3 電泳檢定法 Electrophoresis

- 3.1 電泳原理 Basic principles

  帶有電荷的分子都可在電場中泳動

- 3.2 聚丙烯醯胺膠体電泳 PAGE

  是蛋白質最常用的電泳介質

- 3.3 其它相關技術 Related techniques

  另有一些與電泳相關的檢定技術

3.1 電泳原理 Basic principles for electrophoresis

- 3.1.1 蛋白質的泳動率 Protein mobility

  泳動率與蛋白質所帶的電荷及大小有關

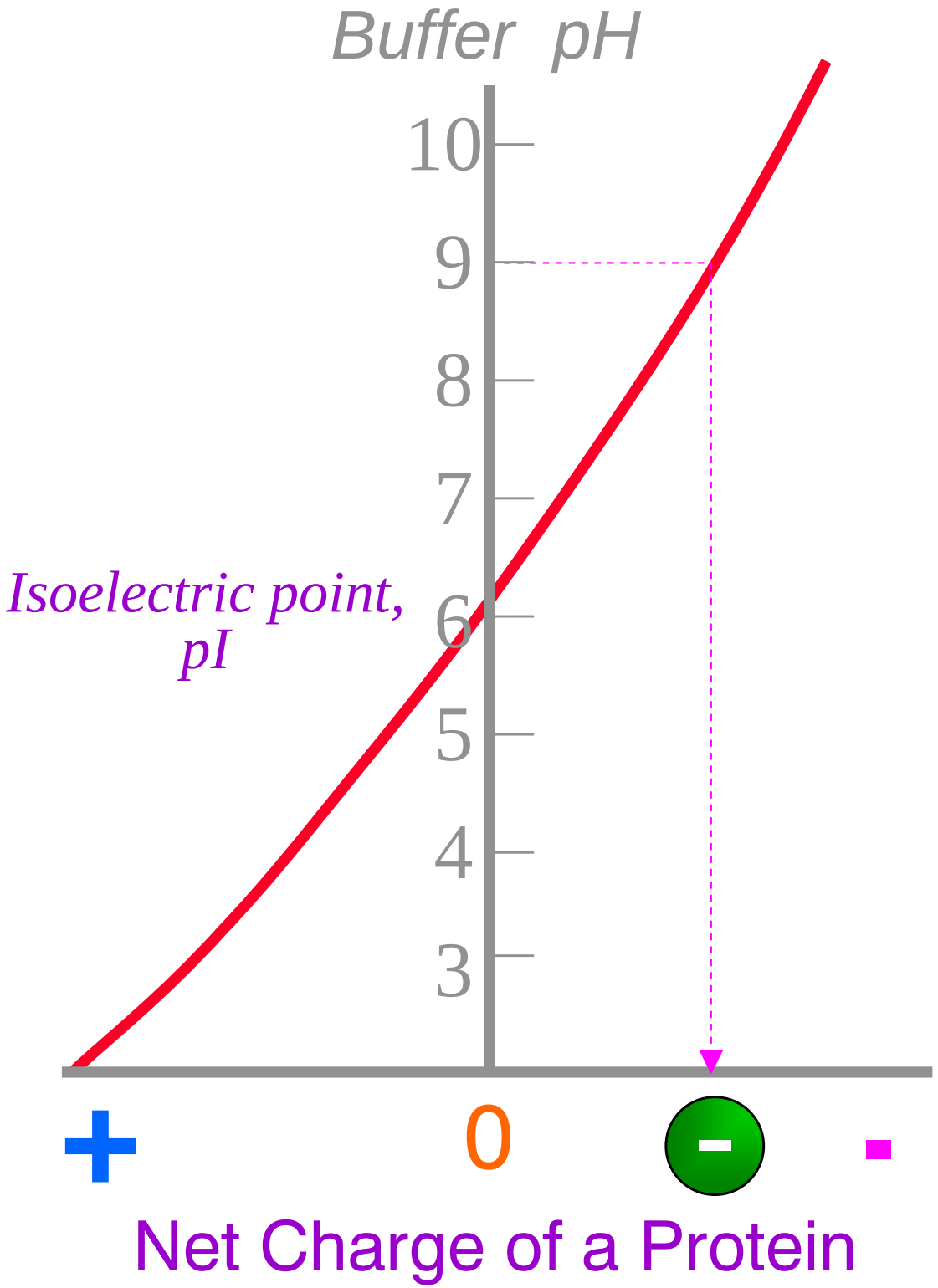
- 3.1.2 電泳的種類 Types of electrophoresis

  電泳的種類與方式很多，但原理是不變的

- 3.1.3 電泳設備及系統選擇 Available systems

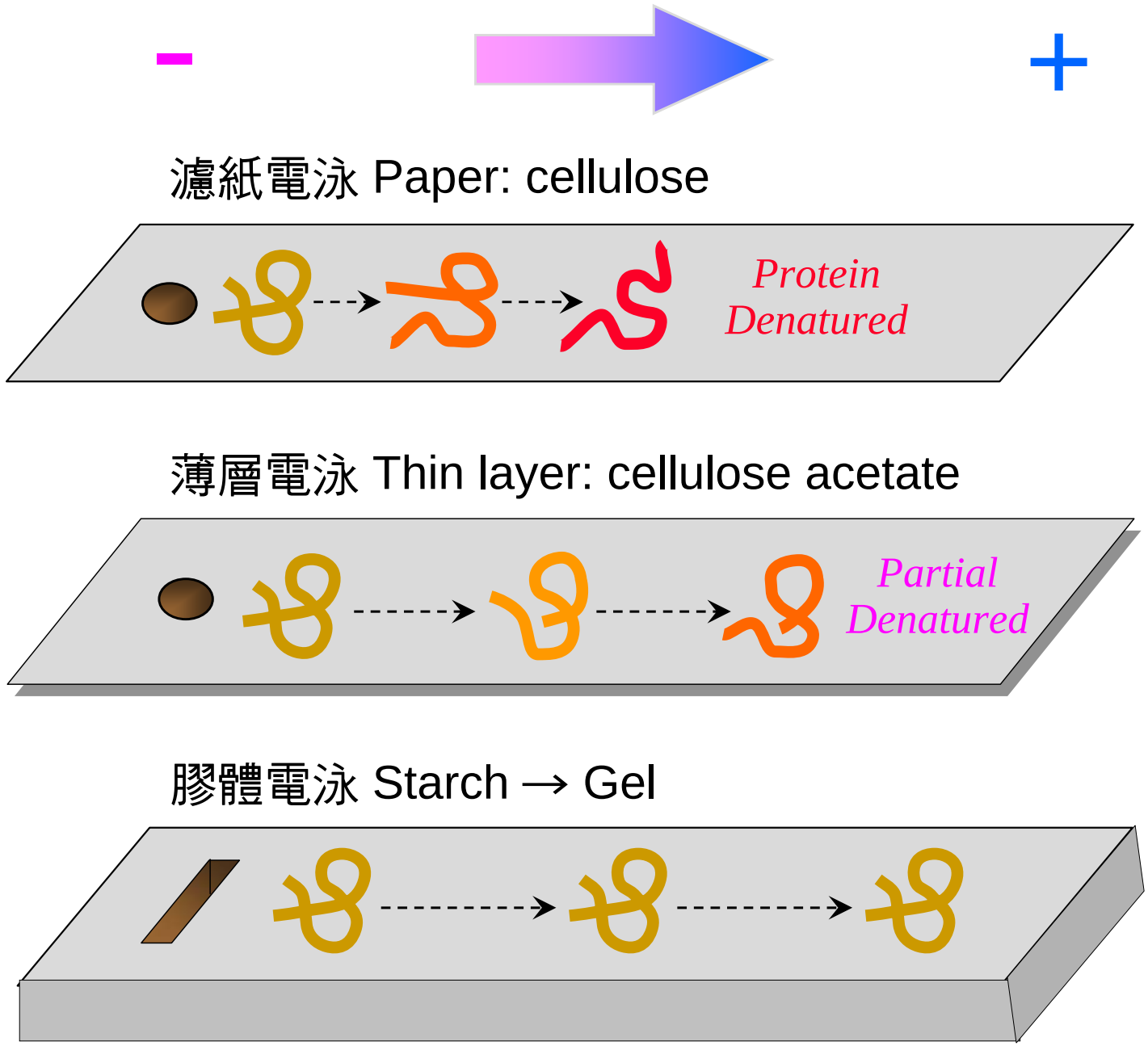
  要選擇正確的系統及適用的設備

環境影響分子的帶電性質



Juang RH (2005) EPA

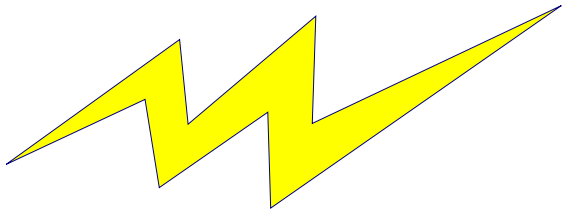
電泳形式的演進



Historical evolution of electrophoresis design

Juang RH (2005) EPA

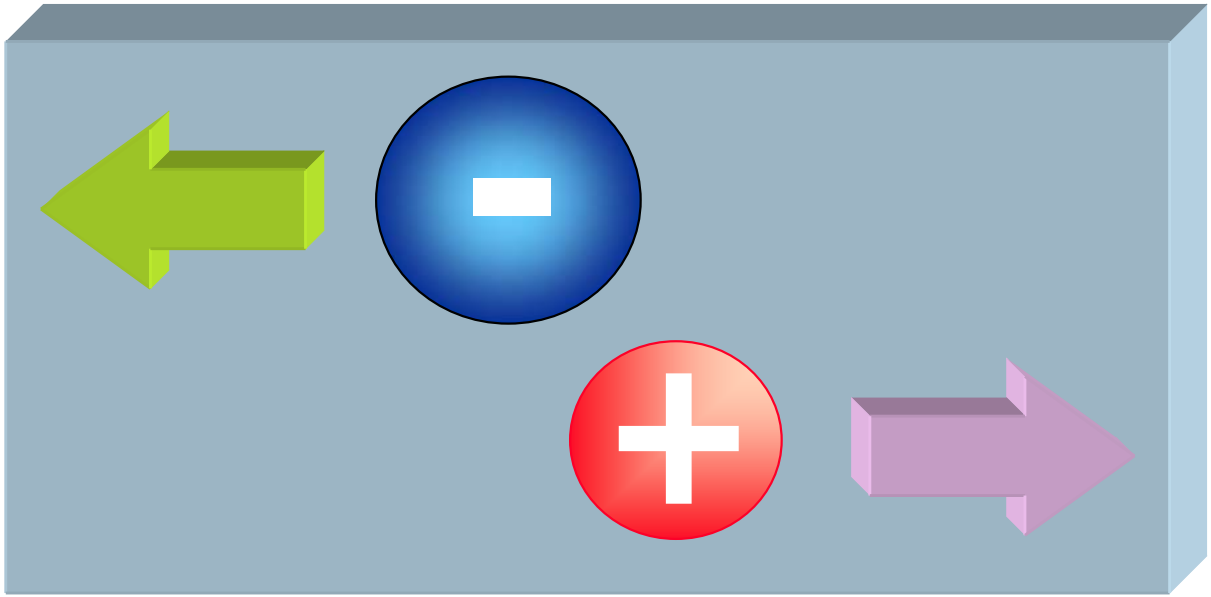
影響泳動率的因素 Factors affecting mobility



外加電流電壓　　Input *current, voltage*

ANODE

+



CATHODE

-

Friction　　***Charge***

分子量 分子形狀
Molecular weight, shape

分子的等電點
Isoelectric point

Juang RH (2005) EPA

3.2 聚丙烯醯胺膠體電泳 PAGE

- 3.2.1 PAGE 種類 PAGE categories

  依蛋白質樣本是否變性可分成兩大類

- 3.2.2 PAGE 膠体組成 Gel composition

  是單体小分子的聚合反應

- 3.2.3 PAGE 系統解剖 System anatomy

  說明膠体的構成及電泳的分離機制

- 3.2.4 結果不佳時 Trouble shooting

  舉出電泳操作時可能出現的問題

膠體的聚合反應 Gel polymerization

Ammonium persulfate (free radical initiator)

$(O_4S\text{-}SO_4)^{2-} \longrightarrow 2\ SO_4^-$

Free radical producer

Acrylamide (monomer)

Basic unit of the gel polymer

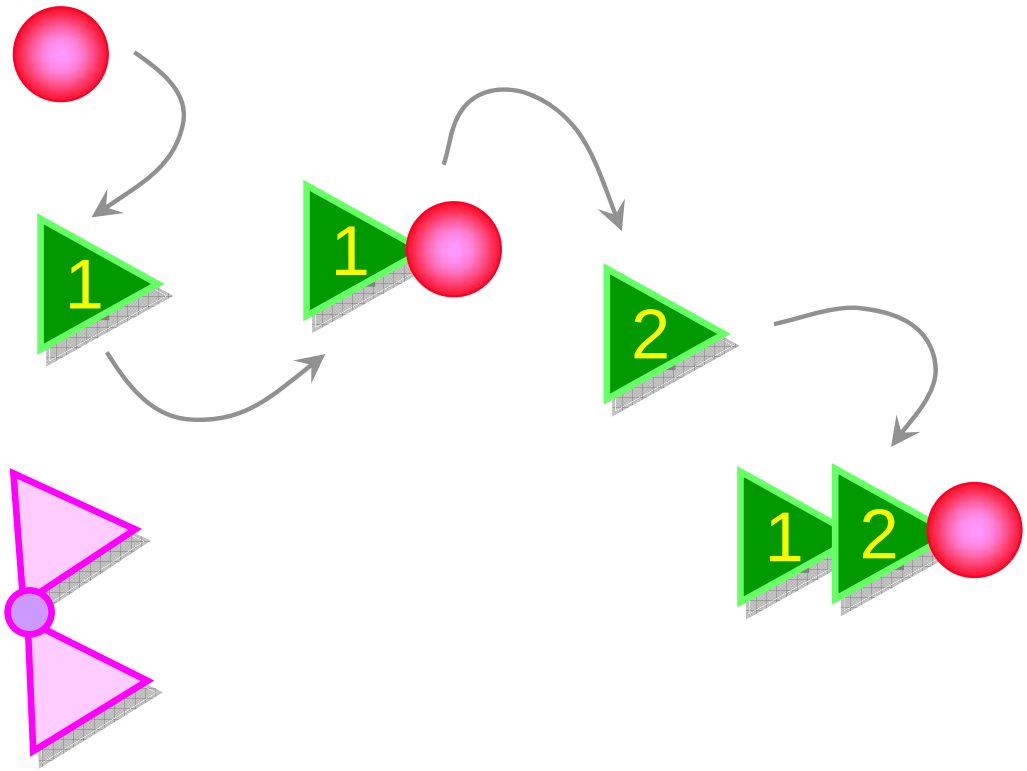
Bis(acrylamide) (bridge)

Cross-linking the gel

TEMED (catalyst)

Help the transfer of radical

SDS (Sodium dodecyl sulfate)



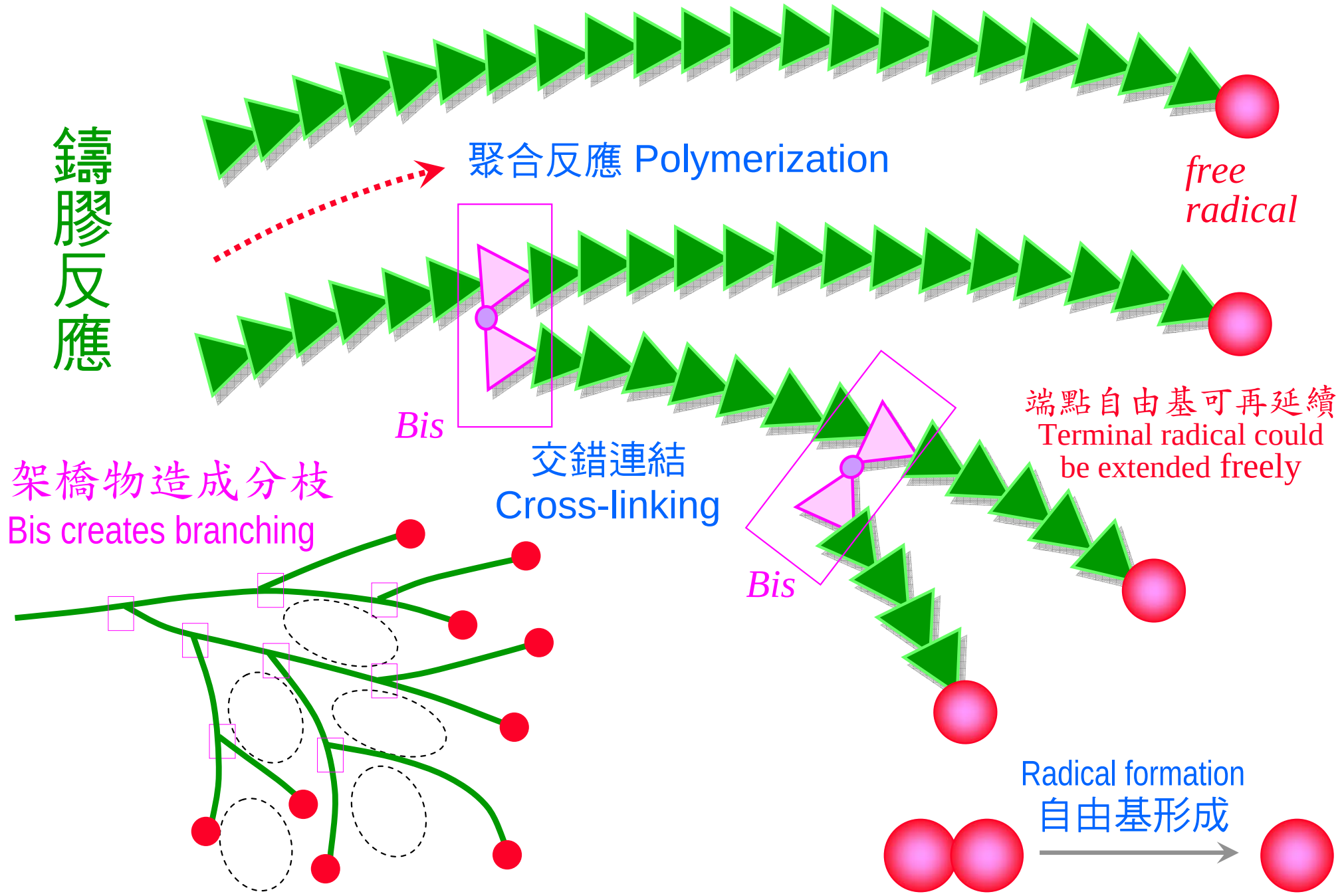
$CH_2=CH\text{-}CO\text{-}NH_2$

Acrylamide is toxic

Heating starch might produce it

Juang RH (2005) EPA

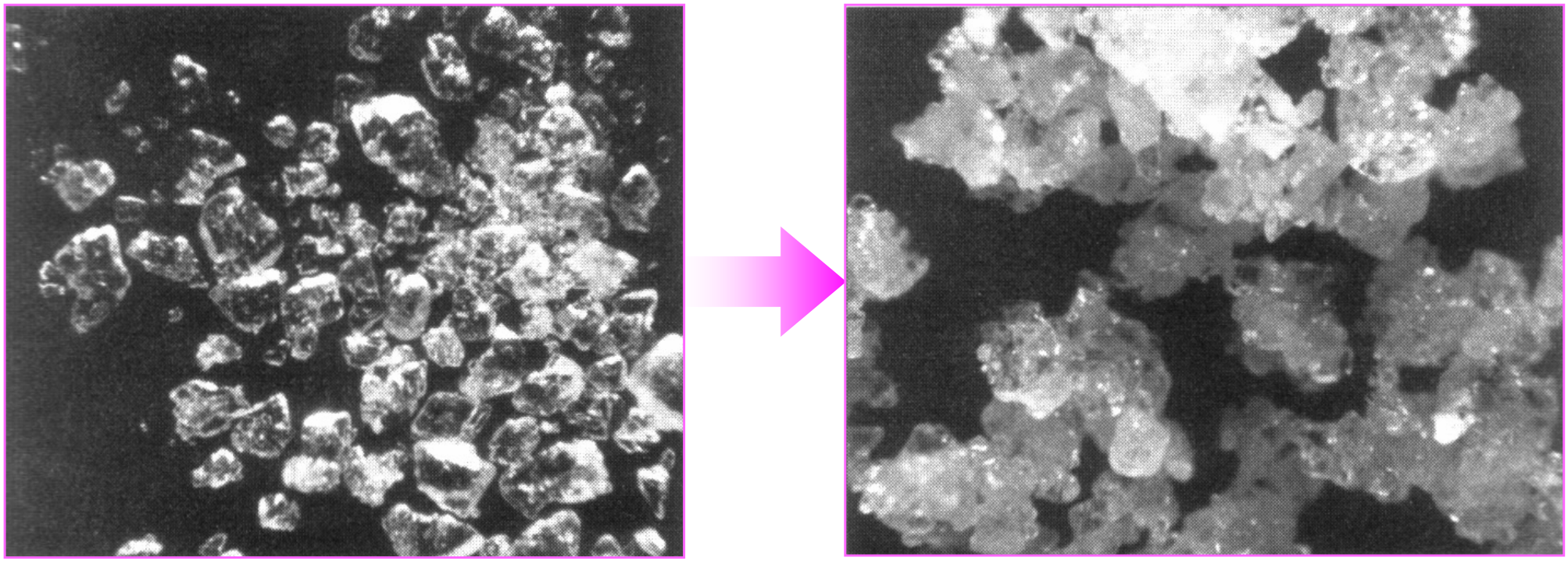
單體聚合反應 Polymerization from the monomer



Sample protein migrates in the space created by polymerization

Juang RH (2005) EPA

凝膠反應注意 A problem in gel polymerization

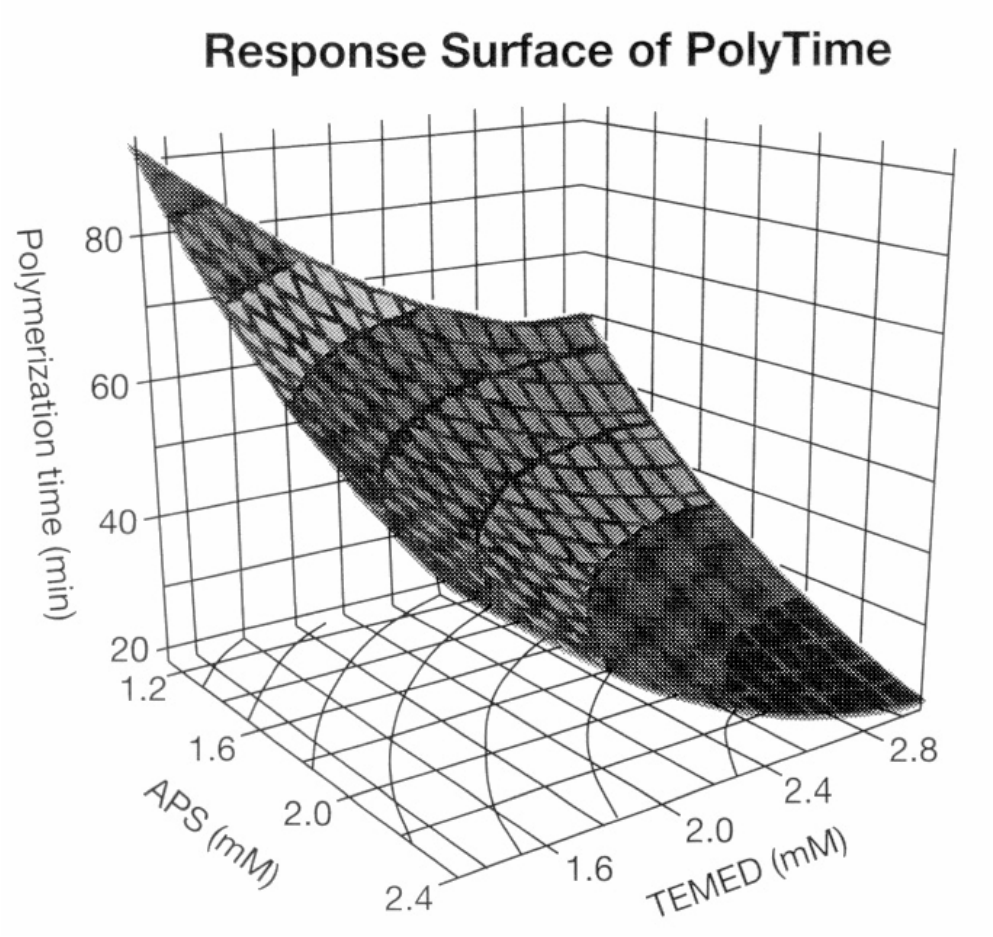


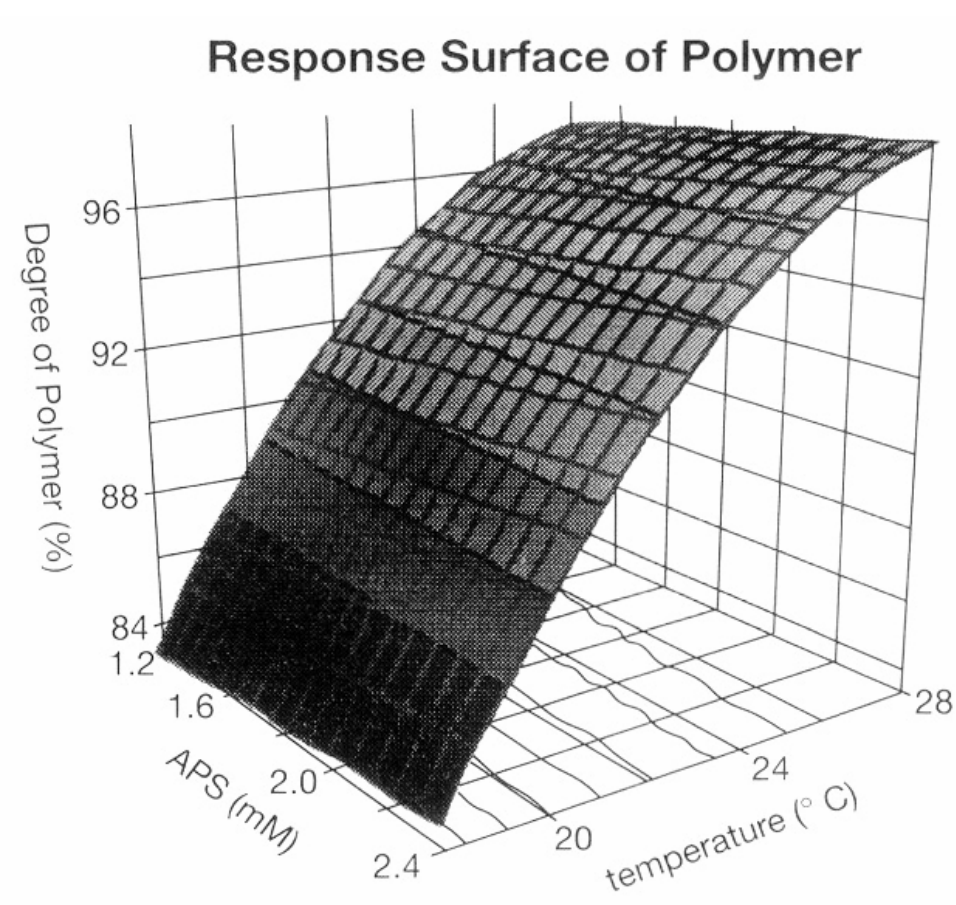
- APS 很容易因潮解而失去效用

APS is highly hygroscopic, and therefore loses its function

Pharmacia: Acrylamide Gel Casting Handbook p.11

凝膠反應條件 Examine polymerization conditions





- 凝膠時間與 APS 或 TEMED 量成反比
  Polymerization time is reduced when APS or TEMED increases

- 凝膠程度 (%) 與溫度成正比但與 APS 濃度無關
  Percentage of polymerization is proportional to the temperature, but not related to APS concentration

Pharmacia: Acrylamide Gel Casting Handbook p.12, 13

電泳膠體系統組成 Composition of PAGE system

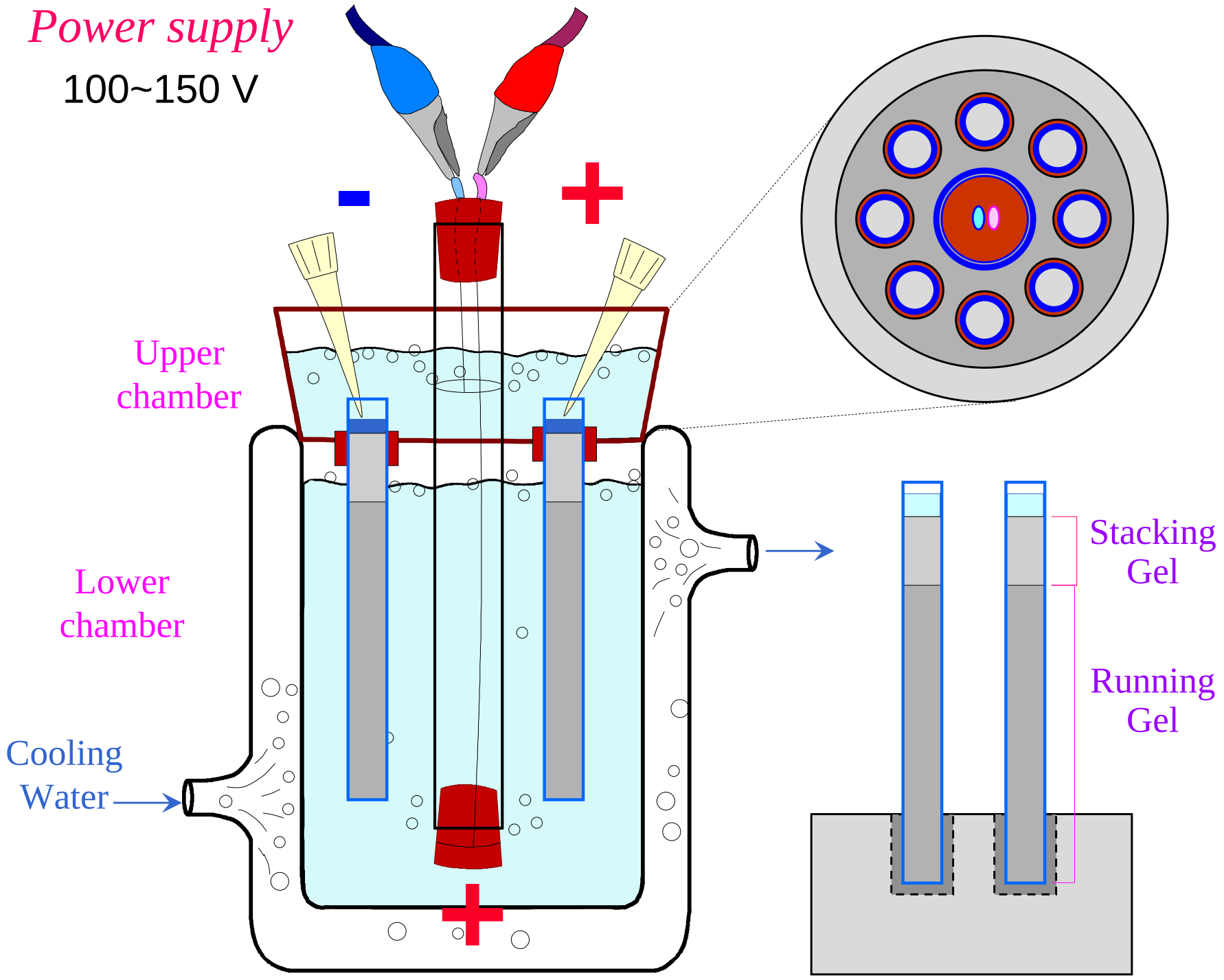
	PAGE system		Buffer	pH	Gel %
1	Cathode buffer		Tris-glycine	8.3	-
2	Sample		Tris-glycine	8.3	-
3	Gel	Stacking	Tris-HCl	6.9	5%
4		Running	Tris-HCl	8.3	7.5~20%
5	Anode buffer		Tris-glycine	8.3	-

- 膠體不連續性有焦聚樣本的作用

The gel discontinuity results in the stacking effect for sample molecules

Juang RH (2005) EPA

直立式電泳裝置



The vertical rod gel is the prototype of modern electrophoresis Juang RH (2005) EPA

焦集膠體主要角色 Key molecules in stacking gel

Glycine: Negative charged

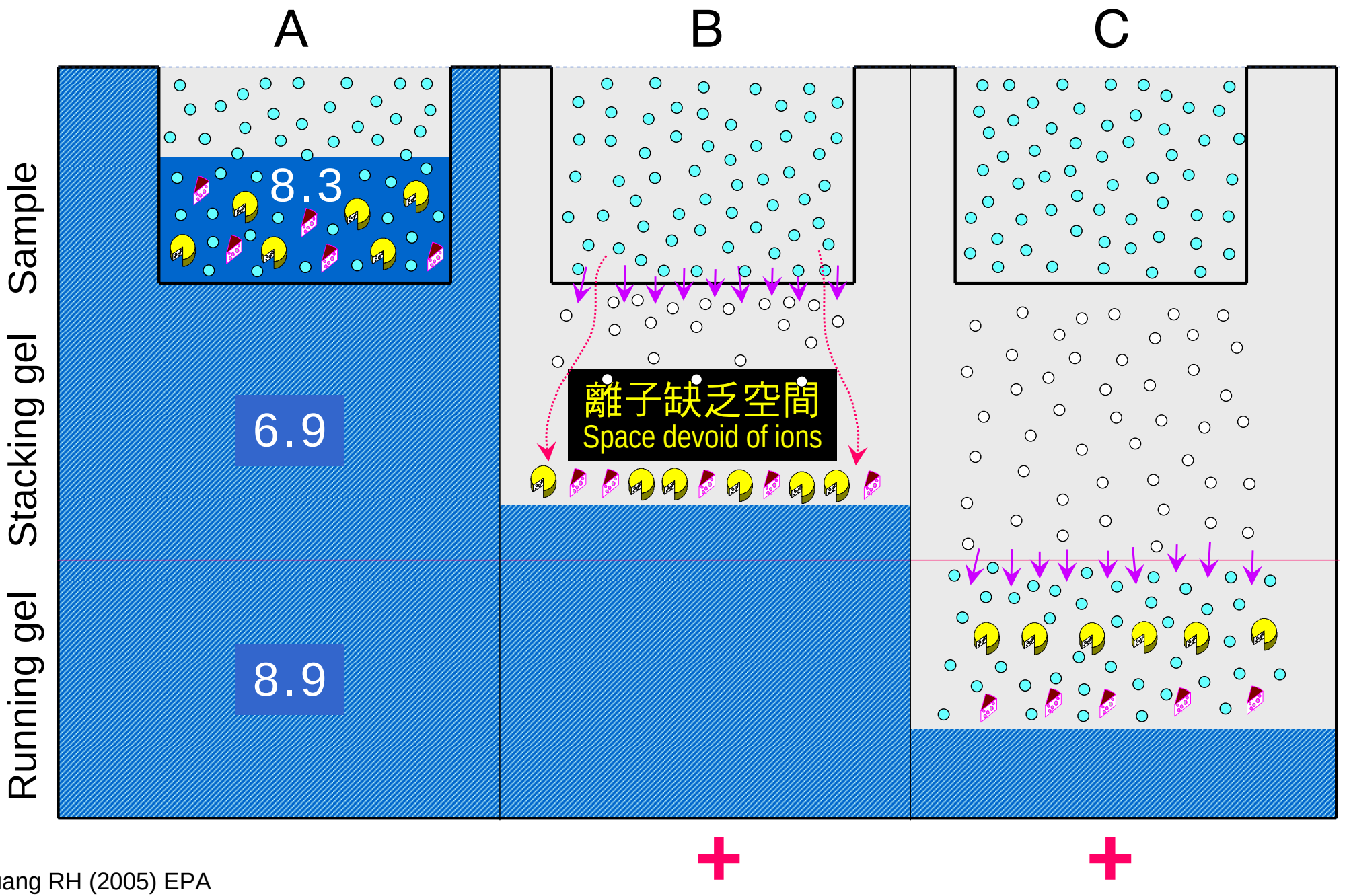
No net charge

Chloride ion:

Proteins: Small molecule

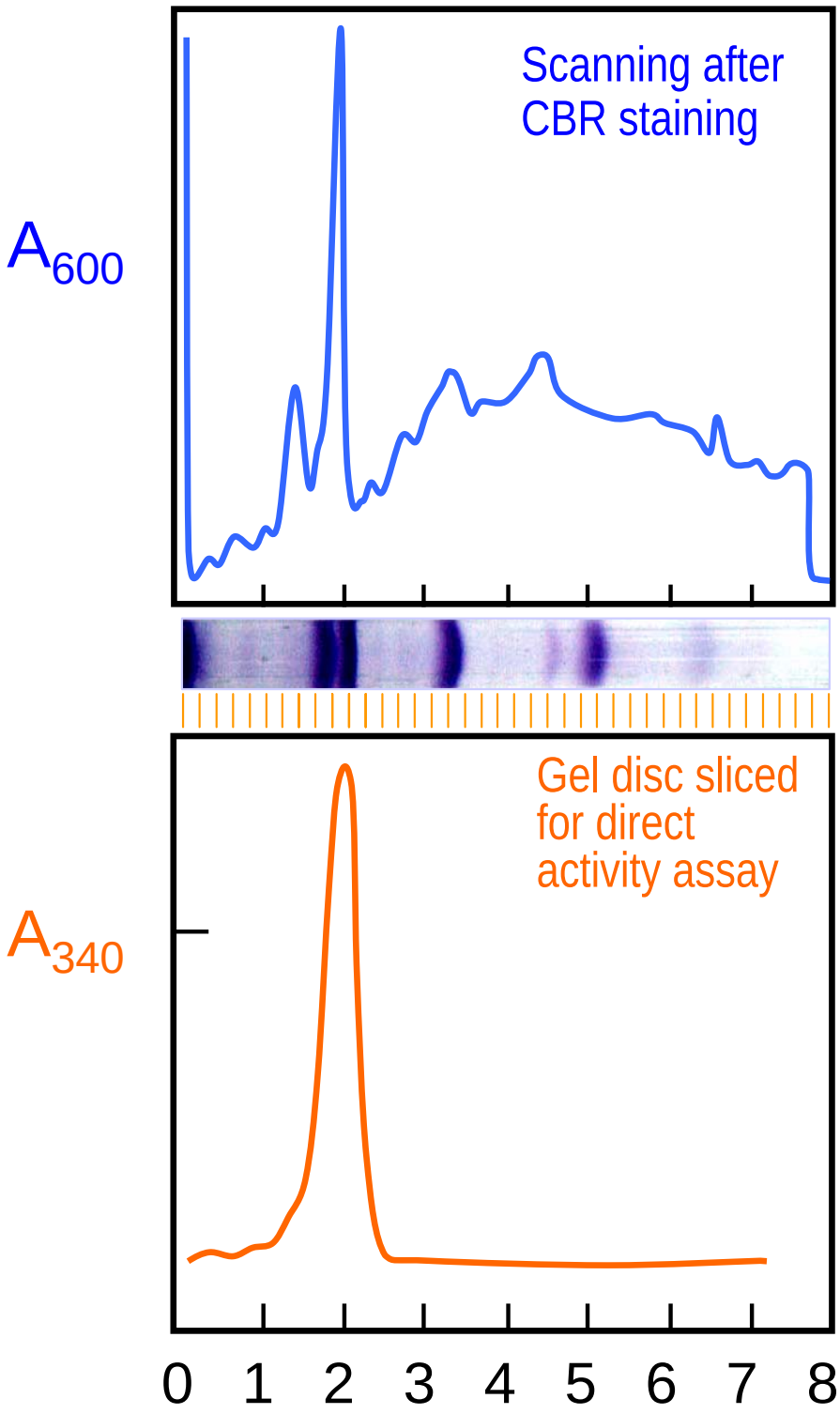
Large molecule

焦集膠體的作用原理 How stacking effect works



Juang RH (2005) EPA

電泳膠體中酵素活性測定

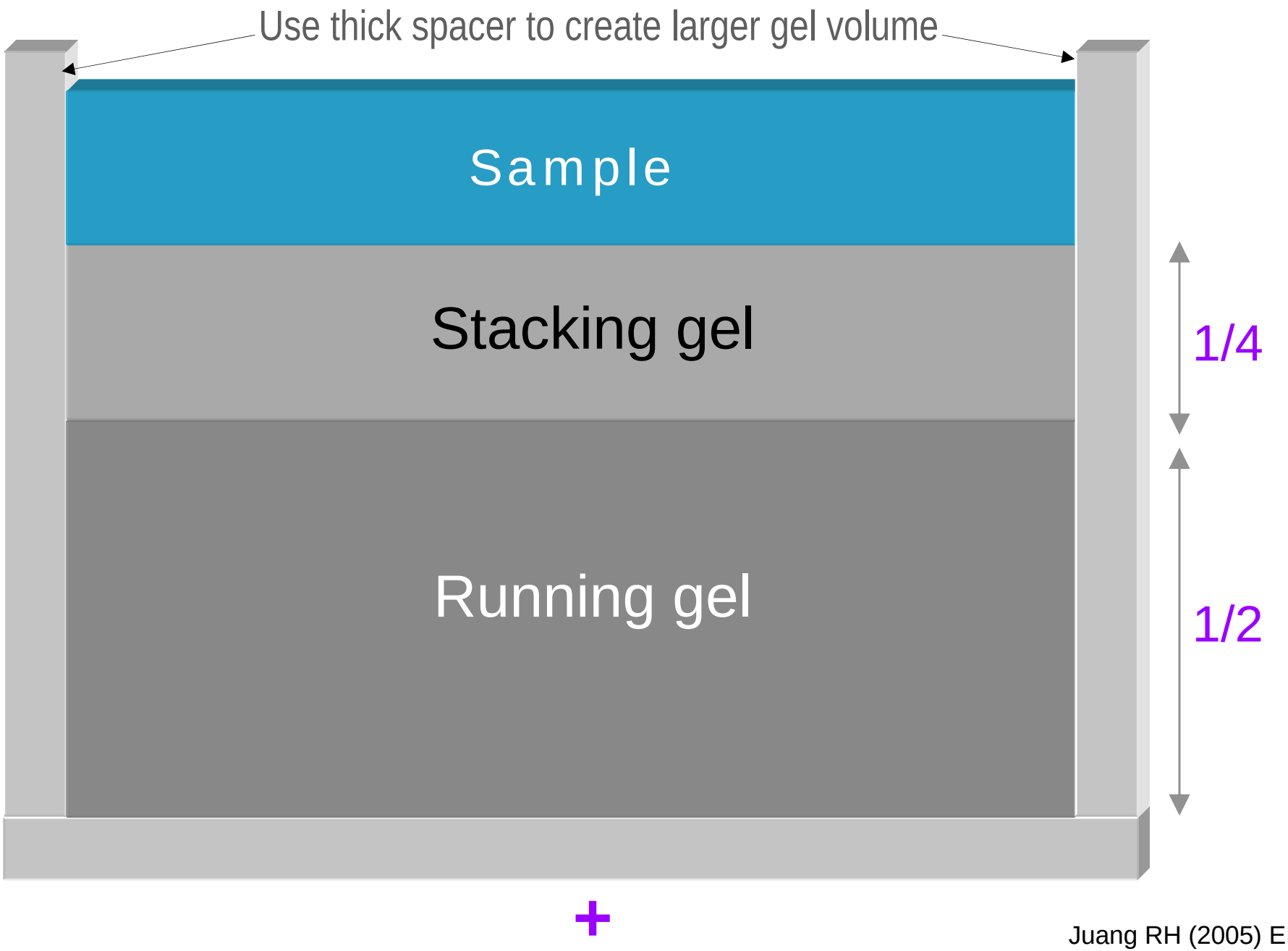


Native PAGE
Non-denaturing PAGE

SDS-PAGE

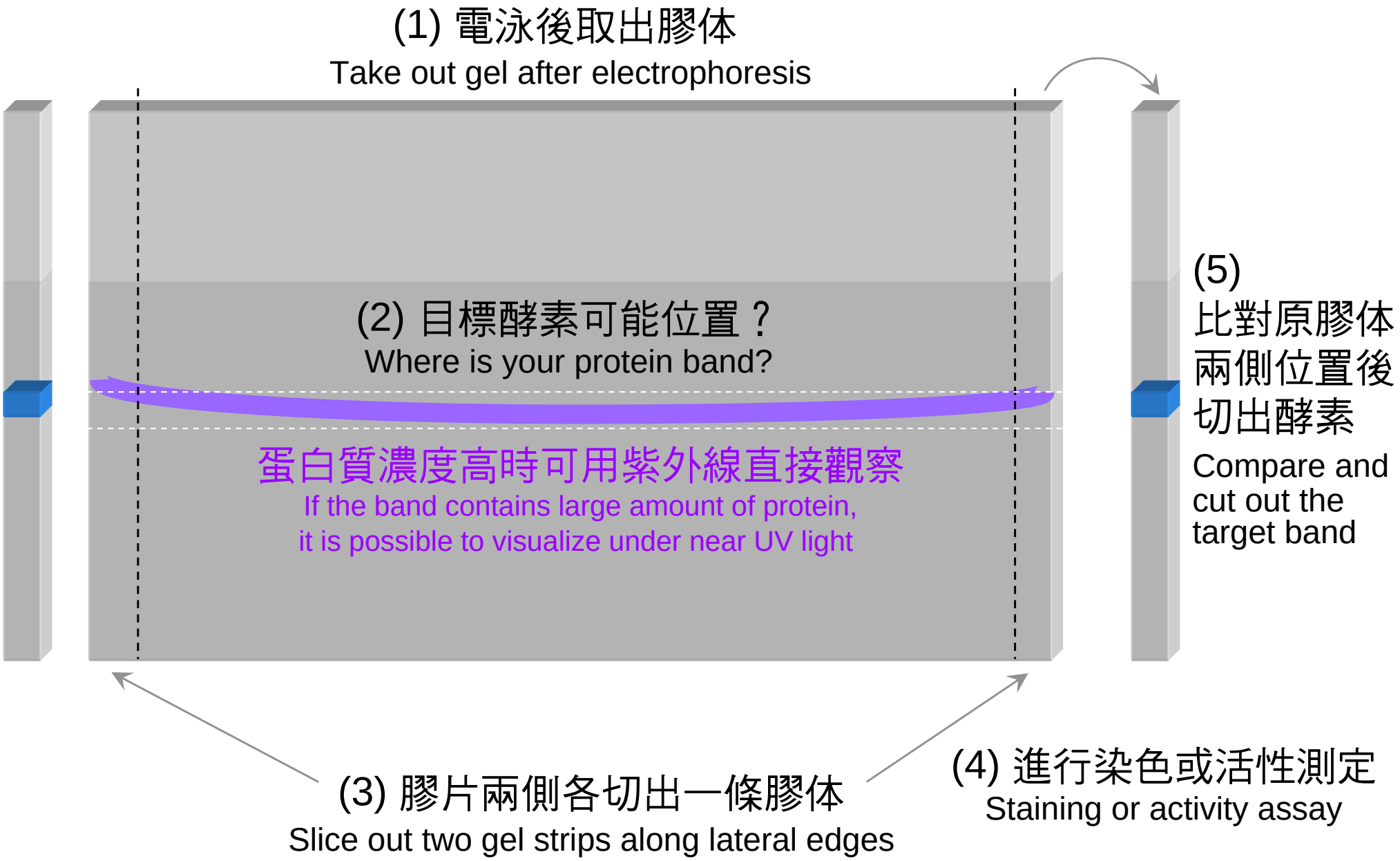
Juang RH (2005) EPA

製備式電泳膠片 Preparative gel



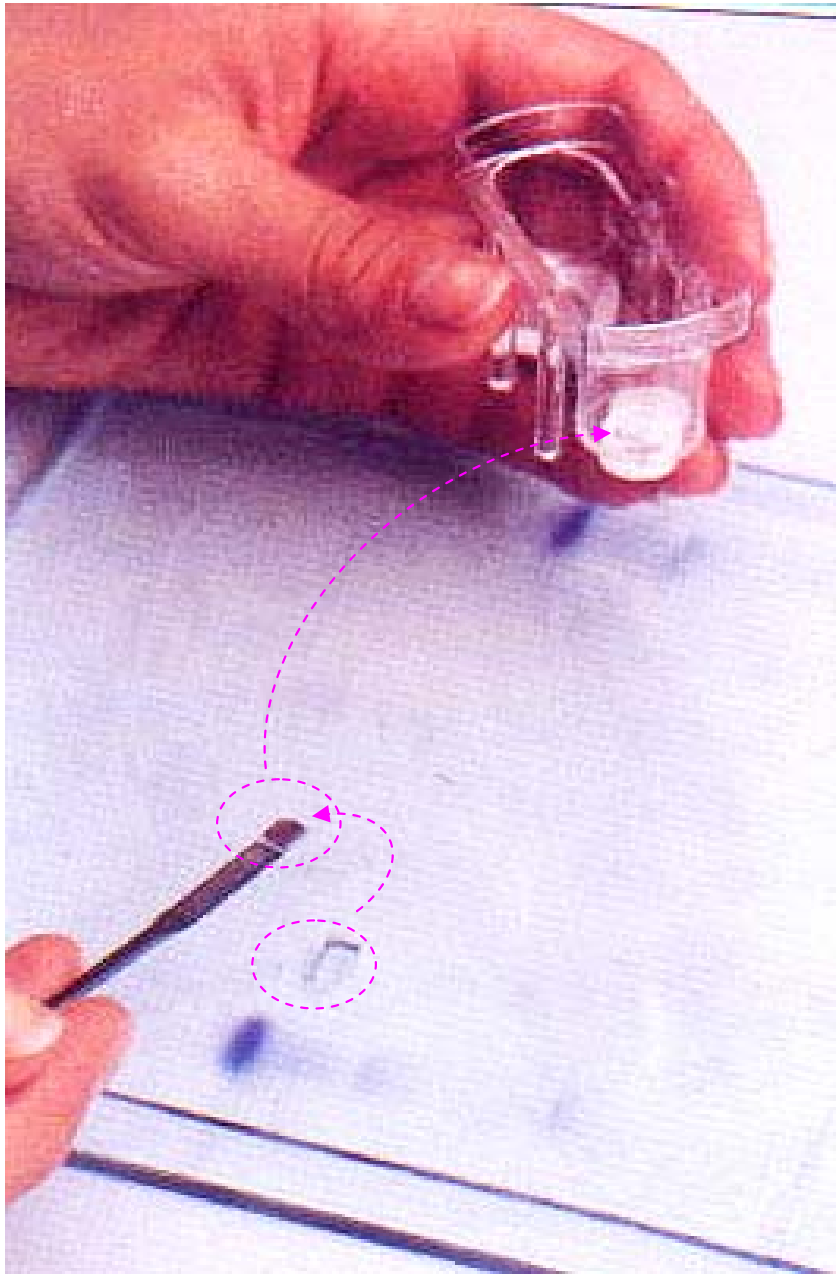
Juang RH (2005) EPA

製備式電泳操作 Detect protein band on the gel



Juang RH (2005) EPA

電泳膠體蛋白質溶離 Protein eluted from the gel

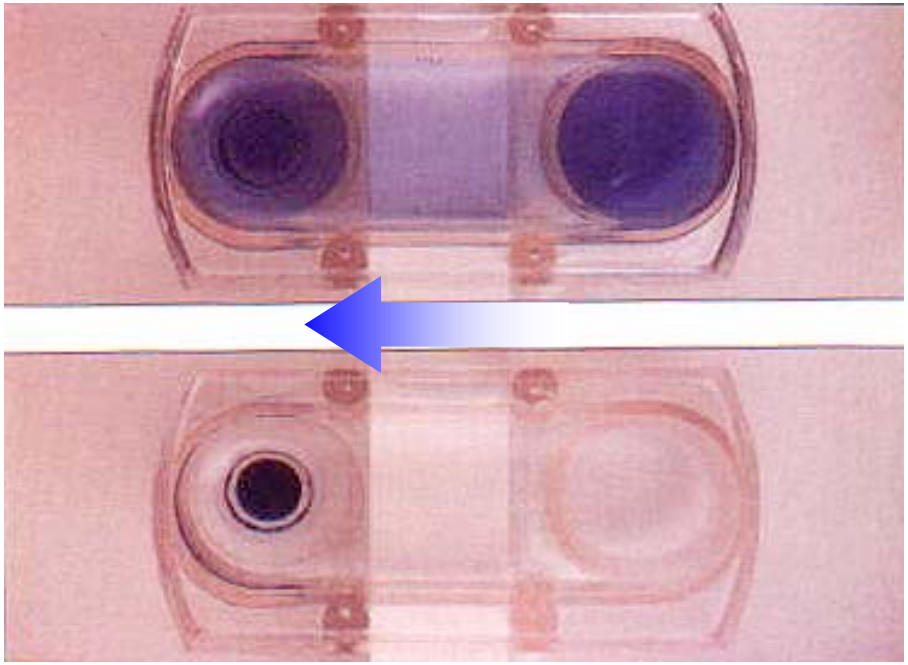


ISCO: Little Blue Tank Concentrator

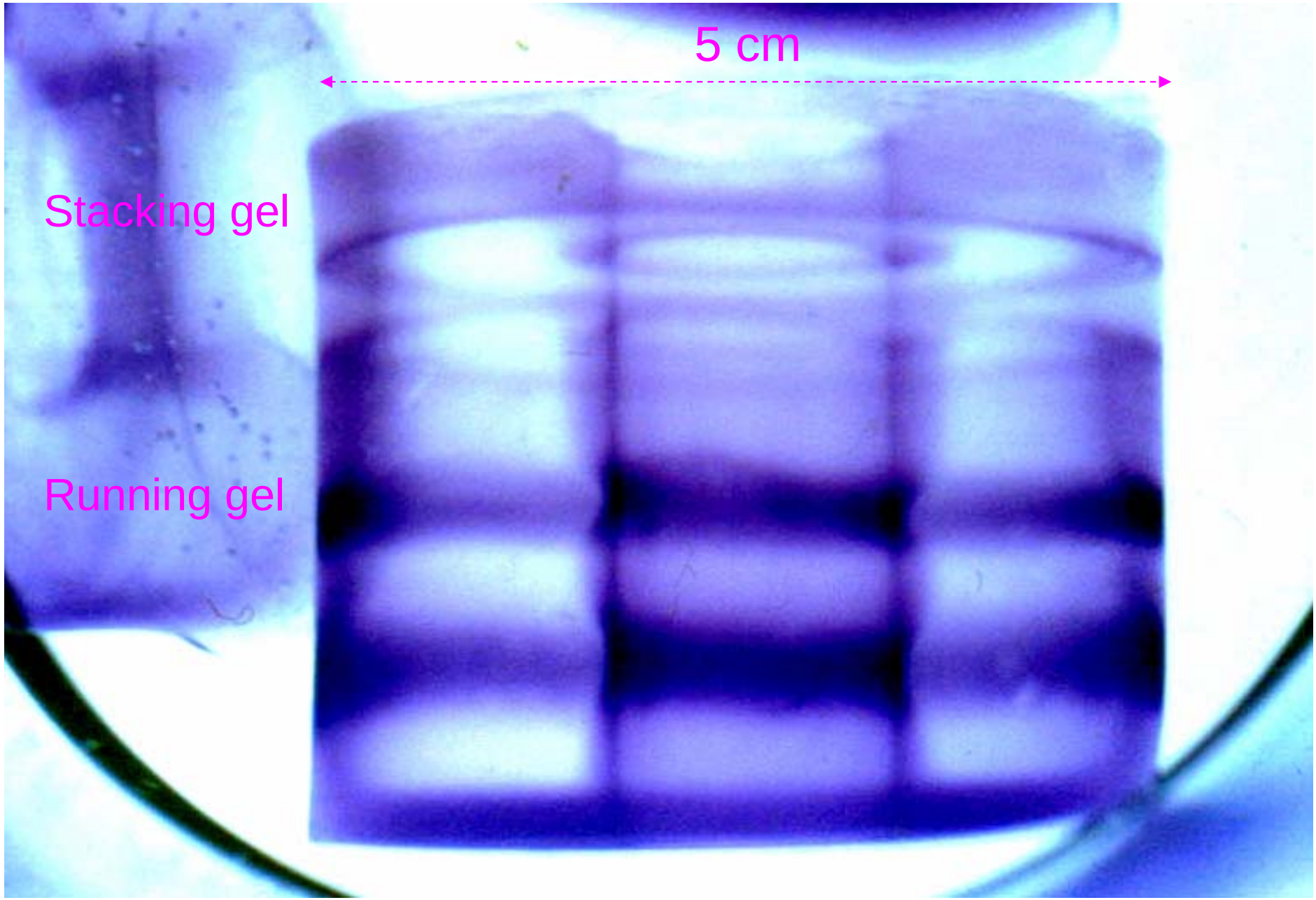
- 直接挖出膠體進行溶離

Cut out and eluted

Little Blue Tank



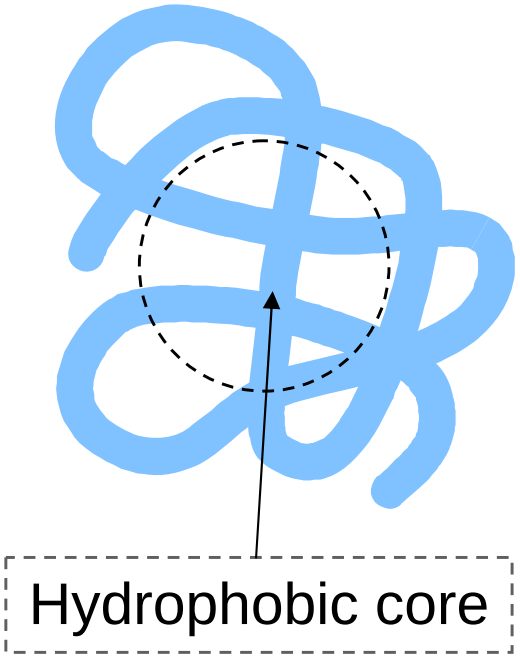
巨無霸的製備式電泳 Jumbo size preparative gel



Juang RH (2005) EPA

SDS 在蛋白質表面均勻敷上一層負電

Native protein

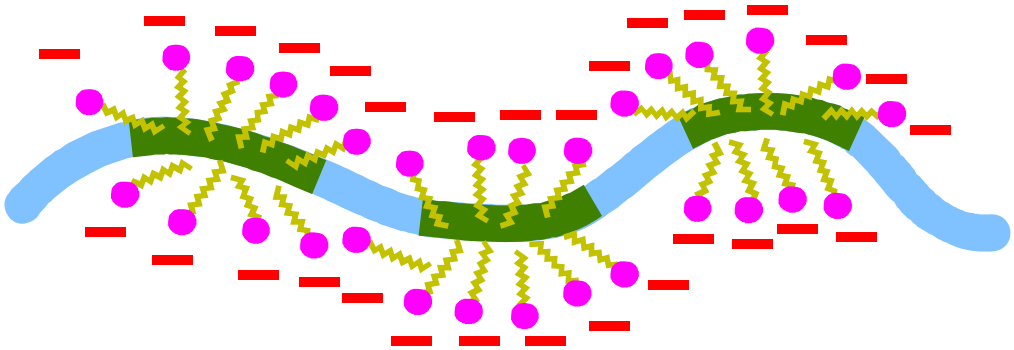


0.1%

SDS

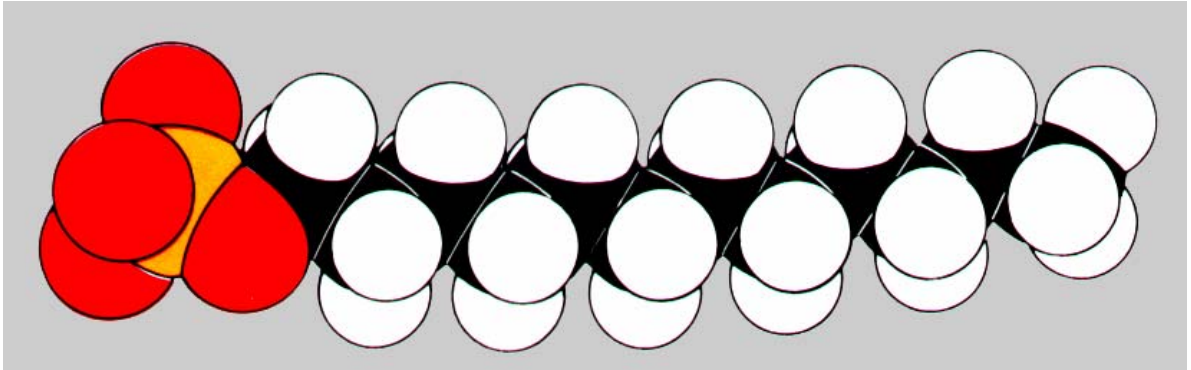
boiling

Protein is denatured to linear form



Its surface covered with negatively charged SDS uniformly

Polar head

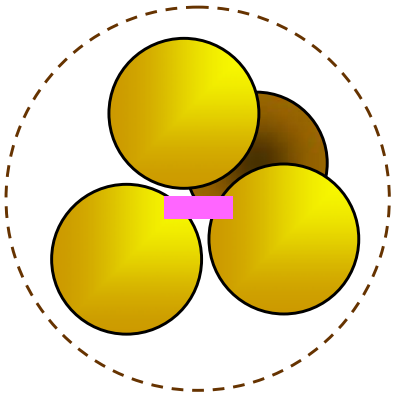


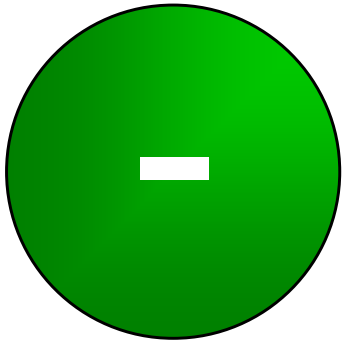
Non-polar tail

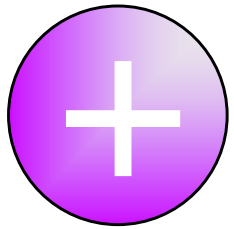
Juang RH (2005) EPA

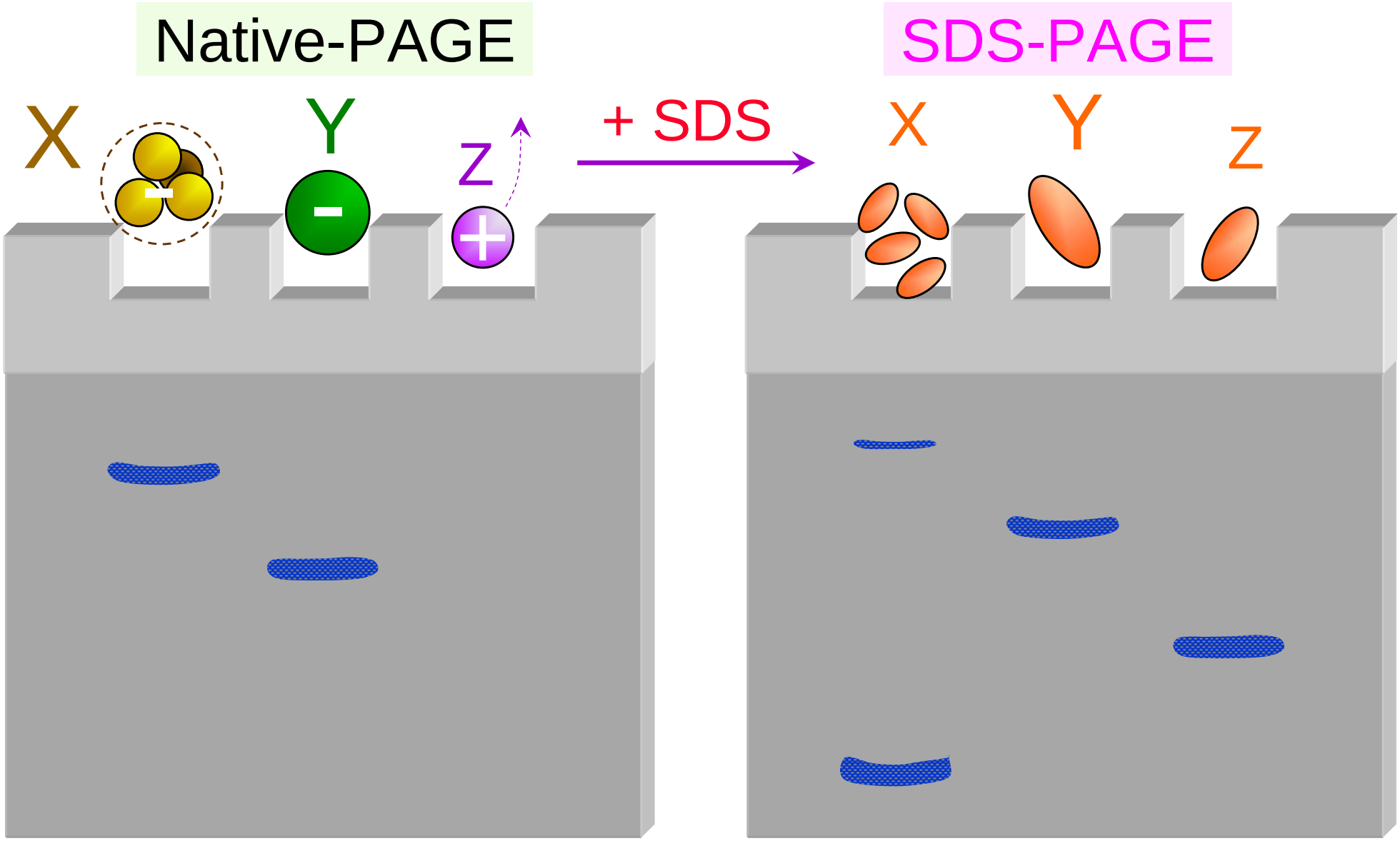
三種不同性質蛋白質的電泳比較 An example

Protein	Quaternary Structure	Molecular Weight	pI	Mobility	
				Native PAGE	SDS-PAGE
X	Tetramer	(40,000)x4	5.8	Slow	Fast
Y	Monomer	88,000	5.2	Fast	Slow
Z	Monomer	60,000	9.3	Upward	Medium

X 

Y 

Z 



+

分子量 及 淨電荷密度

均影響泳動率

molecular weight and net charge density

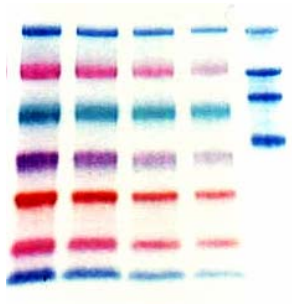
+

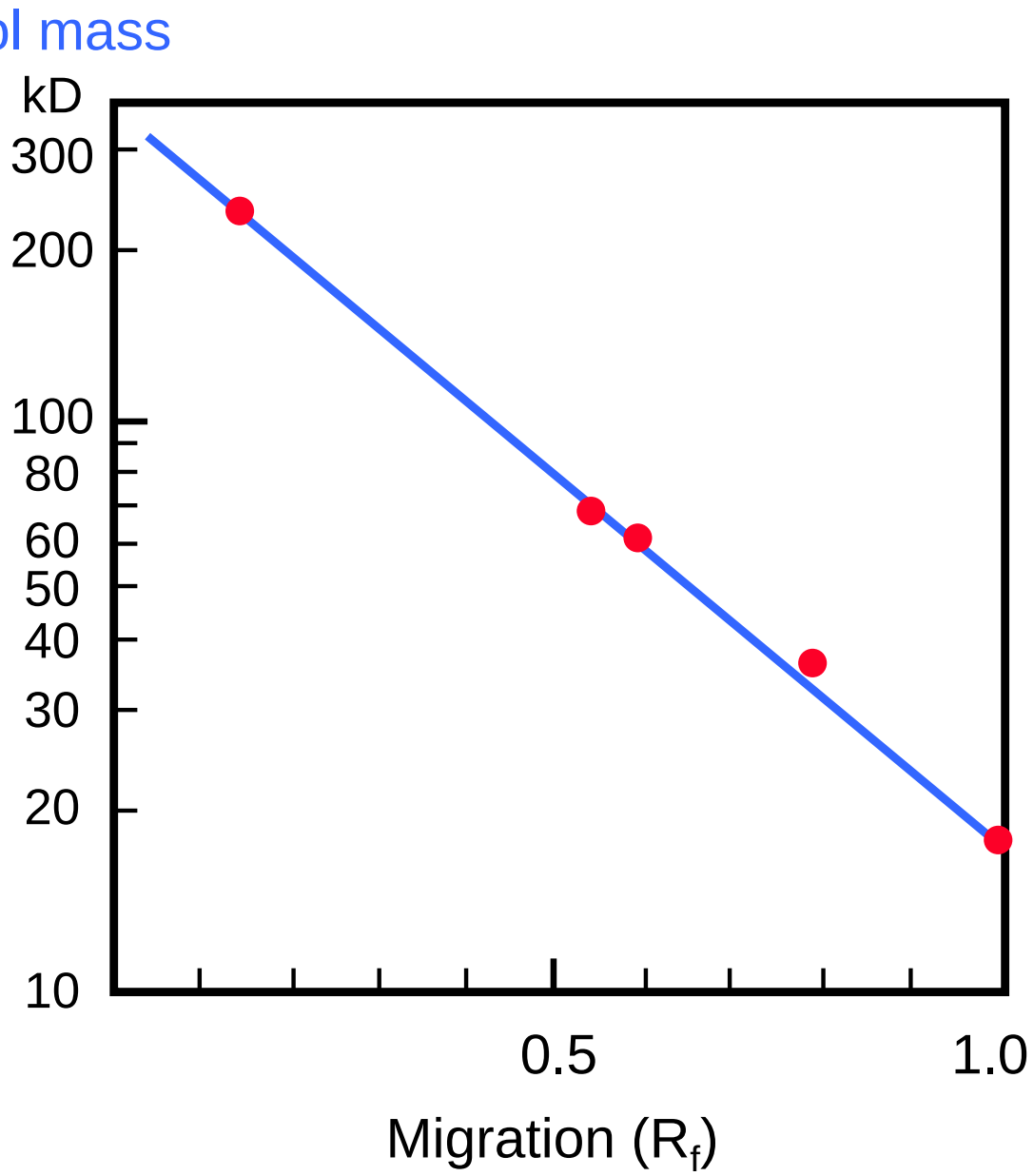
只有 分子量 影響泳動率

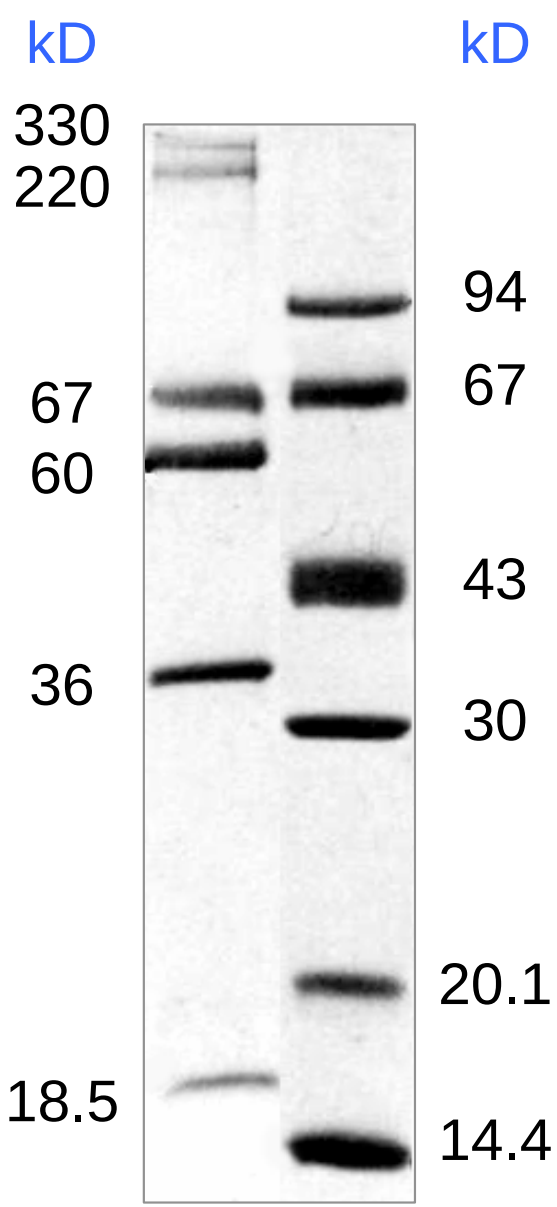
only molecular weight

Juang RH (2005) EPA

測定單元體分子量 SDS-PAGE for subunit MW

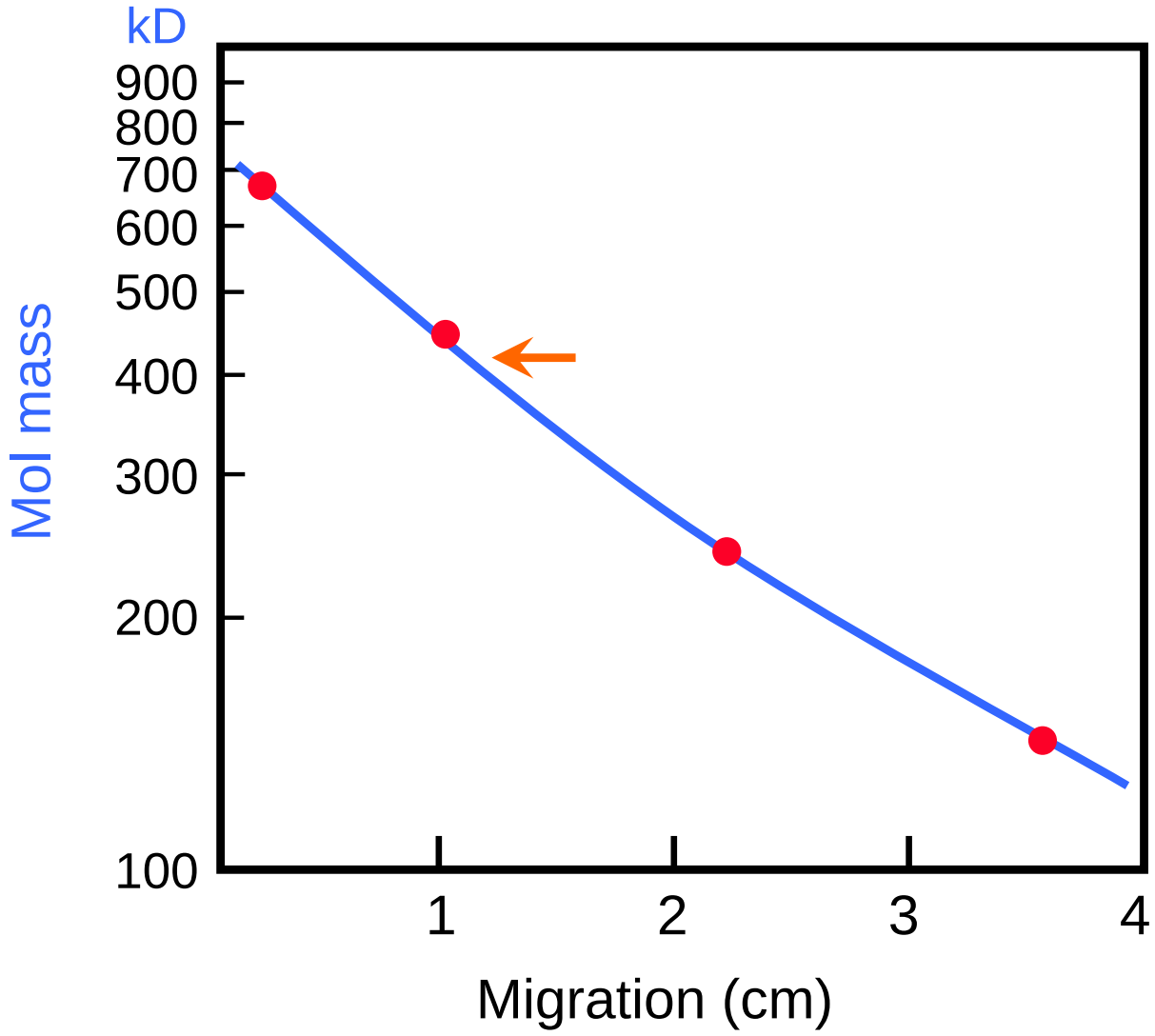


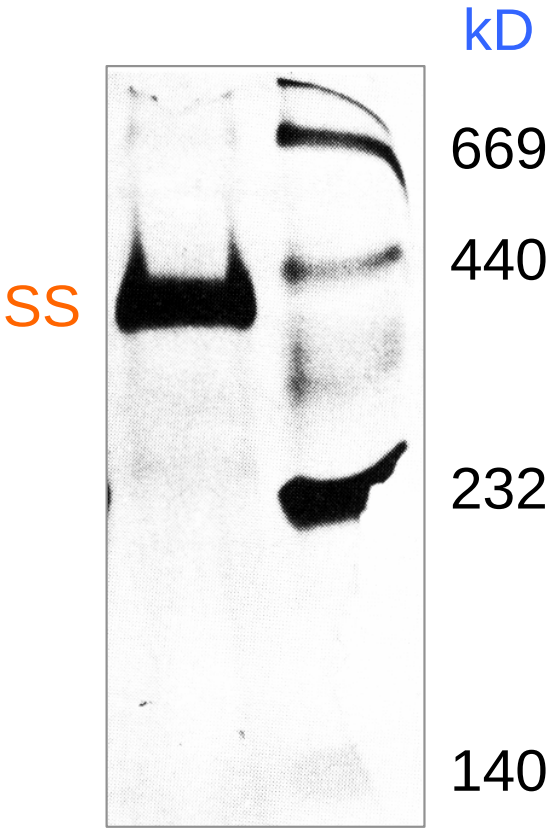




Pharmacia: Molecular Markers for electrophoresis

原態分子量測定 Disc-PAGE for native MW





- 不能以 disc-PAGE 為唯一實驗依據

Don’t take disc-PAGE MW determination as your only evidence

Juang RH (2005) EPA

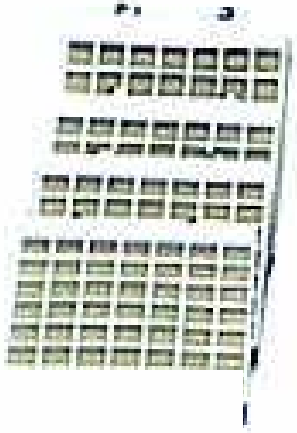
電泳系統的選擇 Choose your systems

電源供應器 Power Supply	● 100 - 250 V	● 100 - 500 mA	
電泳槽 Electrophoretic Unit	Vertical vs Horizontal	Rod vs Slab	Regular vs Mini gel

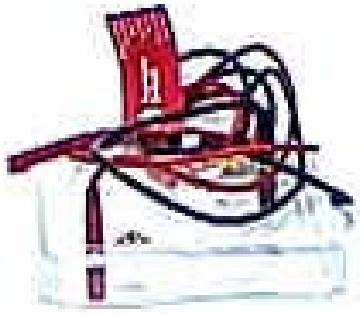
電泳系統選擇 System Choice	■ Gel casting	■ Materials	■ Samples
Vertical	Rod of slab	Acrylamide	Protein
Horizontal	Slab	Agarose	NA Isozyme
Vertical	Vertical slab	Mixed type	DNA sequencing

電泳槽及相關設備 Instruments and equipments

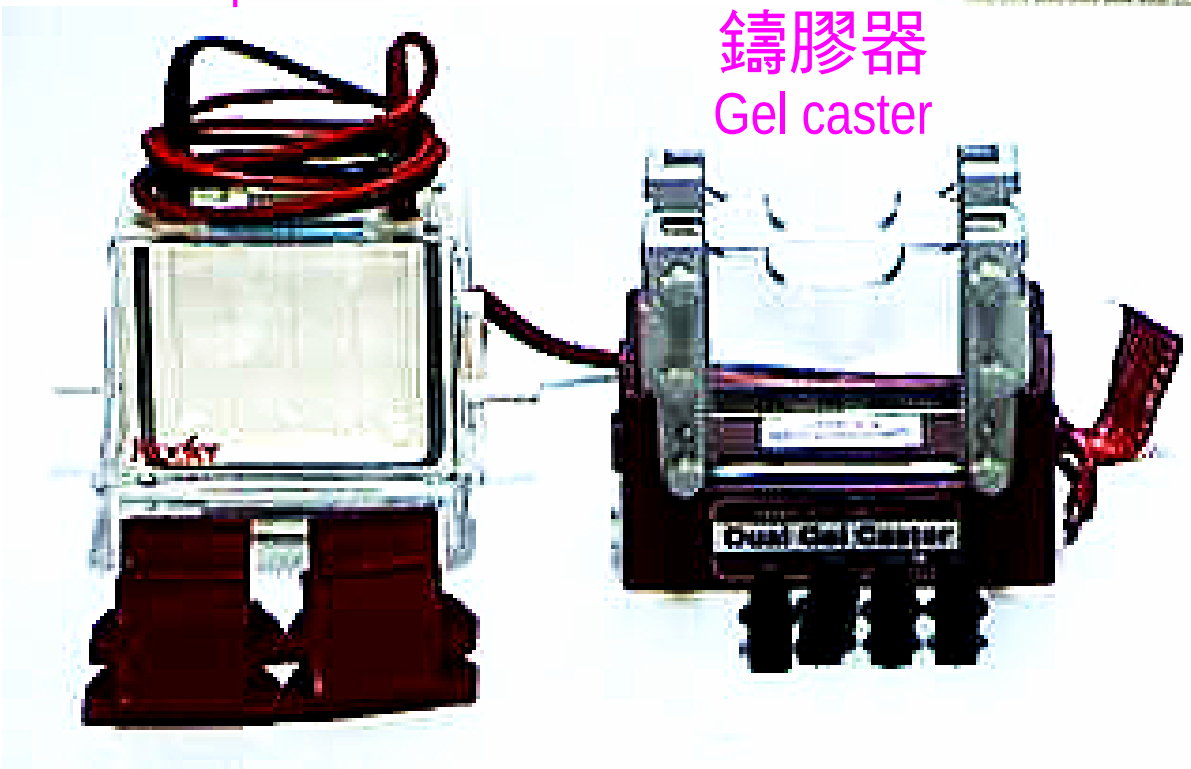
轉印三明治
Transfer sandwich



轉印槽
Gel transfer



電泳槽
Electrophoresis unit



鑄膠器
Gel caster

供電器 Power supply

Juang RH (2005) EPA