4 其它純化或分離方法 Other purification methods

- 4.1 製備式電泳 Preparative electrophoresis

  蛋白質色帶由原態電泳中直接切除出來

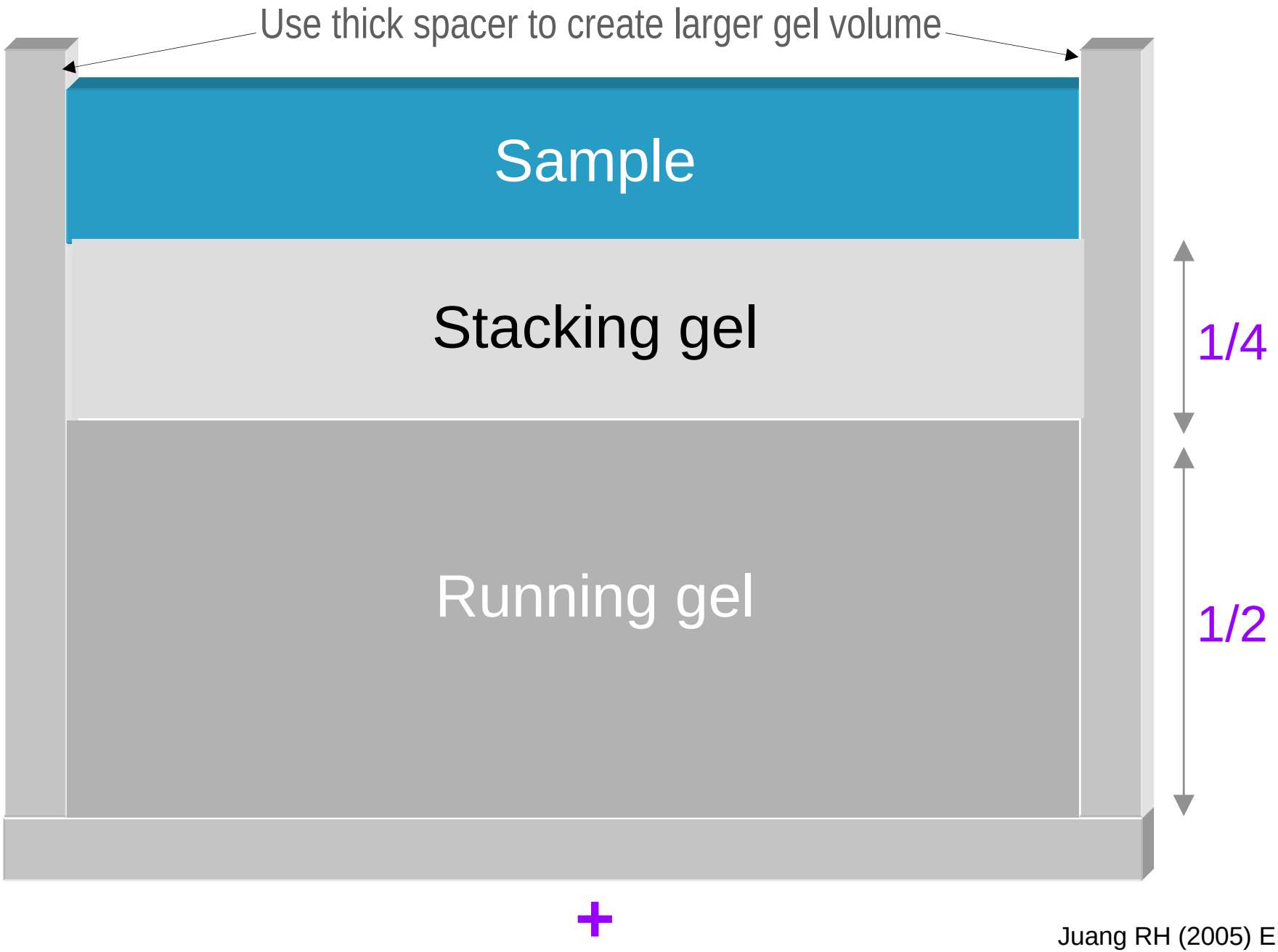
- 4.2 超高速離心法 Ultracentrifugation

  各種分子的沉降係數不同來進行分離

- 4.3 超微薄膜過濾法 Ultrafiltration

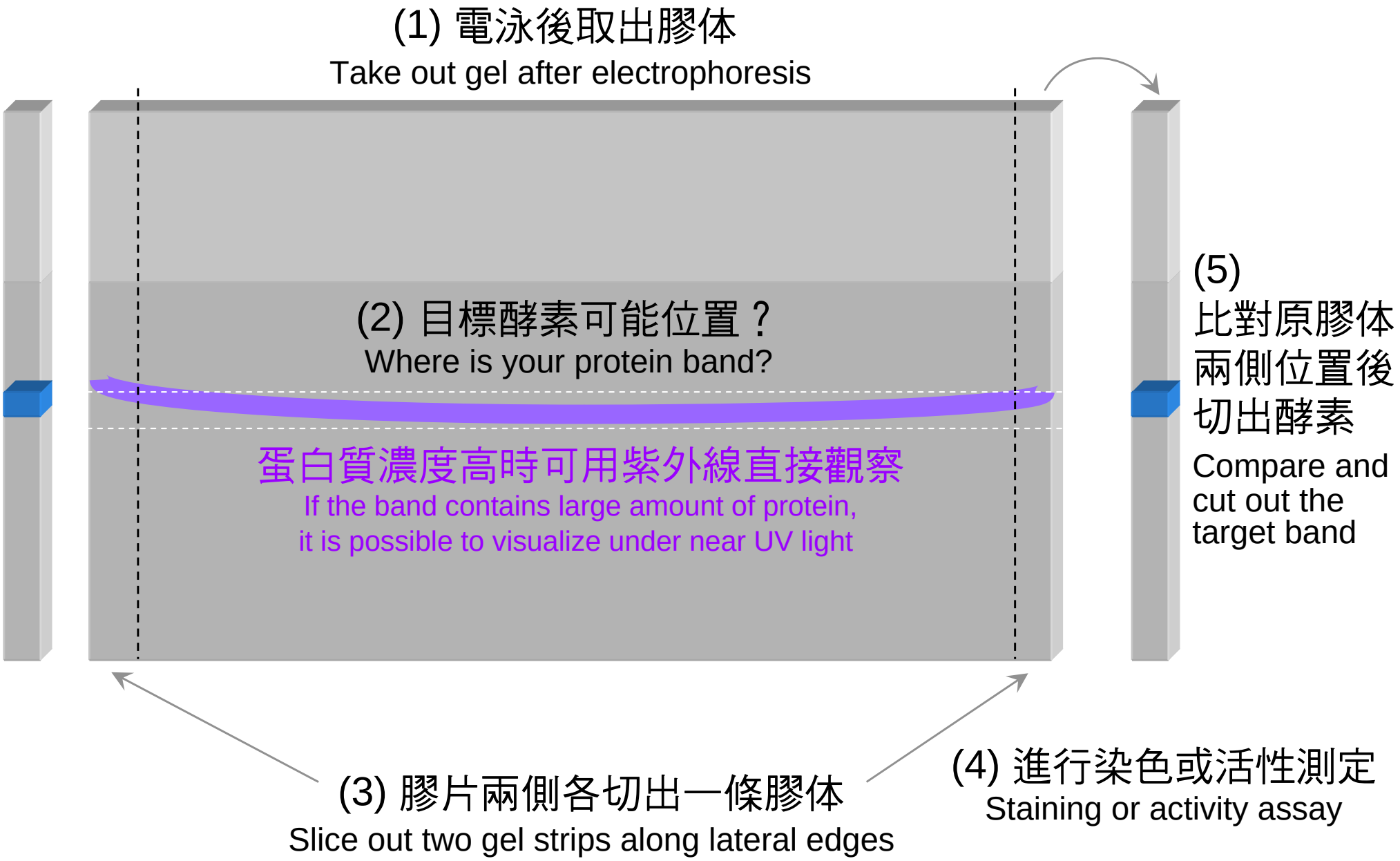
  超微薄膜可以用來脫鹽及濃縮蛋白質

製備式電泳膠片 Preparative gel format



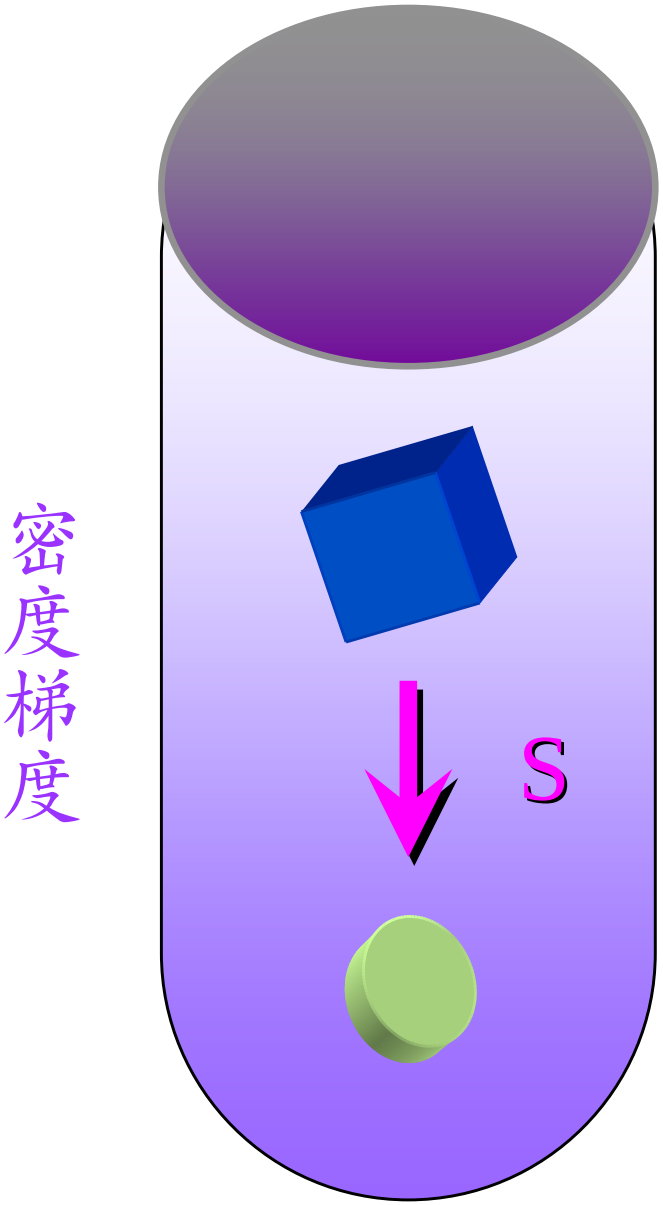
Juang RH (2005) EPA

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



Juang RH (2005) EPA

超高速離心 Sedimentation coefficient



Svedberg unit

粒子在密度梯度離心時的沈降速率

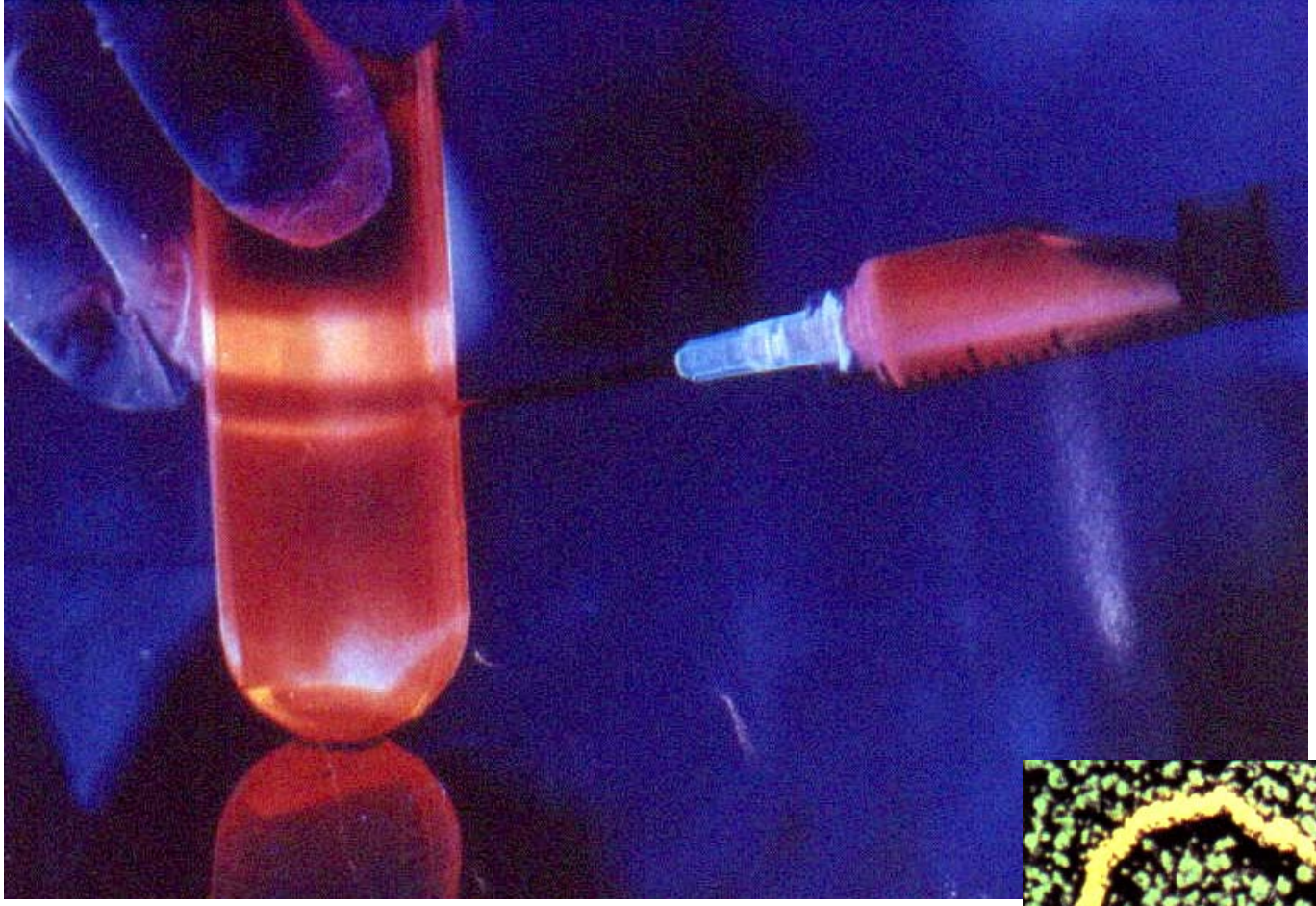
The sedimentation velocity of a particle when it is centrifuged in a density gradient

分 子 量 molecular weight

分子密度 molecular density

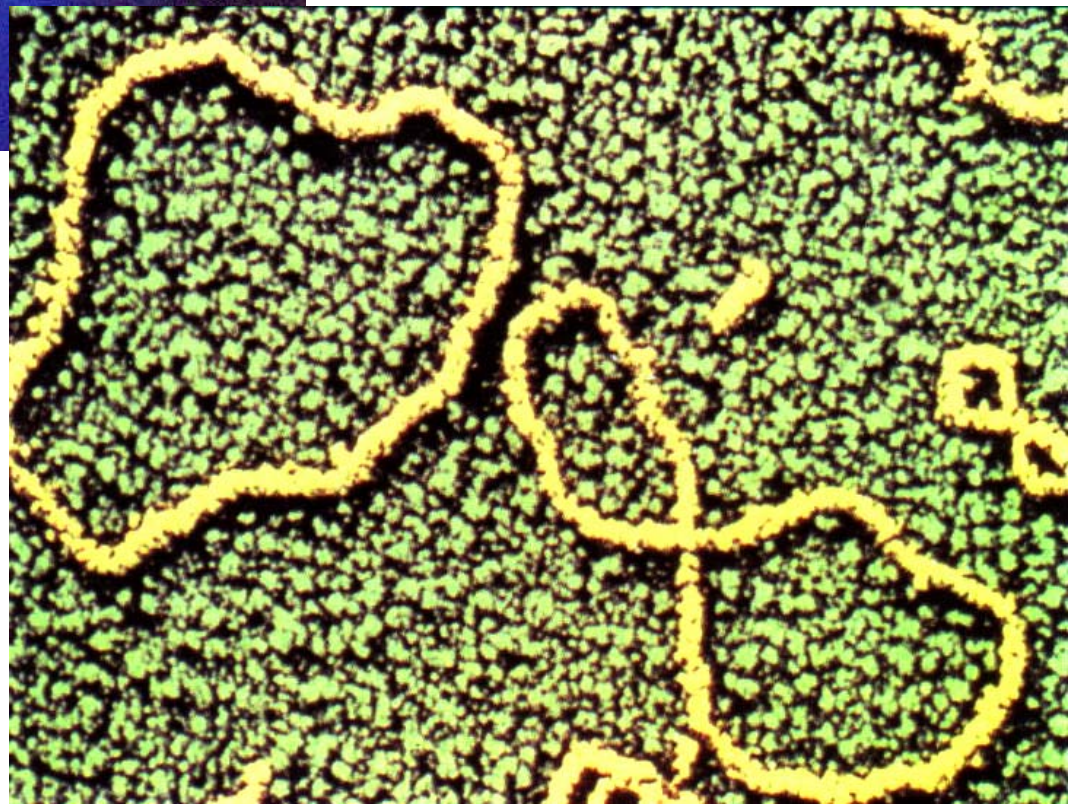
分子組成 molecular composition

分子形狀 molecular shape



以超高速離心大量製備質體

Ultracentrifugation is used to prepare plasmid in large scale



兩種超高速離心比較 Two ultracentrifuge types

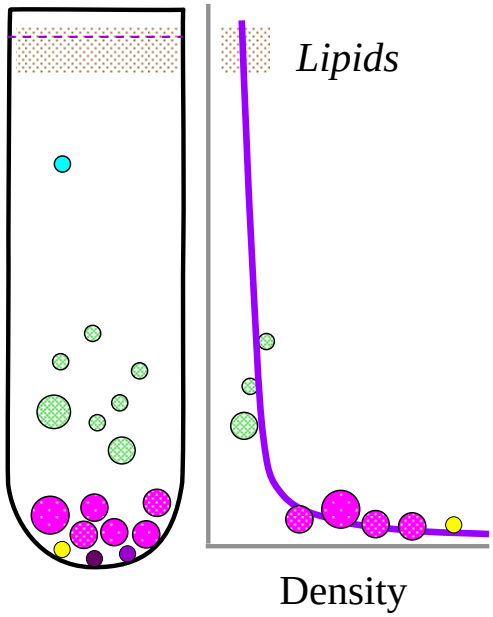
Centrifuge	Sedimentation Velocity	Sedimentation Equilibrium
also called →	Zone Centrifugation	Isopycnic Equilibration
Gradient formation	Precast (sucrose, glycerol)	During centrifugation (CsCl)
	Shallow gradient, lower density	Steep gradient, higher density
Suitable samples	Similar density, different MW	Similar MW, different density
	Protein	Nucleic acid / cell organelle
Centrifugation conditions	Lower speed, not complete sedimented, stop at proper time	Completely sediment to where the density is equilibrated, high speed, long running time
	區帶離心法	等密度平衡離心法

Juang RH (2005) EPA

各種高速離心法比較 Comparison of centrifuges

High speed | Ultracentrifugation

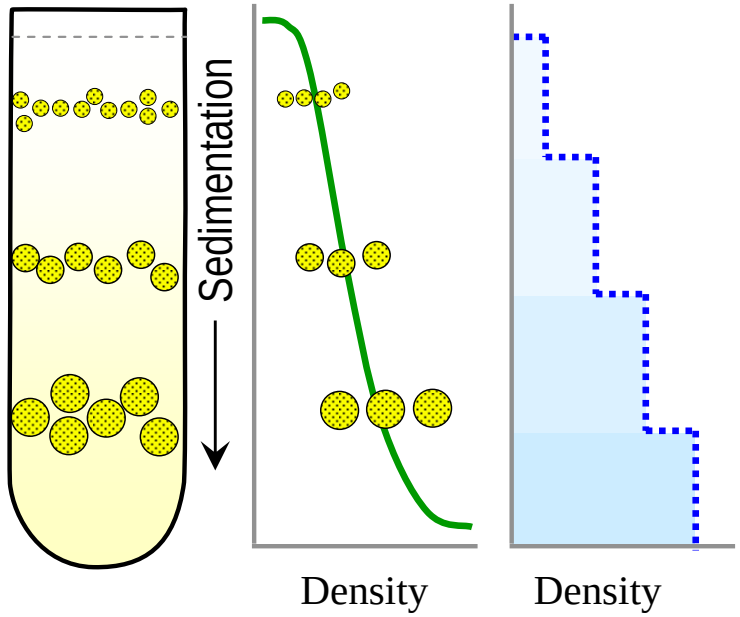
Gravity Centrifugation
(No density)



一般的重力離心僅把顆粒與溶液分離開來

Utilize gravity force to separate particles from the solution

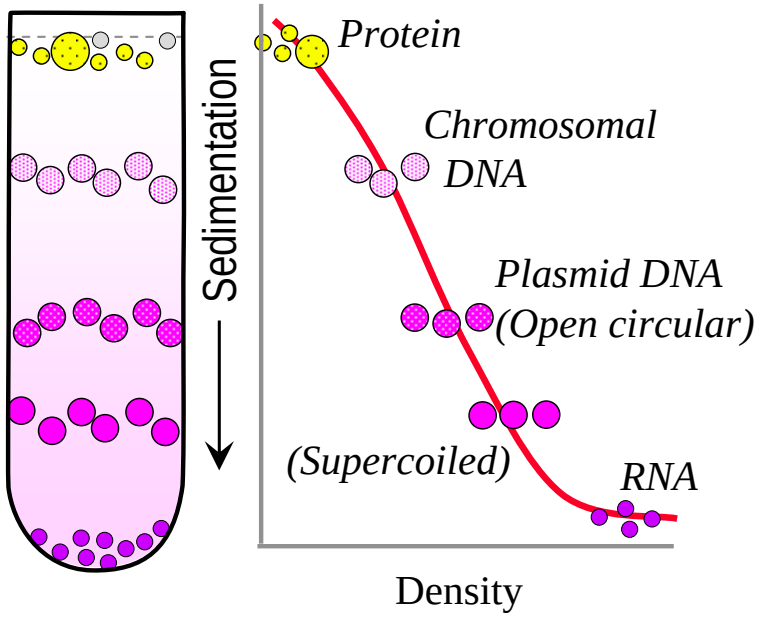
Zone Centrifugation
(Precast) → (step-wise)



樣本：多為蛋白質
密度相似、分子量不同者

Sample: protein (similar density, but different in MW)

Isopycnic Equilibration
(CsCl gradient forming)



樣本：多為核酸
密度不同、分子量相似者

Sample: nucleic acid (similar MW, but different in density)

Juang RH (2005) EPA

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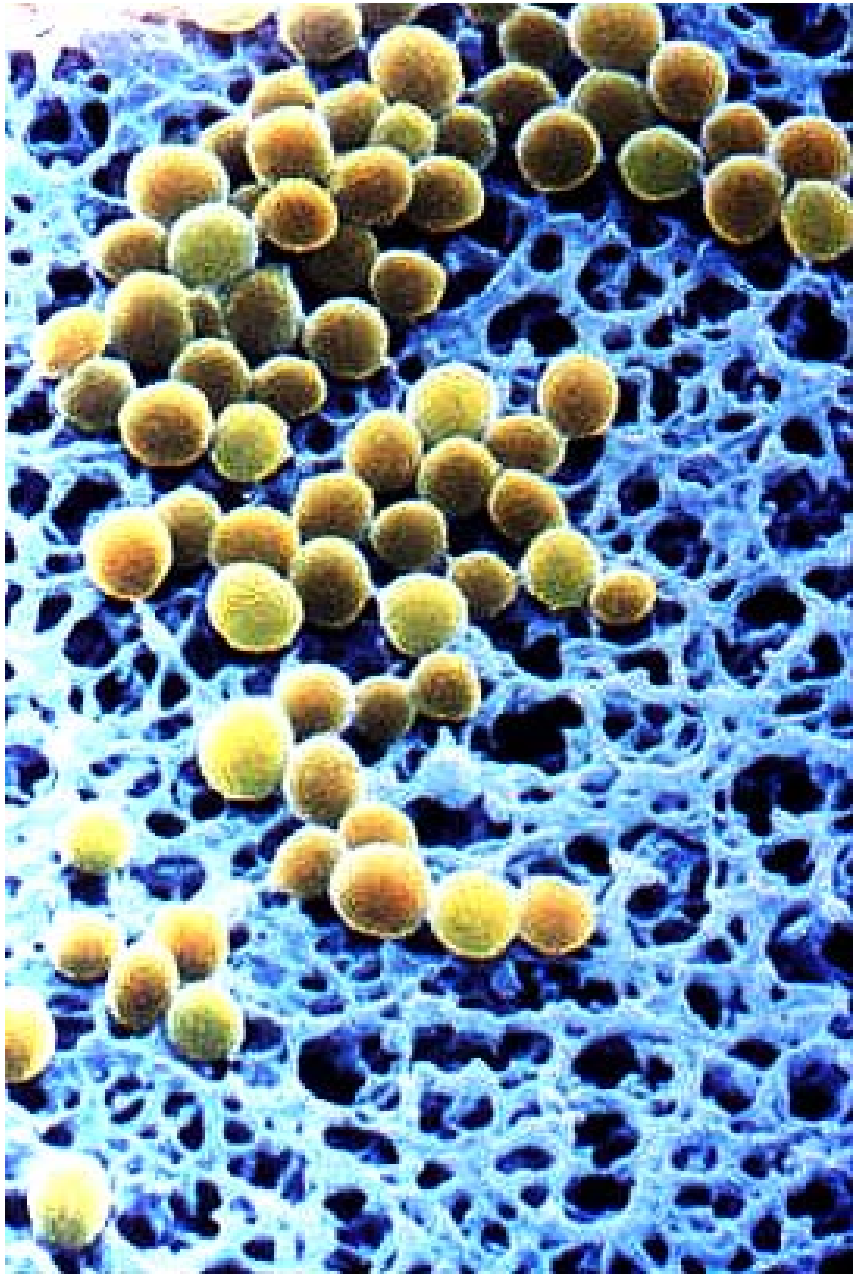
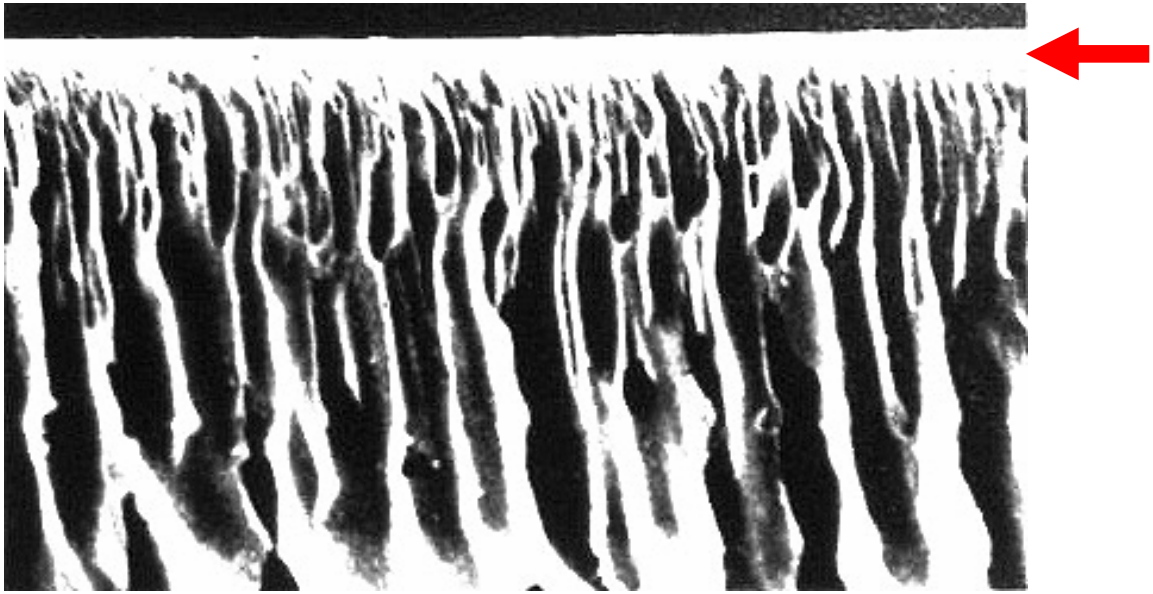
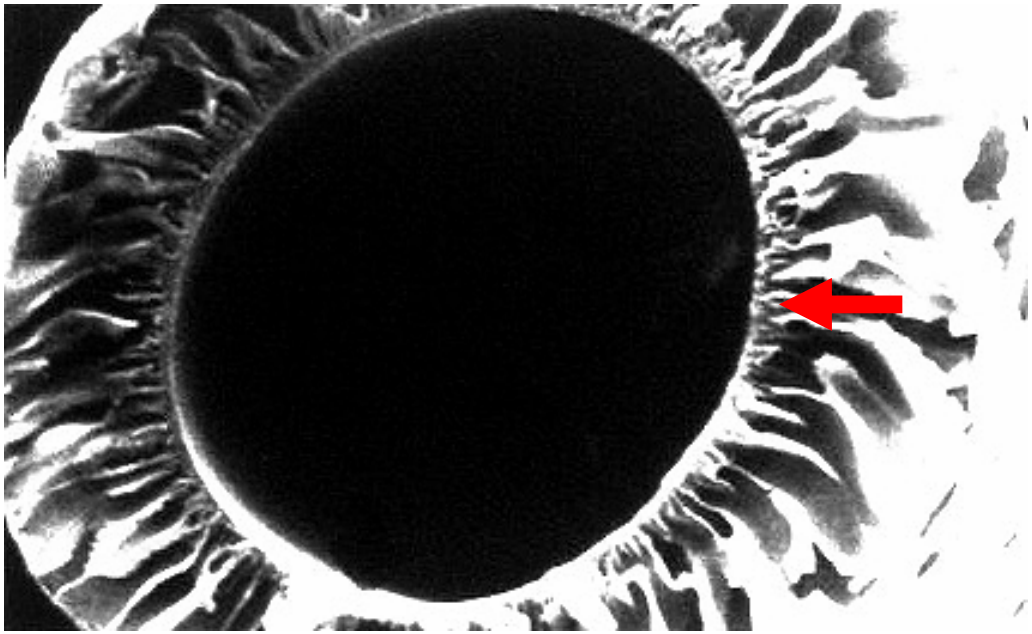
超微薄膜技術 Ultrafiltration technology

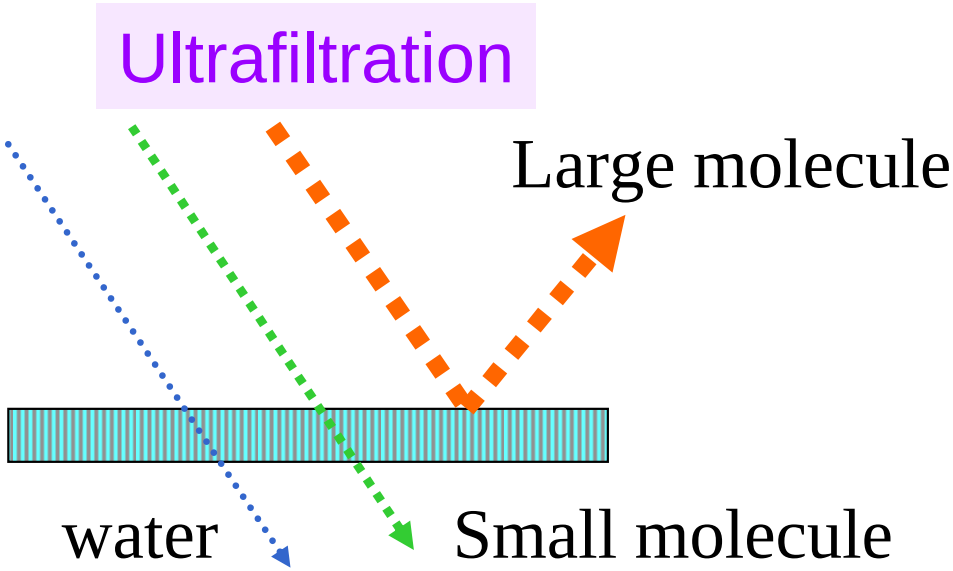


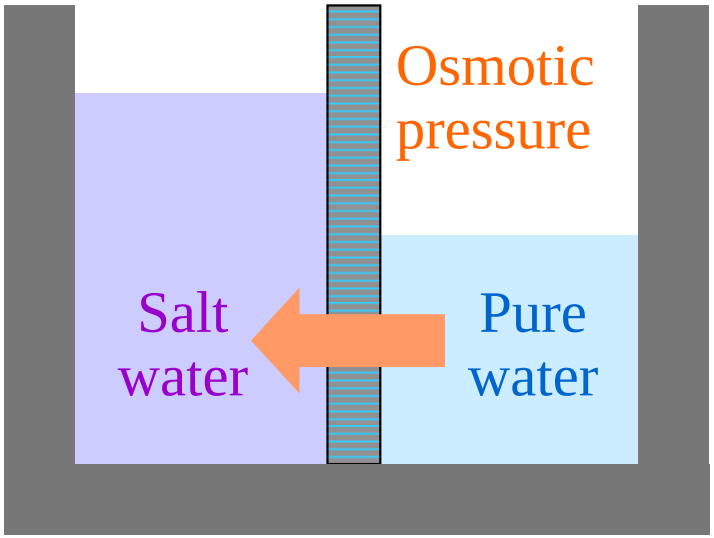


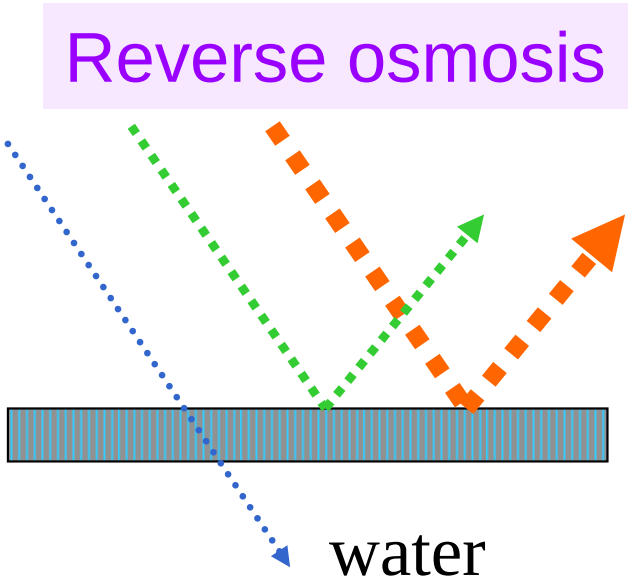


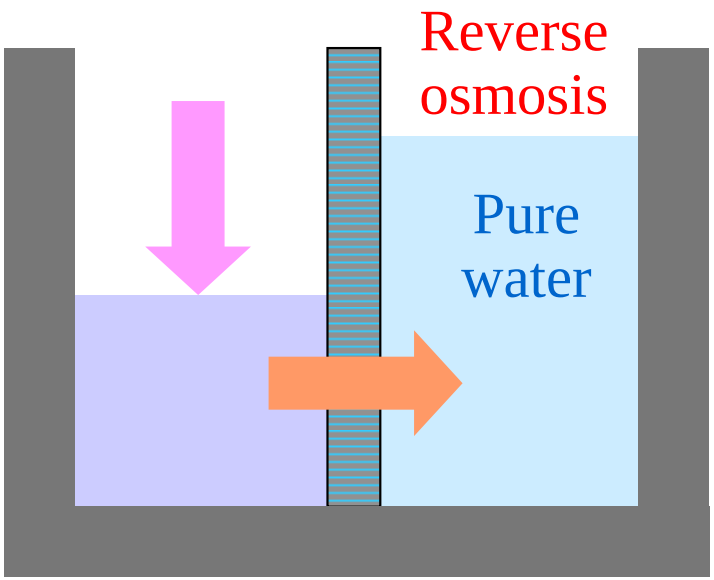
Amicon catalogue

超微薄膜及逆滲透 Ultrafiltration and RO



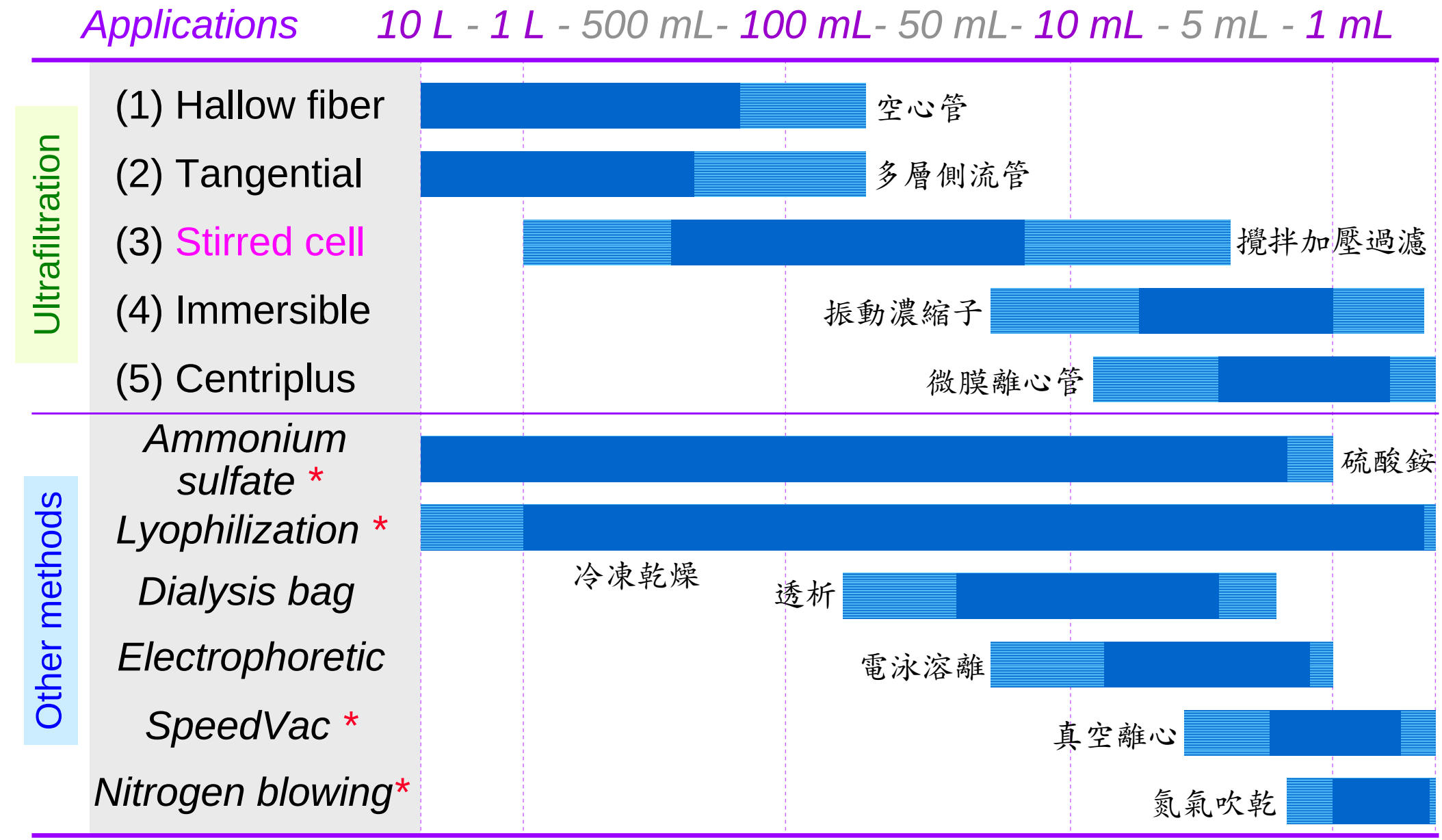






Juang RH (2005) EPA

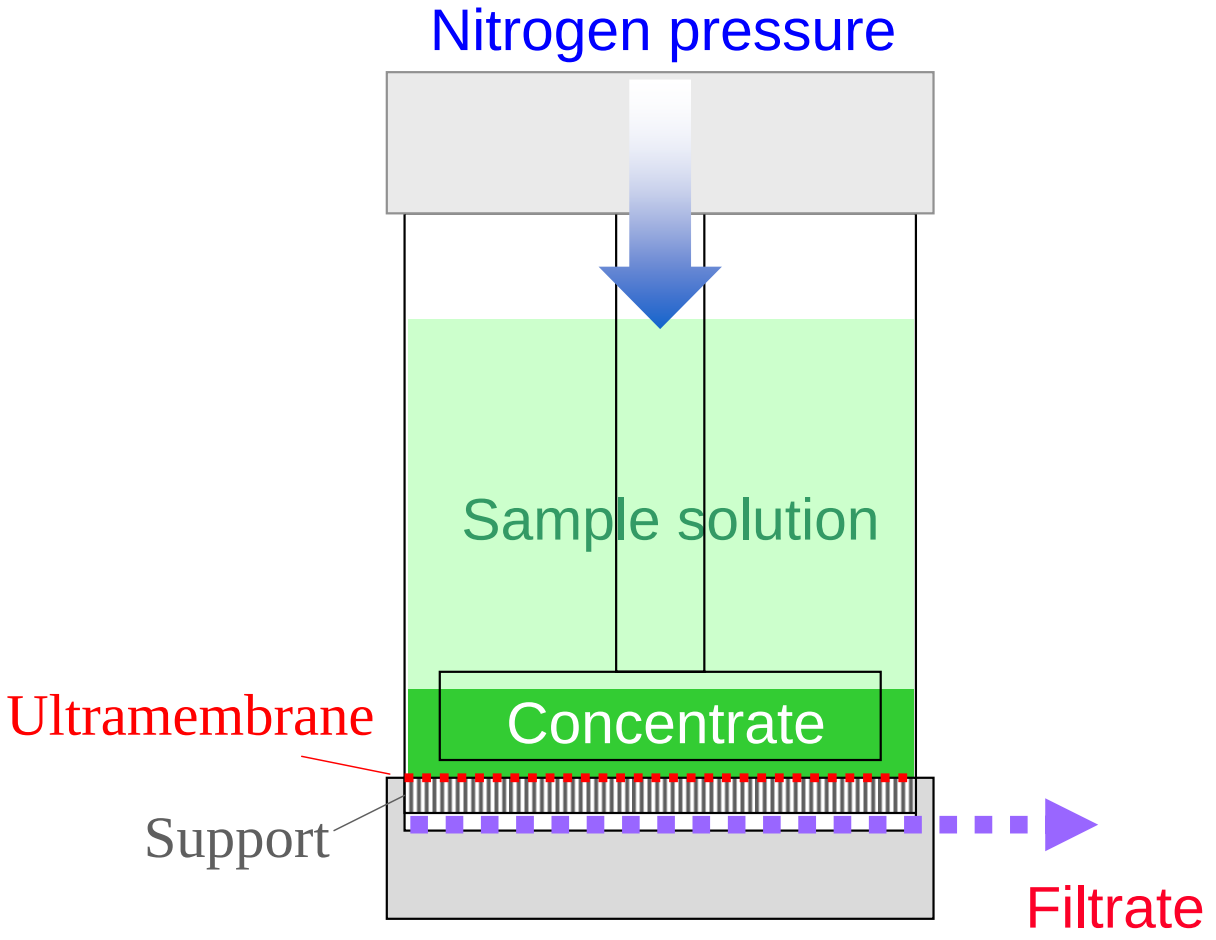
各種濃縮方法的使用範圍 Useful ranges



* The salt concentration increases in the sample

Juang RH (2005) EPA

超微薄膜濃縮裝置 - Stirred cell



Amicon Stirred Cells

Juang RH (2005) EPA

5 純化策略 Purification strategy

5.1 純化步驟設計 Design a purification protocol

一邊進行純化工作，一邊改進純化方法或步驟

摸著石頭過河 (Trial and error)

5.1.1 影響純化的因素 Critical factors in purification

要求高活性、高回收率、高純度、方便快速、經濟

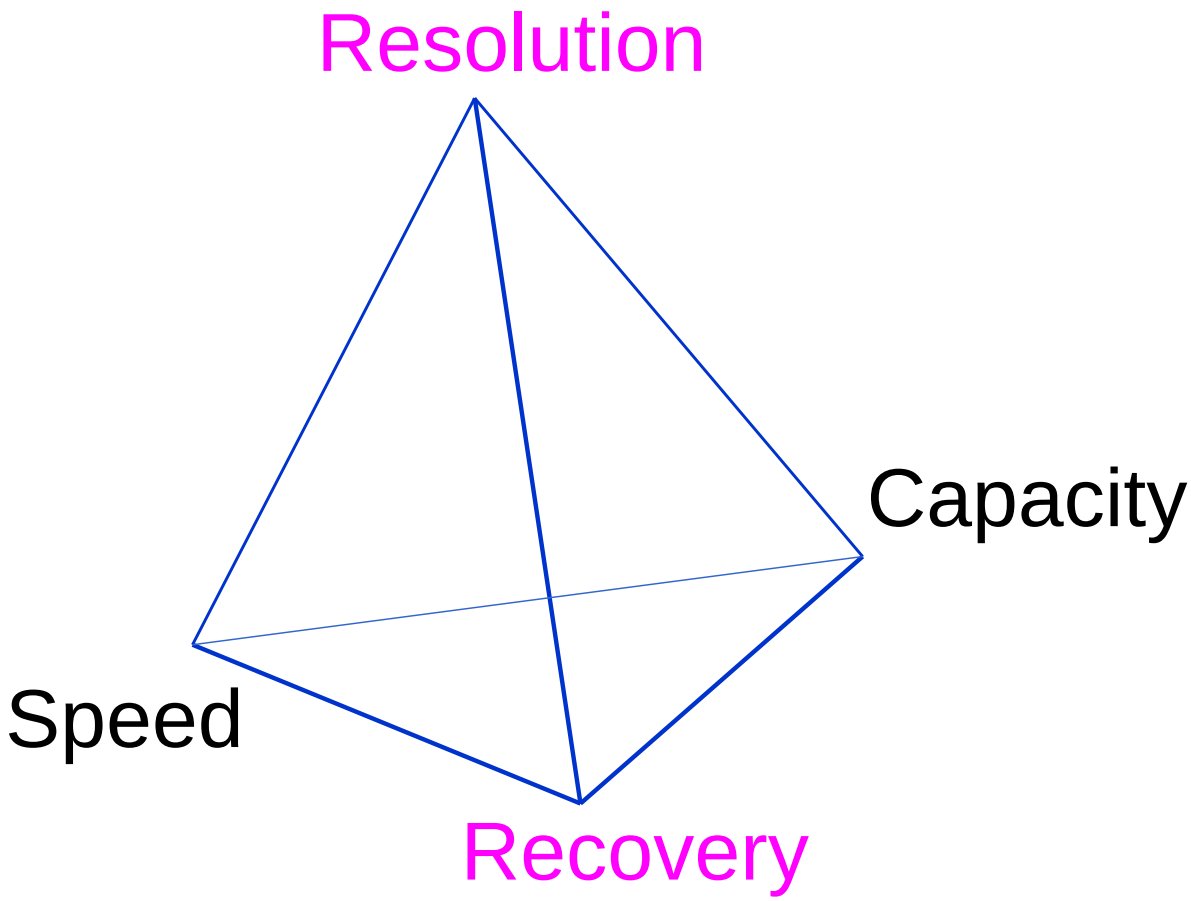
5.1.2 組合純化步驟 Set up purification steps

組合各種方法以達純化效率

5.2 純化結果 Purification table

以純化表來檢討純化效果

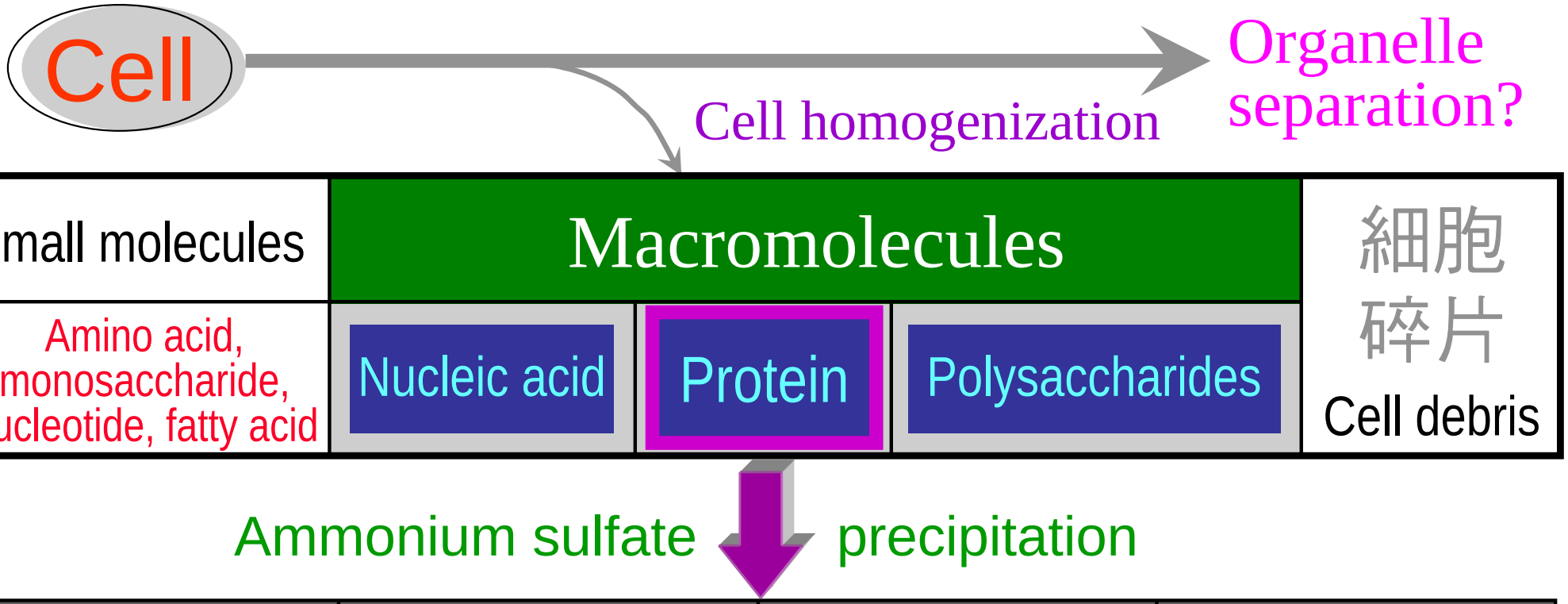
檢討純化效果 Critical factors



- 高活性
  High activity
- 高回收率
  High recovery
- 高純度
  High purity
- 方便與快速
  Rapid
- 經濟
  Economy

Juang RH (2005) EPA

各種純化或分析方法的原理



Ammonium sulfate precipitation

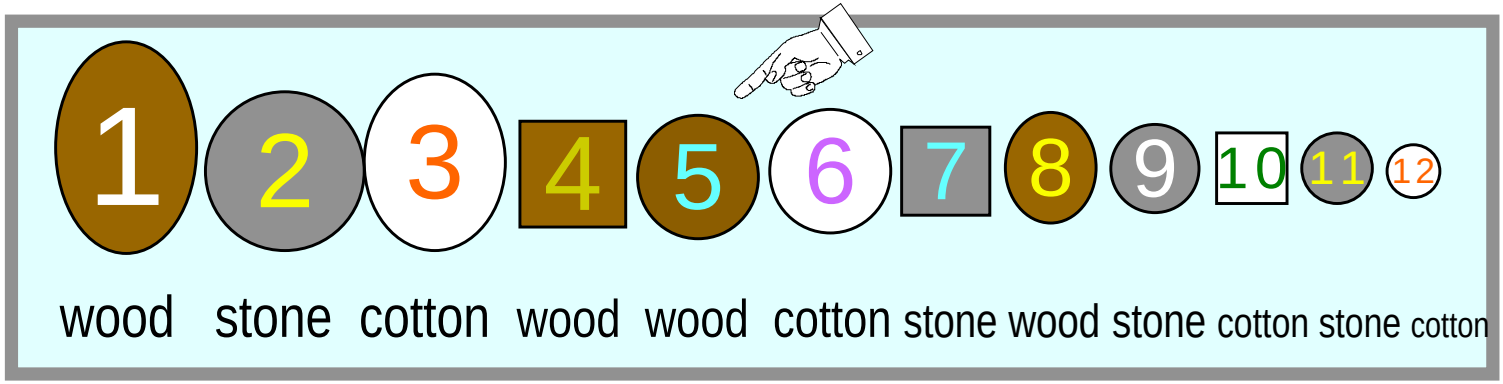
Molecular size	Molecular charge	Molecular polarity	Affinity
Gel filtration, SDS-PAGE, Ultrafiltration	Ion exchange, Chromatofocusing, Disc-PAGE, Isoelectric focusing	Reverse phase chromatography, HIC, Salting-out	Affinity chromatography, Hydroxyapatite

Basic protein properties useful for planning separation steps

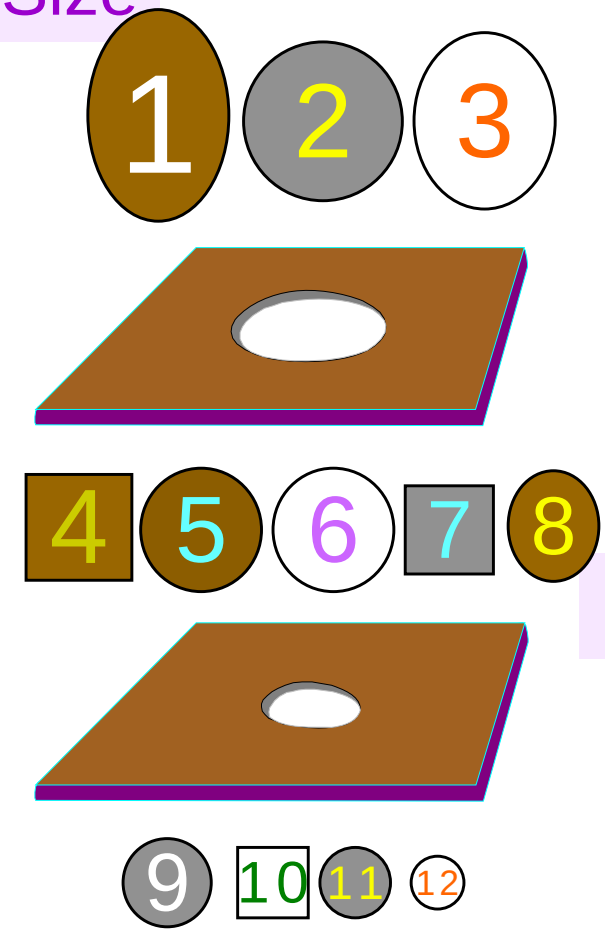
Juang RH (2005) EPA

如何分離這些大小物件

shape
size
density

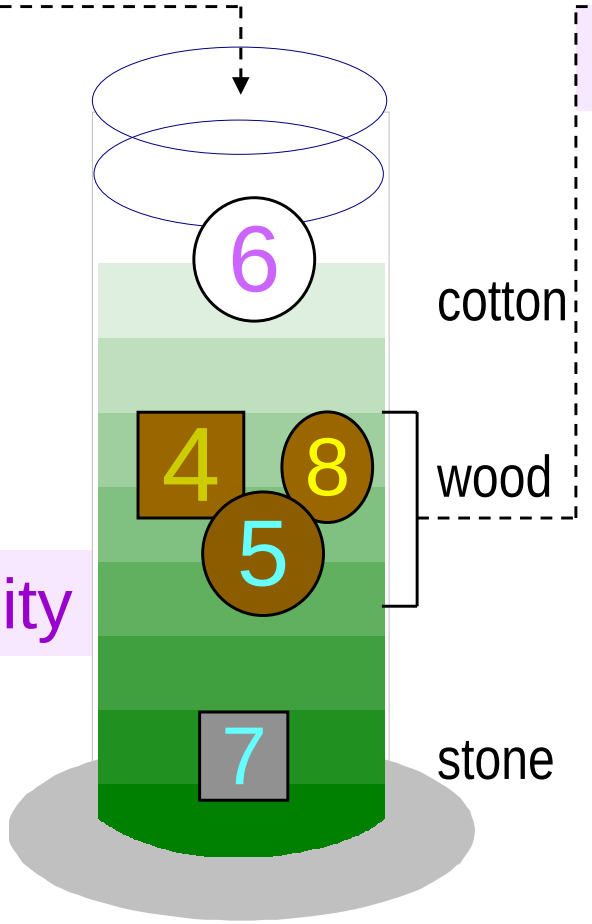


Size



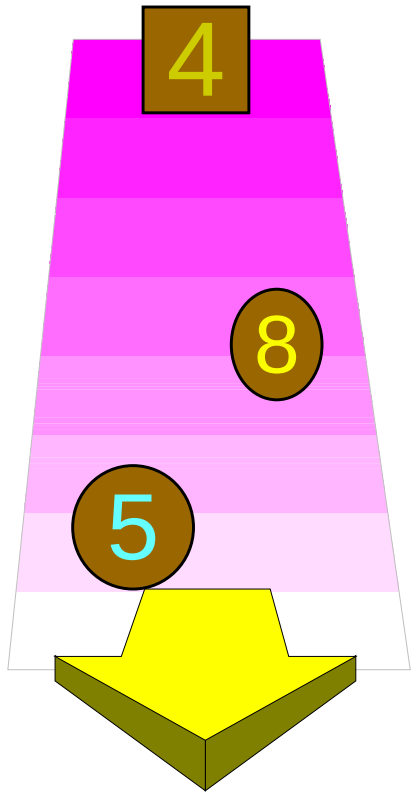
Sieving different sizes

Density



Sedimentation in different speed

Shape



Rolling down in Different speed

How to separate these 12 objects?

Juang RH (2005) EPA

組合純化步驟 Set up your purification protocol

純化流程基本骨幹

Basic backbone for purification

硫酸銨分劃
Ammonium sulfate fractionation

分子表面極性不同
The polarity of the protein

離子交換法
Ion exchange

分子淨電荷不同
The charge of the protein

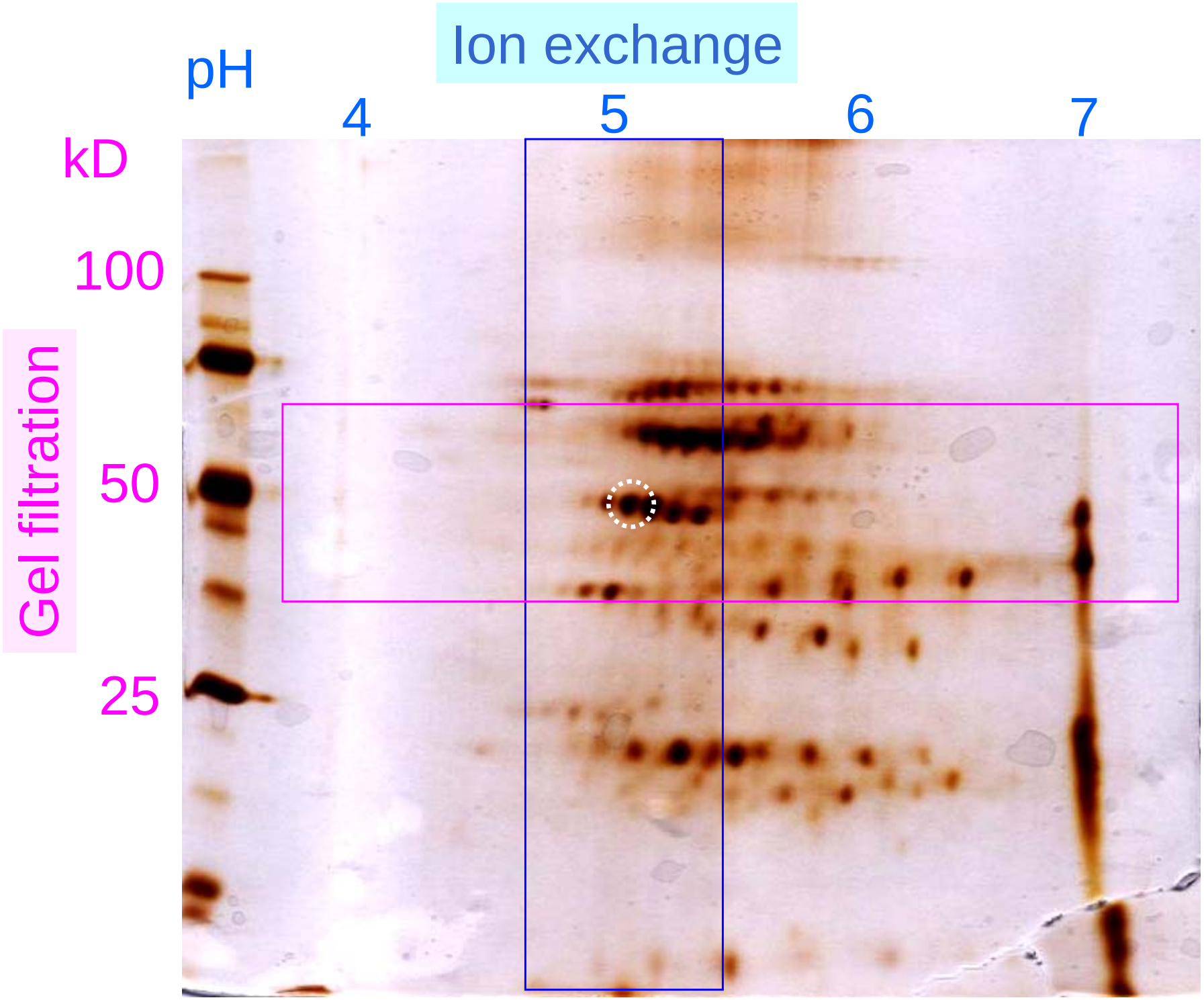
膠體過濾法
Gel filtration

分子量大小不同
The molecular size of the protein

HIC? Hydroxyapatite? Affinity chromatography? Another ion exchange?

Juang RH (2005) EPA

純化過程的鳥瞰

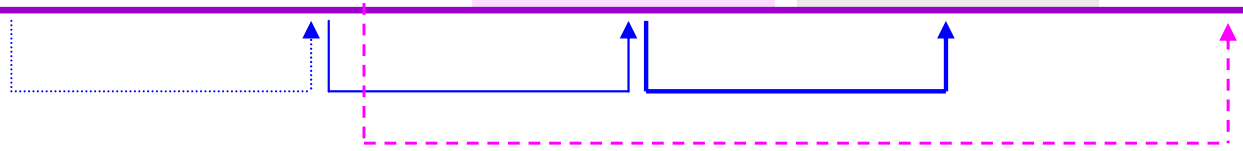


The result of purification after two chromatographic steps

Juang RH (2005) EPA

蔗糖合成酵素之純化表 Purification table

Step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification fold (fold)	Recovery (%)
Crude extraction	1,070	9,672	9.0	1.0	100
Protamine sulfate precipitation	800	12,555	15.7	1.7	130
Ammonium sulfate (35-55% sat)	250	6,610	26.4	2.9	68
Sepharose CL-6B gel filtration	53	5,789	111.3	12.4	60
DEAE Sepharose ion exchange	8.6	2,960	344.2	38.2	31



From 100 g rice grain at its milky stage (乳熟期)

Juang RH (2005) EPA