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

3.1 製備式電泳 Preparative electrophoresis

蛋白質色帶由原態電泳中直接切除出來

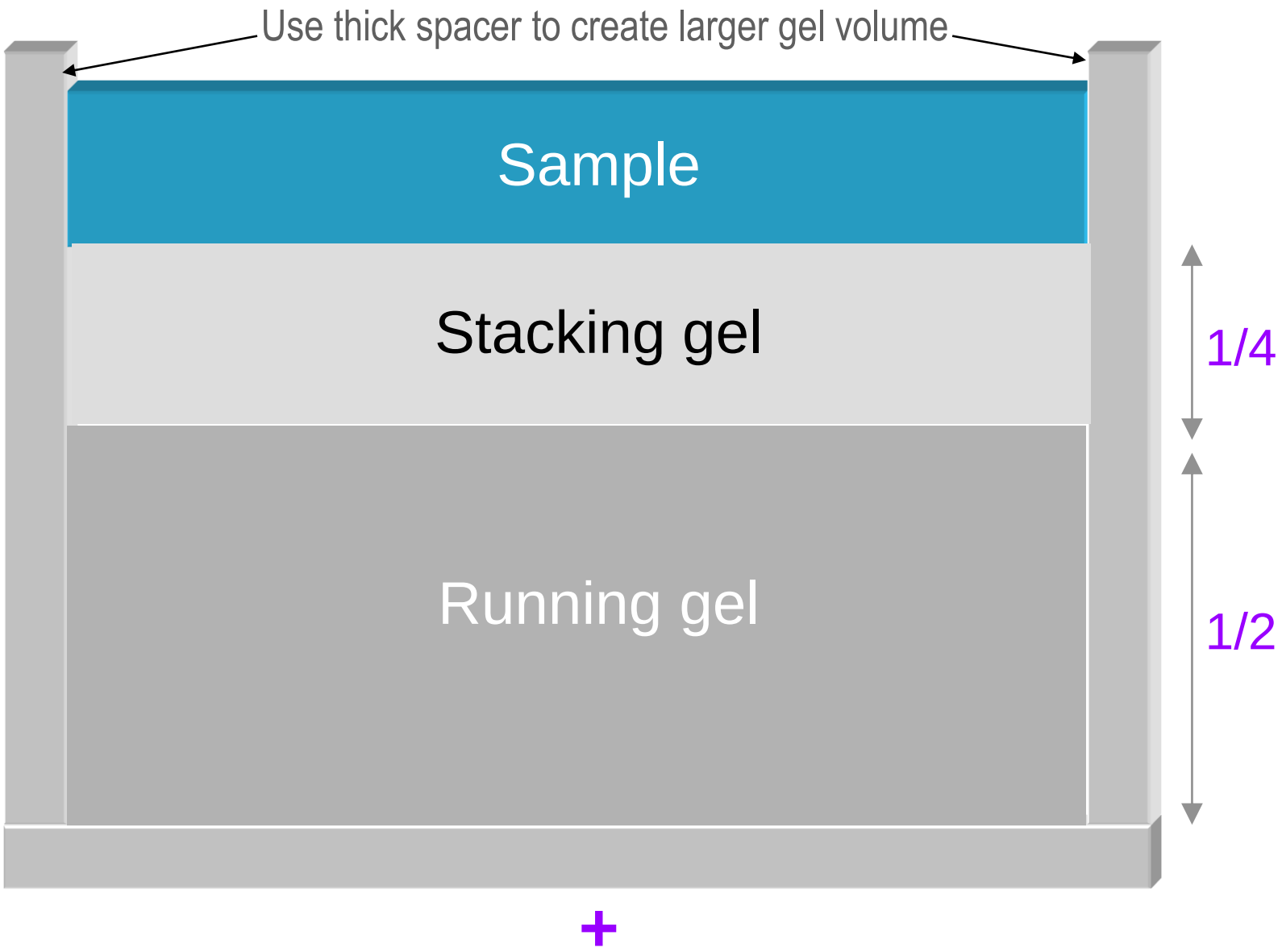
3.2 超高速離心法 Ultracentrifugation

以各種分子的沉降係數不同來進行分離

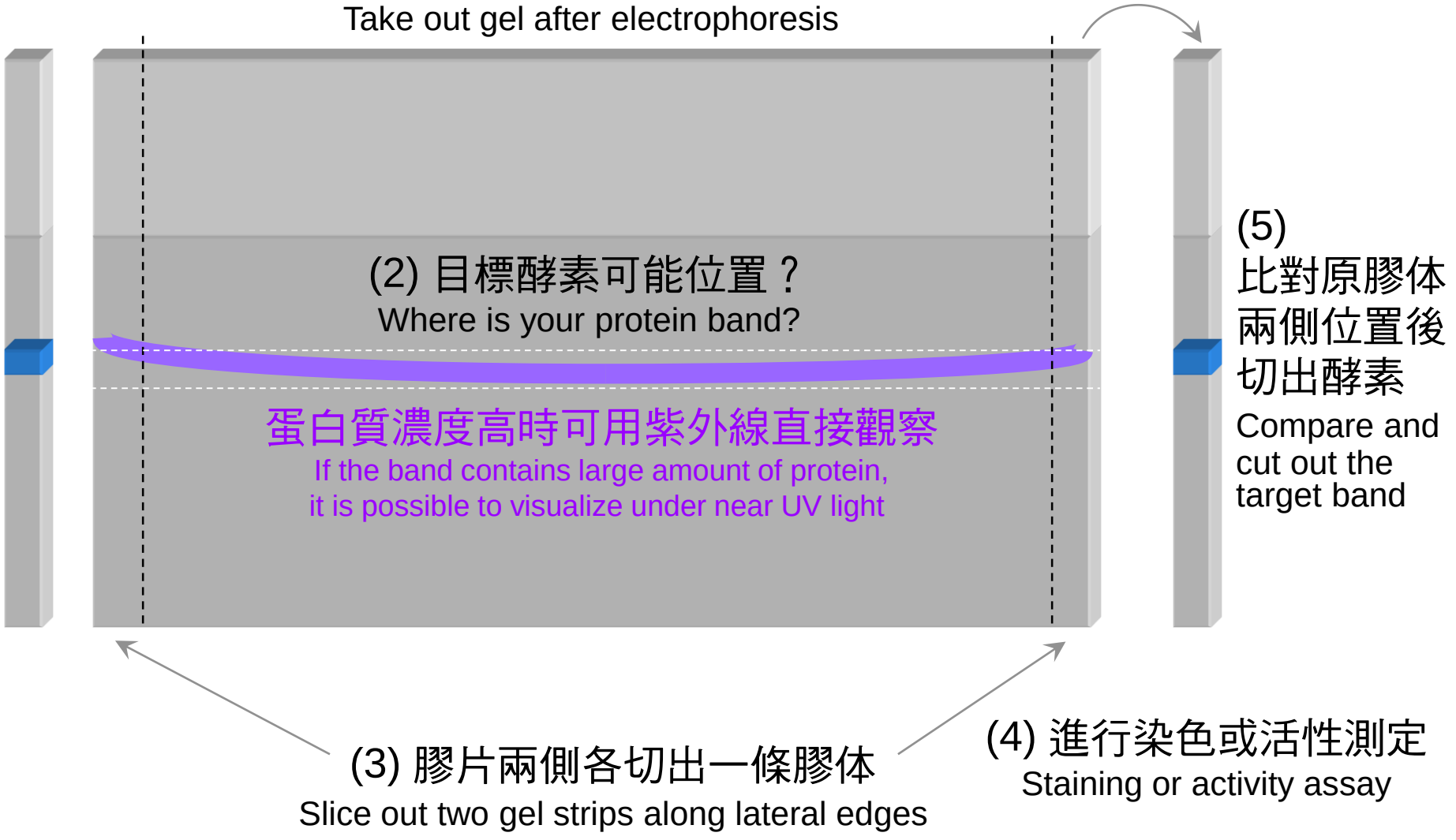
3.3 超微薄膜過濾法 Ultrafiltration

超微薄膜可以用來脫鹽及濃縮蛋白質

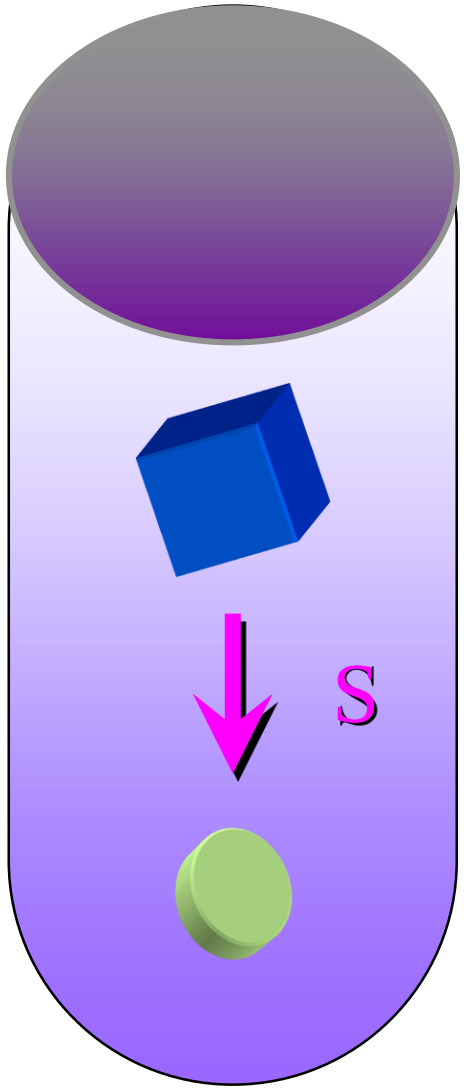
製備式電泳膠片 Preparative gel format



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



超高速離心 Sedimentation coefficient



Svedberg unit

粒子在密度梯度離心時的沈降速率

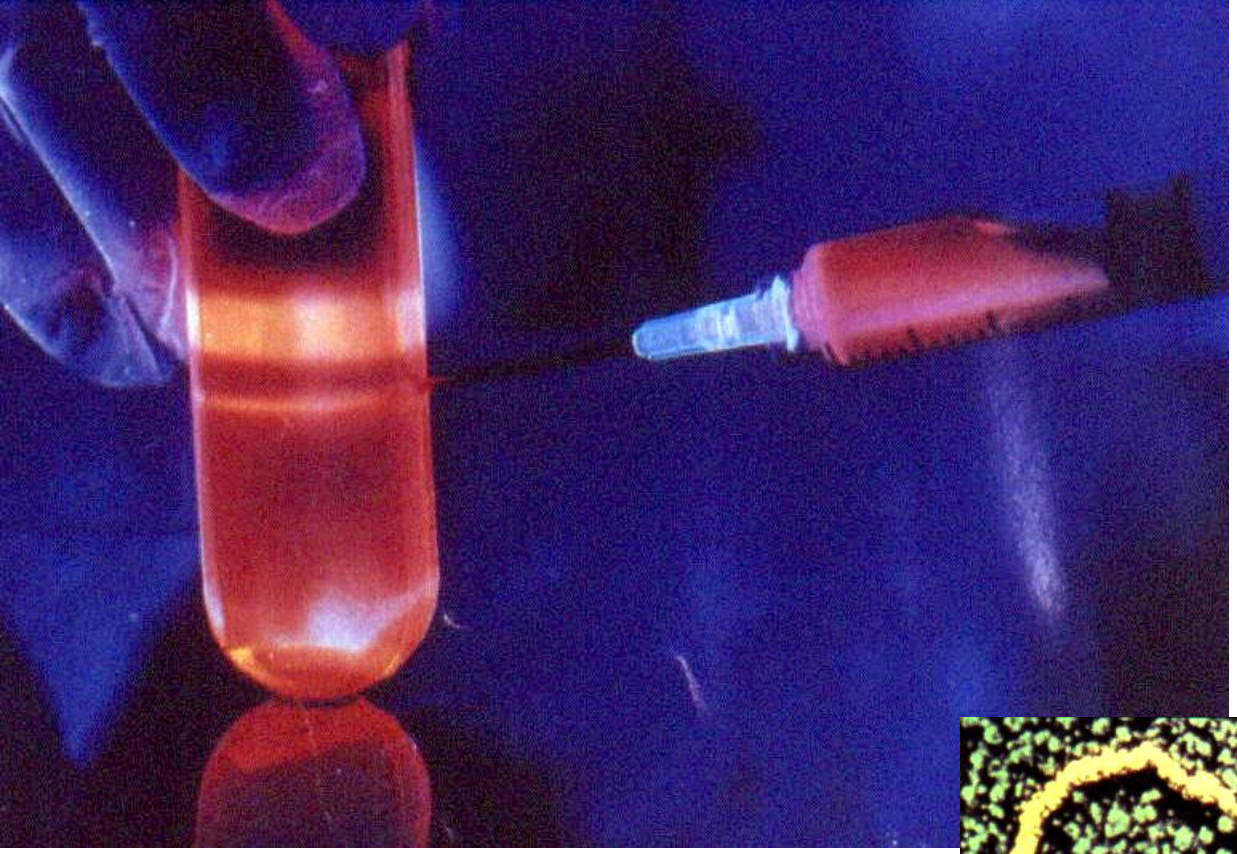
The sedimentation velocity of a particle when it is centrifuged in a density gradient

分 子 量 molecular weight

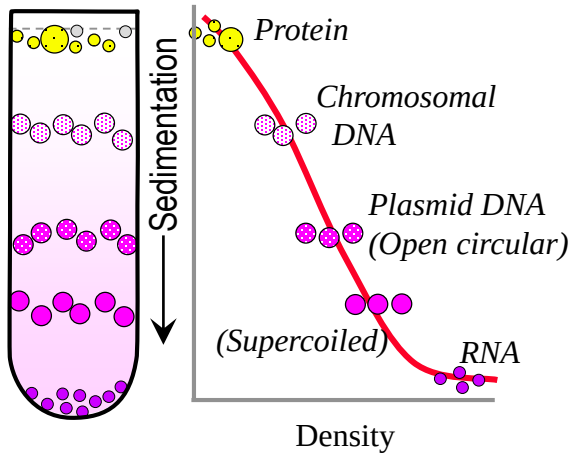
分子密度 molecular density

分子組成 molecular composition

分子形狀 molecular shape



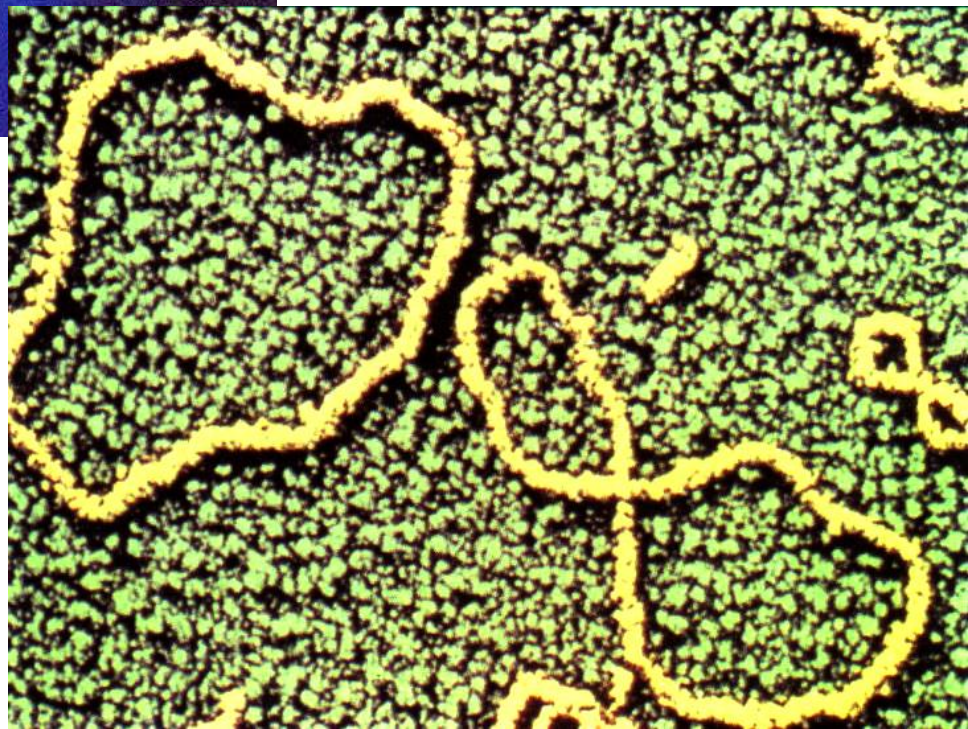
CsCl gradient ultracentrifugation



以超高速離心大量製備質体

Ultracentrifugation is used to prepare plasmid in large scale

Plasmid DNA (Open circular)



兩種超高速離心比較 Two ultracentrifuge types

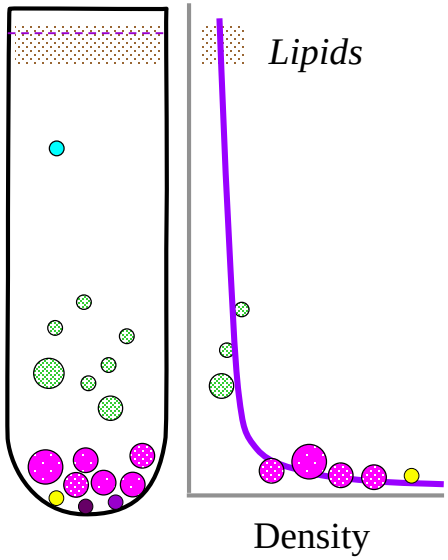
Centrifuge	Sedimentation Velocity	Sedimentation Equilibrium
also called →	Zone Centrifugation	Isopycnic Equilibration
Gradient formation	Precast (sucrose, glycerol)	During centrifugation (CsCl)
	Shallow gradient, lower density	Steep gradient, higher density
Suitable samples	Similar density, different 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
中文名稱	區帶離心法	等密度平衡離心法

表 3.1

各種高速離心法比較 Comparison of centrifuges

High speed

Gravity Centrifugation
(No density)



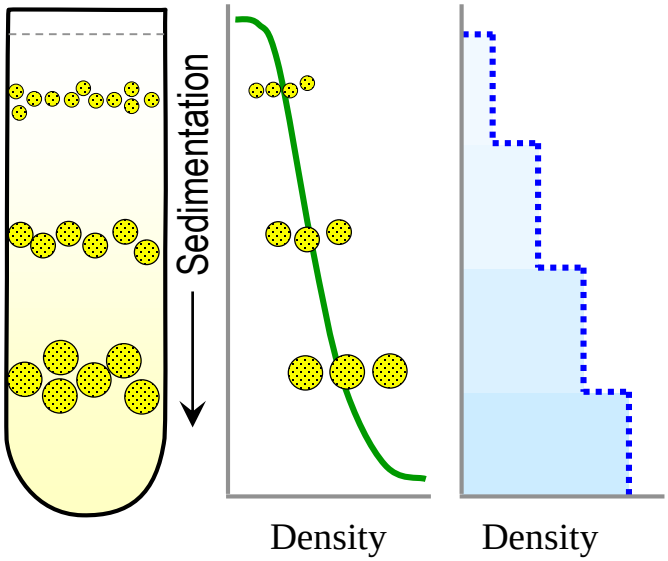
一般的重力離心僅把顆粒與溶液分離開來

Utilize gravity force to separate particles from the solution

Ultracentrifugation

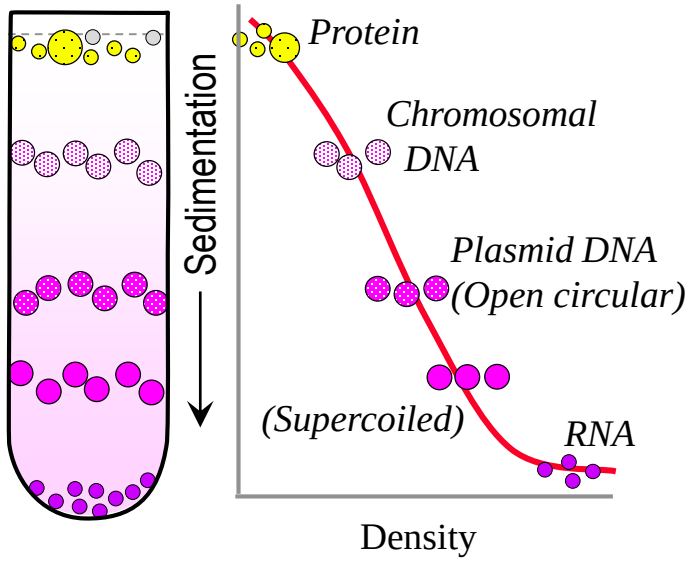
Zone Centrifugation
(Precast) → (step-wise) ← (CsCl gradient forming)

Isopycnic Equilibration



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)



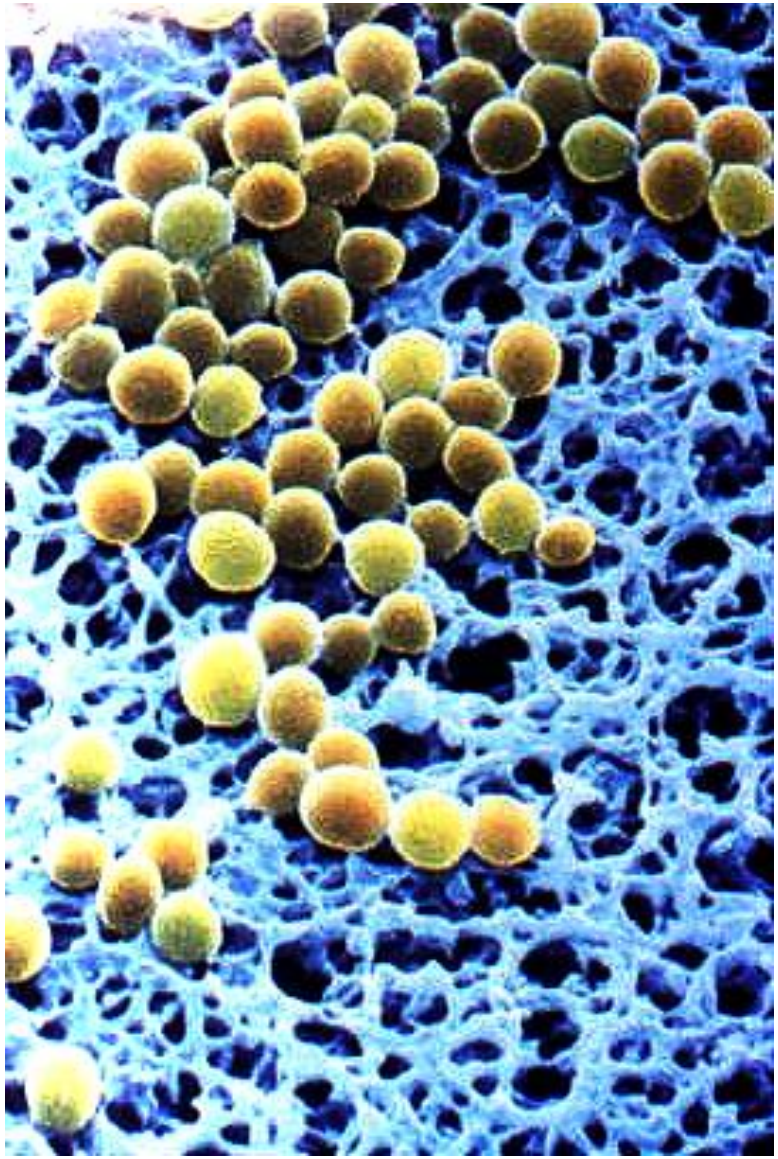
樣本：多為核酸
密度不同、分子量相似者

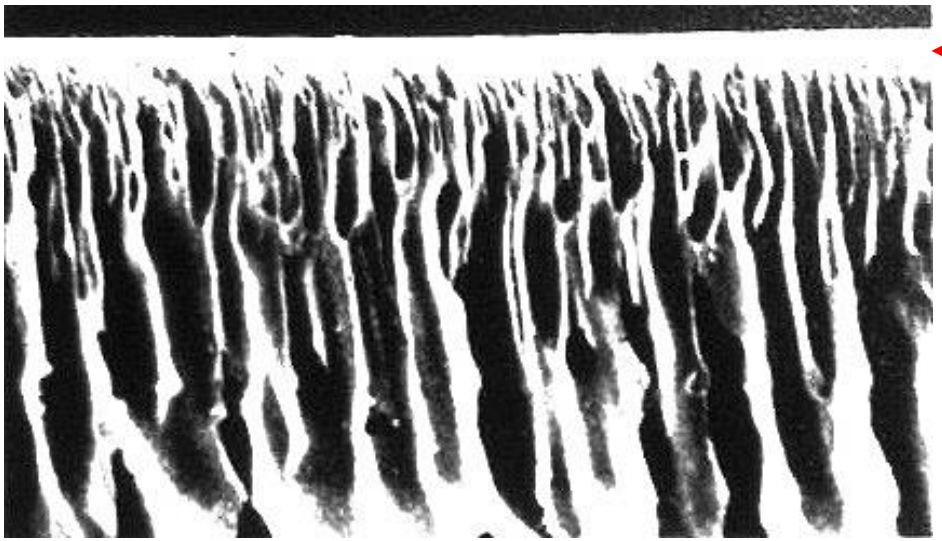
Sample: nucleic acid (similar MW, but different in density)

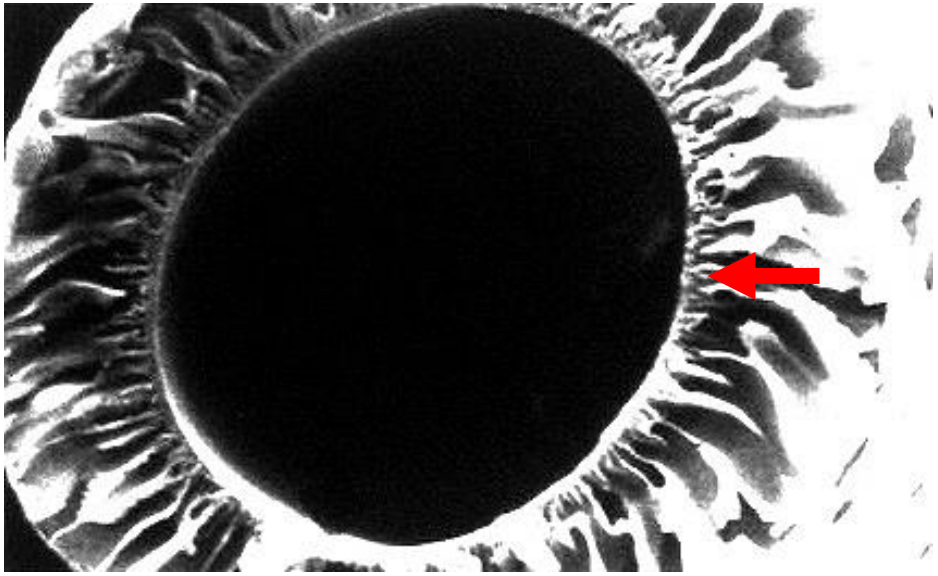
Lower density ⟷ Higher density

圖 3.1

超微薄膜技術 Ultrafiltration technology

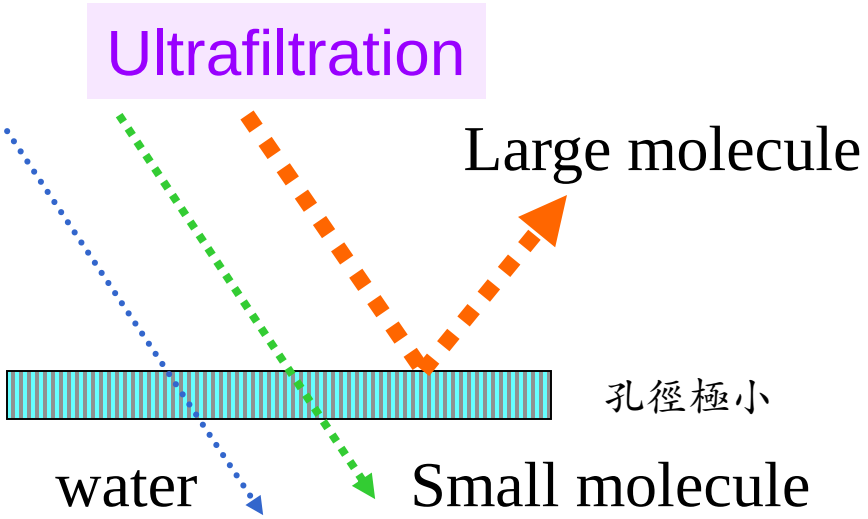


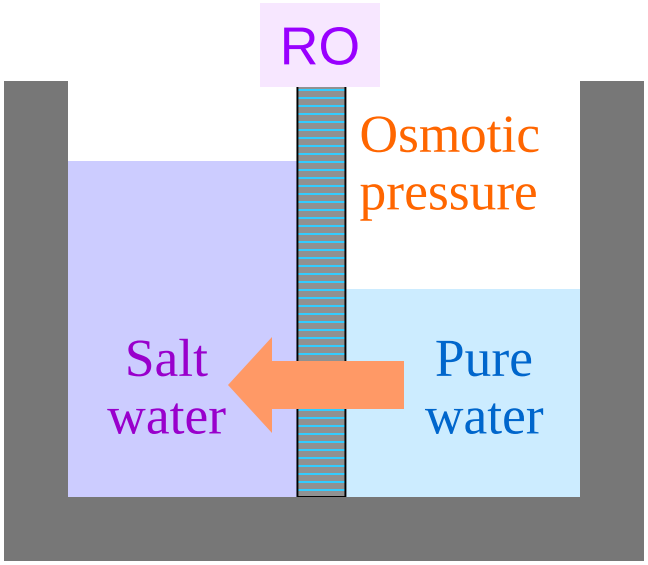


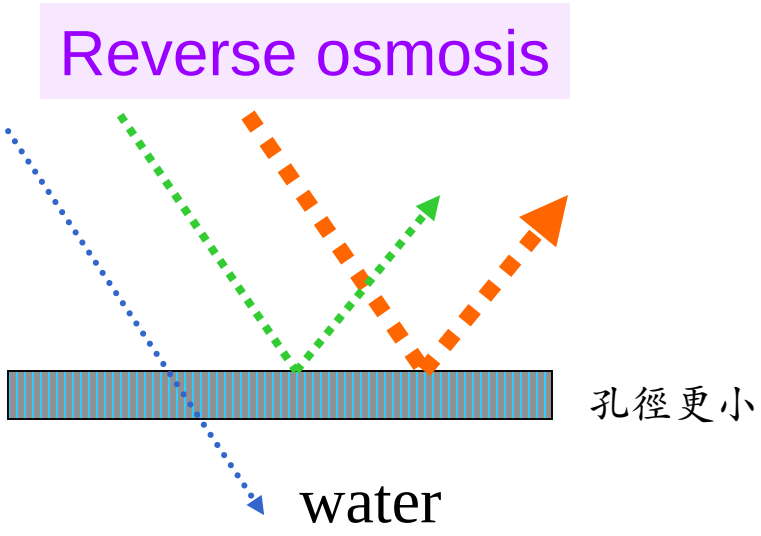


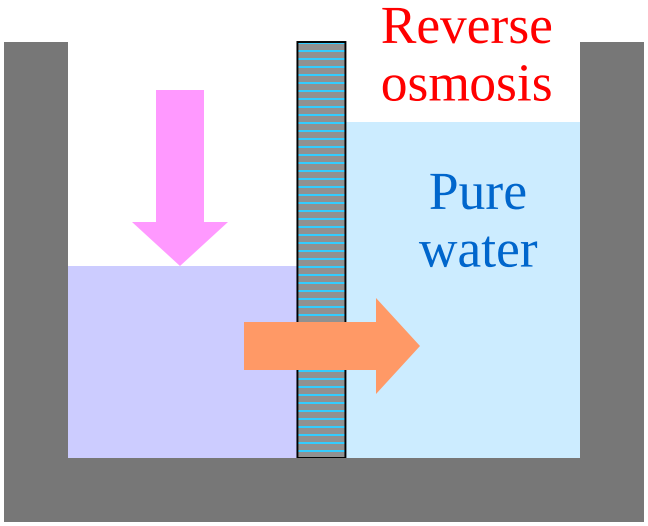
Amicon catalogue

超微薄膜及逆滲透 Ultrafiltration and RO

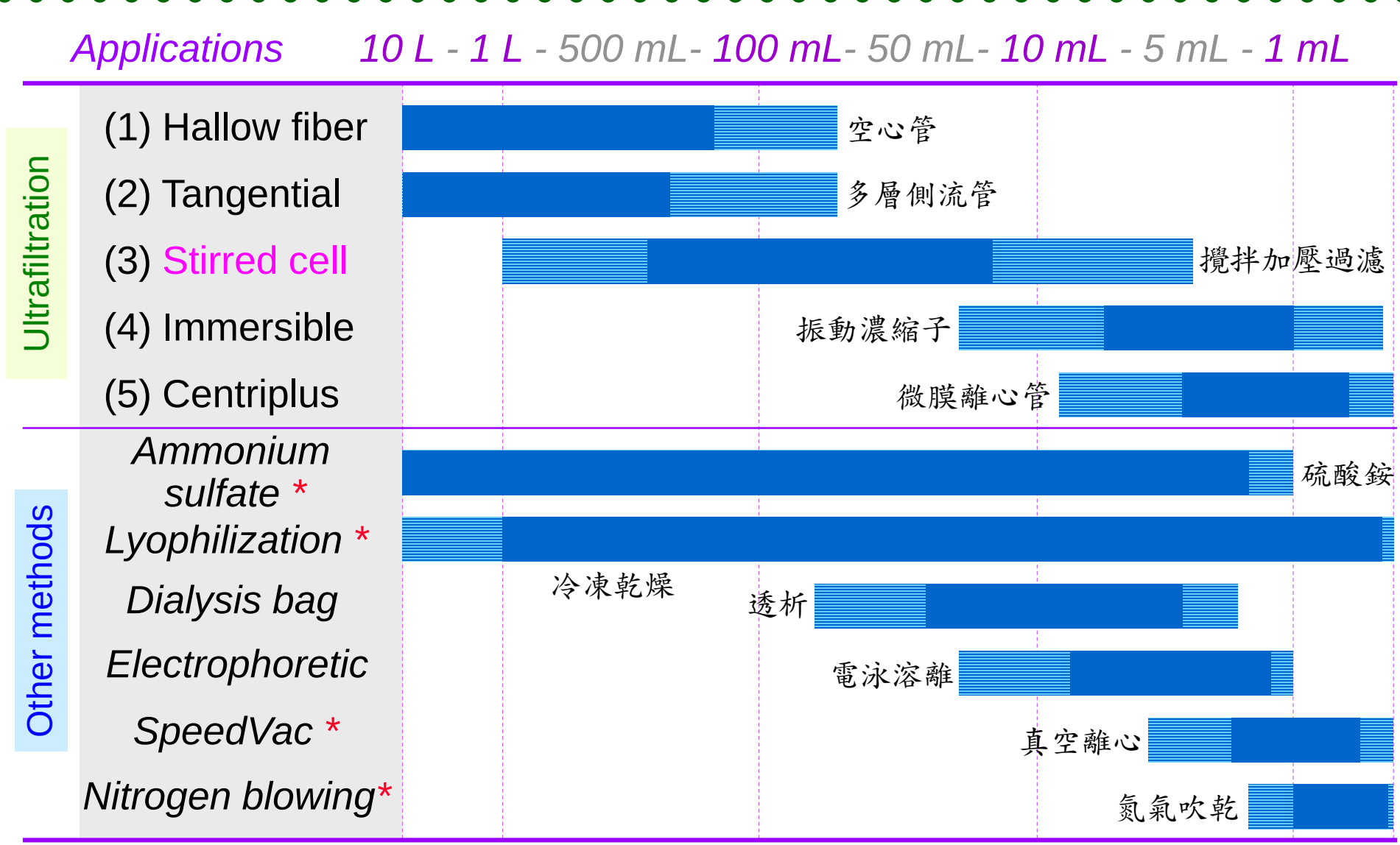








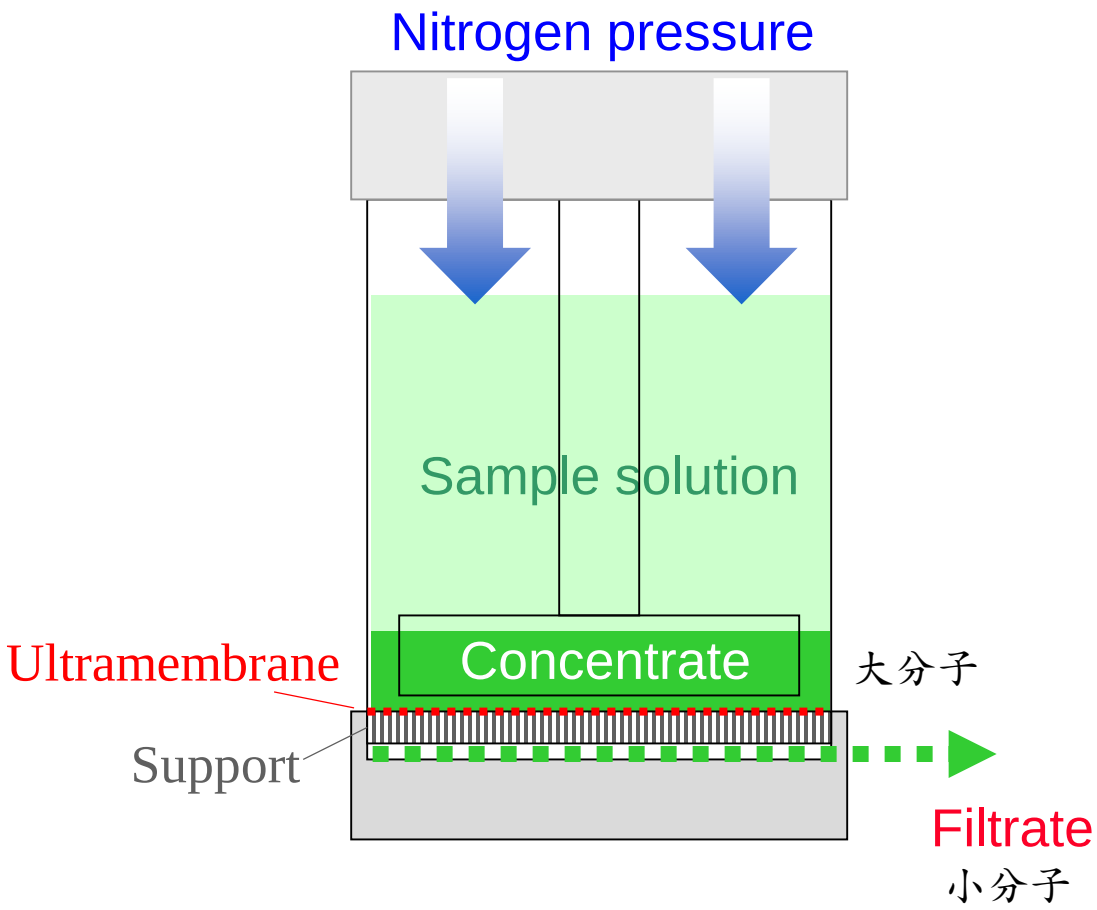
各種濃縮方法的使用範圍 Useful ranges



* The salt concentration increases in the sample

圖 3.2

超微薄膜濃縮裝置 - Stirred cell



Amicon Stirred Cells