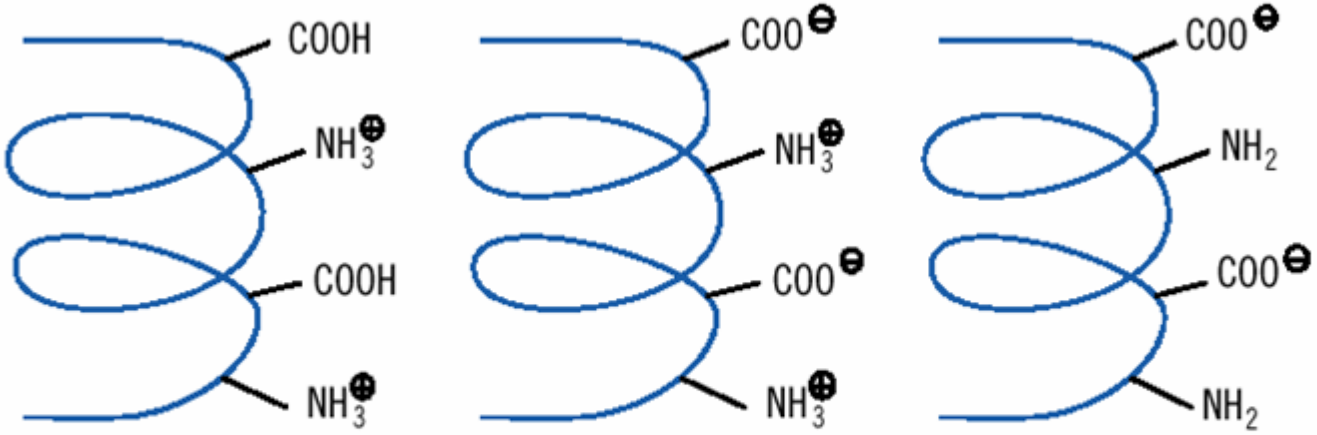
FIGURE 2.4. Chemical structures of acrylamide, bisacrylamide, and polyacrylamide. (Adapted, with permission, from Hames 1998 [©Oxford University Press].)

利用起始劑自由基反應聚合:

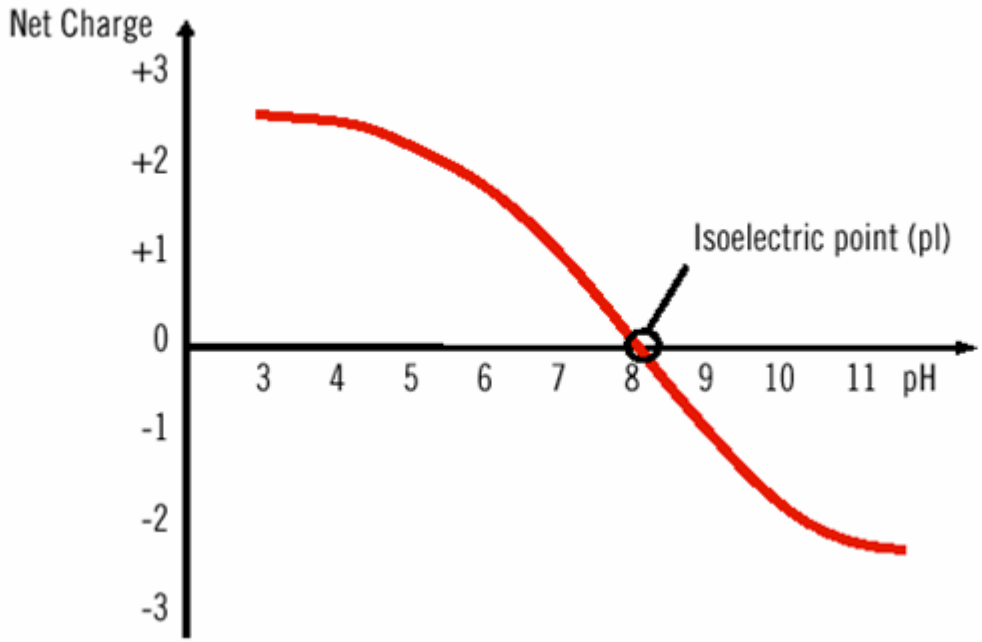
- Chemical: TEMED and APS.
- Photochemical: riboflavin-5'-phosphate, or methylene blue.

電泳膠孔徑(由單體濃度及比例決定):

- %, and ratio: acrylamide and Bis-acrylamide
- Polymerization temp.
- Gel additives:
  - Urea (smaller pore size)
  - Polyethylene glycol: macroporous.

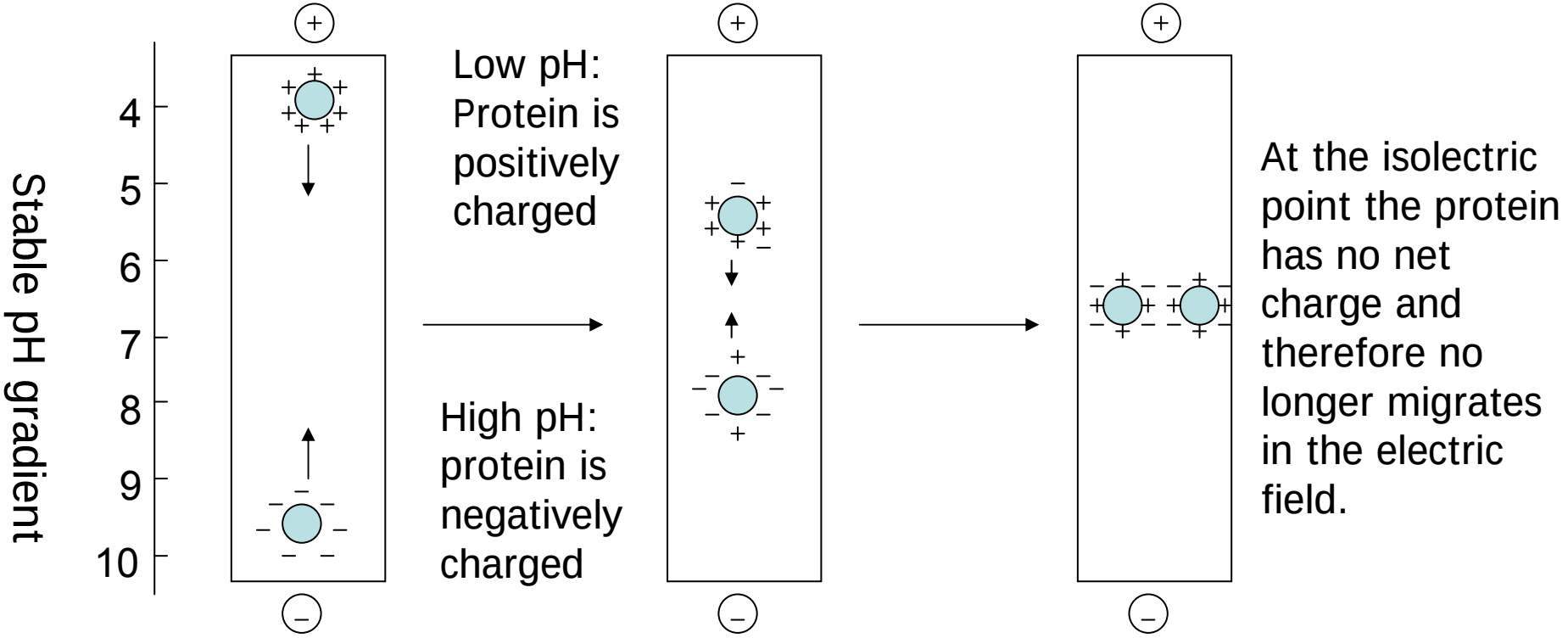


pH<pI pH pI pH>pI



Isoelectric point (pI)

- Separation by charge:



pH值的選擇

- pH梯度差越小,解析度越高.
- 根據不同的樣品選擇不同pH梯度, e.g.,
  - The known extreme pI : acidic glycoprotein of the chimpanzee: pI=1.8, Lysozyme from human placenta: pI=11.7.
- The most used pI range is pH=3 ~ 10.

IEF Ideal Lysis Buffer (標準配方)

- 8M urea (or 7M urea + 2M thiourea in the case of fractions containing hydrophobic proteins)
- 2-4% CHAPS
- 1% IPG buffer (appropriate buffer for pH range to be analysed)
- 2 mg/ml DTT

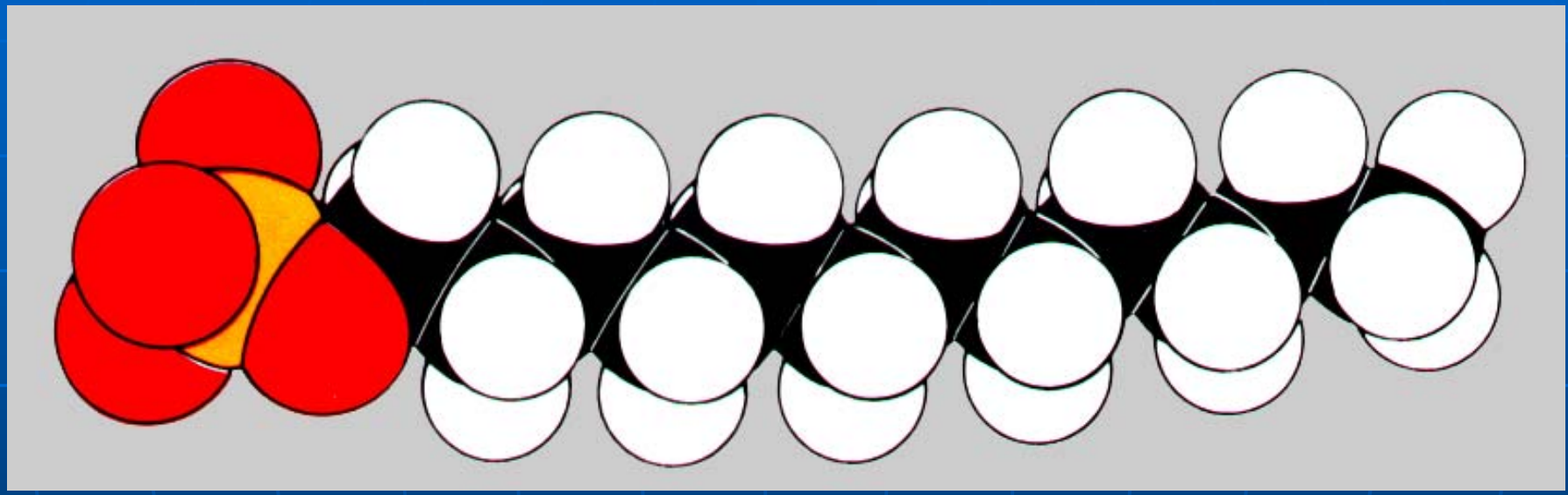
Factors which may affect pI

- amino acid composition
- phosphorylation
- glycosylation
- other post-translational modifications

2nd -Dimensional gel Electrophoresis

- 第二維電泳通常以SDS PAGE進行.
  - 傳統SDS PAGE須使用聚膠電泳(stacking gel)及分離電泳(separating gel)合併使用
  - 通常二維電泳中,因爲第一維已聚膠，不使用stacking gel.

(sodium dodecyl sulfate)



極性頭部　　非極性尾巴

切勿吸入 SDS 塵埃以免引起呼吸困難

SDS 在蛋白質表面均勻附上一層負電：

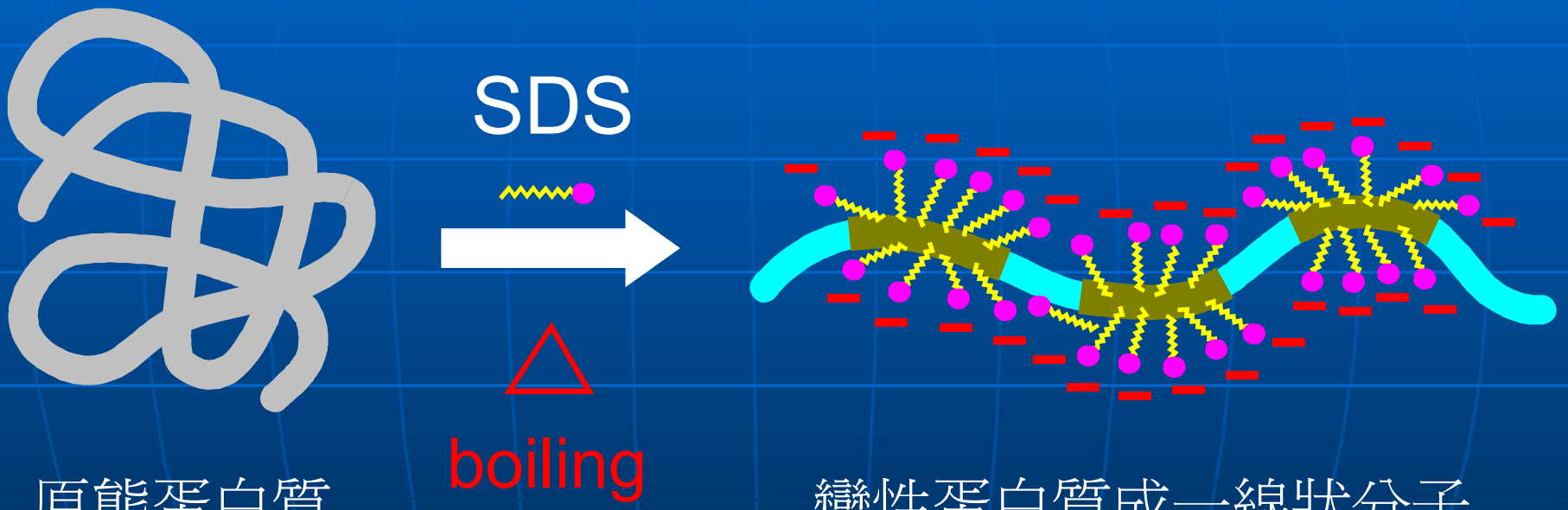
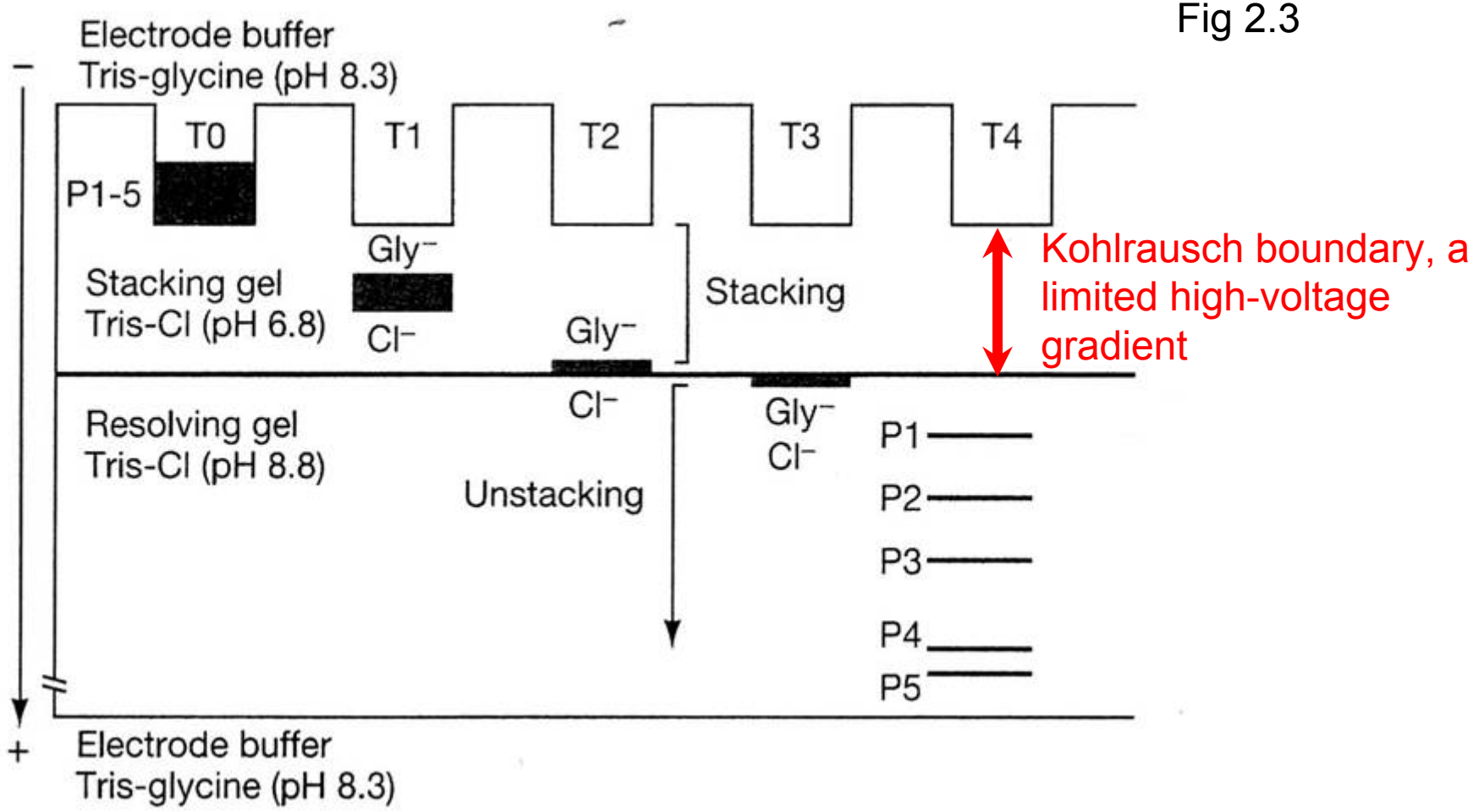


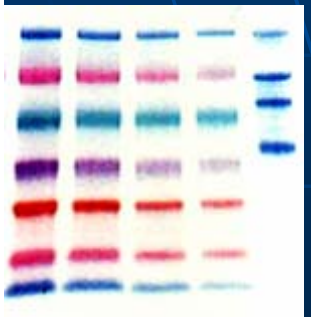
Fig 2.3

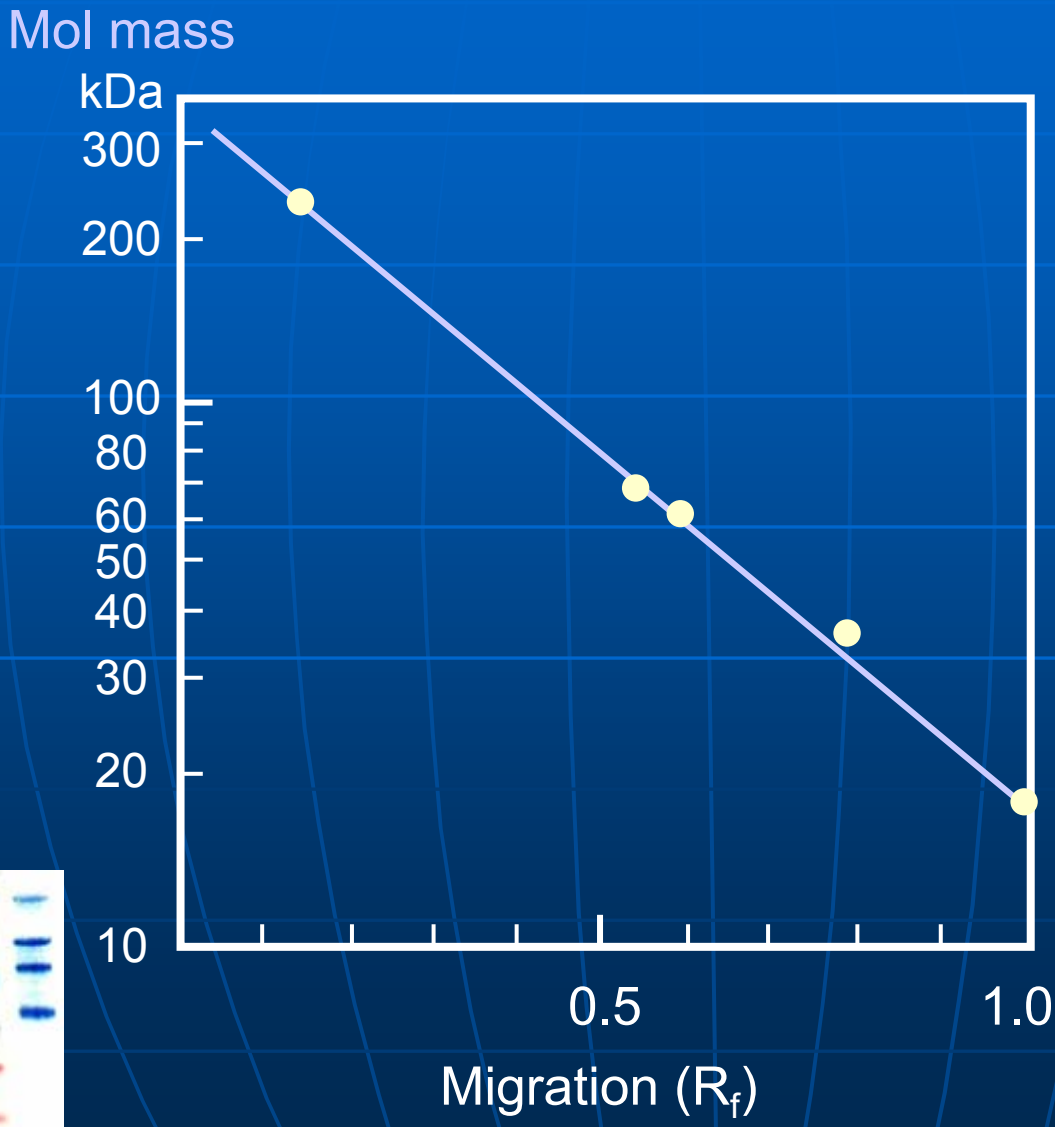


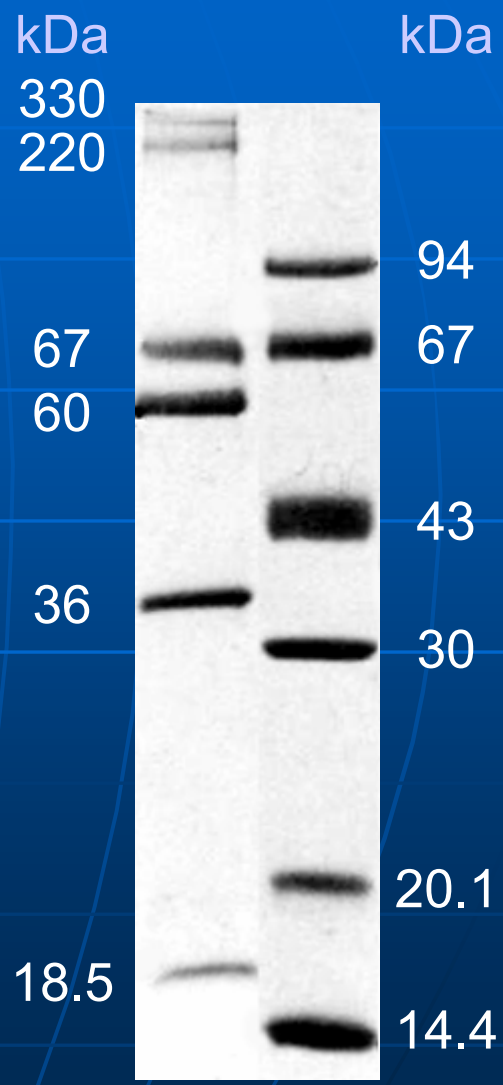
Stacking gel: pH6.8, resolving gel: pH8.8, electrode buffer, pH8.3 (Tris-glycine)
Sampel(P1-P5) running between two solution: Cl-(leading) , and trailing (Gly-)

Laemmli system

單元體分子量的測定： SDS-PAGE







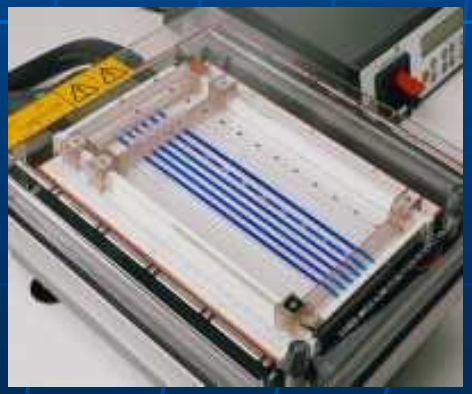
Pharmacia: Molecular Markers for electrophoresis

Multiphor™ II IEF System

- Perform first- and second-dimension separations with the same system.
- Run up to 12 IPG strips (7-24 cm) simultaneously.
- With DryStrip Reswelling Tray, samples can be loaded during IPG strip rehydration by including rehydration buffer.

Step 1. Rehydration
Use DryStrip Reswelling Tray.

Step 2. IEF
Use the parallel grooves



Cap loading

Ettan IPGphor IEF System

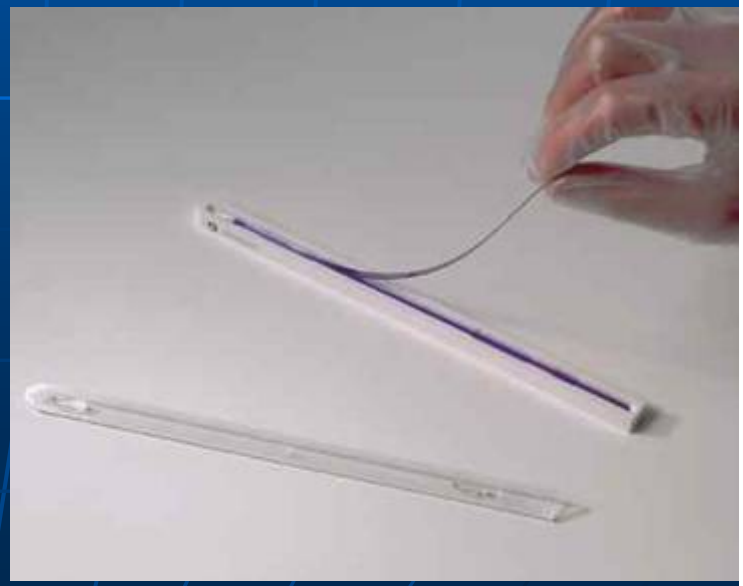
- *Accommodates all lengths of Immobline DryStrip gels (7, 11, 13, 18, and 24 cm) and can run 12 gels simultaneously.*
- *8,000 V Max*

BioRad system

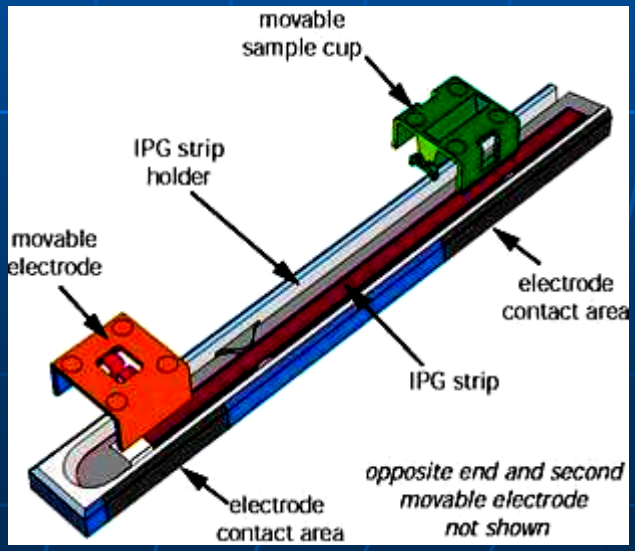
- Isoelectric focusing (IEF)
  - Pre-cast IPG strips
    - Variable pH range (*e.g.* 3-10, 4-7, 3-6, 6-12, 4-5)
    - Variable strip length (7, 11, 17, 24 cm)
    - Optimised protein loading
    - 10,000V Max

- *IPGphor ceramic strip holders are available in five lengths: 7, 11, 13, 18, and 24 cm.*

- *IPG strips are rehydrated with IPG Buffer in regular strip holders. The sample can be added to the buffer for efficient loading during rehydration.*

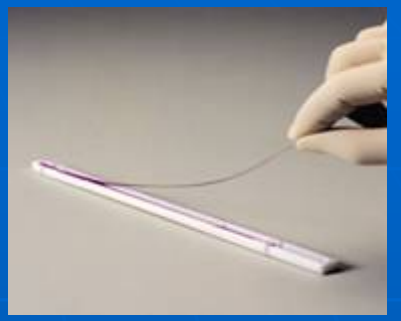


- *With movable electrodes and sample cup, the Cup Loading Strip Holder accommodates IPG strips up to 24 cm long.*



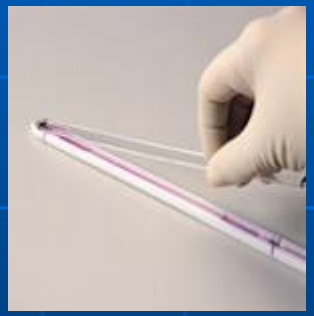
1. Apply rehydration solution.

2. Remove protective film from DryStrip gel.



3.Drystrip in strip holder

4. Apply Cover oil



5. Cover on strip holder.

6. Place assembled strip holder on Ettan™ IPGphor™ platform.

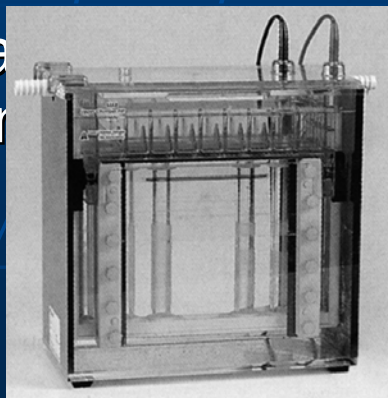
TECHNICAL SPECIFICATIONS	
Instrument	Integrated Peltier cooling and high-voltage power supply
Voltage	0–8000 VDC
Current	0–1.5 mA
Platform temperature	18–25 ˚C
IPG strip lengths	7, 11, 13, 18, or 24 cm Immobiline™ DryStrip Gels
Number of strips	12 maximum
Reswelling	In strip holder or reswelling tray when using Cup Loading Strip Holder
Strip holder material	Base – aluminum oxide Cover – clear acrylic
Sample application	During rehydration or after rehydration when using Cup Loading Strip Holder
Software features	10 protocols, 9 voltage ramps or steps each; set rehydration delay; set maximum current limit; set temperature
Ports	Serial port (RS232)
Operating temperature	15–35 ˚C
Relative humidity	0–90% non-condensing
Line voltage	90–260 VAC
Maximum power	100 W
Dimensions (H x W x D)	14 x 25 x 46 cm
Weight	6.8 kg
Safety features	Automatic voltage shut-off when safety lid is opened
Safety certifications	CE 73/23/EEC (LV directive); UL3101-1; CSA22.2 1010-1

Multiphor™ II and ExcelGel™ System

- ***Perform second-dimension separations using convenient precast gels and 7 11 18 or 24 cm IPG strips.***
- Choose from ExcelGel precast SDS-polyacrylamide gels available as 12.5% homogeneous and 12-14% gradient gels.
- Prepare multiple 2-D maps on a single gel using three 7 cm or two 11 cm IPG strips side-by-side.
- Configure Multiphor II with MultiTemp III Therm Circulator and the programmable EPS 1001 Pow for reproducible running conditions.

Hoefer™ SE 600 Ruby™ Vertical System

- ***Perform second-dimension separations using 7 or 13 cm IPG strips.***
- Prepare multiple 2-D maps on a single gel using two 7 cm IPG strips side-by-side.
- Run up to four gels simultaneously and perform eight second-dimension separations at once.
- Configure SE 600 with MultiTemp III Thermostatic Circulator and the programma EPS 601 Power Supply for reproducible runn conditions.



影像分析

- Complex protein patterns in 2D electrophoresis gels are captured in digital format to allow accurate, reproducible and quantifiable comparisons.
  - Image Master (Melanie) (Pharmacia)
  - PDQuest (BioRad)

Image Scanner

ImageScanner has an extremely broad optical density range to give the high resolution required for reliable quantification of Coomassie and silver stained gels. Fast scanning and image acquisition rates ensure short scan times.

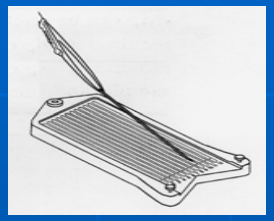
Typhoon

- Typhoon unites four-colour fluorescence, filmless autoradiography and chemiluminescence into a single system.
- Its broad dynamic range ensures accurate results at the first attempt.
- Highly sensitive optics facilitate extremely low limits of fluorescence detection and enable direct chemiluminescence imaging without intermediate exposures to film or screens.

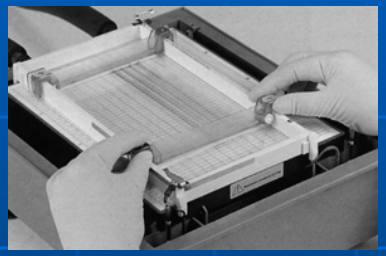
蛋白質體分析流程

2-DE

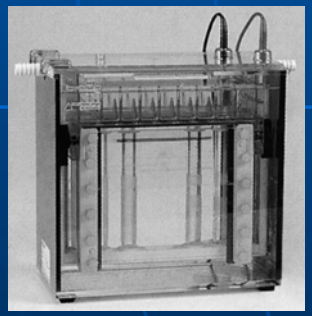
Sample prep.



Rehydration



IEF



SDS-PAGE

Gel -Image Analysis

In-Gel Digestion

Reduction

Alkylation

Extraction

Peptide

MALDI-TOF

Protein identify from database

Mass