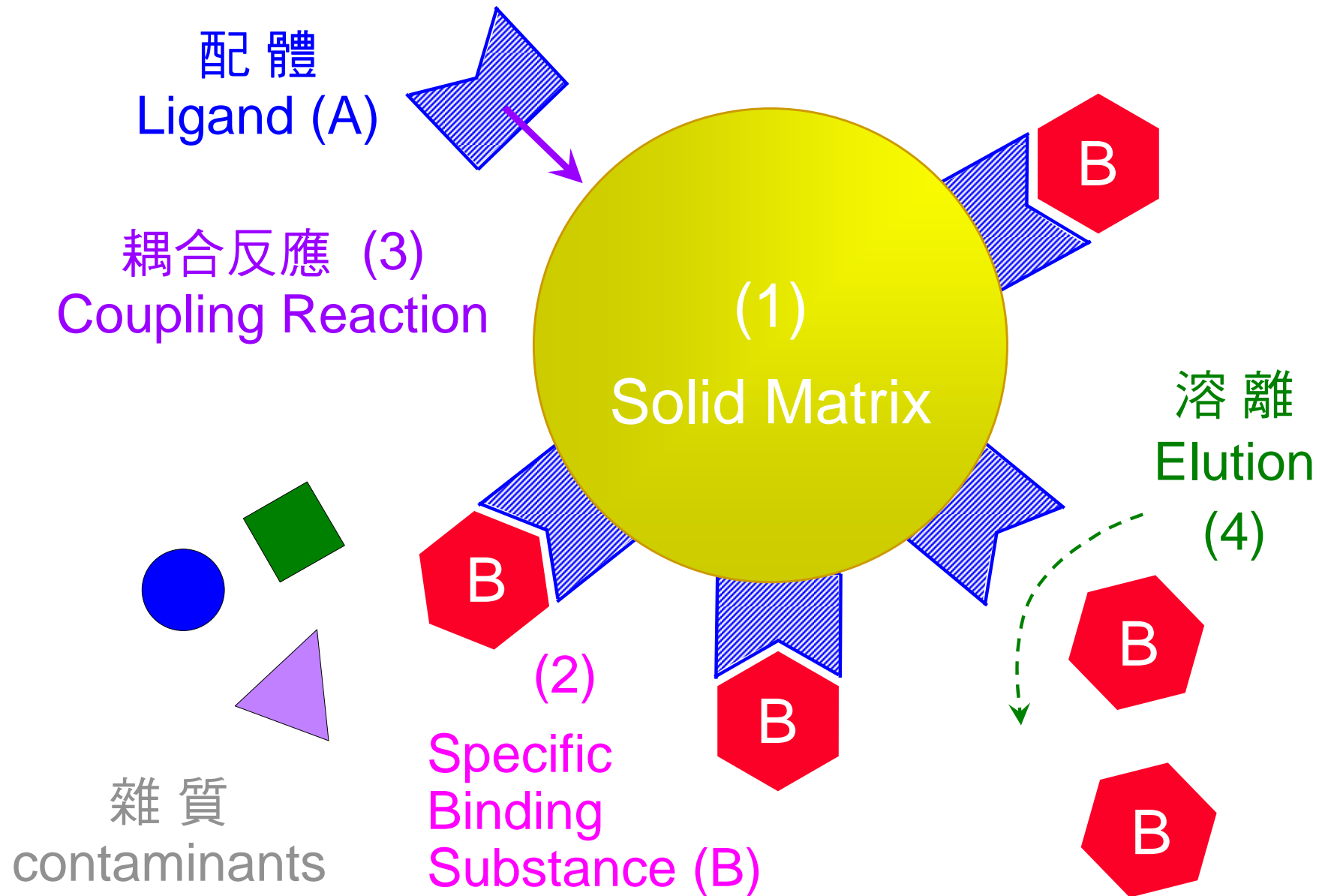


3.4 親和層析法 Affinity chromatography

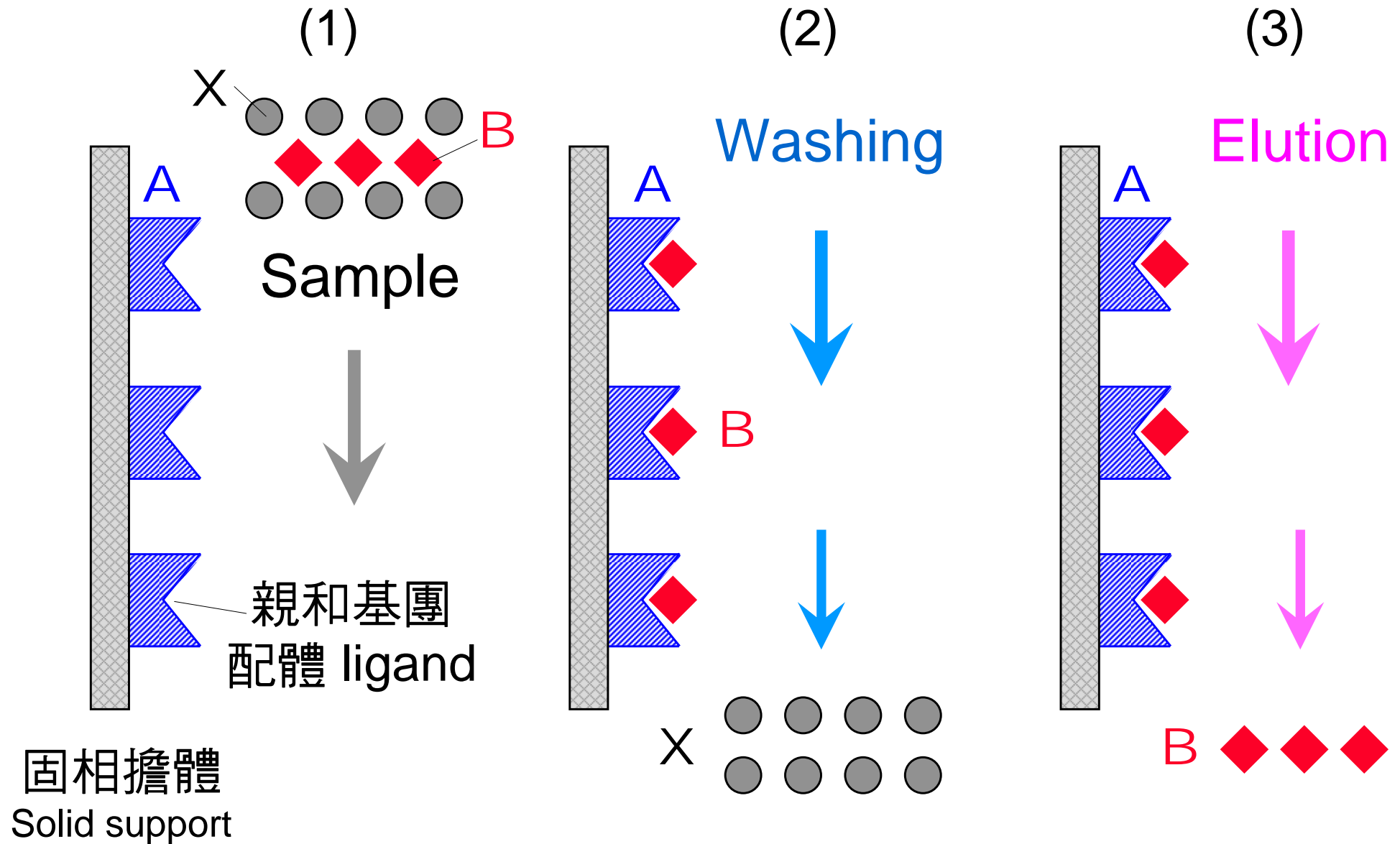


- 3.4.1 原理概述 Basic principles
要有一對具有高親和性的分子
- 3.4.2 親和吸著劑 Affinity adsorbent
有很多親和性吸著劑可利用
- 3.4.3 金屬螯合層析法 Metal chelating
利用分子與金屬的吸引力
- 3.4.4 疏水性層析法 Hydrophobic interaction
利用分子間的疏水性引力
- 3.4.5 液相分配 Liquid partition
分子在兩液相間的分配比例不同

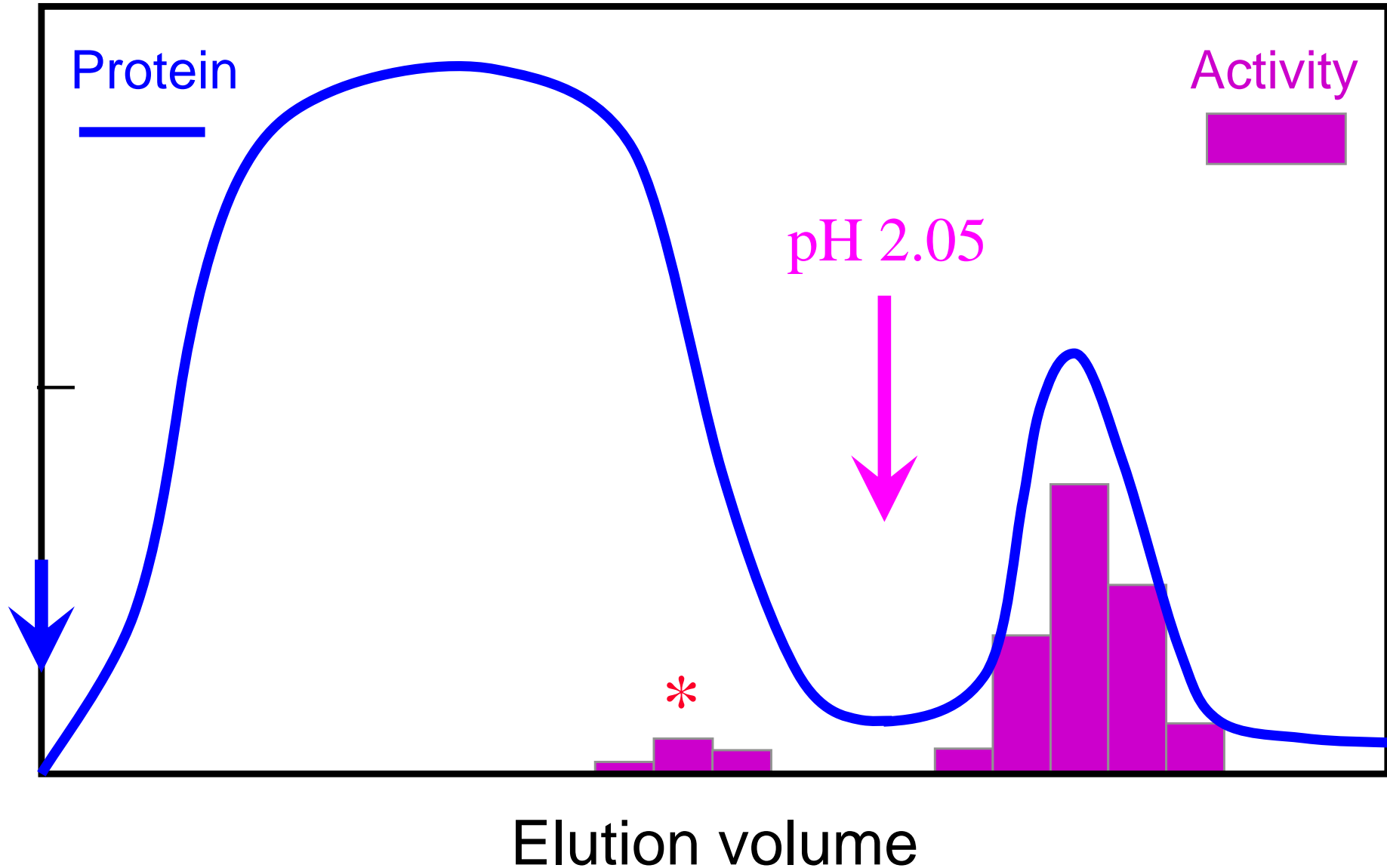
■ 親和層析法的四項要素 Four essential factors



■ 親和層析法的作用機理 How it works



■ 典型的親和層析操作 Typical elution pattern



■ 專一性結合力量的構成因素 Specific interactions

I. Conformational Match: II. Interaction Forces:

Van der waals interaction

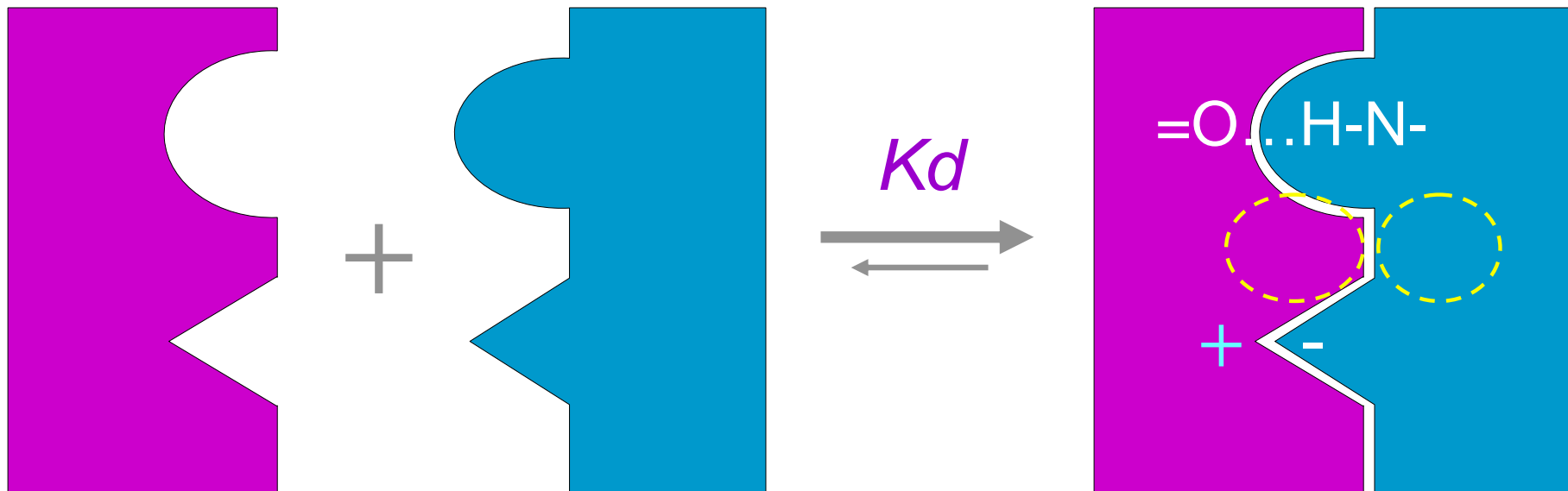
兩分子間因構形互補所造成的吸引力是由凡得瓦爾力所貢獻

(1) Hydrogen bond

(2) Hydrophobic interaction

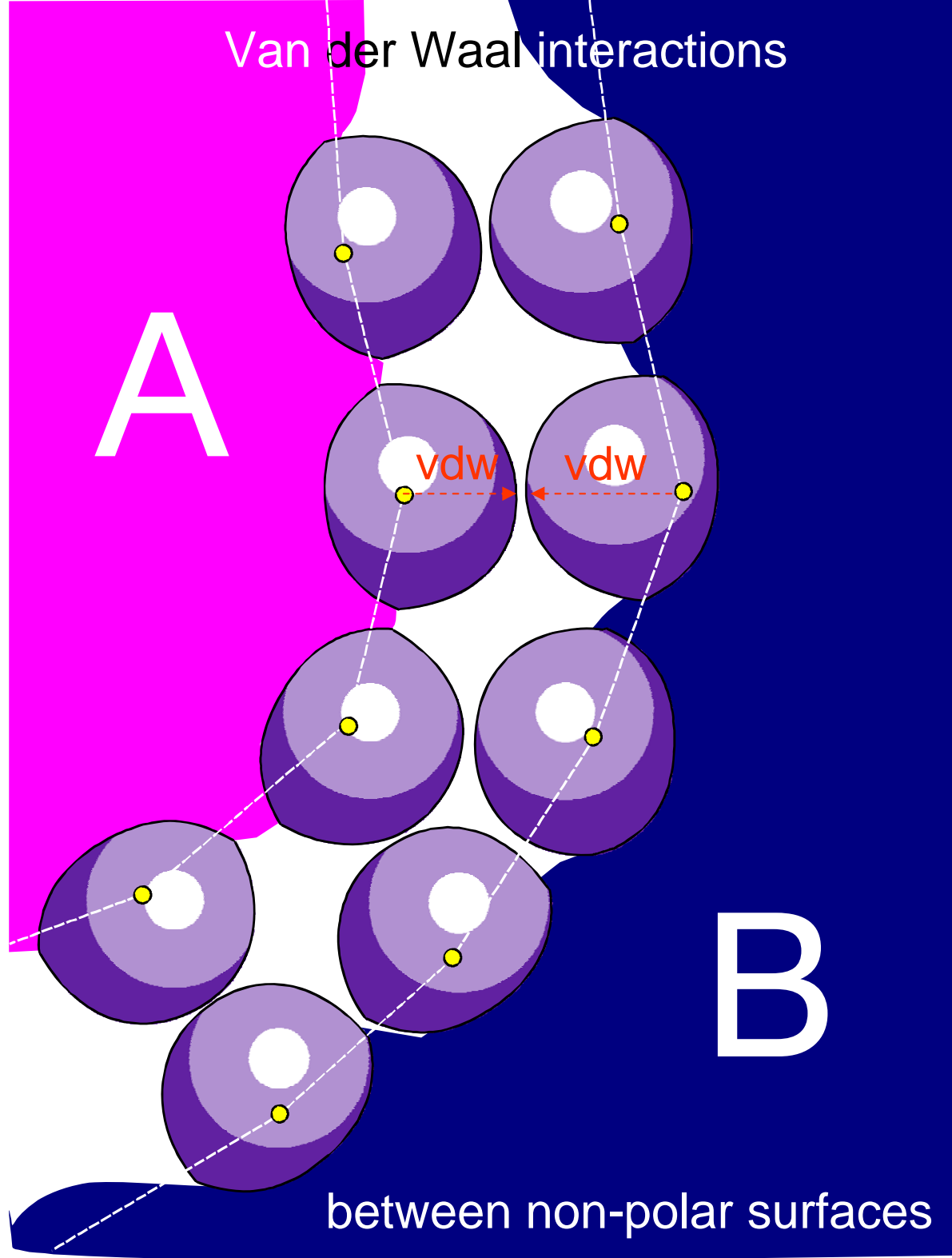
(3) Electrostatic interaction

(4) Van der waals interaction



■ 構形互補所造成的吸引力

凡得瓦爾鍵數目夠多即足以造就親和力



■ 生化分子的反應基團 Some functional groups

氰基 $-C\equiv N$ ●●●●●

醛基 $-HC=O$ ●●●

酸基 $-COOH$ 胺基 $-NH_2$ ●●

醇基 $-OH$ 硫醇基 $-SH$ ●

醚 $-O-$ ●

烷基 $-CH_3$ X

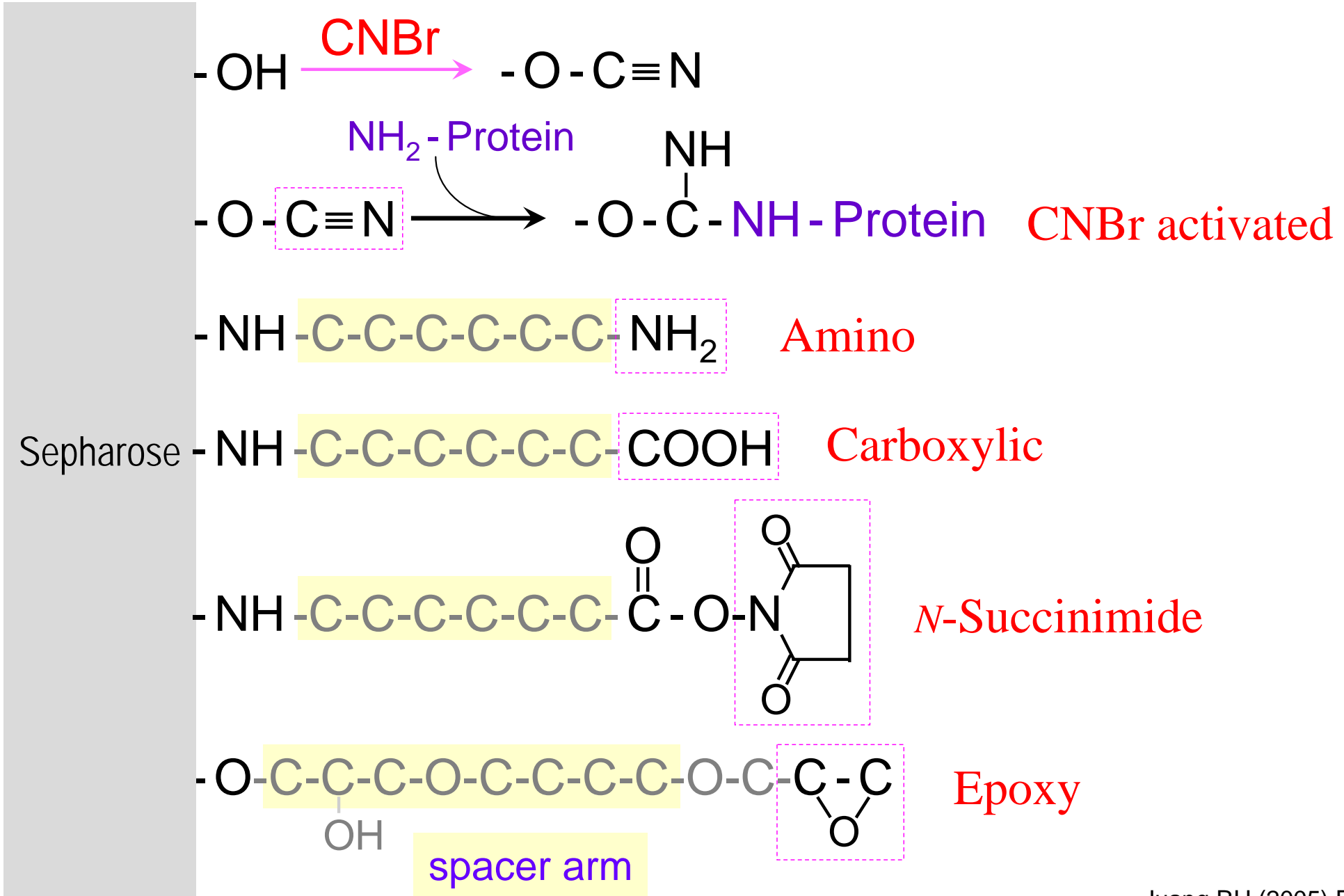
可與各種配體基團反應的介質

Pharmacia

Ligand	Affinity materials	Reaction	How
-NH ₂	CNBr-activated Sepharose 4B	-C≡N	Directly
	CH-Sepharose 4B 或活化型	-COOH	+ EDC*
		N-OH-succinimide	Directly
	Epoxy-activated Sepharose 4B	oxirane	Directly
-COOH	AH Sepharose 4B	-NH ₂	+ EDC*
-OH	Epoxy-activated Sepharose 4B	oxirane	Directly
-SH	Epoxy-activated Sepharose 4B	oxirane	Directly
	Thiopropyl-Sepharose 4B	-S-S-R	+ DTT
	Activated thio-Sepharose 4B	-G-S-S-R	Directly

* EDC = *N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide HCl

■ 親和性介質的反應基團 Functional groups



各種親和性介質及其專一性基團

Pharmacia

Ligand	Target	Remarks
Antibody	Specific antigen	Immunosorbent (custom made)
Substrate/inhibitor	Specific enzyme	Enzyme-substrate binding
Protein A	Part of IgG	mAb purification
Con A	Glycoprotein	Specific to α -D-glc and mannose
Heparin	Fibrin et al	Heparin Sepharose CL-6B
Oligo (dT)	mRNA	Oligo (dT)-cellulose
Cibacron-Blue	NAD(P) ⁺ binding	Blue Sepharose CL-6B
AMP, ADP	NAD(P) ⁺ binding	5'AMP-, 2', 5'ADP-Sepharose 4B
Oligosaccharides	Lectin	Purify lectins

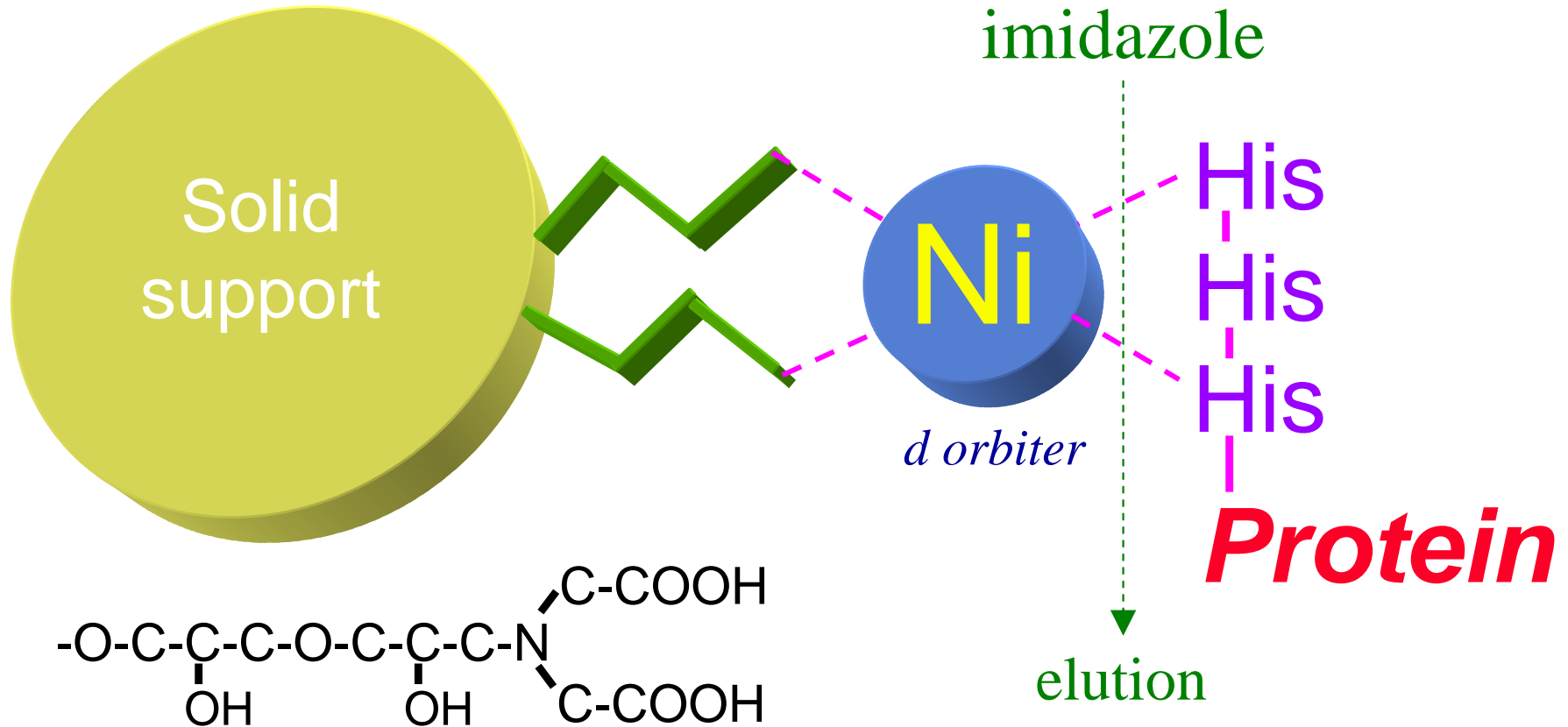
(There are more other affinity adsorbents from commercial sources)

金屬螯合層析法 Using metal-chelating affinity



Transition metals 過渡元素

Tag

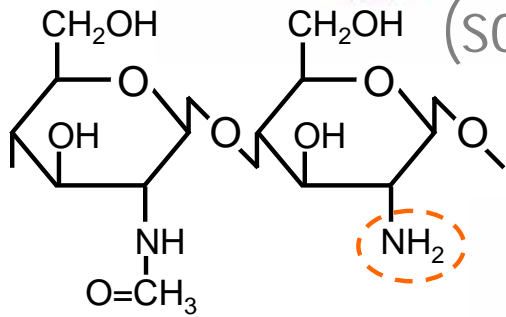


Metal Chelate Affinity Chromatography

■ 親和層析法實例 Materials used in an example



Chitin
(solid support)



glutaraldehyde

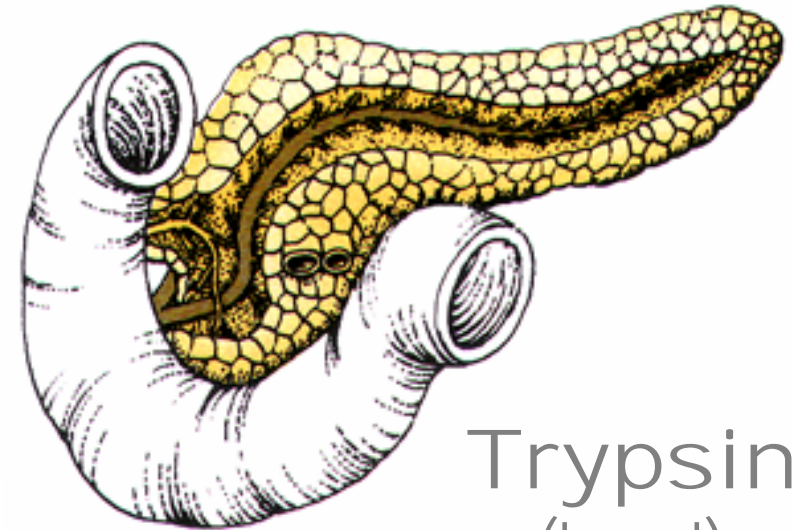
CHOM

(ligand, bait)

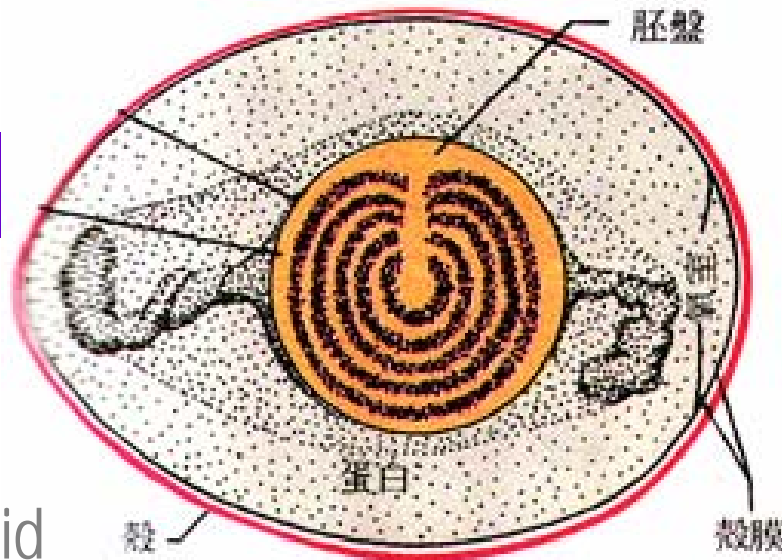
雞蛋粘多糖蛋白

Chicken ovomucoid

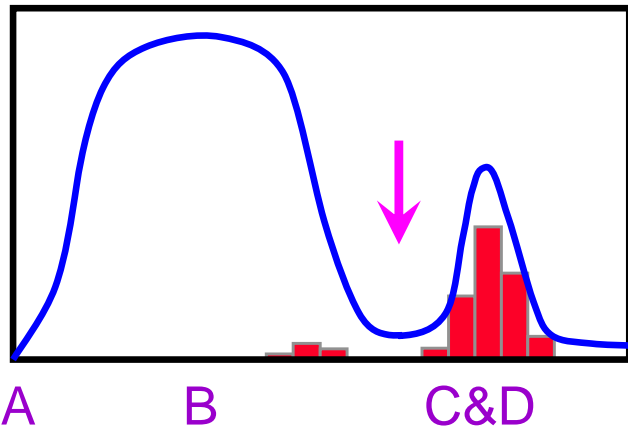
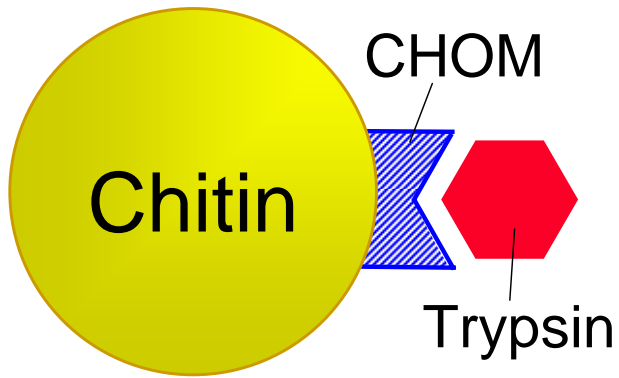
Trypsin inhibitor



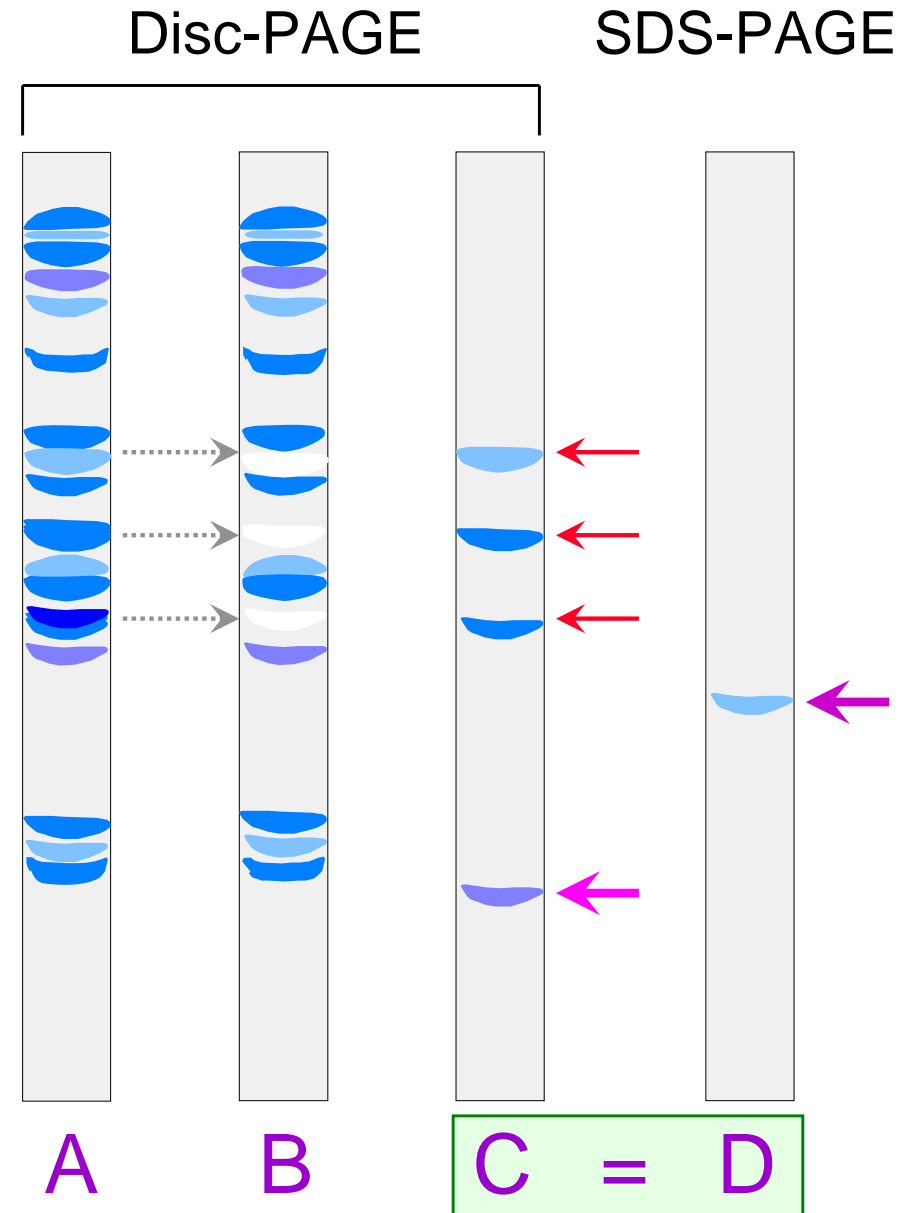
Trypsin
(target)



以親和層析法純化 Trypsin purification

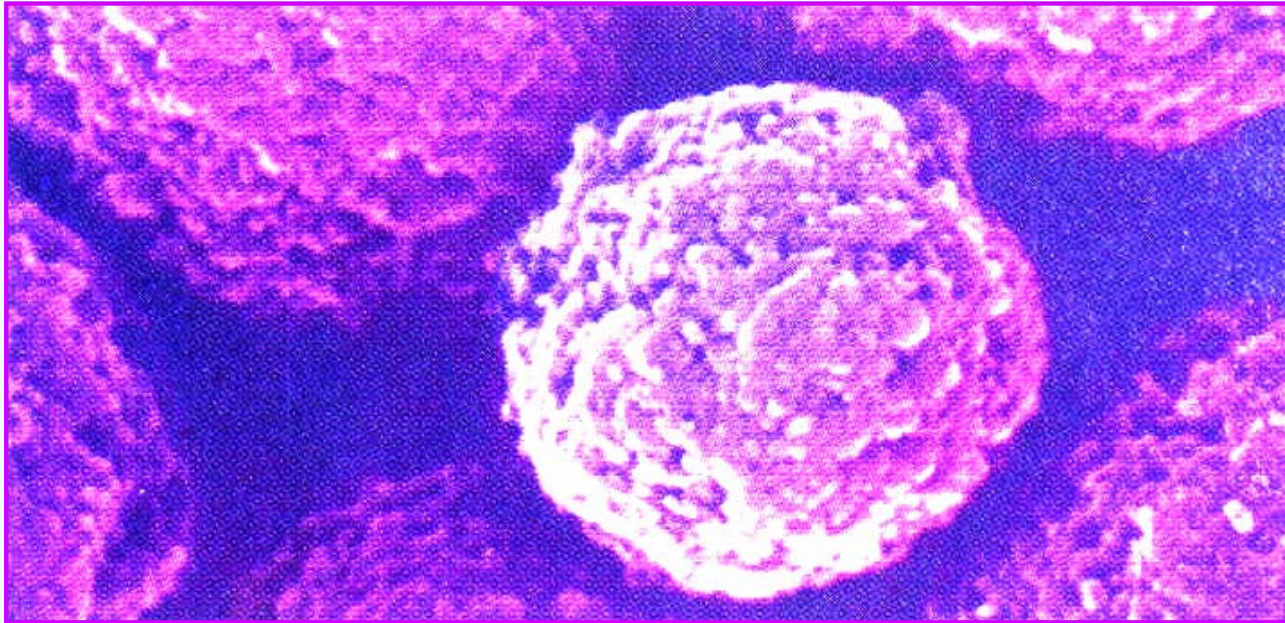


以膠體電泳檢定親和層析
操作過程各步驟的樣本
Check by PAGE →



Hydroxyapatite 吸著劑

磷酸鈣陶土 $(\text{Ca}_5(\text{PO}_4)_3\text{OH})_2$



可選擇 **NaCl** 或 **磷酸** 等不同溶離條件

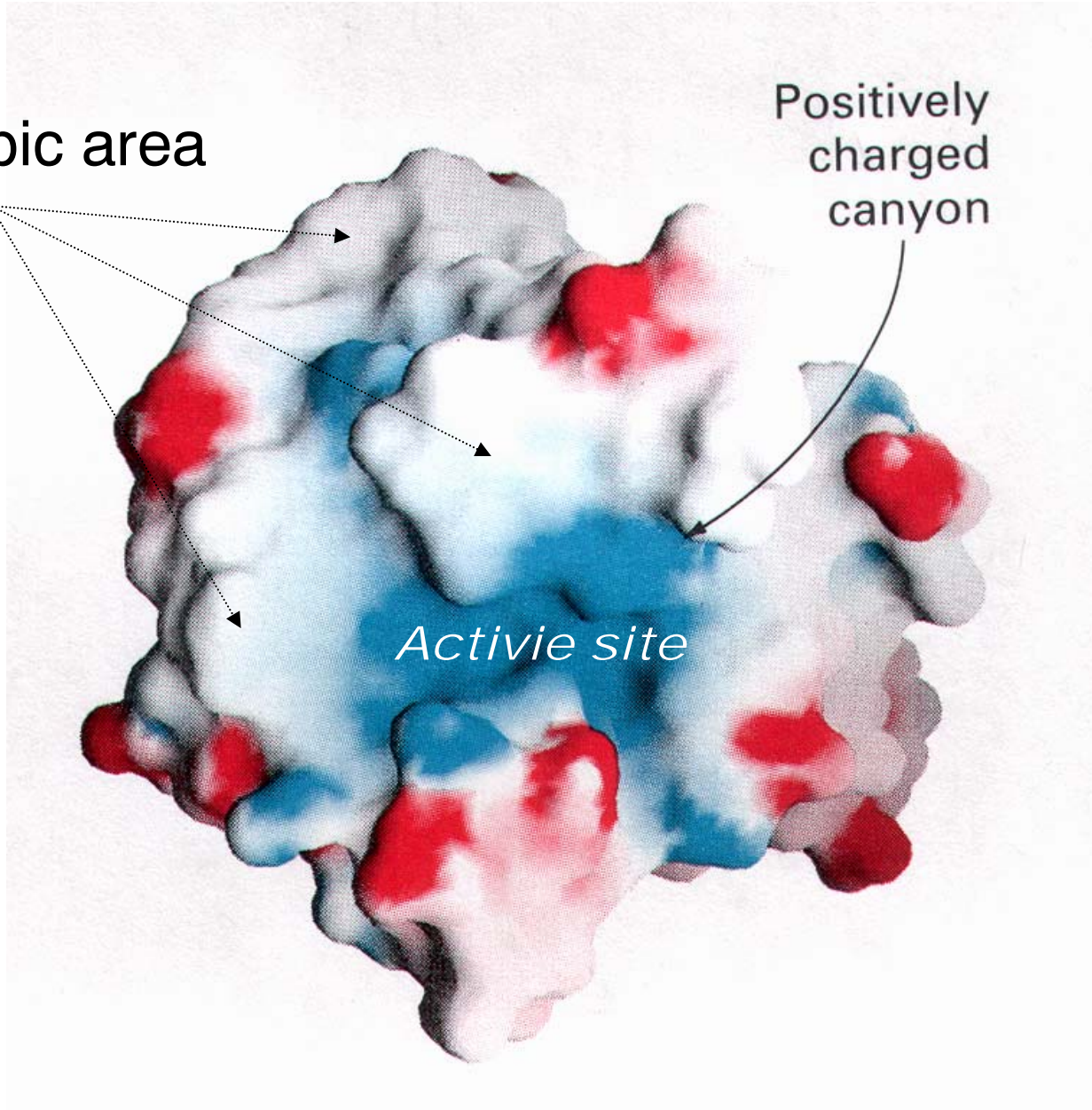
Elution by NaCl or phosphate gradient leads to different results

蛋白質表面的極性或非極性分布

Hydrophobic area

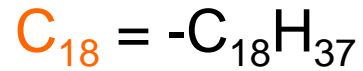
Positively charged canyon

Active site



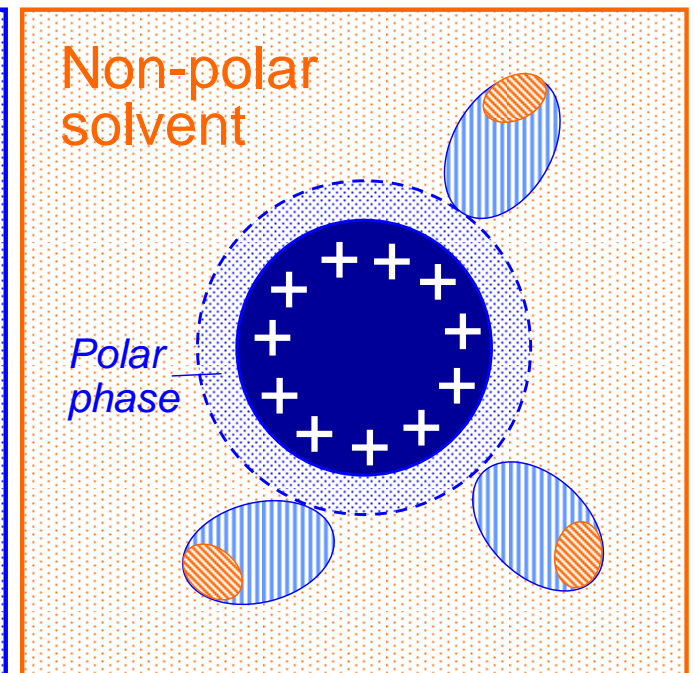
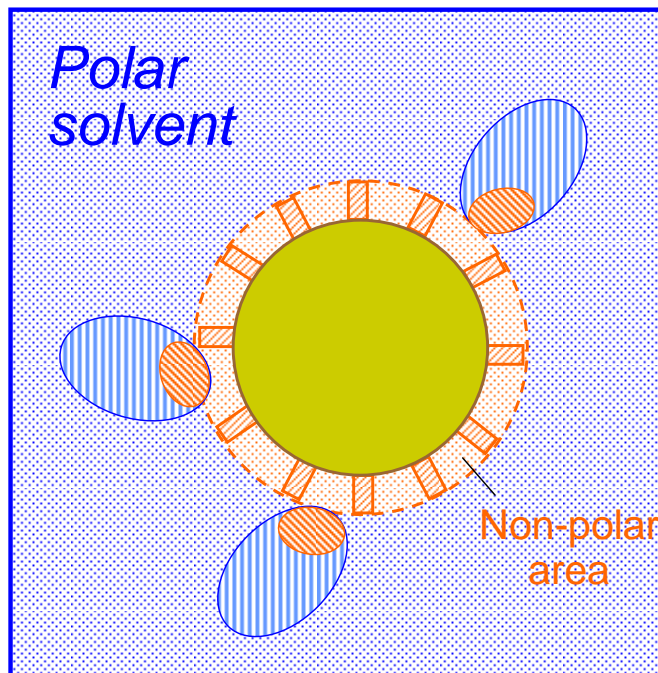
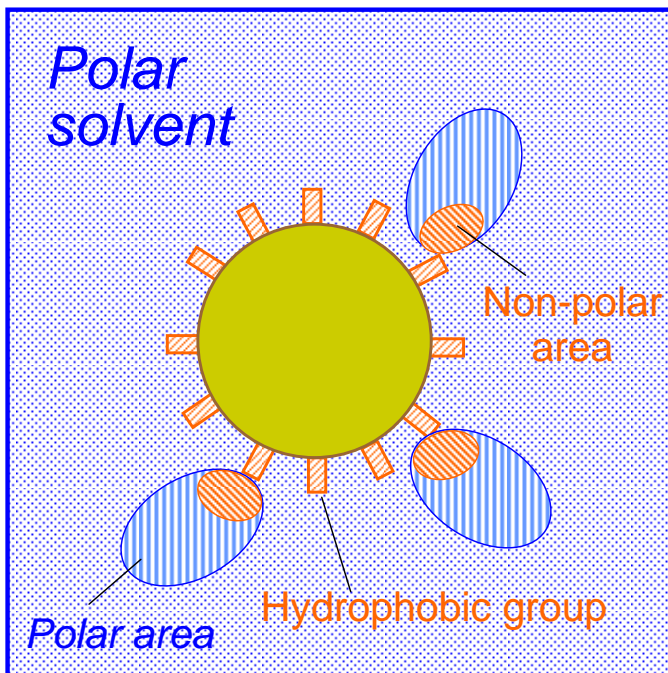
Superoxide
dismutase
(SOD)

■ 疏水性及反相層析法 Hydrophobic interaction



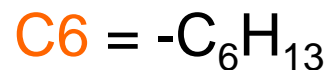
HIC (liquid-solid)

Reverse Phase Chromatography (liquid-liquid)



Using **non-polar groups** as a stationary phase

Using ion-exchanger



Hydrophobic interaction chromatography (HIC)

