3.3 離子交換法 Ion exchange

- 3.3.1 原理概述 Basic principles

  離子與固相擔体帶電基團間的爭奪戰 (Ion wars)

- 3.2.2 交換介質 Exchange materials

  是帶有電荷基團的多醣長鏈聚合物 (膠球)

- 3.3.3 緩衝液與層析系統 Buffer system

  緩衝液的影響極大

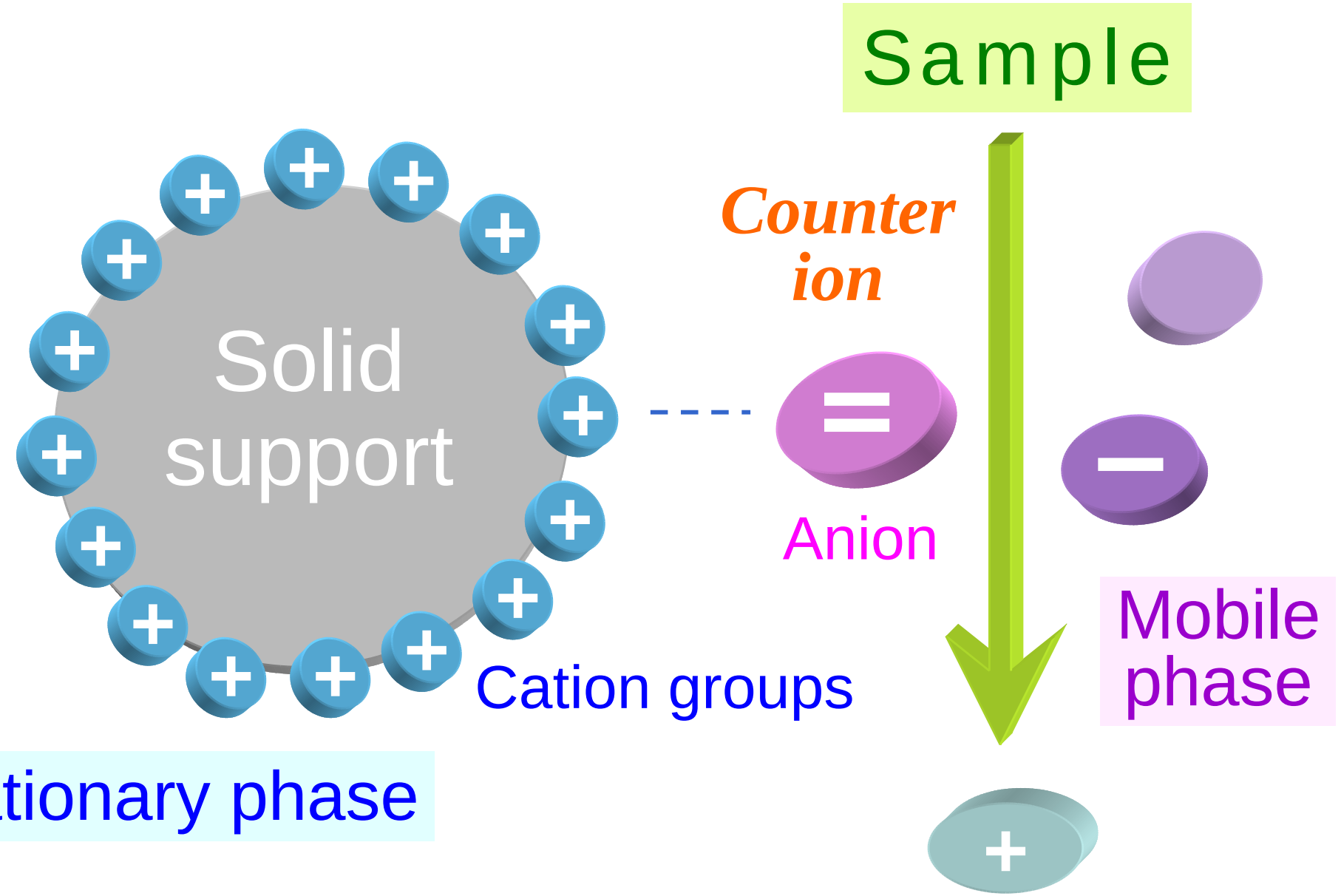
- 3.3.4 管柱操作方法 Column operation

  如何操作一支離子交換管柱

- 3.3.5 色層焦集法 Chromatofocusing

  依蛋白質等電點之差異來進行分離

陰離子交換法 Anion Exchange

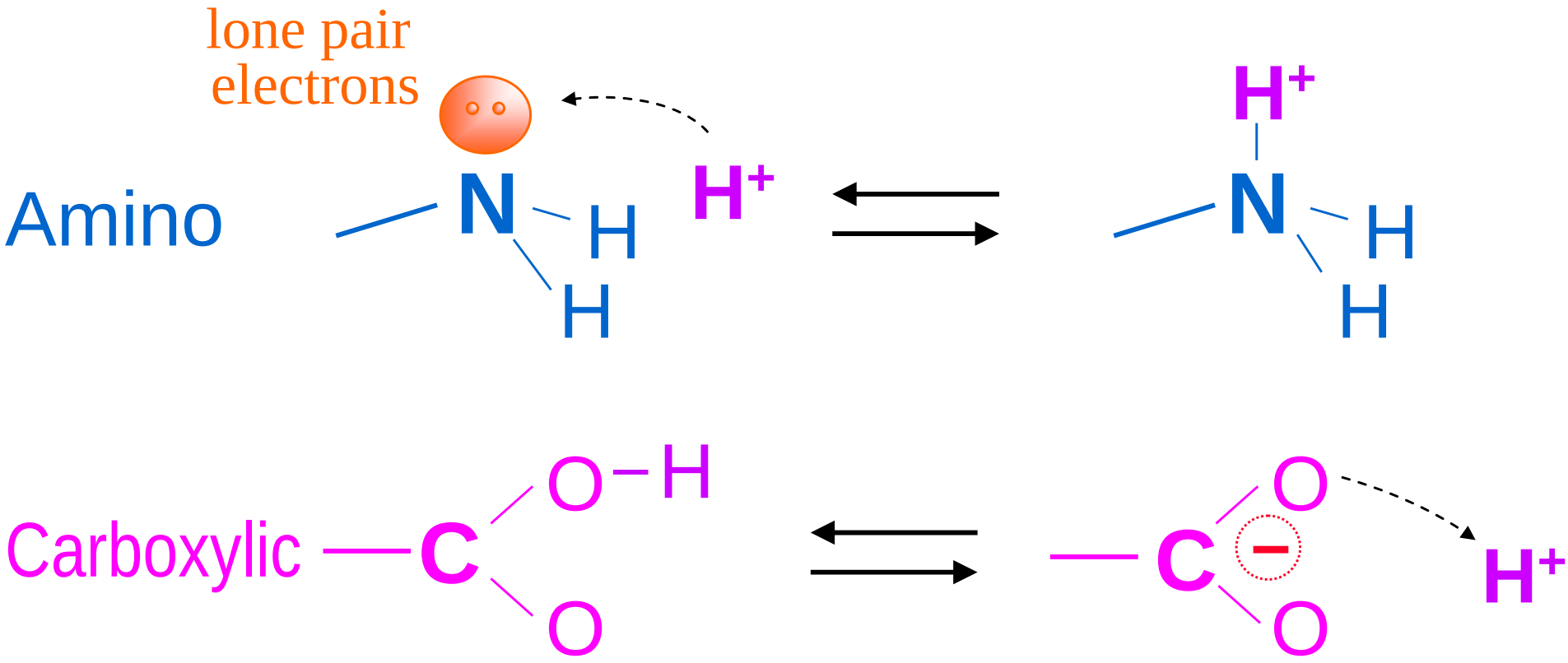


Ion exchange is an adsorption chromatography

Juang RH (2005) EPA

質子可以吸著或脫離一基團

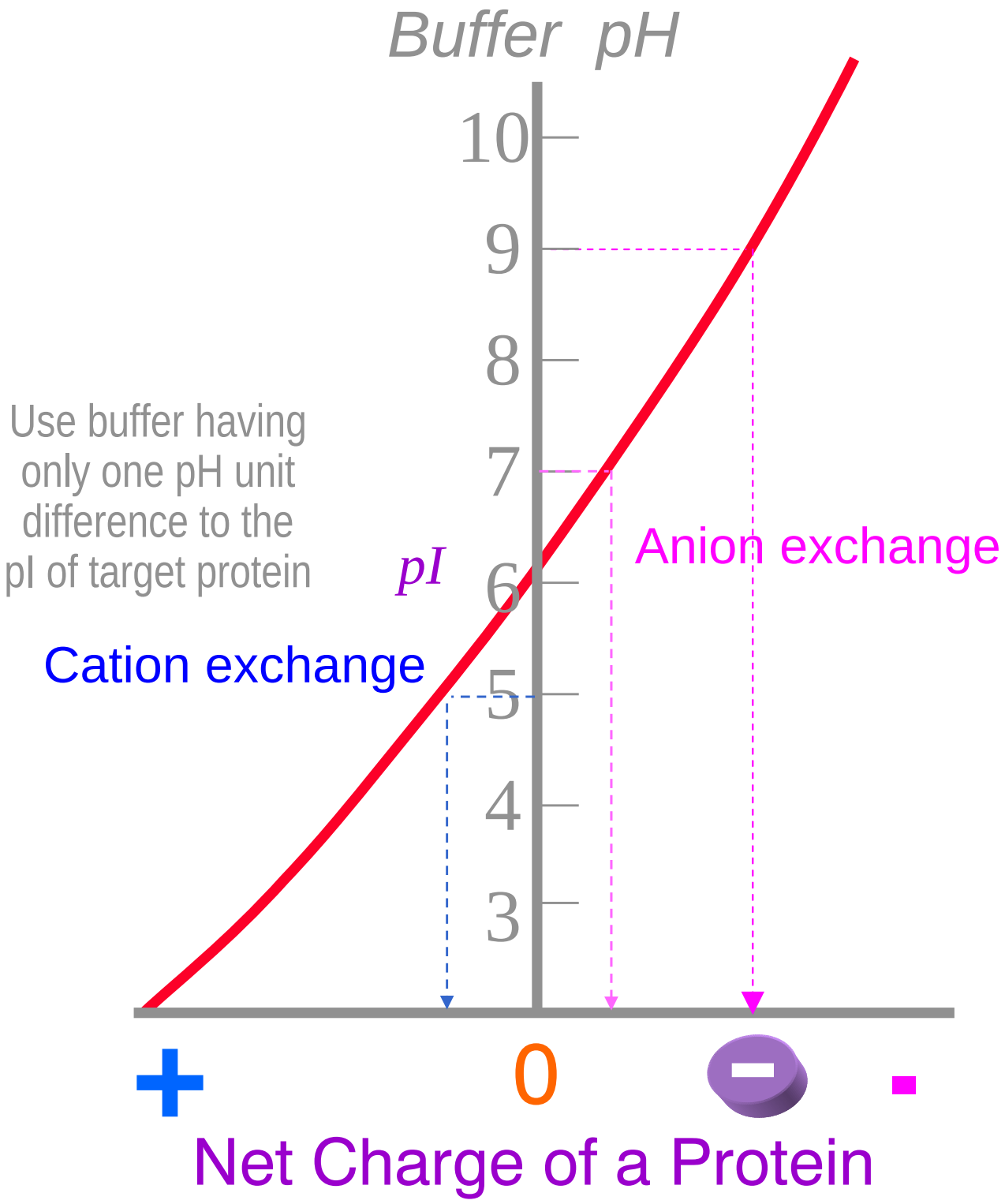
Proton： the smallest and most abundant particle in the living cell controlling the pH and the charge property of a molecule



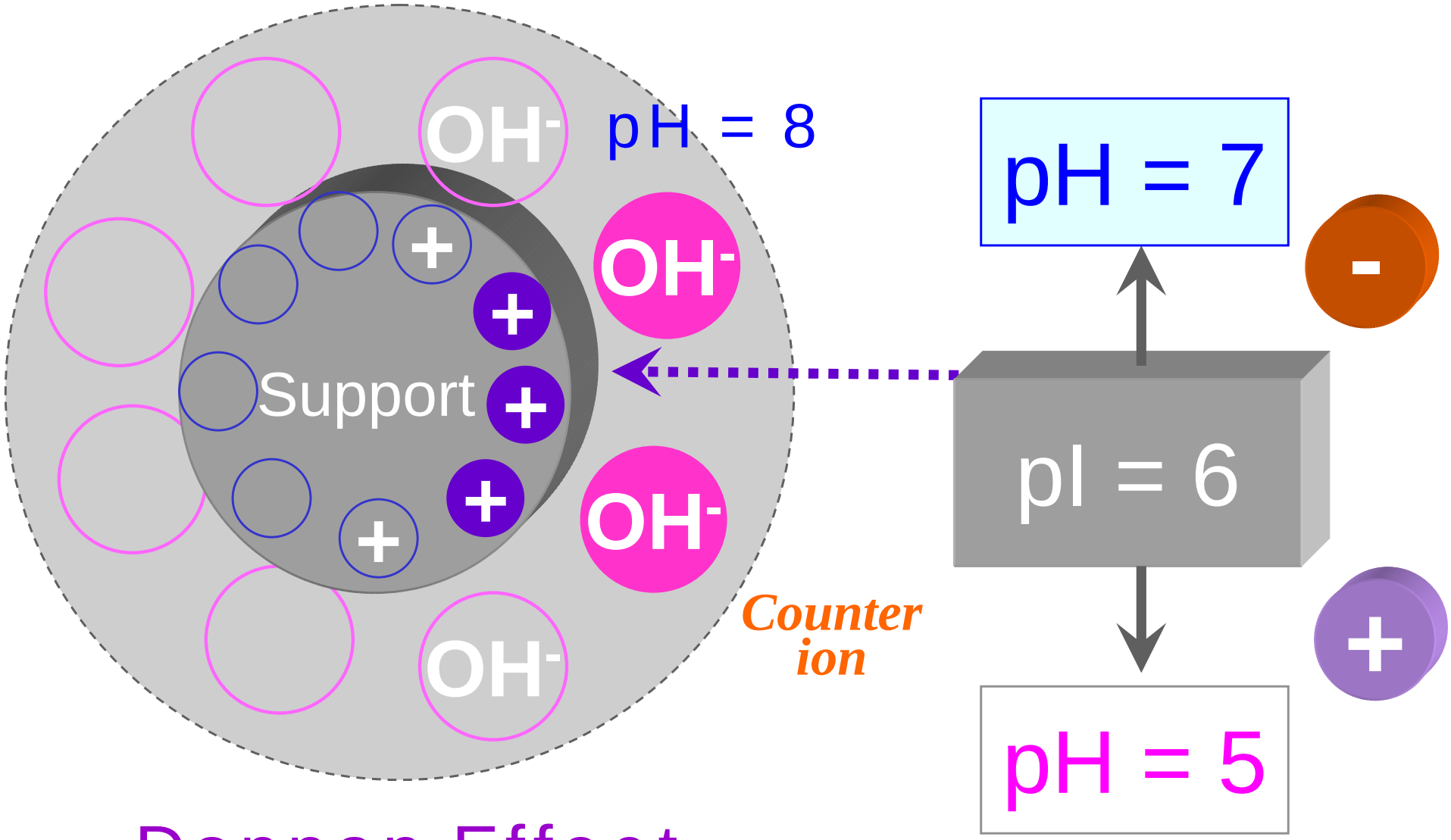
Ampholyte: a molecule contains both positively and negatively charged groups

Proton can enter or leave a functional group relatively freely

選擇離子交換介質


Buffer pH
10
9
8
7
6
5
4
3
Use buffer having only one pH unit difference to the pI of target protein
pI
Anion exchange
Cation exchange
+
0
-
Net Charge of a Protein

陰離子交換膠體的 pH 變化

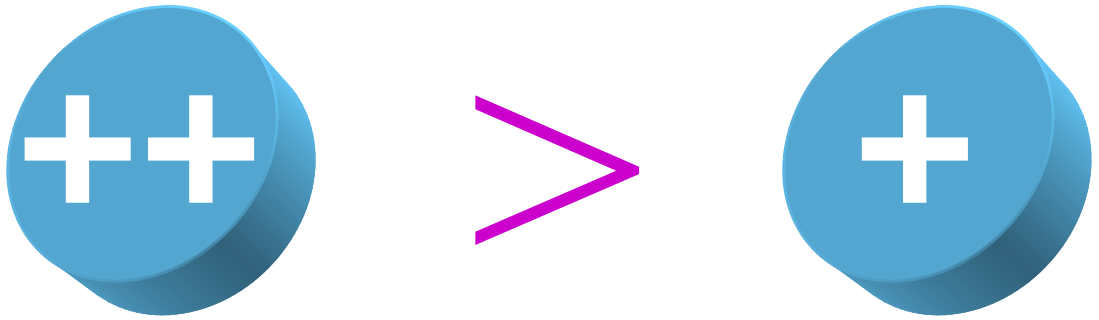


The pH change of the microenvironment surrounding the ion exchange gel particle

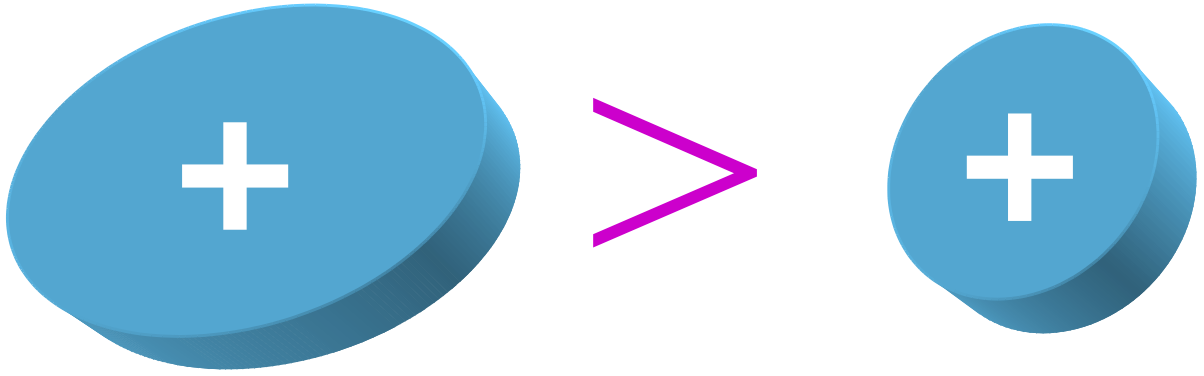
Juang RH (2005) EPA

離子取代優先順序 Displacing order of ions

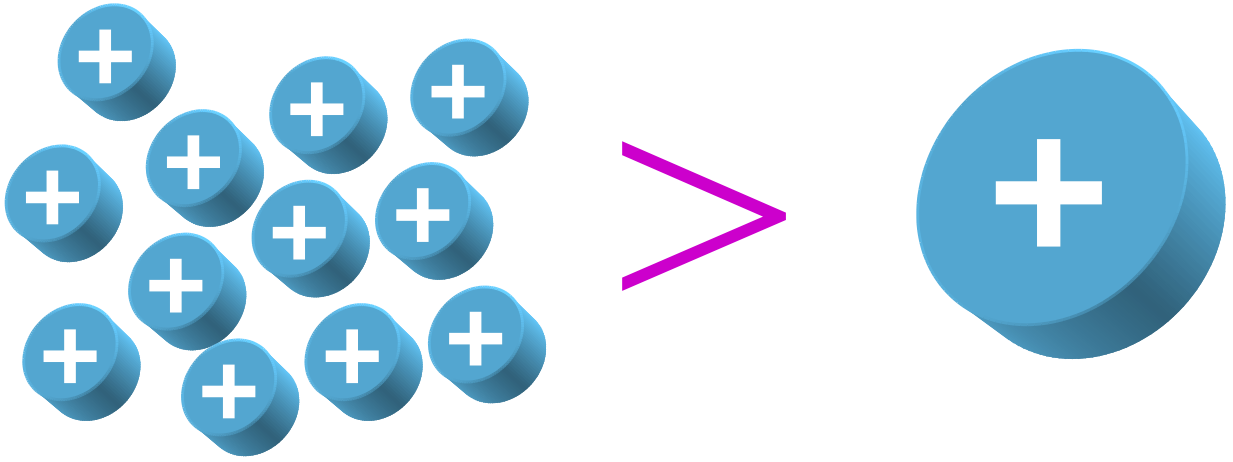
電荷高者
(higher charge)



離子大者
(larger ion)



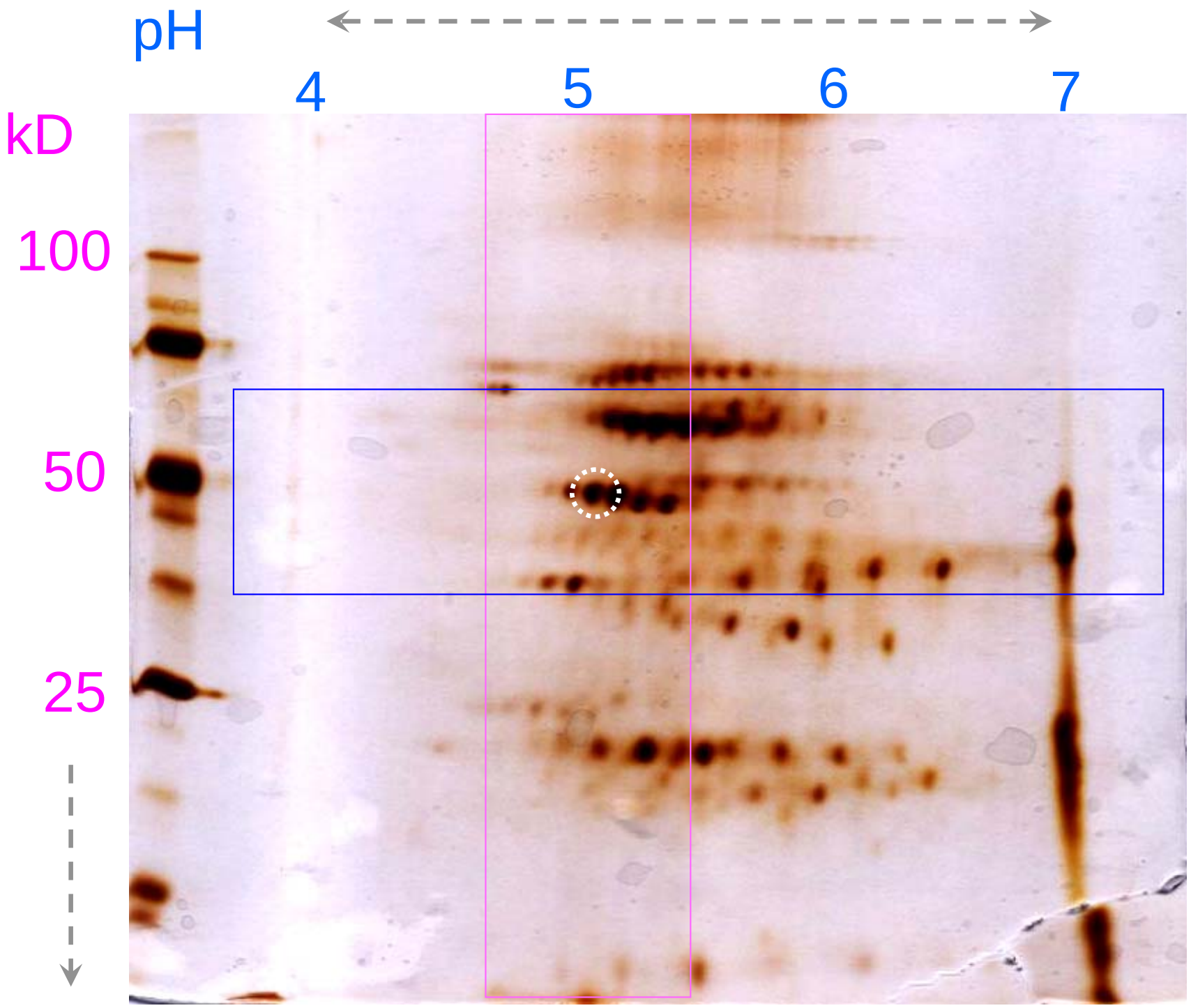
濃度大者
(higher concentration)
change pH, NaCl gradient



Key properties determining the order of ion replacement

Juang RH (2005) EPA

陰離子交換法的分離範圍



The approximate range of proteins isolated by chromatography

Juang RH (2005) EPA

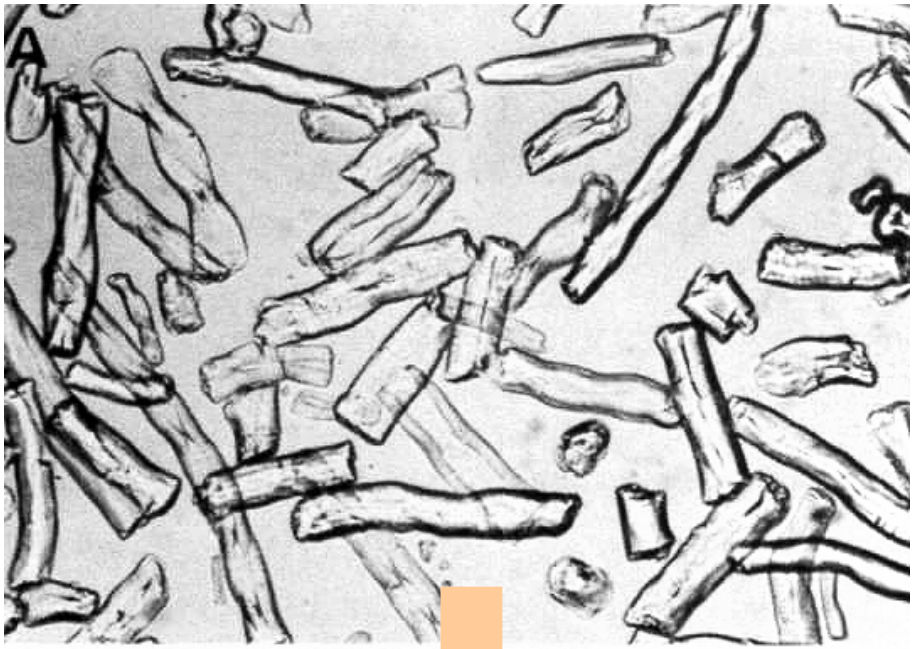
離子交換介質 Common ion exchange materials

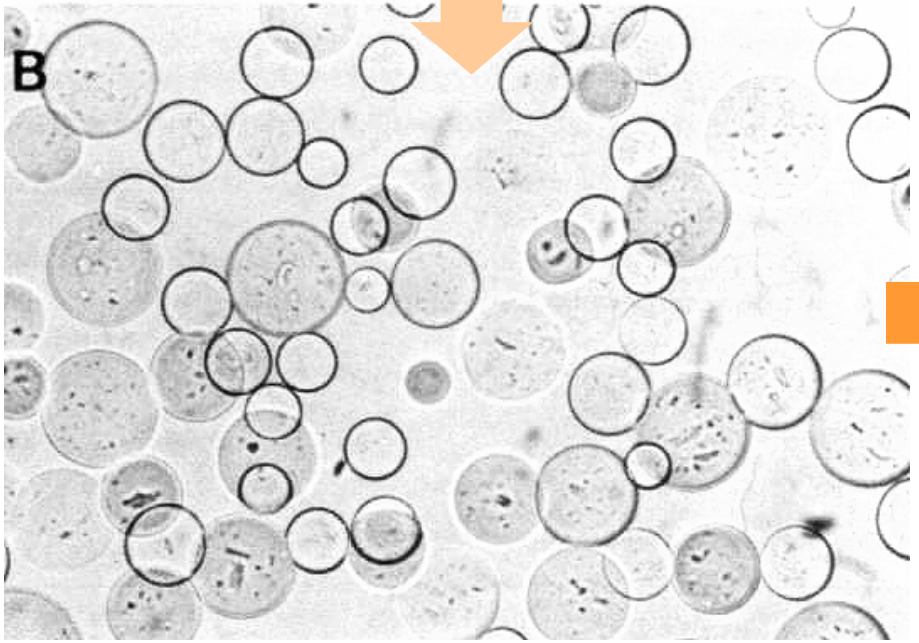
Classification		Resin / Polystyrene		Glycan / Cellulose = X		Mono bead
Anion Exchanger	Strong	Dowex-1 Dowex-2	$-\overset{+}{N}R_3$	TEAE-X (QAE-X)	$-\overset{+}{N}R_3$	Q
	Weak	Dowex-3 IR-45	$-\overset{+}{N}HR_2$	DEAE-X	$-OCH_2CH_2\overset{+}{N}HR_2$	
Cation Exchanger	Strong	Dowex-50	$-SO_3^-$	Phospho-X	$-PO_4^{2-}$	S
	Weak	IRC-150	$-COO^-$	CM-X	$-OCH_2COO^-$	

X = Sephadex, Sepharose, Sephacel or cellulose

Juang RH (2005) EPA

膠體的組成與外型 Support material and bead





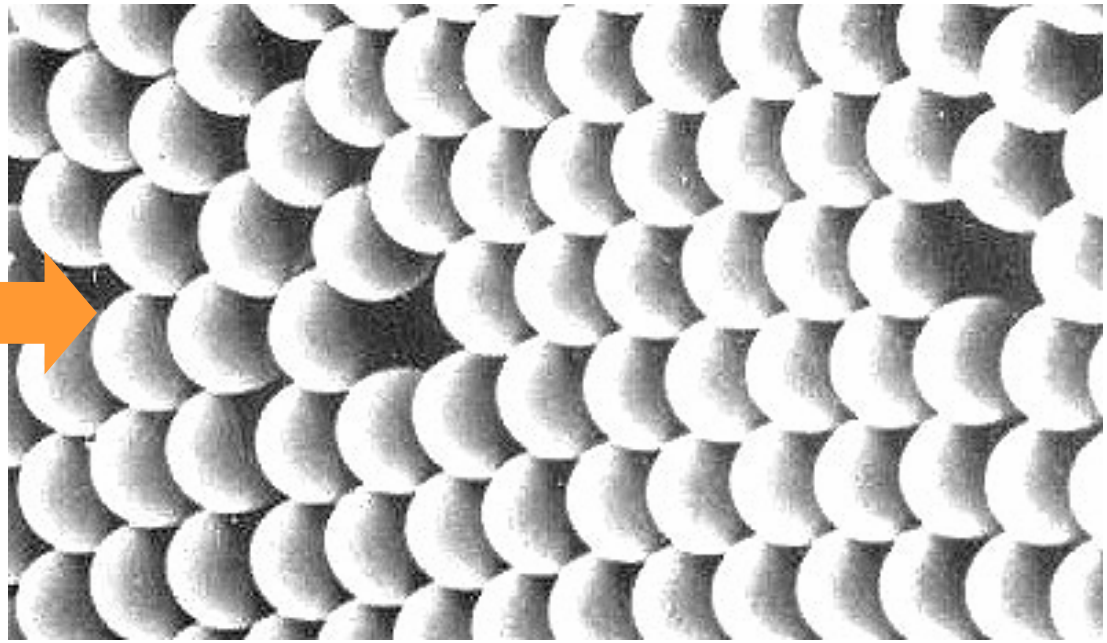
Pharmacia (1980) Separation News: 5

Cellulose

Sephacel

Monobead

- Homogeneous bead shape is critical to its resolution and flow rate



Pharmacia (1991) Ion Exchange Chromatography – Principles and Methods p.24

不同膠體的吸著容量有很大差異

Adapted from Pharmacia (1991) Ion Exchange Chromatography – Principles and Methods p.64

	Lactalbumin	Albumin	Ferritin
DEAE-Sephadex A-25	191	31	2
DEAE-Sephadex A-50	10	102	1
DEAE-Sepharose CL-6B	45	115	4.3
DEAE-Sephacel	38	86	8.6

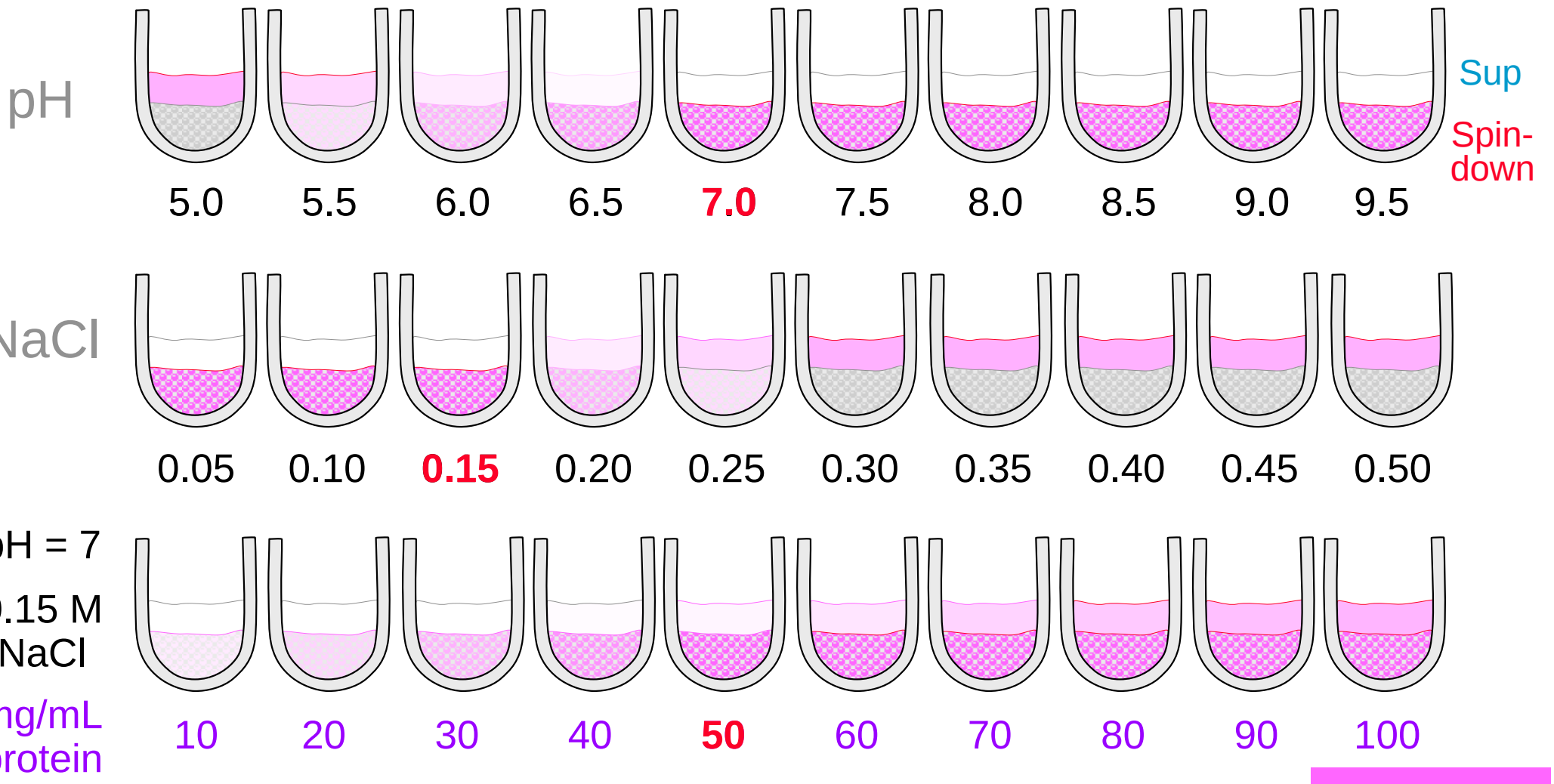
Buffer: 0.01 M Tris-HCl, pH 8.0

mg / mL gel

Each gel has different adsorption capacity toward different target proteins

離子交換法預備試驗 Determine the conditions

Enzyme + Buffer + Gel → Incubation → Spin → Sup + Spindown



Capacity

Adapted from Pharmacia (1991) Ion Exchange Chromatography – Principles and Methods p.70

膠柱裝填方法 Packing column step by step

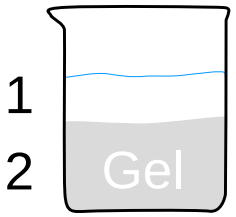
清洗膠體
Wash gel well

預估體積
Estimate
gel volume

緩衝液
平衡
Equilibrated
in buffer

靜置膠體
Gel stands o/n

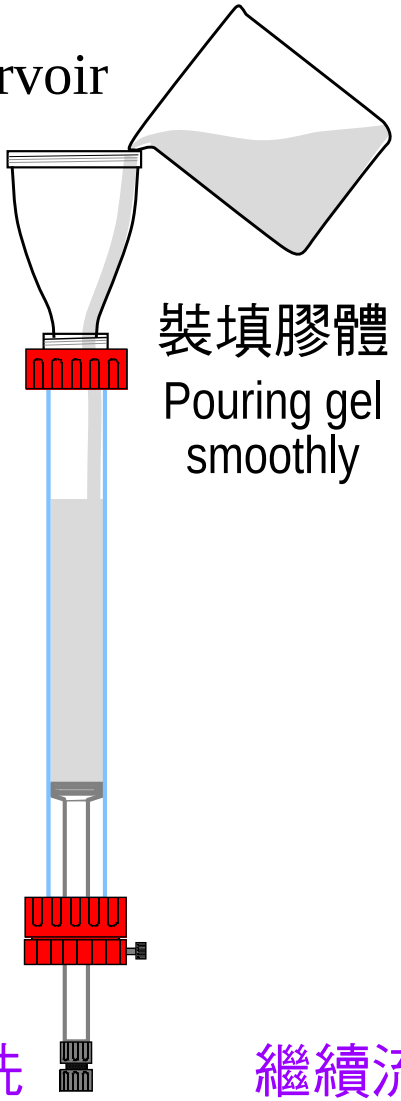
溫度平衡
Temperature
equilibrated



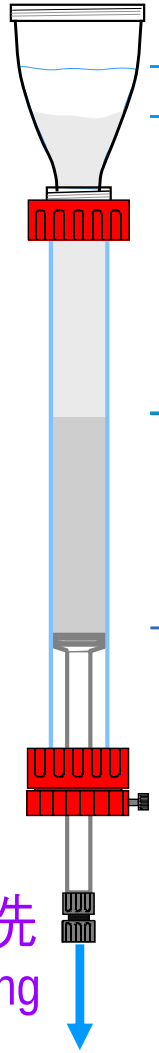
Put on reservoir

檢查管柱
是否暢通
Check flow
rate of empty
column

裝填膠體
Pouring gel
smoothly

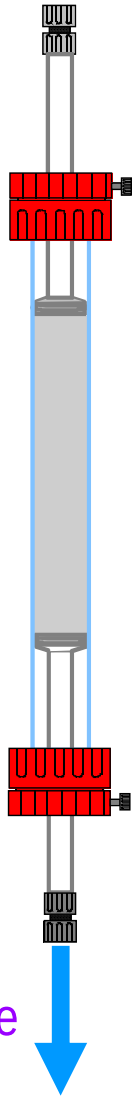


暫停流洗
Stop elution
X



繼續流洗
Keep eluting

Put on adaptor



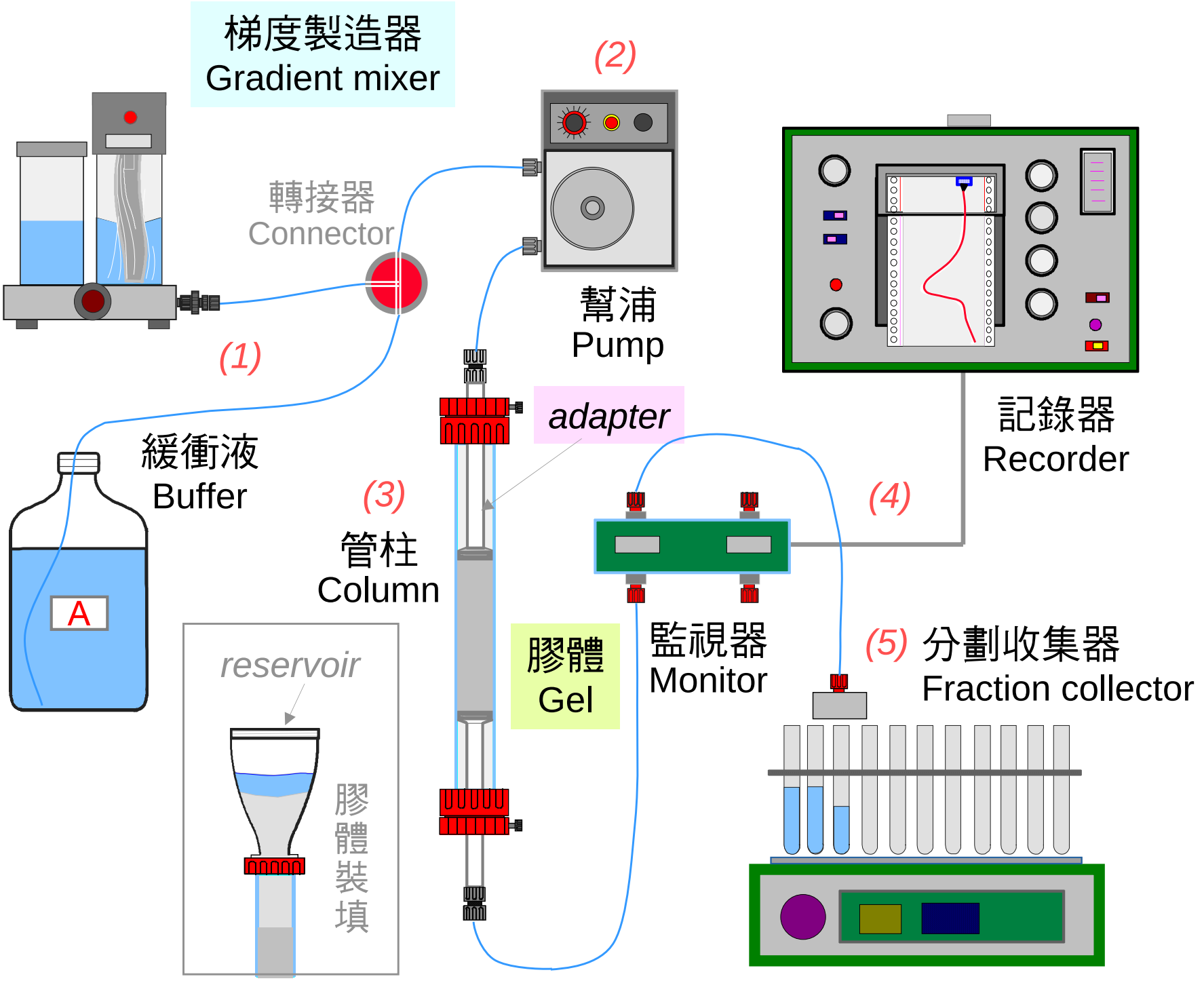
緊密堆積

加壓流洗
Elute under
High pressure

Gel should be equilibrated completely before packing

Juang RH (2005) EPA

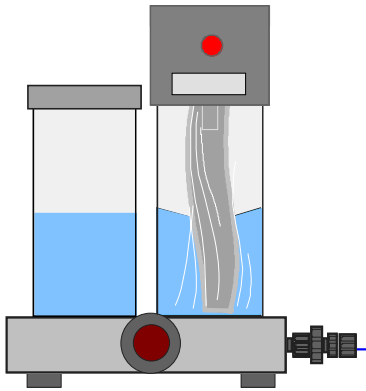
液相層析管柱系統



The whole family of liquid chromatography apparatus

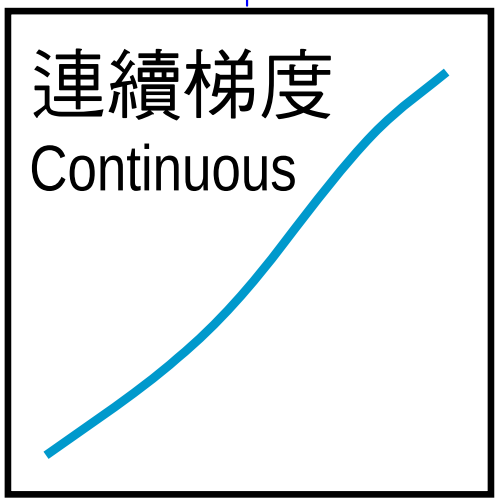
Juang RH (2005) EPA

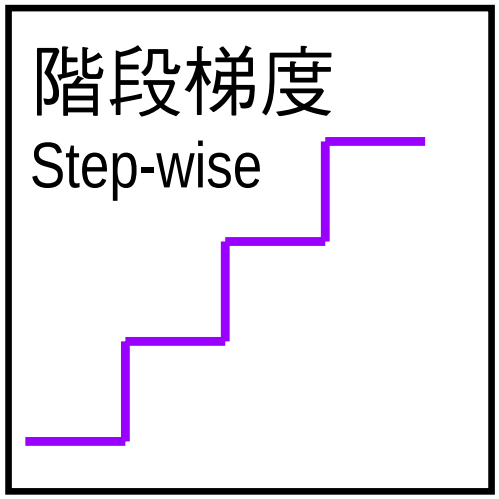
鹽梯度的兩種方式 Two ways for making gradient

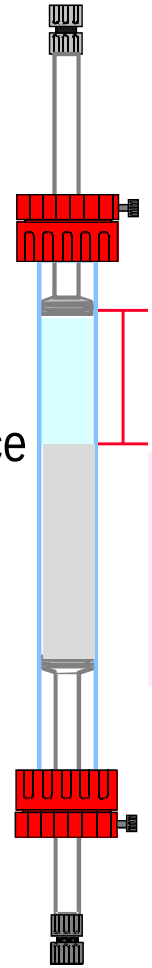


高限溶液 低限溶液

Upper-limit Lower-limit







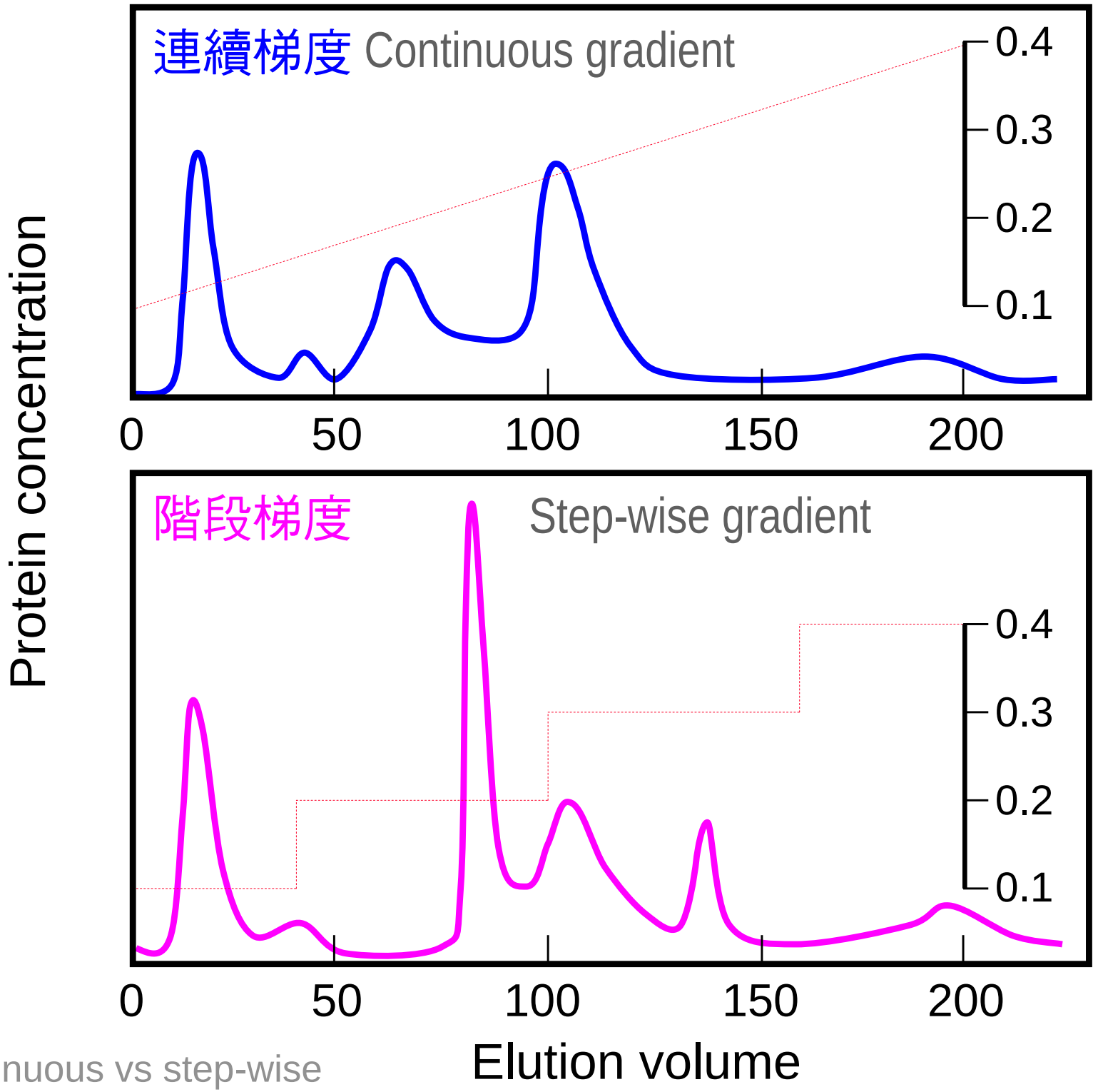
兩種方式均可，各有特點。

Both methods have their specific applications

Pharmacia

Juang RH (2005) EPA

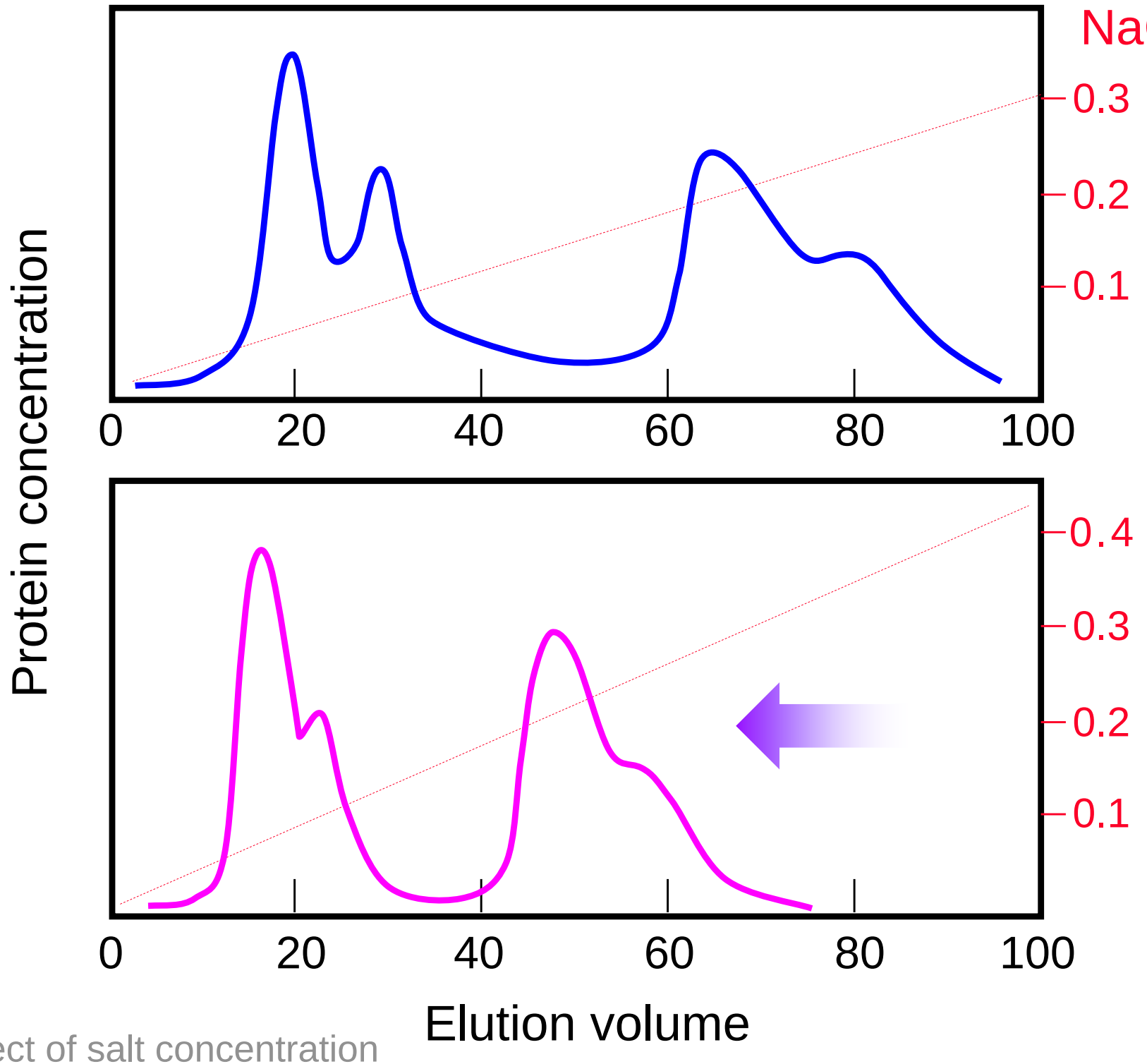
連續與階段梯度比較



Continuous vs step-wise

Adapted from Pharmacia: Ion Exchange Chromatography – Principles and Methods

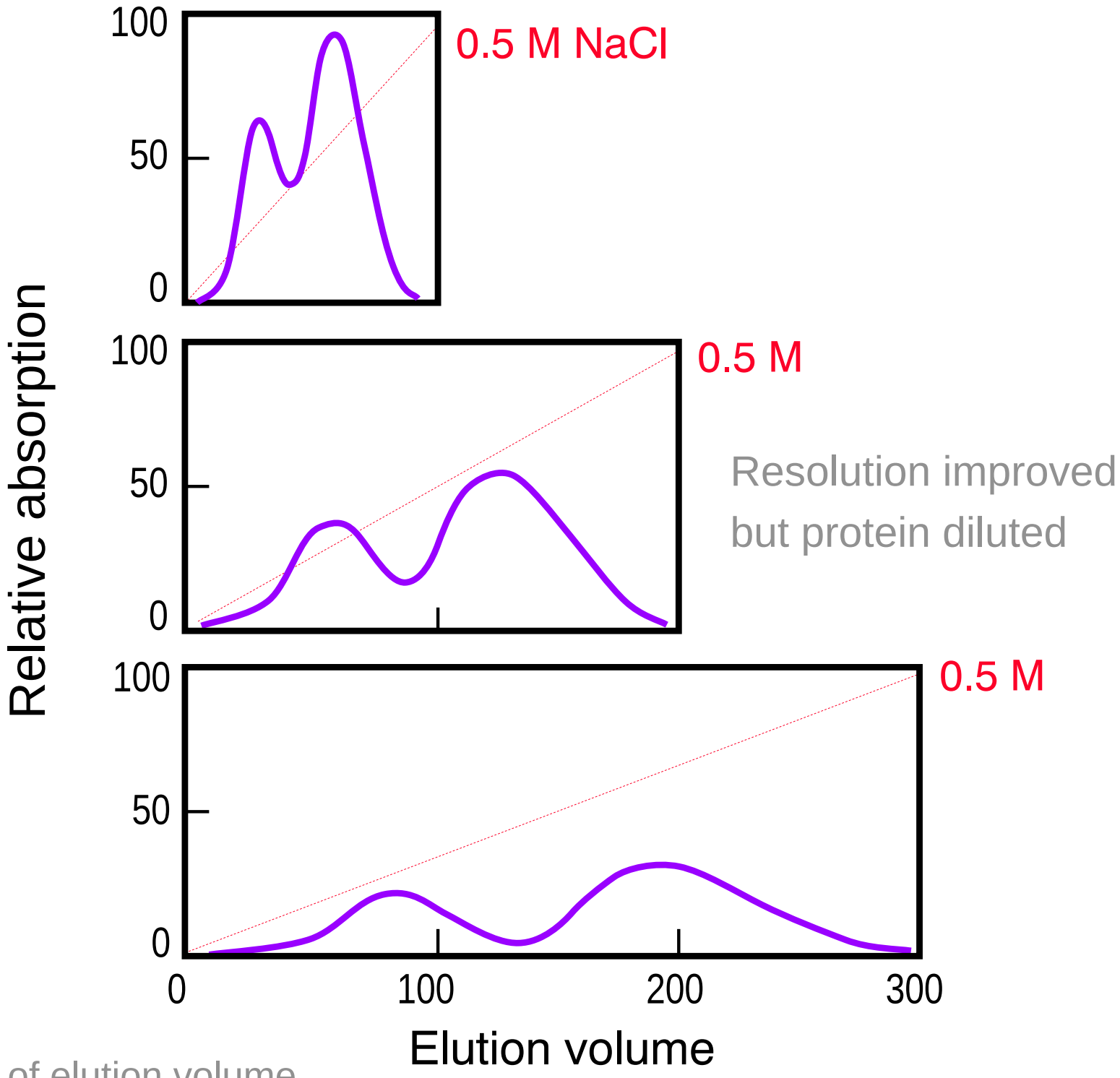
鹽濃度比較



Effect of salt concentration

Adapted from Pharmacia: Ion Exchange Chromatography – Principles and Methods

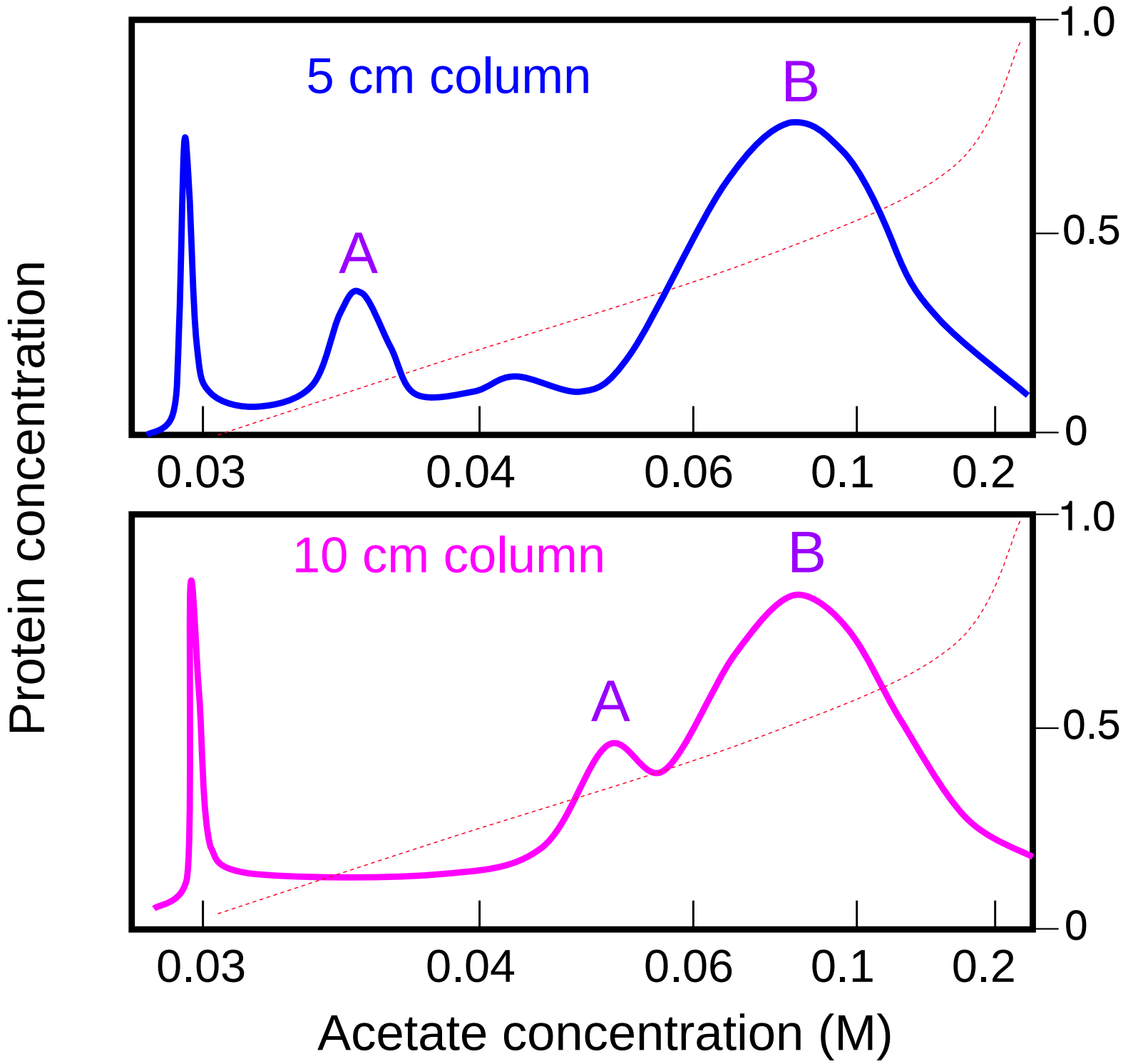
溶離體積會影響解析度



Effect of elution volume

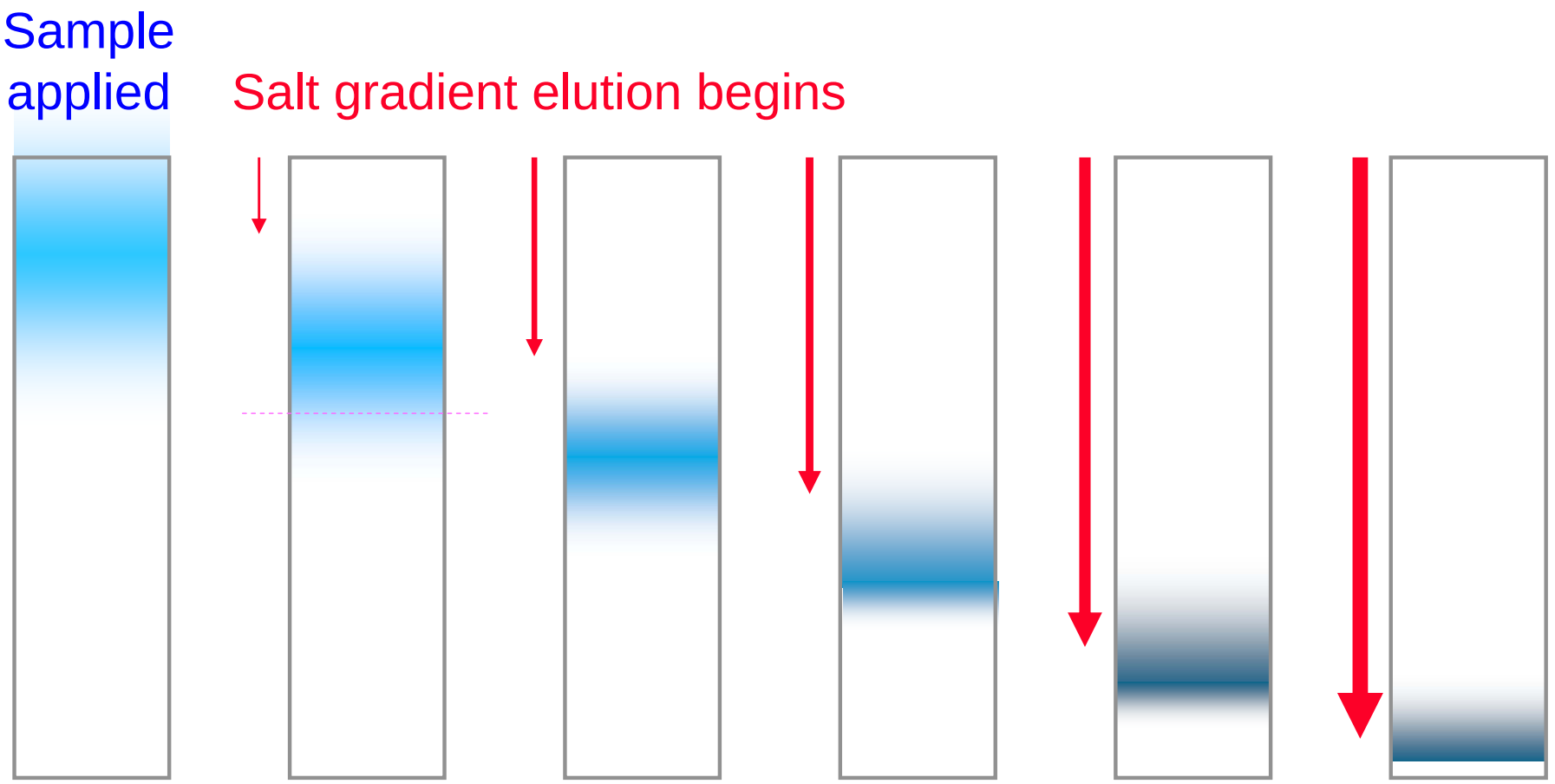
Adapted from Pharmacia: Ion Exchange Chromatography – Principles and Methods

管柱長短的影響



Effect of column length

離子交換的梯度溶離有濃縮效果



Salt elutes and brings the proteins moving forward,
but proteins are adsorbed again in the front and retarded

Salt gradient of ion exchange can concentrate the proteins in sample elution

Adapted from Scope RK (1987) Protein Purification – Principles and Practice p.112

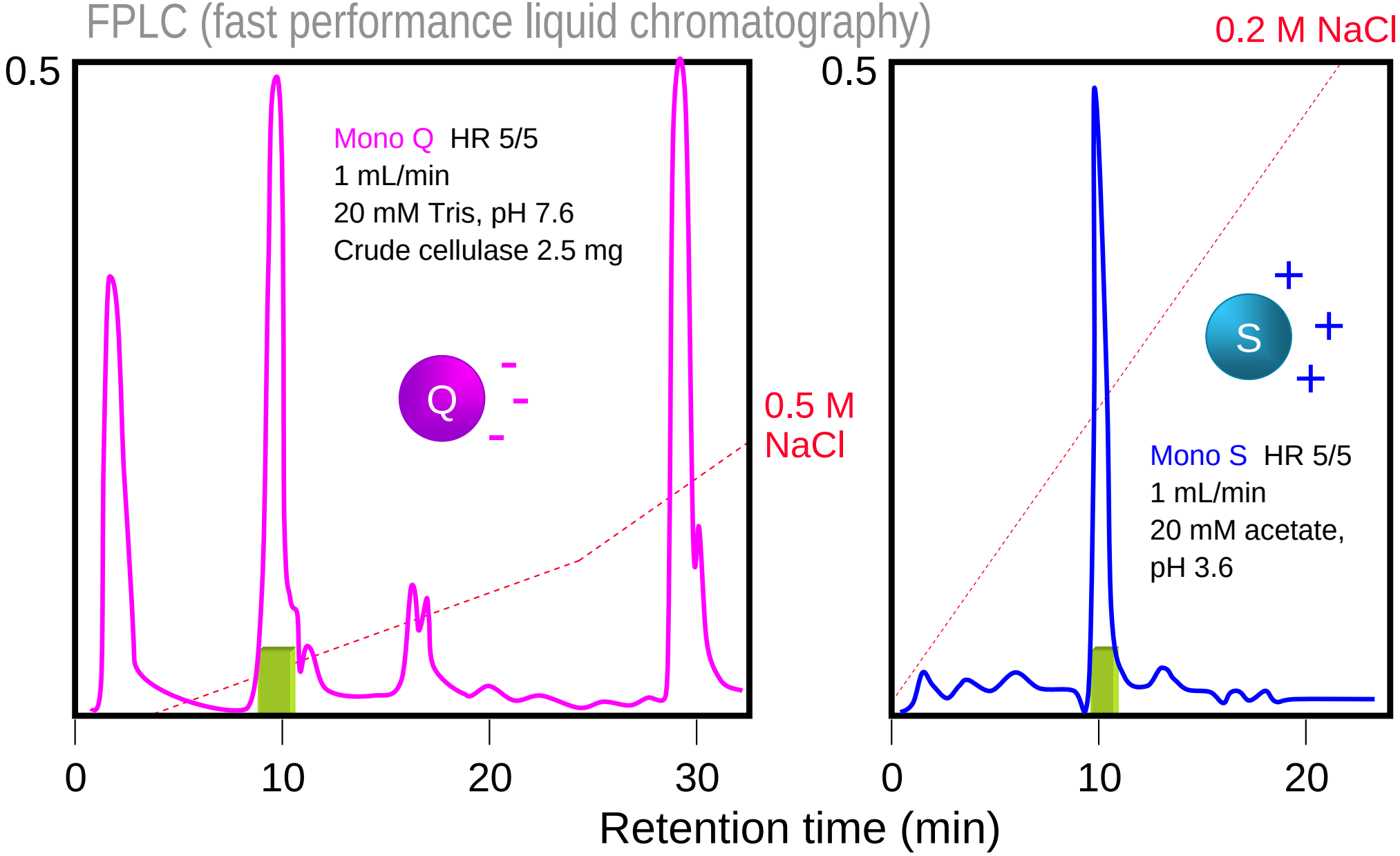
離子交換法操作要點 Summary on operation

Equilibration	Used gel should be regenerated and equilibrated well
Elution	Eluted with NaCl in continuous or step-wise gradient
Gradient	Use proper concentration and volume in elution
Buffer pH	Keep target proteins in weak charged state
Non-specific	Wash out contaminants with low NaCl concentration
Sample	Equilibrated in buffer, don’t overload column capacity
Elute through	Flow target protein through and adsorb others
Dead volume	Eliminate any dead volume in the column

Juang RH (2005) EPA

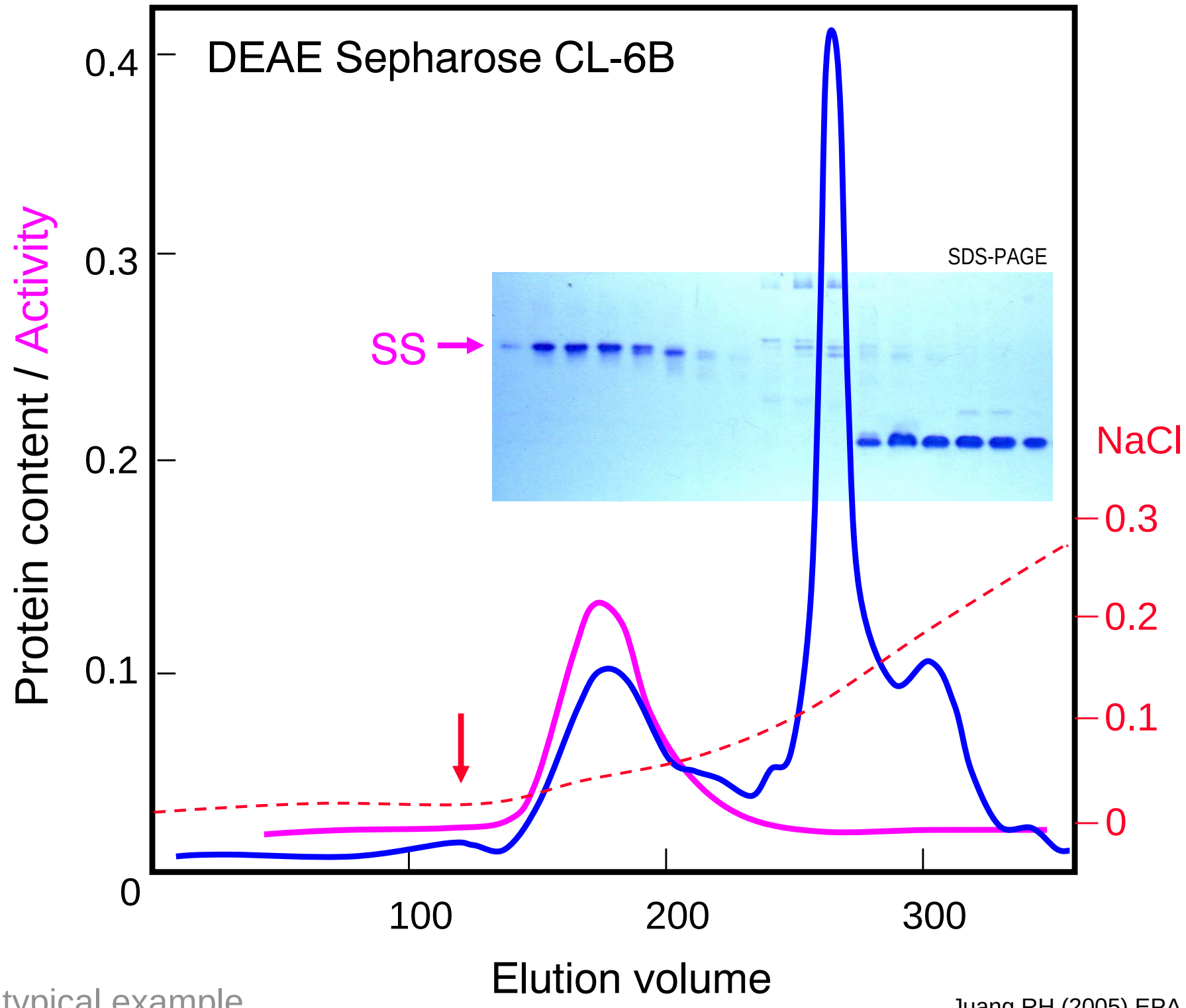
離子交換法實例 Two-step cellulase purification

FPLC (fast performance liquid chromatography)



Adapted from Pharmacia (1991) Ion Exchange Chromatography – Principles and Methods p.127

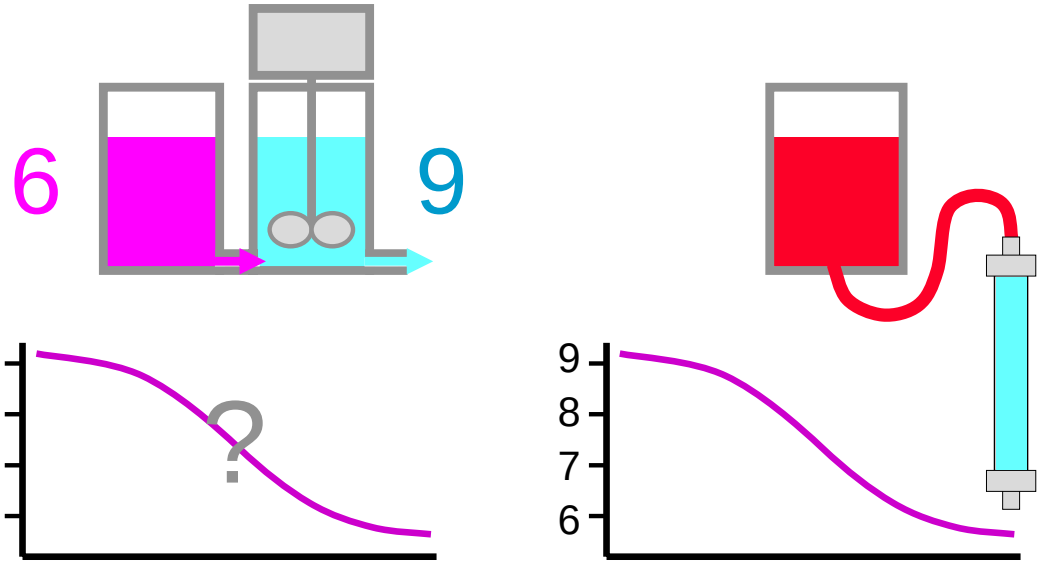
離子交換法實例



A typical example

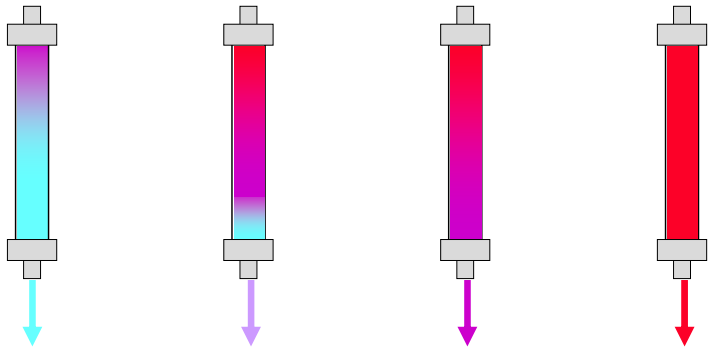
Juang RH (2005) EPA

色層焦集法如何拉出 pH 梯度

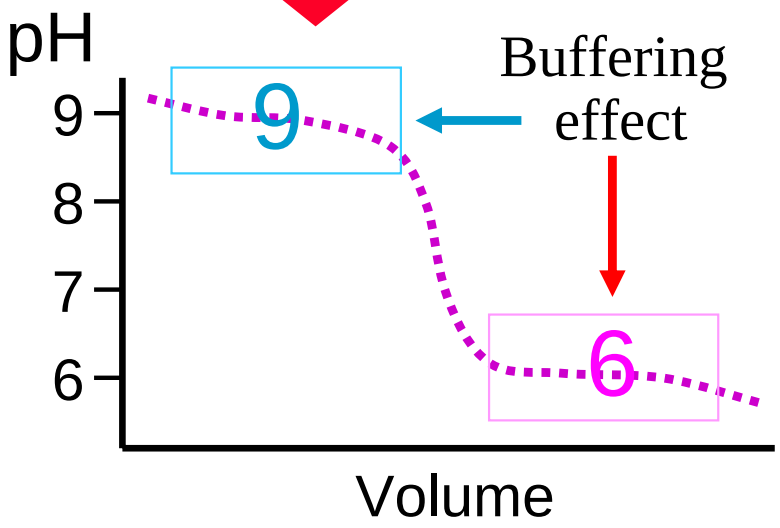


● Polybuffer 含有連續 pK_a 的緩衝分子 (ampholyte) 可拉出連續 pH 梯度

● Polybuffer contains *ampholyte* which is a mixture of chemicals having continuous pK_a

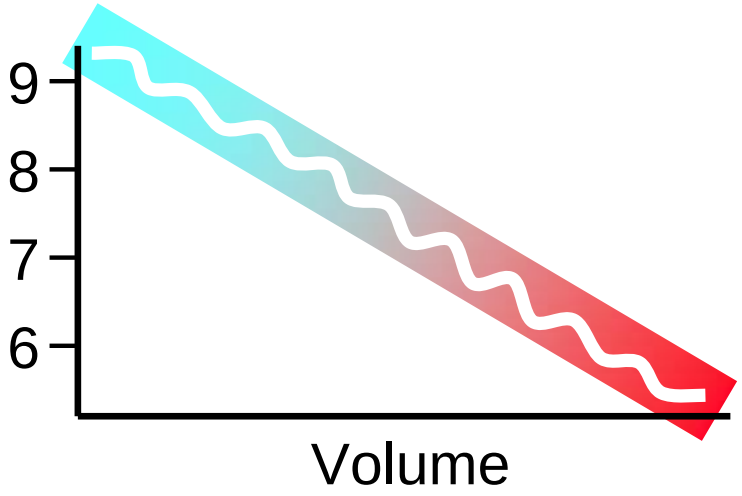


實際結果 ➡ 無法拉出 pH 梯度



假如含有許多緩衝分子 ➡

If there are many buffering molecules in the buffer…..



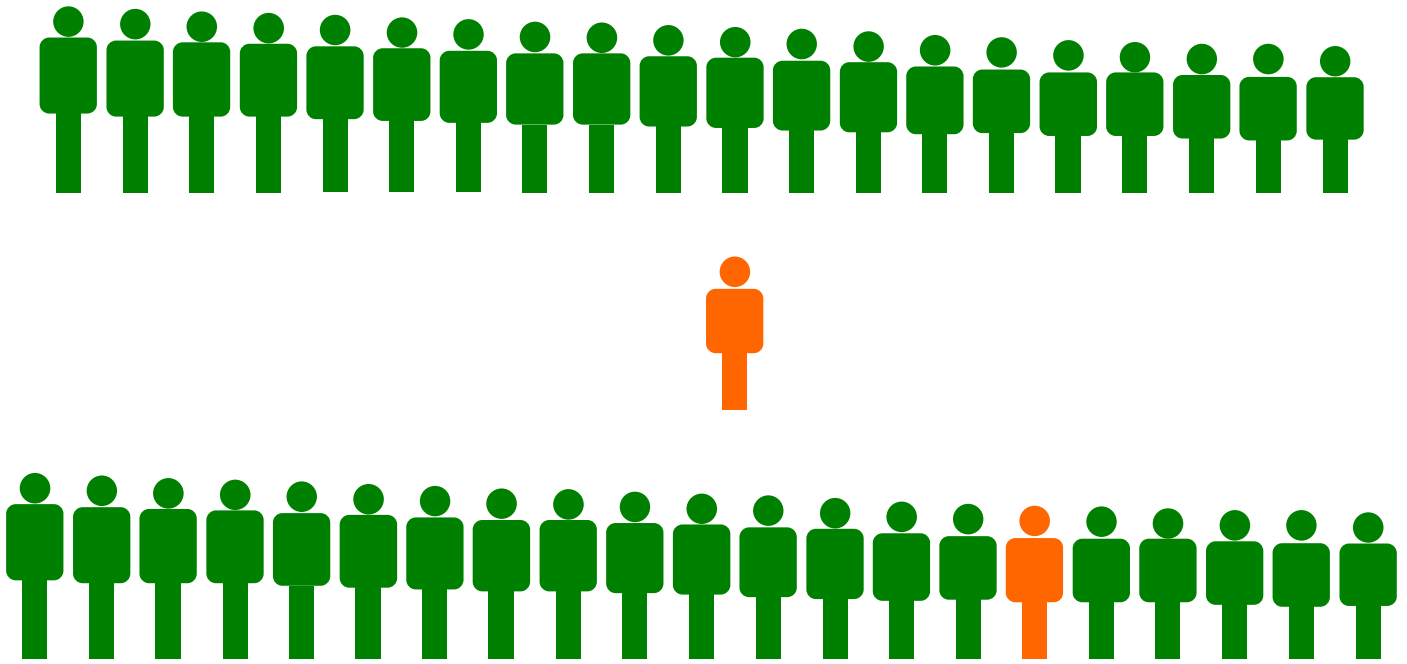
How chromatofocusing creates its pH gradient

Juang RH (2005) EPA

3.3.5 色層焦集法 Chromatofocusing

其介質也是一種離子交換介質

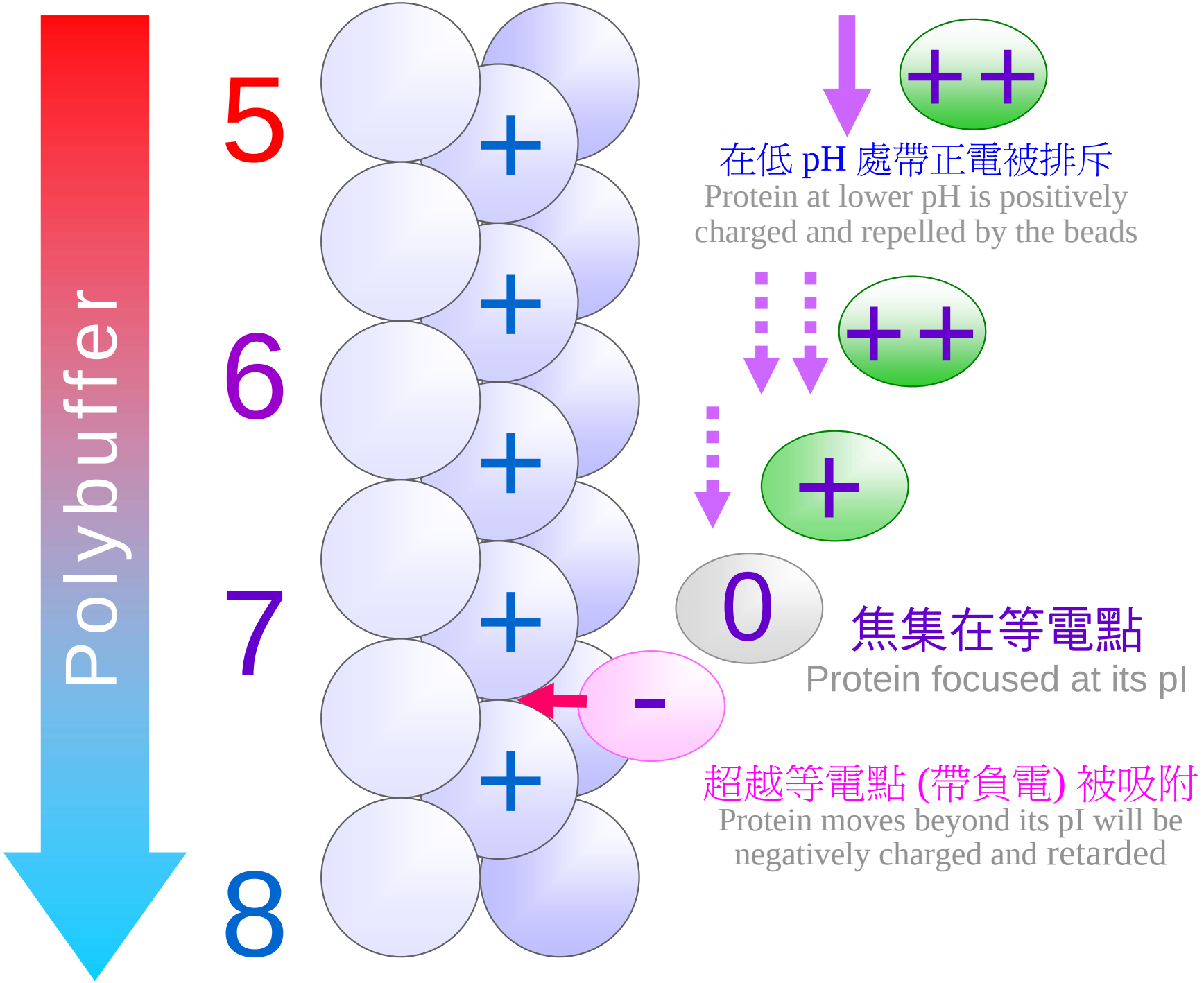
但所使用的 Polybuffer 可拉出穩定的 pH 梯度



Chromatofocusing using ion exchange bead, but eluted with Polybuffer

Juang RH (2005) EPA

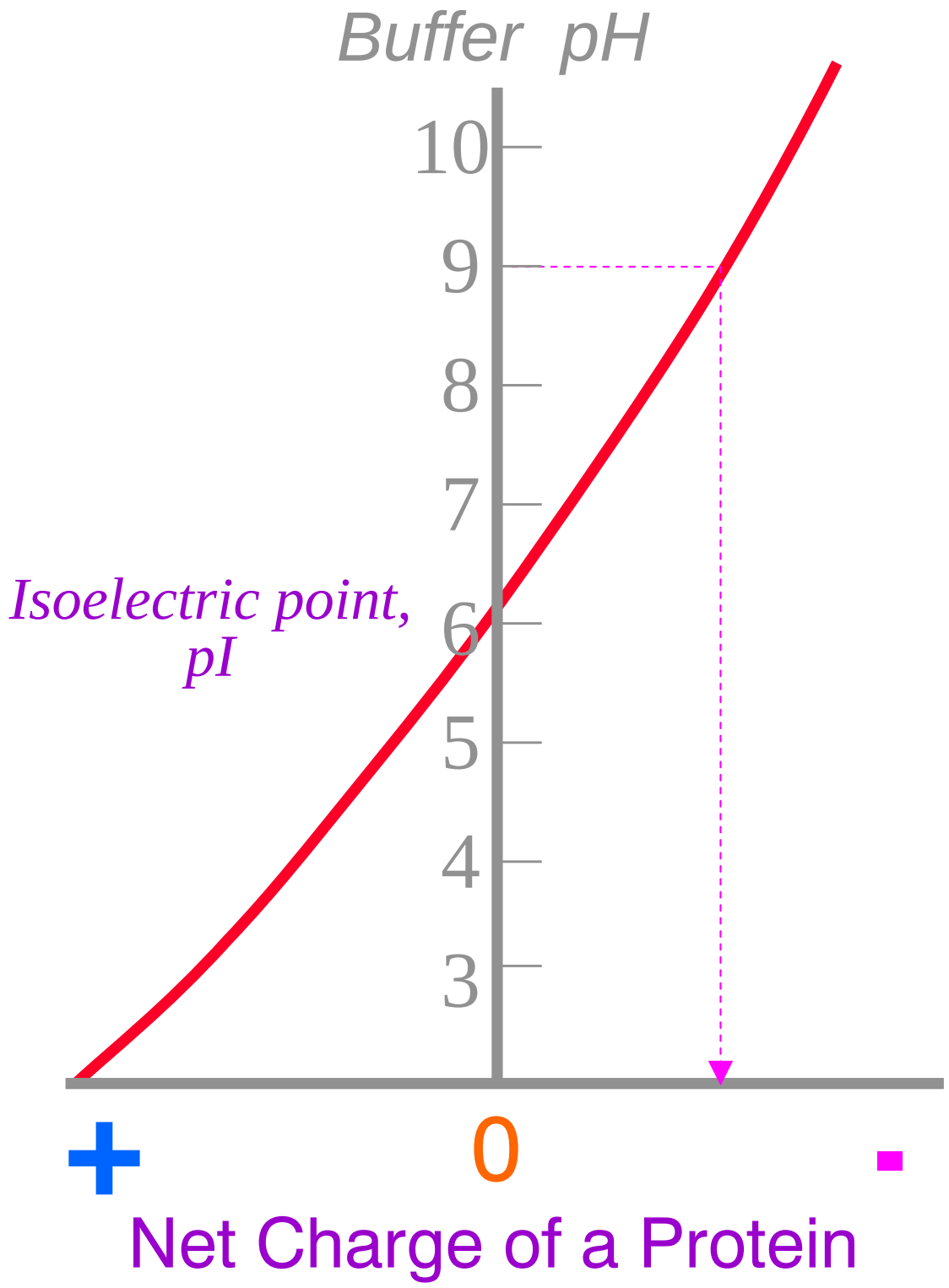
色析焦集法的焦集機制



Focusing mechanism in chromatofocusing

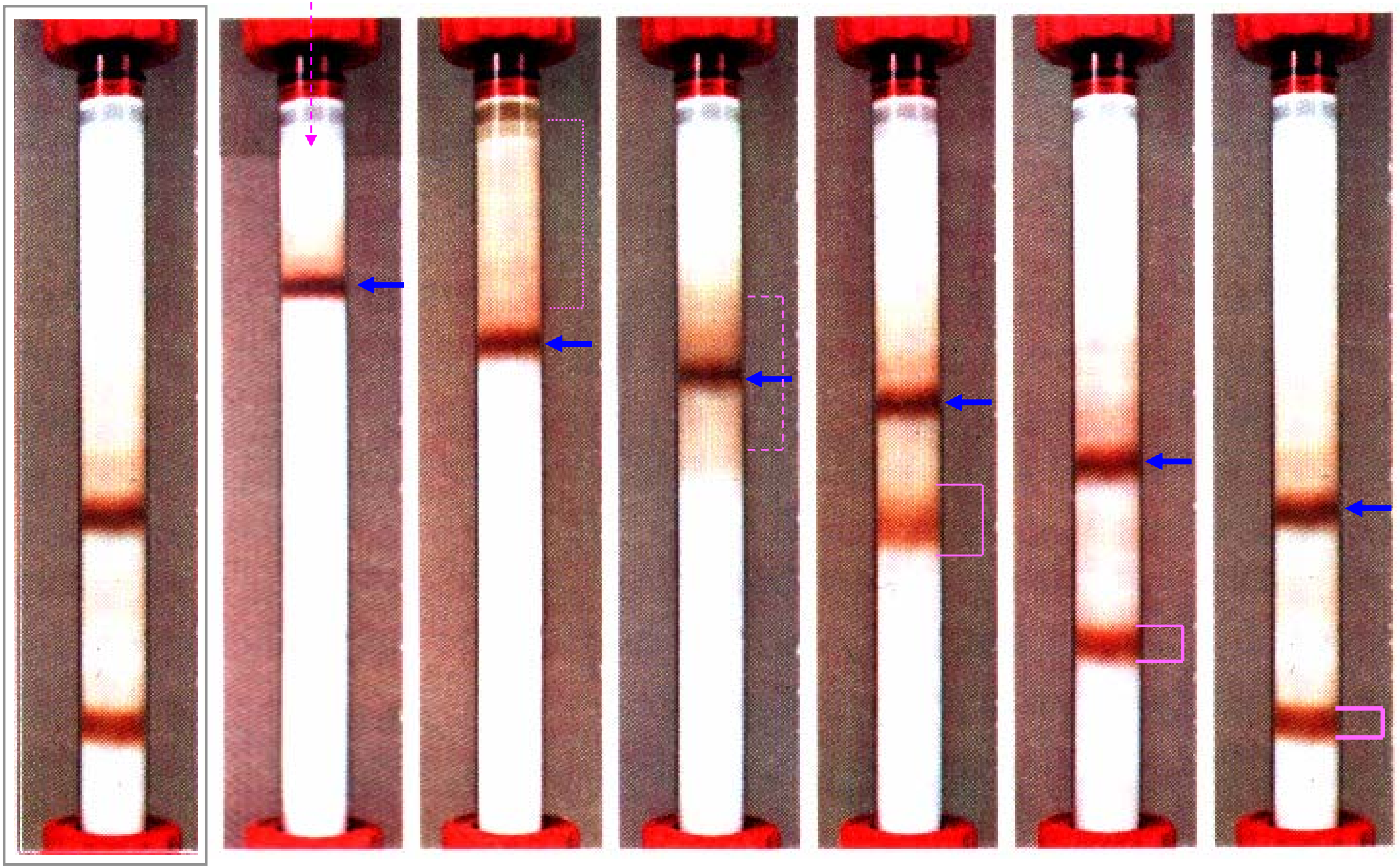
Juang RH (2005) EPA

環境影響分子的帶電性質



Juang RH (2005) EPA

● 抹香鯨肌紅蛋白 (pI = 8.2) 追過 馬肌紅蛋白 (pI = 7.4)



A molecule might be caught up by another protein in chromatofocusing

Pharmacia