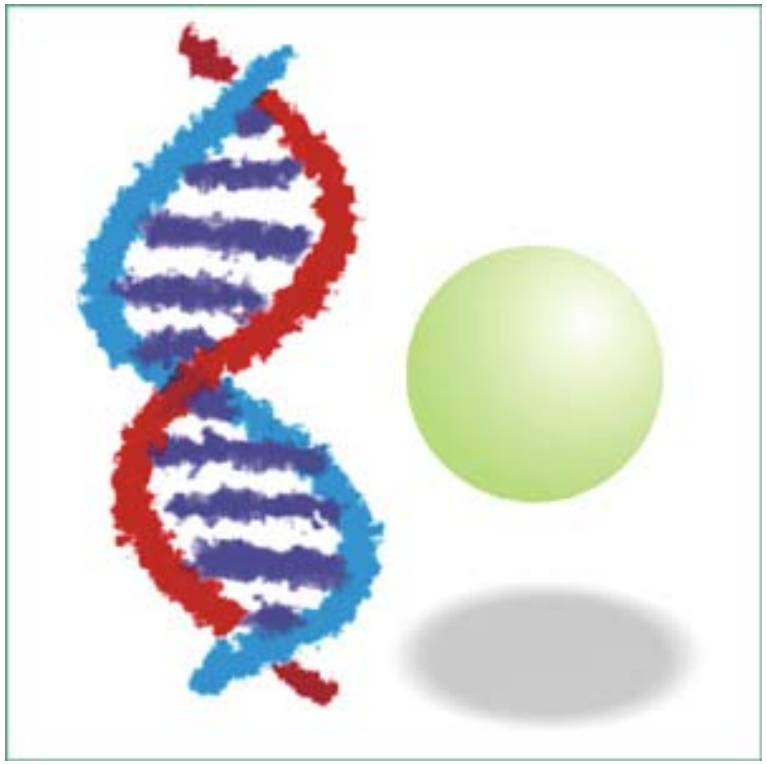
BST

生化科技系

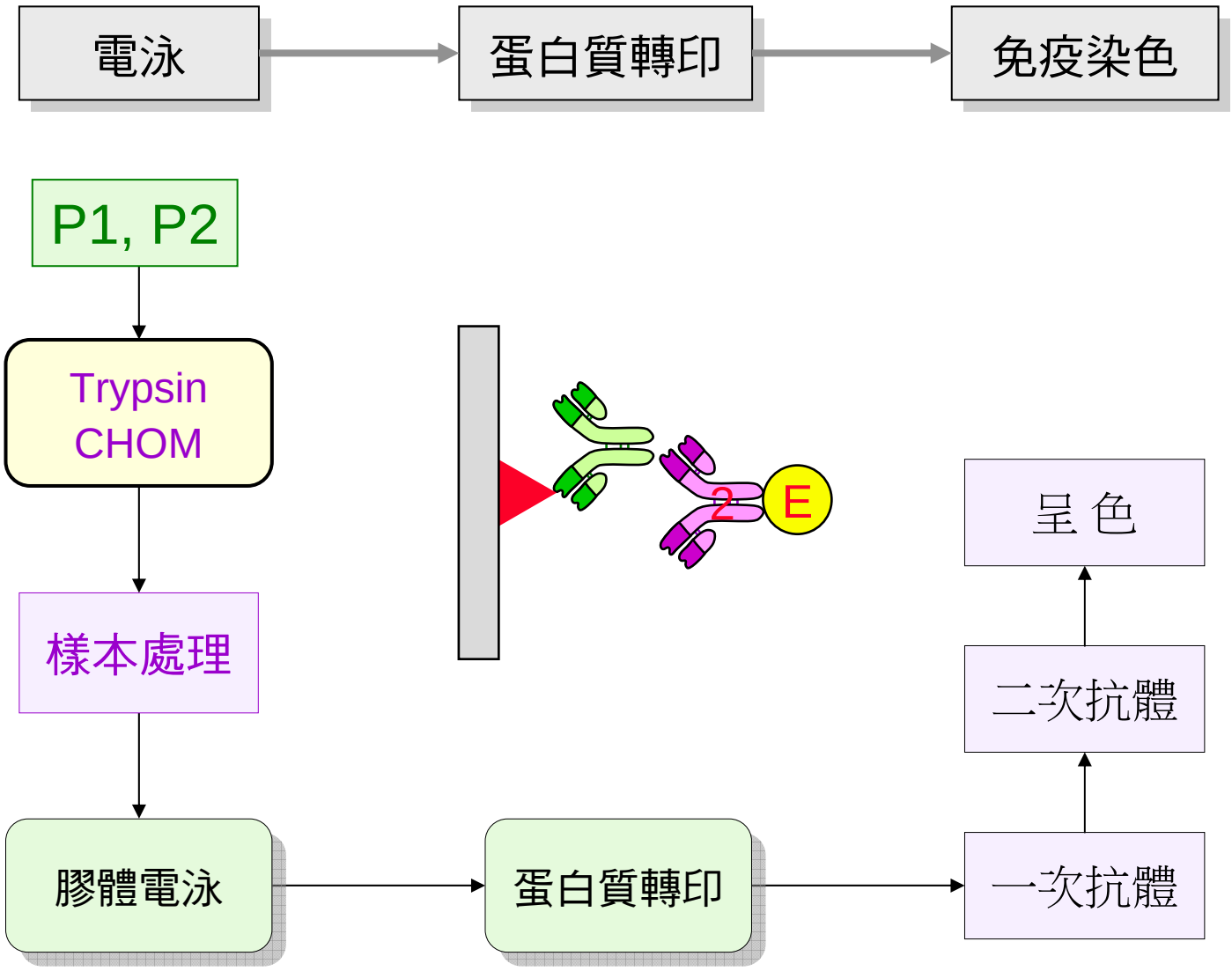


BCX S2

# 生物化學實驗

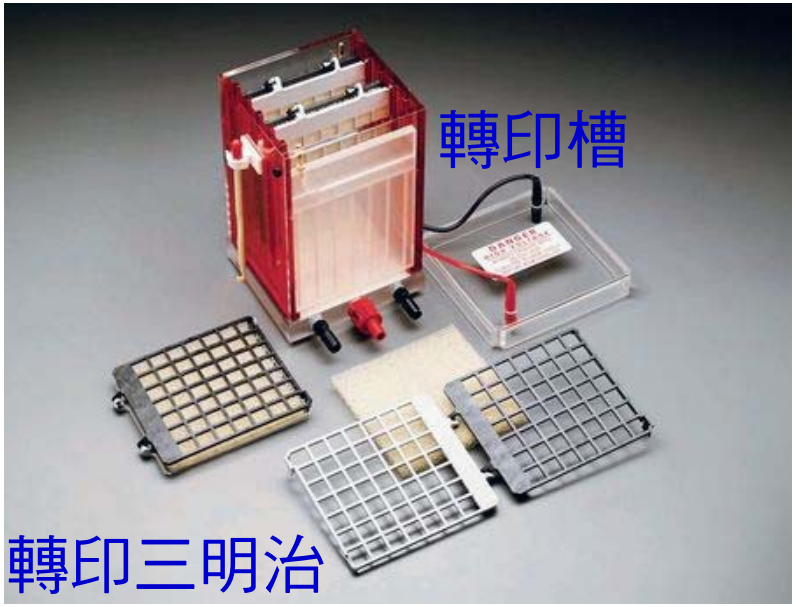
## 免疫染色法

# S2 免疫轉印法


電泳
蛋白質轉印
免疫染色
P1, P2
Trypsin
CHOM
樣本處理
膠體電泳
蛋白質轉印
一次抗體
二次抗體
呈 色
2
E

# 電泳槽及相關設備：

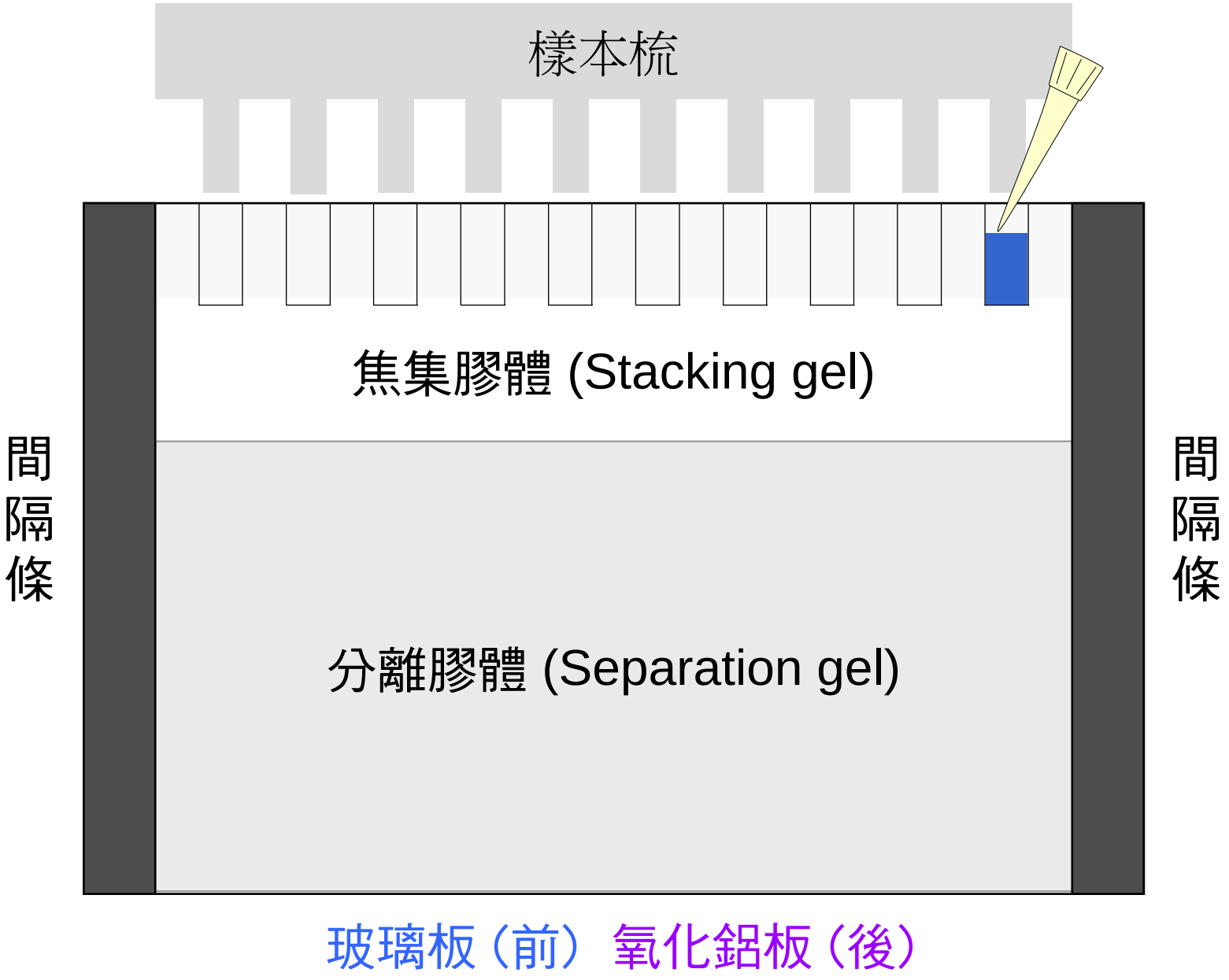
電泳槽




鑄膠器

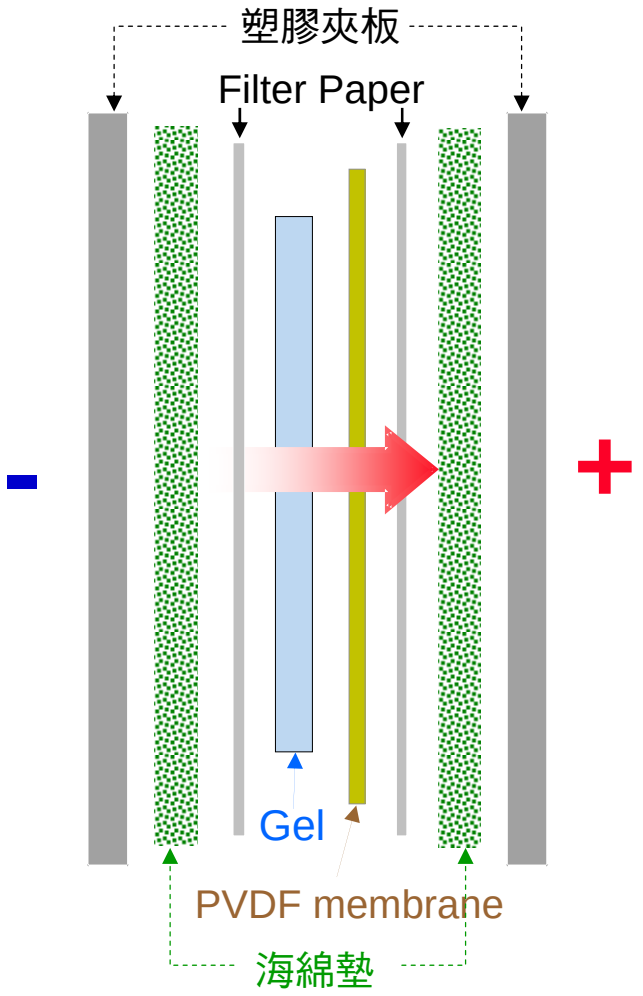

供電器

# 鑄膠過程與注入樣本

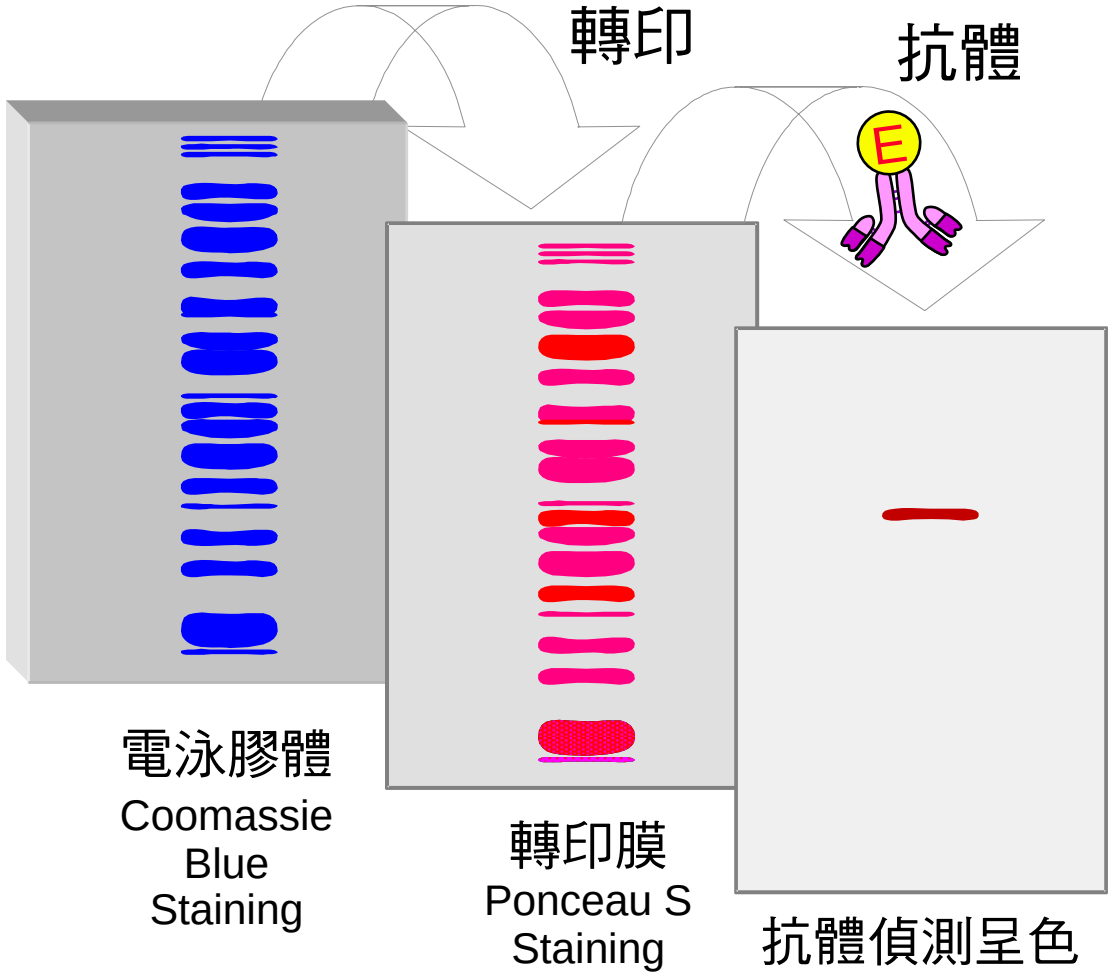

樣本梳
焦集膠體 (Stacking gel)
分離膠體 (Separation gel)
間隔條
間隔條
玻璃板（前）
氧化鋁板（後）

# 轉印及免疫染色流程：

A 轉印三明治

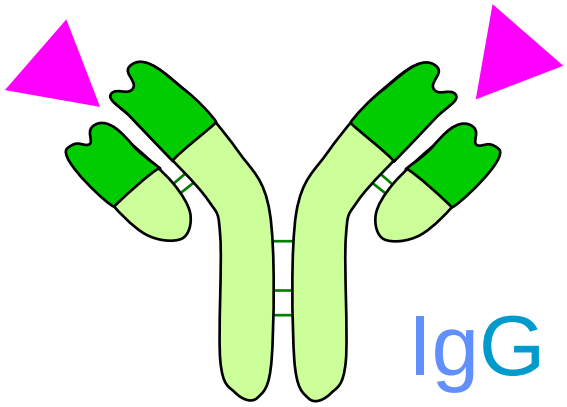



B 免疫染色流程及結果



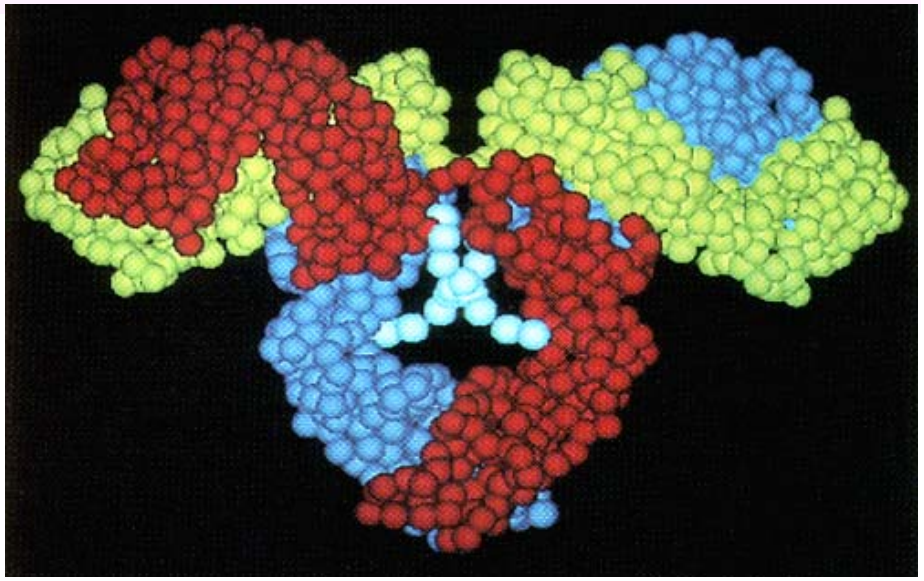
# ■ 抗體 是一種蛋白質

Davies et al (1977) PNAS / Roitt et al (2001) *Immunology*. p.73



IgG

Immunoglobulin



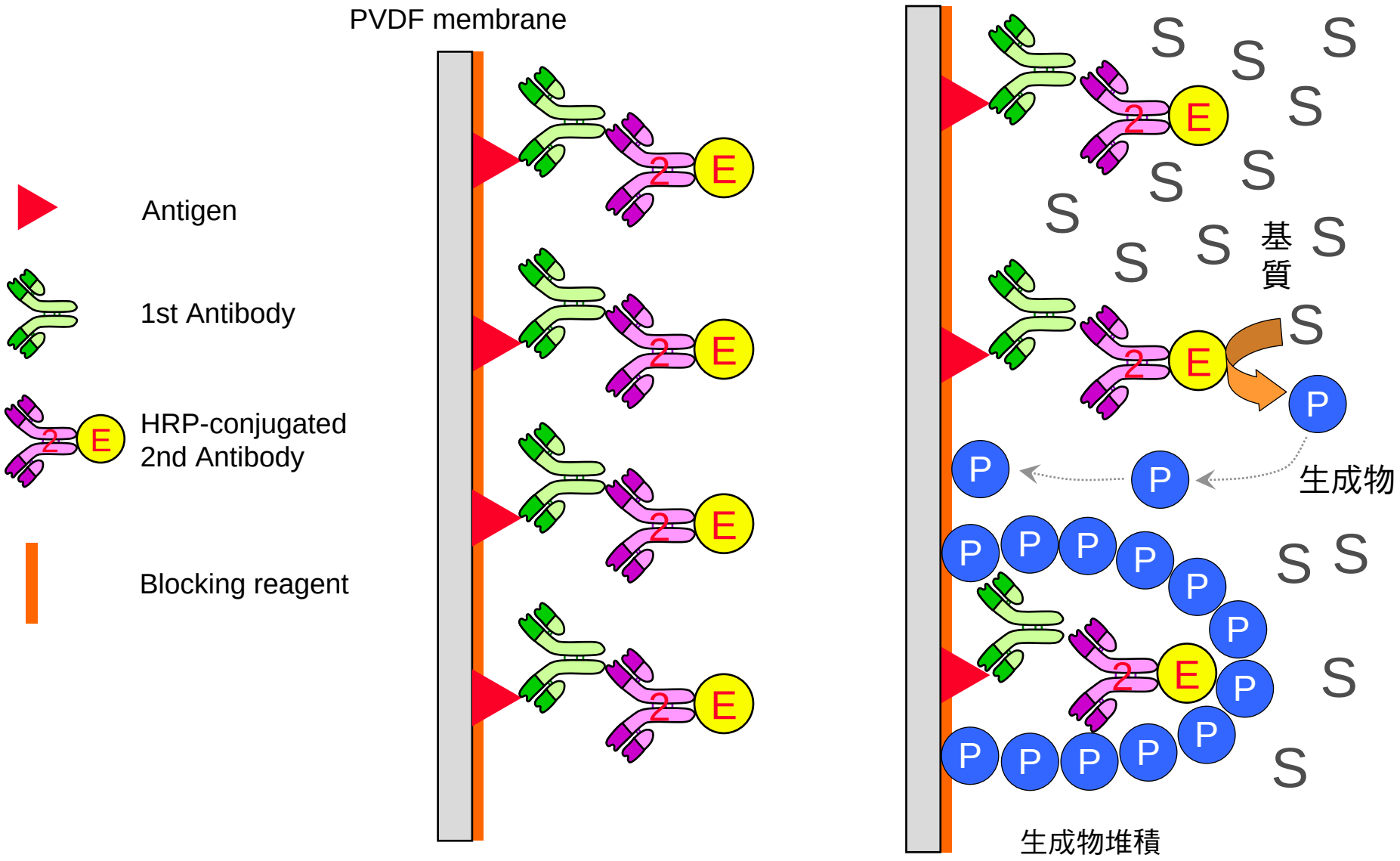
→ 抗體由四條 蛋白質 長短鍊所組成 (兩長兩短)

→ 抗體分子上有兩個 抗原結合區 (二者相同)

→ 抗體與抗原結合是專一性的 (lock & key)

● IgG 是單一個抗體分子，另有 IgM (五元體) 及 IgA (二元體)

# 免疫轉印的種類與呈色機制：



## 標誌酵素及呈色劑：

**Peroxidase**

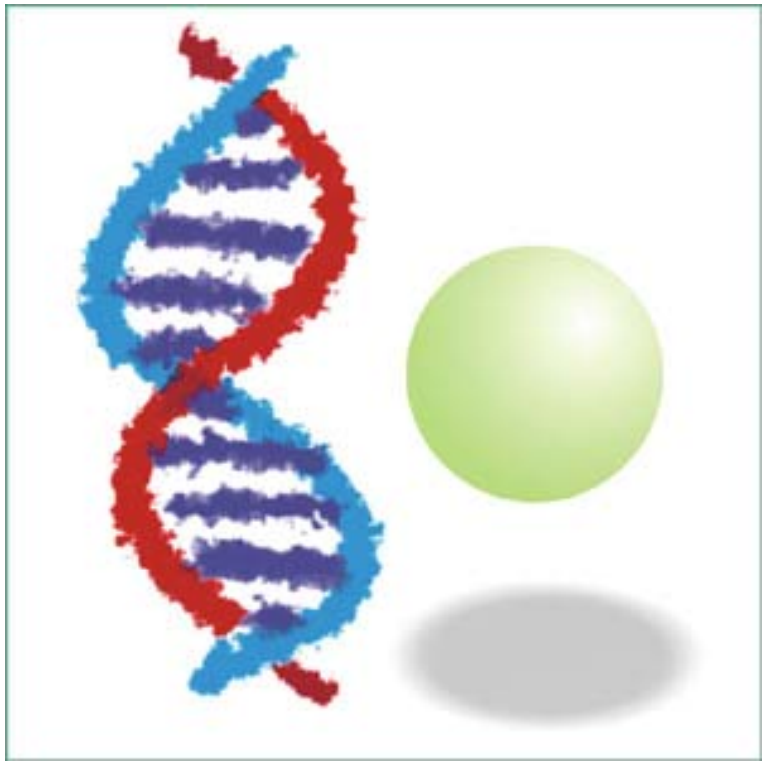
Horse radish peroxidase (HRP) 山葵過氧化酶

基質： $H_2O_2$ + DAB (褐色)

$H_2O_2$ + ECL (冷光)

BST

生化科技系



BCX

P4

# 生物化學實驗

## 膠體過濾法

# P4 膠體過濾法

管柱裝填 → 色析操作 → 分子量測定

標準蛋白質

Trypsin

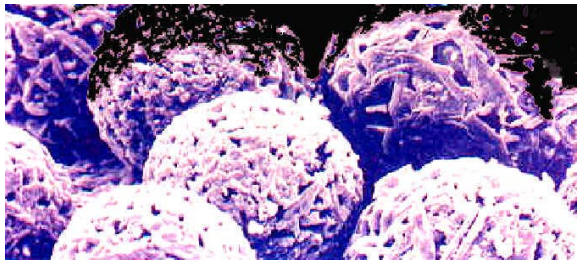
膠體過濾

分子量
標準校正線

分劃收集

Bradford
method

活性分析



α-D-1, 6-glucose

Dextran

α-D-1, 3-glucose

Cross-linkage

$CH_2\text{-}CH\text{-}CH_2Cl$

O

Epichlorohydrin

Sephadex

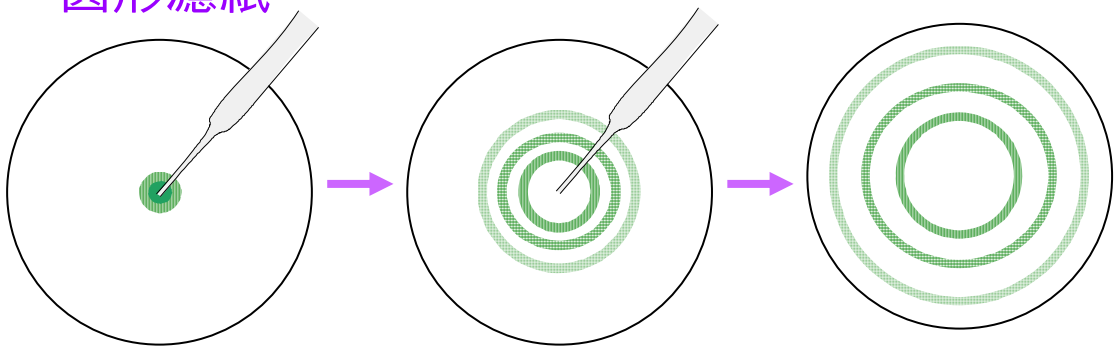
Modified from Amersham Biosciences

# 色析法的演進過程：

Martin, Synge (1952)

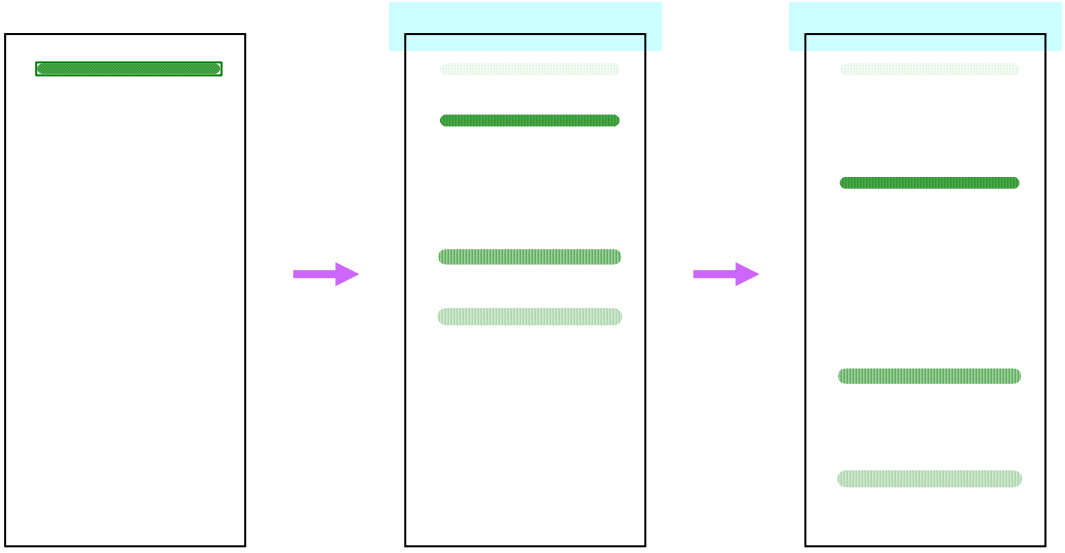
Paper partition chromatography (PPC)

圓形濾紙

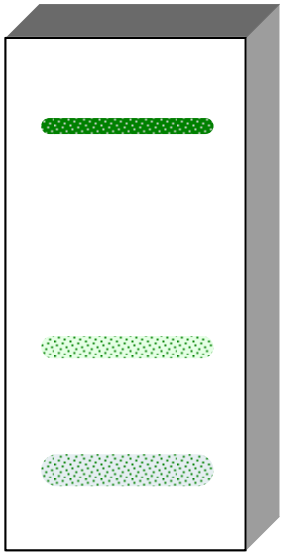


長條濾紙

加展開液

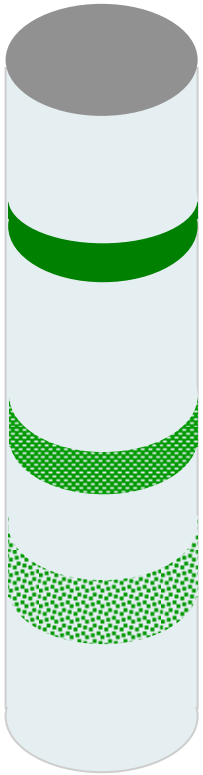


薄層層析
(TLC)



容量變大

管柱層析

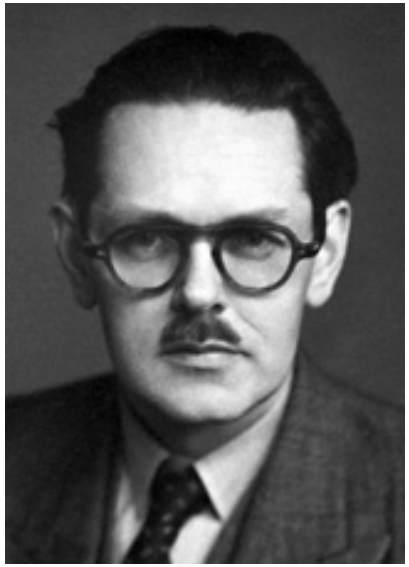


容量更大

Adapted from Scope RK (1987) Protein Purification – Principles and Practice p.9

# The Nobel Prize in Chemistry 1952

## for their invention of partition chromatography



**Archer John Porter Martin**
**National Institute for Medical Research**
**London, United Kingdom**

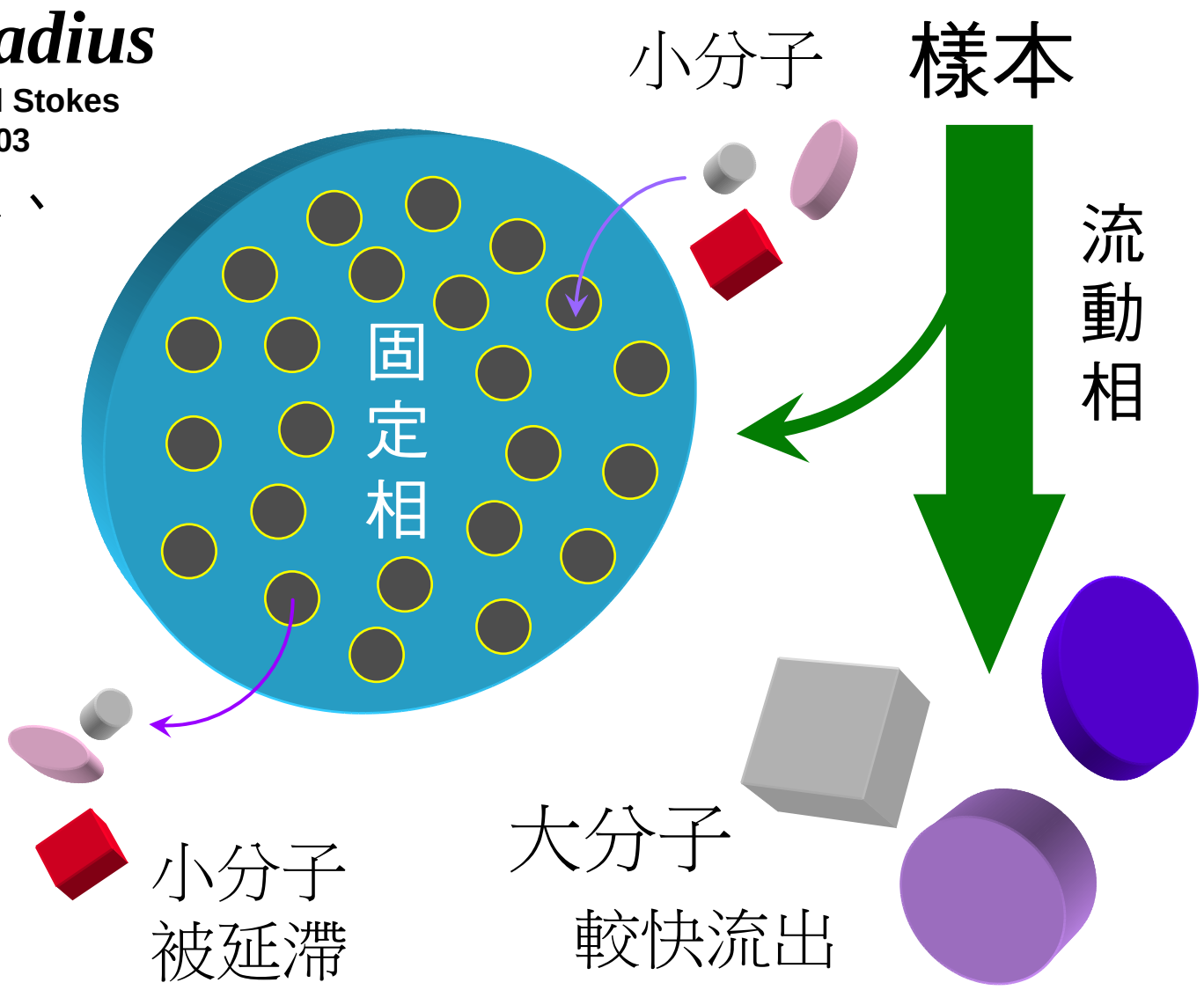
**Richard Laurence Millington Synge**
**Rowett Research Institute**
**Bucksburn (Scotland), United Kingdom**

# 膠體過濾法是一種 Partition 層析法：

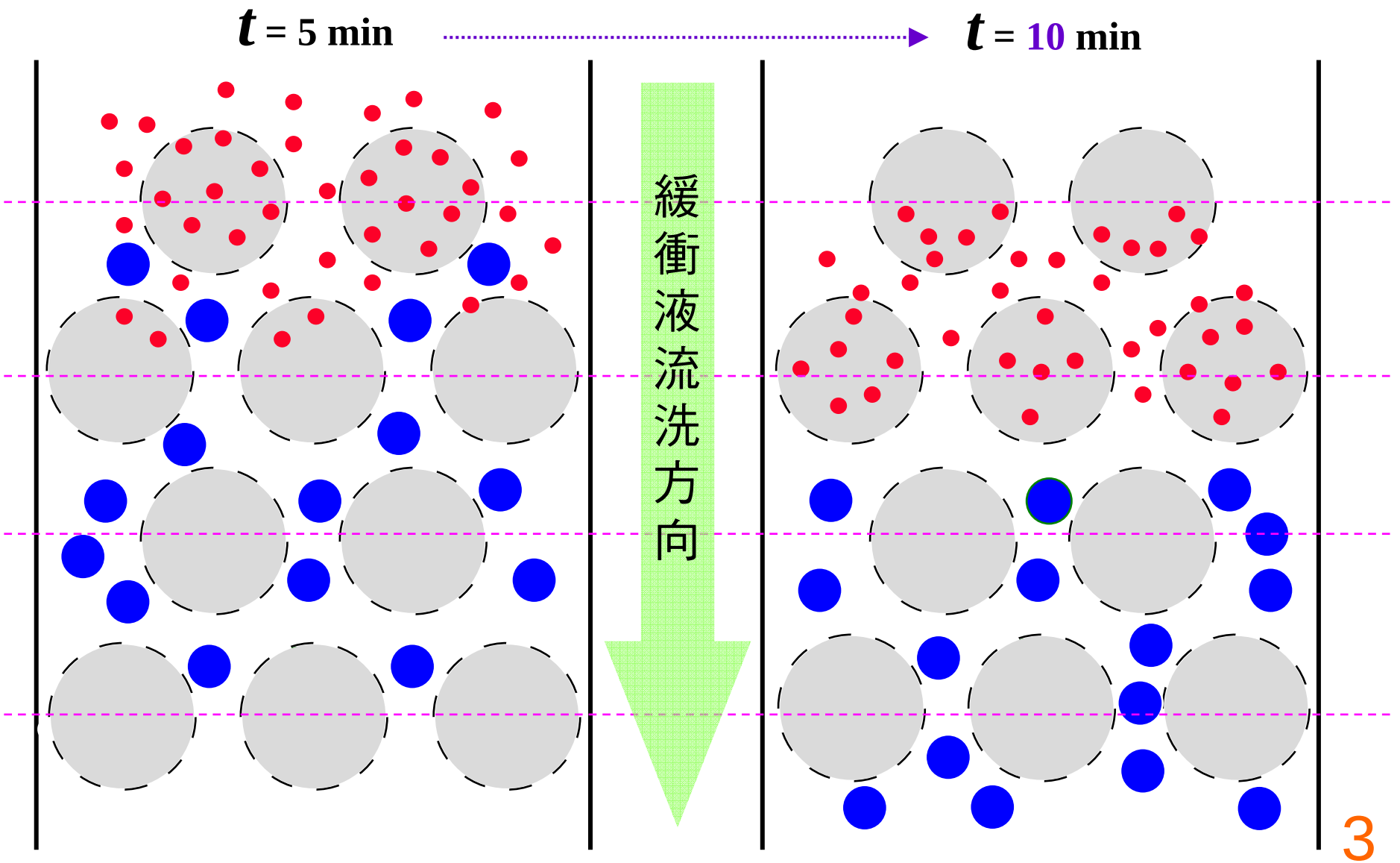
***Stokes radius***

**George Gabriel Stokes**
**1819 - 1903**

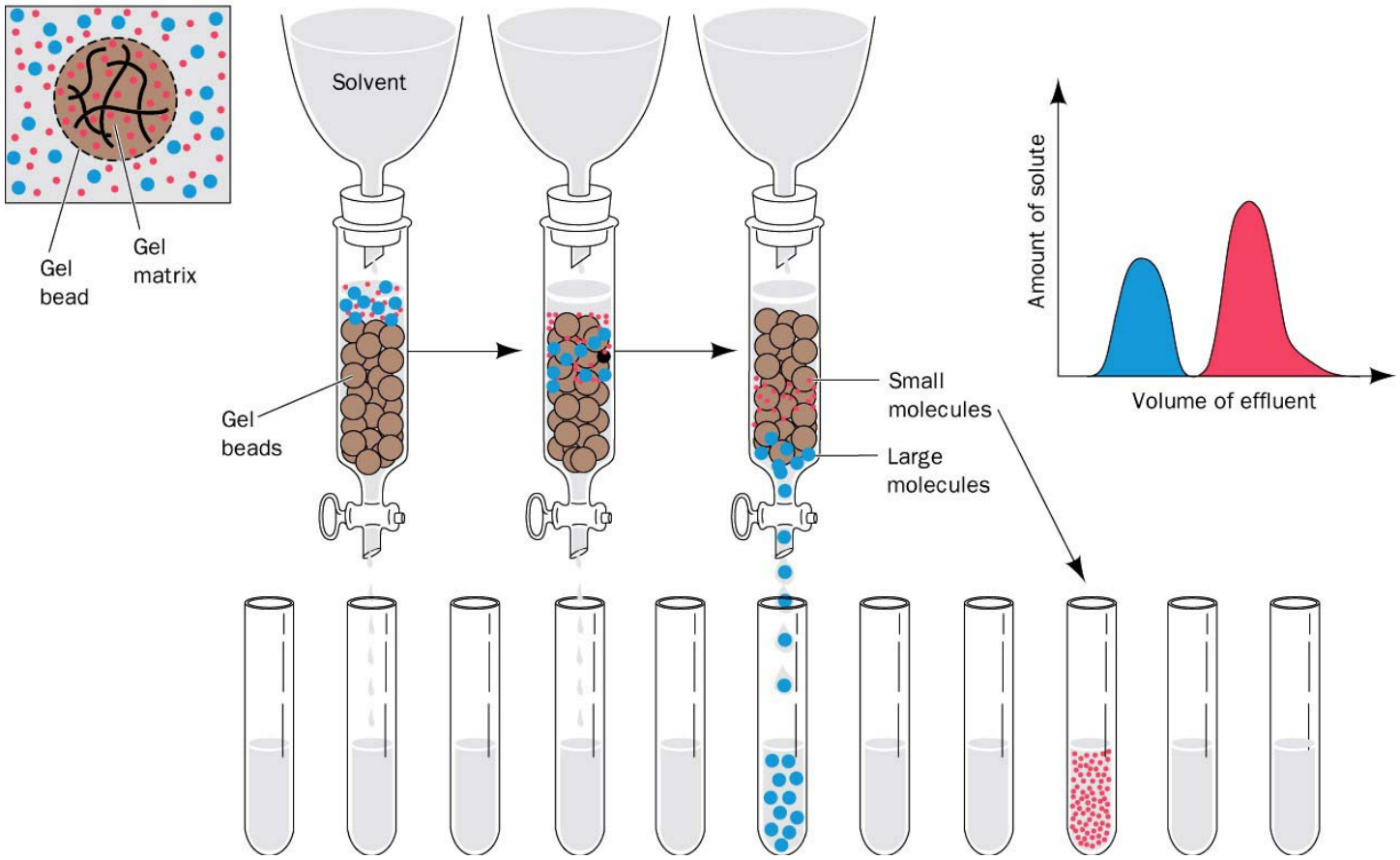
分子大小、
形狀



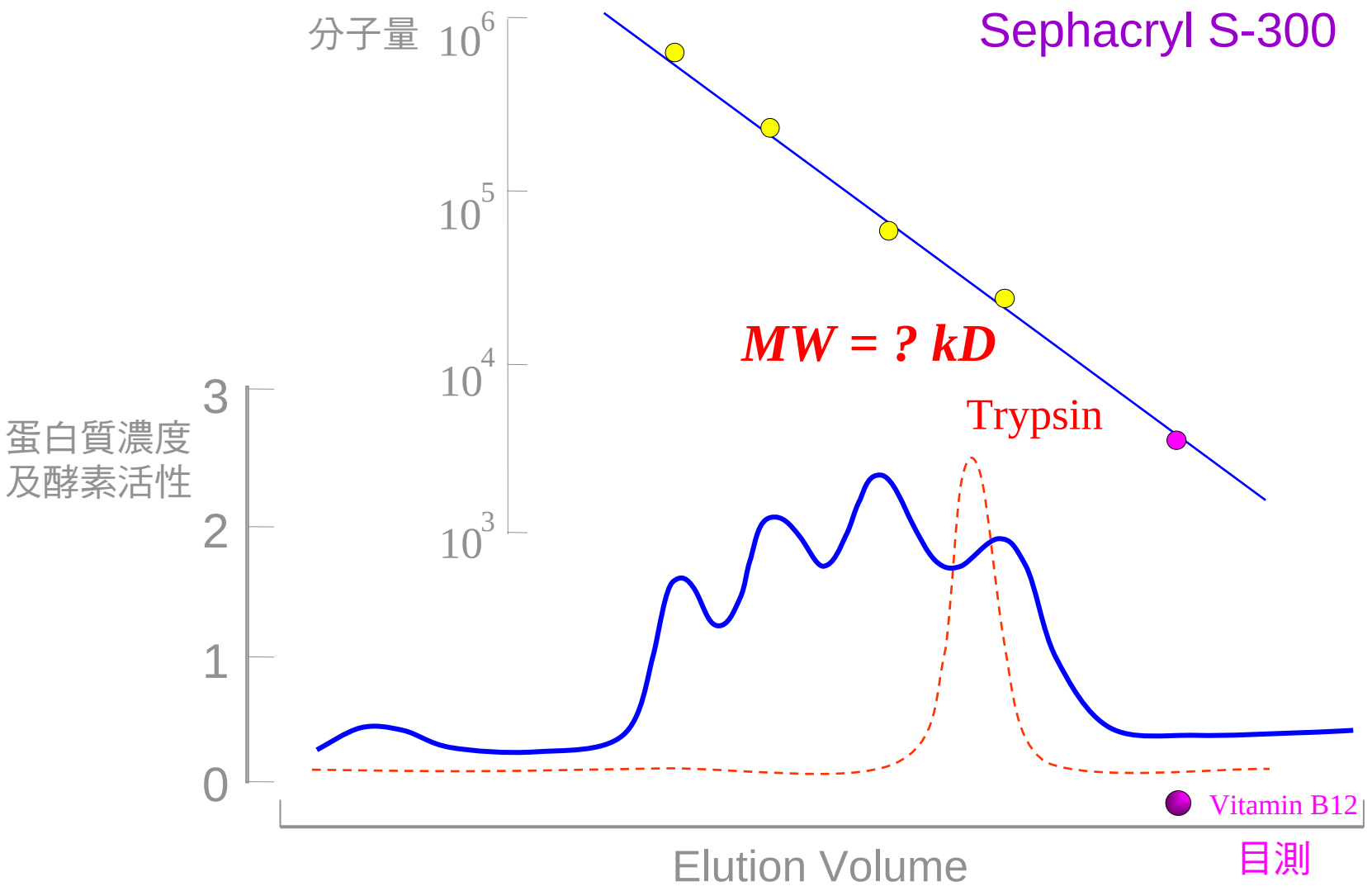
# 膠體過濾法：Separation, Desalting and Exchange buffer



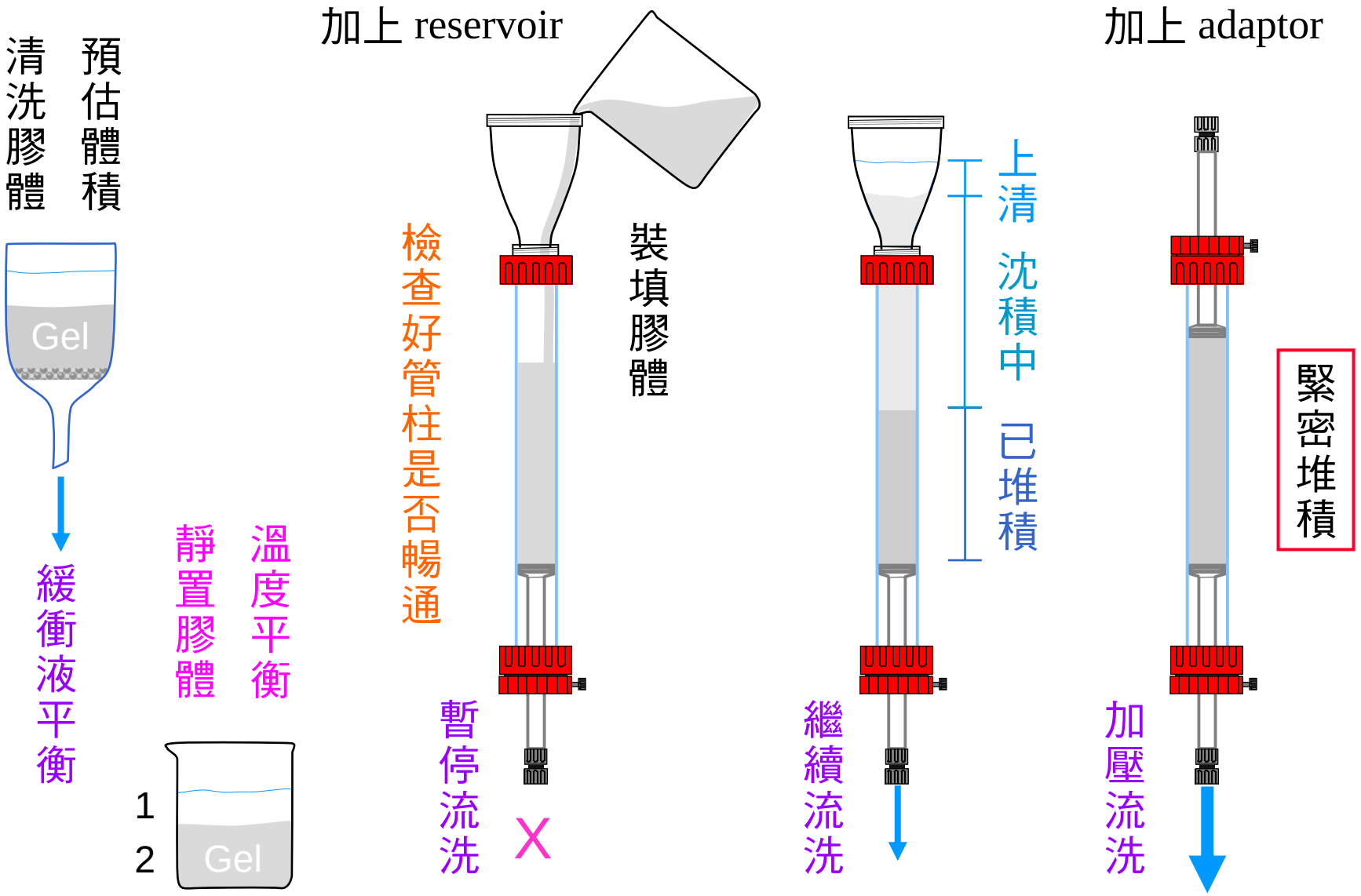
# 膠體過濾法：


Gel bead
Gel matrix
Solvent
Gel beads
Small molecules
Large molecules
Amount of solute
Volume of effluent

# 以膠體過濾法決定蛋白質分子量



# 膠柱的裝填方法：


預估體積
清洗膠體
Gel
緩衝液平衡
溫度平衡
靜置膠體
1
2
Gel
加上 reservoir
檢查好管柱是否暢通
裝填膠體
暫停流洗
X
上清
沈積中
已堆積
繼續流洗
加上 adaptor
緊密堆積
加壓流洗