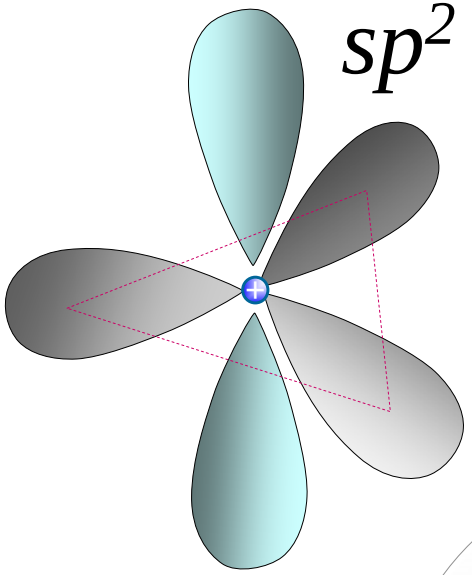
The Chemical Nature of Enzyme Reaction

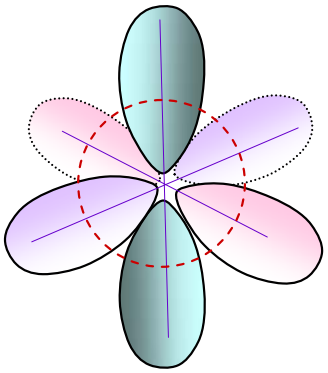
C^6

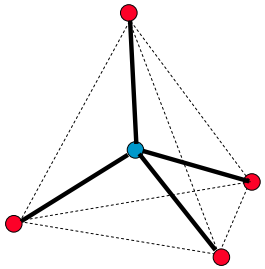
I Chemical reactions
II Enzyme catalytic mechanism
III Identification of catalytic sites

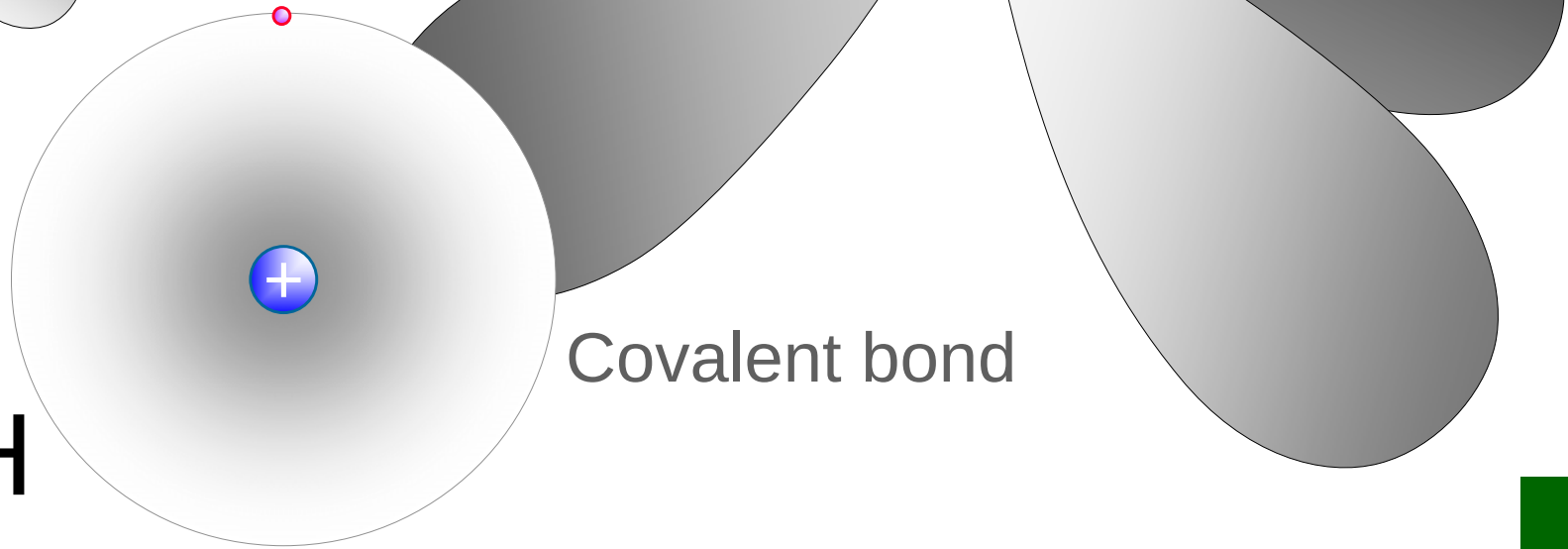
sp^3 *hybrid*

$sp^2 + p$





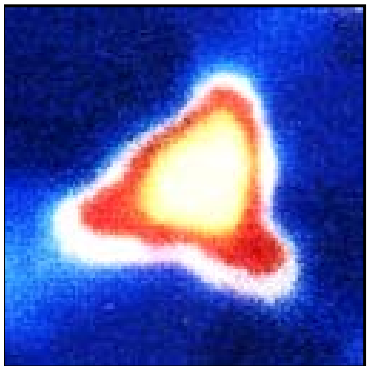




Covalent bond

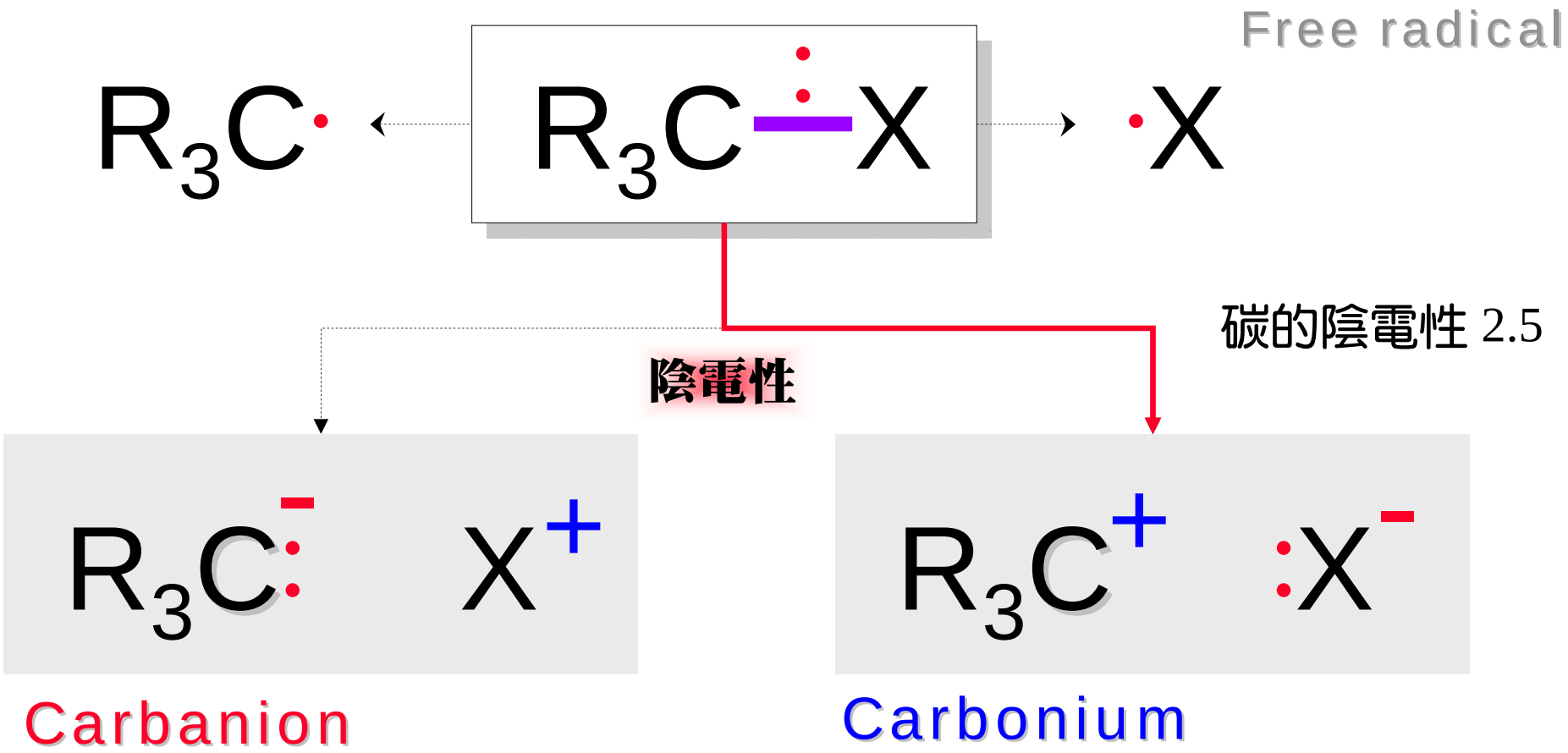
H

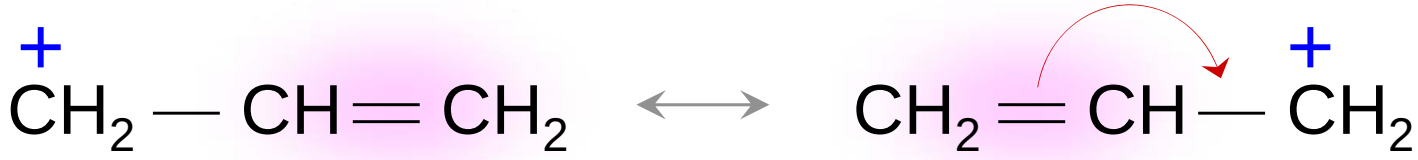
矽的 sp^3 四面體



Campbell (1990) Biology (2e) p.23

I The basic mechanism of chemical reaction





stabilized by resonance

Four main types of reactions

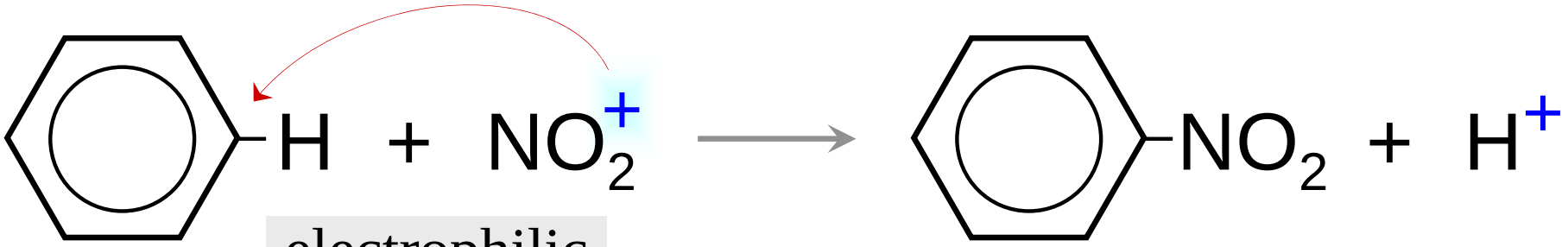
Substitution **nucleophilic**
electrophilic

Addition

Elimination

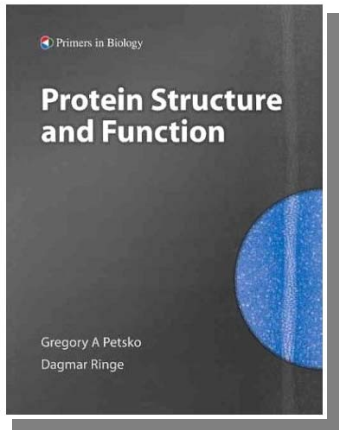
Rearrangement

Oxidation/reduction
Addition/elimination
Hydrolysis
Decarboxylation



electrophilic substitution

(1) 碳原子的軌域與八耦律。 (決定電子如何排列)
(2) 相鄰原子陰電性之大小。 (決定何者搶奪電子)
(3) 反應物與生成物之穩定。 (穩定者較容易形成)



It's a matter of electrons!

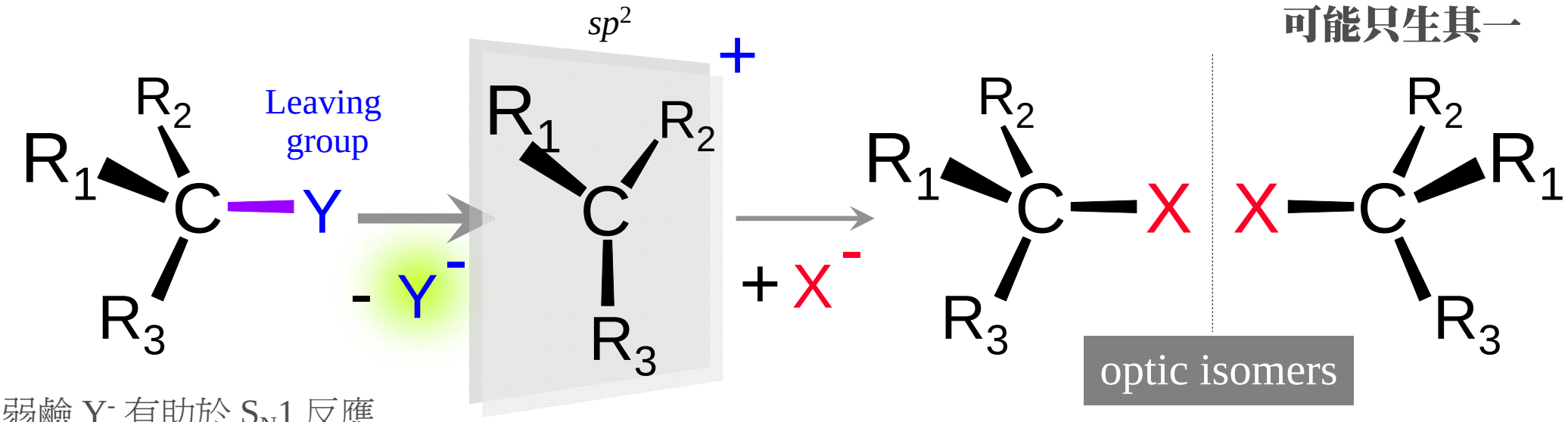
Palmer T (1991) ***Understanding Enzymes***, Chap. 10, 11 (Ellis Horwood, England)

Petsko GA, Ringe D (2004) ***Protein Structure and Function*** (New Science Press, London)

Nucleophilic substitution reactions

Unimolecular nucleophilic substitution (S_N1)

若經酵素催化
可能只生其一



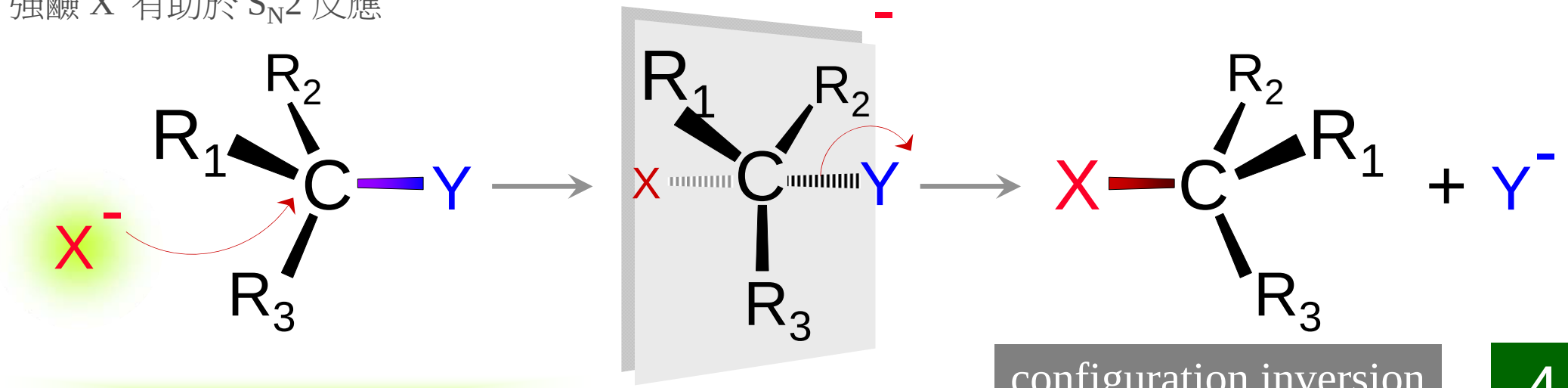
optic isomers

弱鹼 Y^- 有助於 S_N1 反應

Bimolecular nucleophilic substitution (S_N2)

注意 sp^3 - sp^2 變化

強鹼 X^- 有助於 S_N2 反應



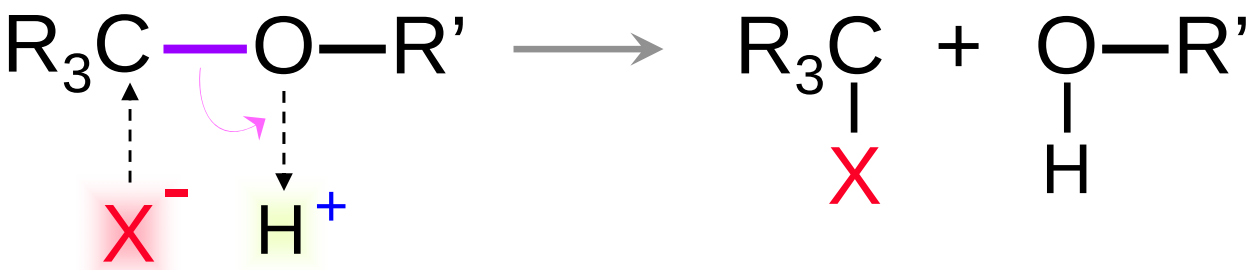
configuration inversion

反應速率決定於形成過渡狀態的難易

II Mechanisms of enzyme catalytic reactions

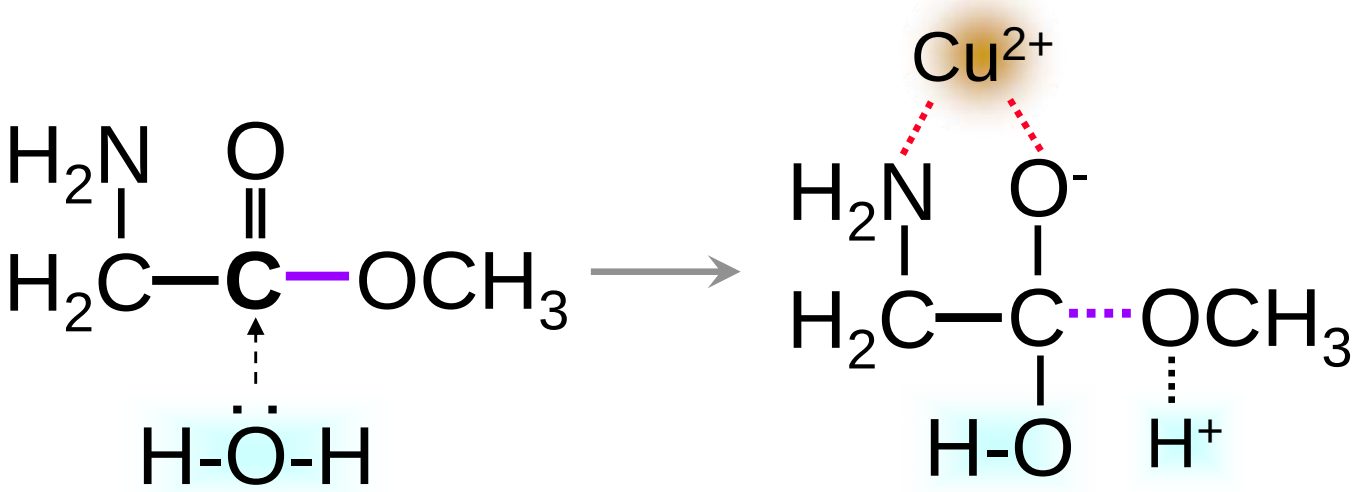
(1) Acid-base catalysis

反應速率受環境 pH 影響
(要注意 buffer 的 pH)

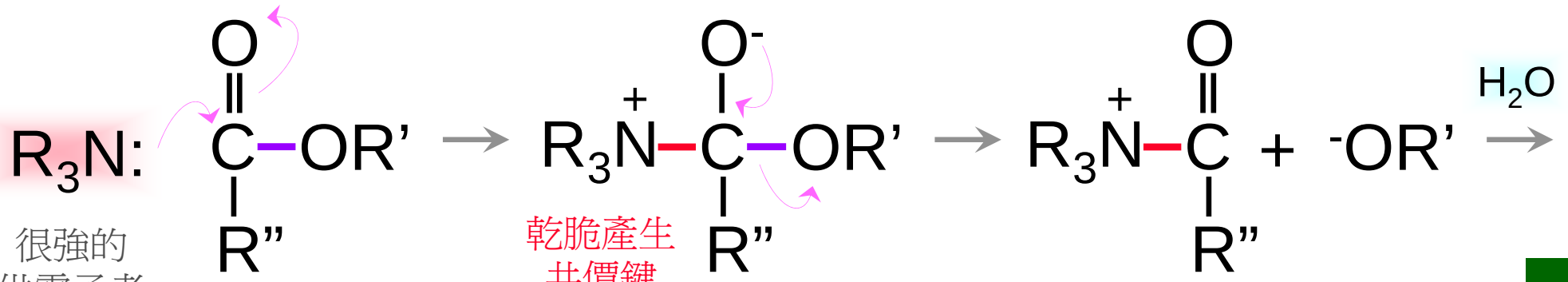


(2) Electrostatic catalysis

以中和電荷穩定過渡狀態



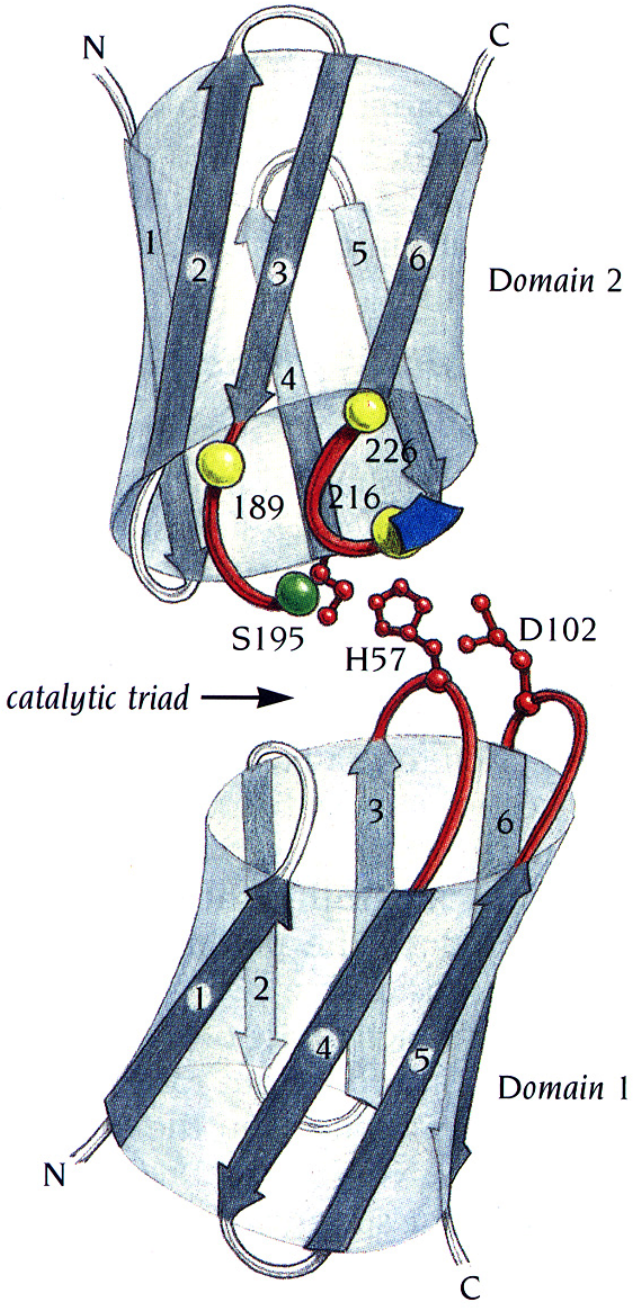
(3) Covalent catalysis

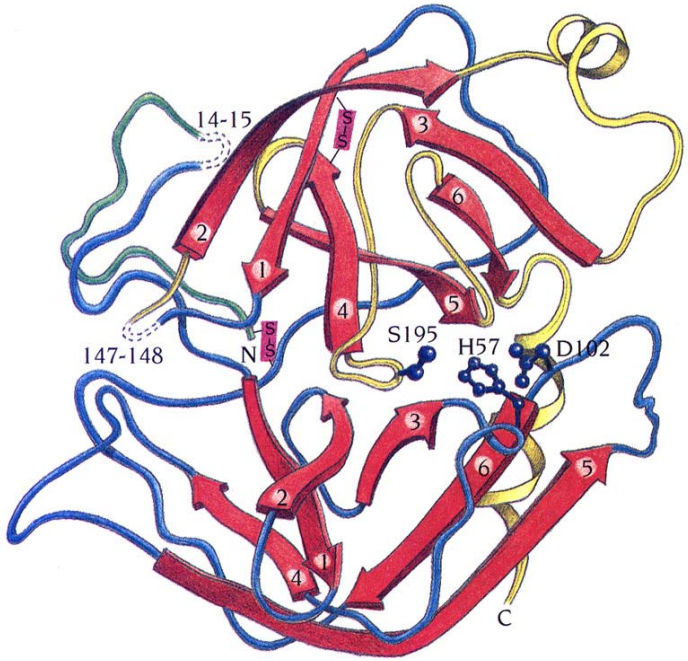


很強的
供電子者

Chymotrypsin 催化過程利用以上三種機制

Chymotrypsin





Catalytic triad

Zymogen activation

pH dependence

Transition state

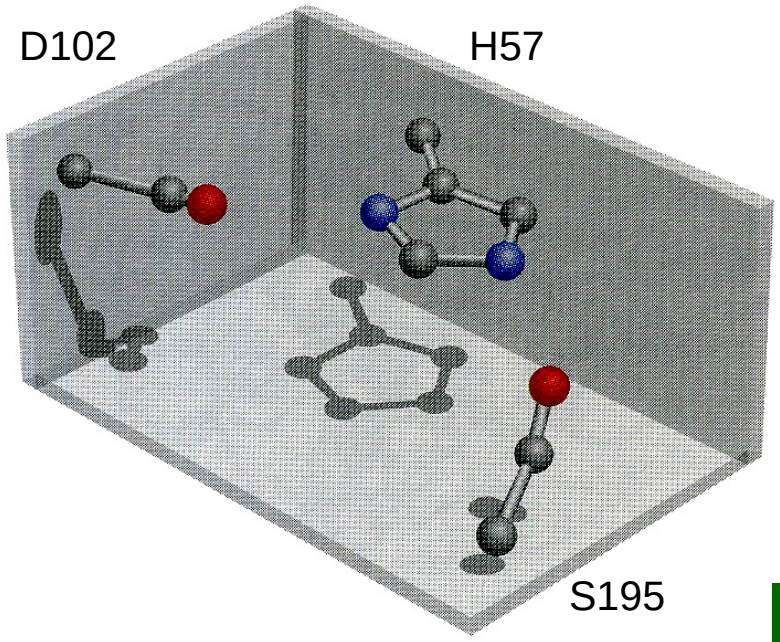
Two-step mechanism

Covalent catalysis

Substrate specificity

← Active site could be created between two domains

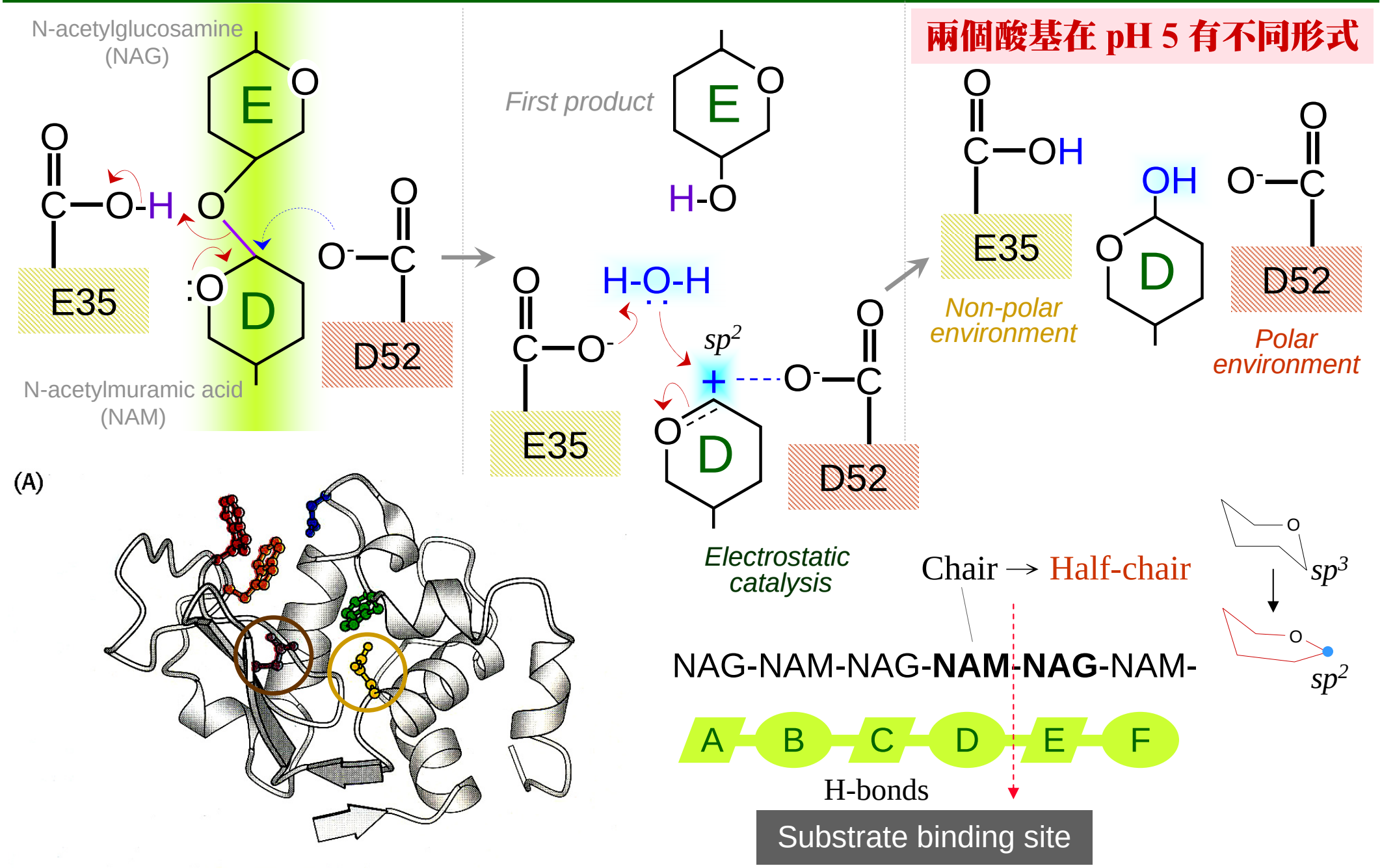
→ The geometry of the catalytic triad of the serine protease as used to locate similar sites in other proteins



Branden & Tooze (1999) Introduction to Protein Structure (2e) p.212, 210

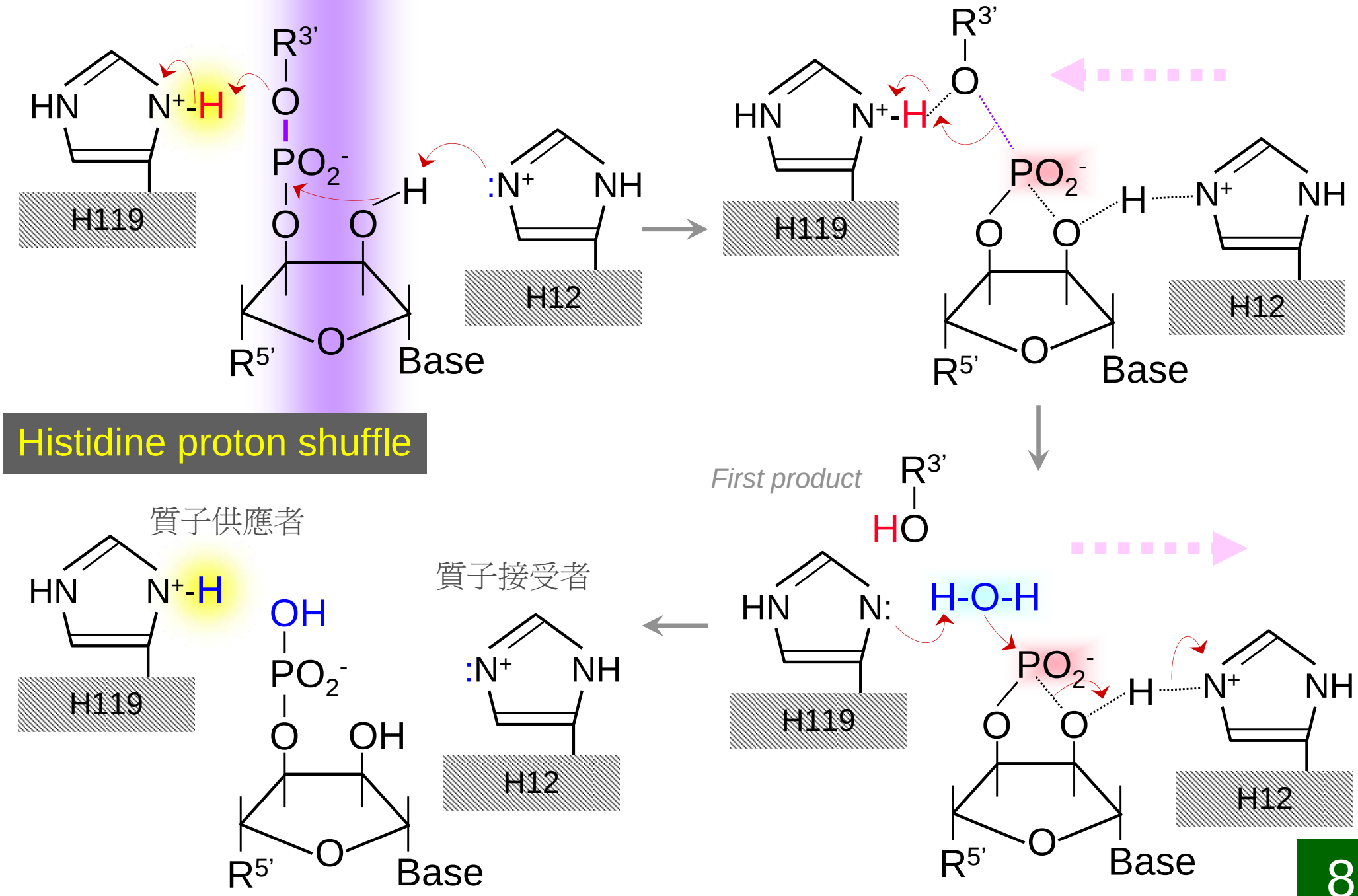
Petsko GA, Ringe D (2004) ***Protein Structure and Function*** F4-35

Lysozyme

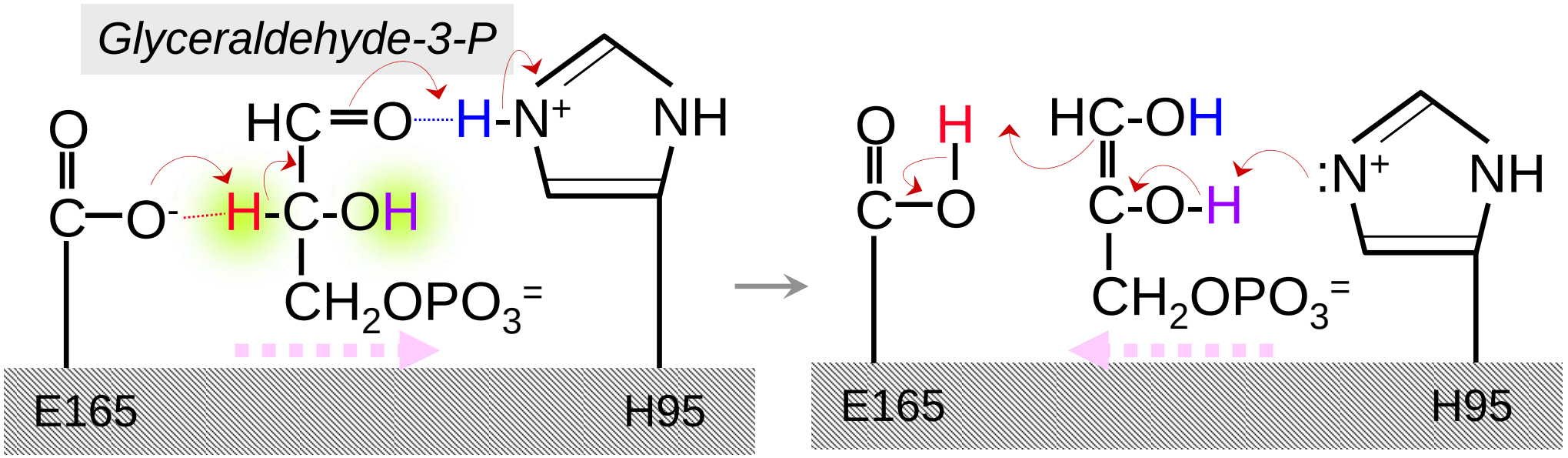


Stryer (2002) Biochemistry (5e) p.199, Fig 8-7

Ribonuclease



Triose phosphate isomerase

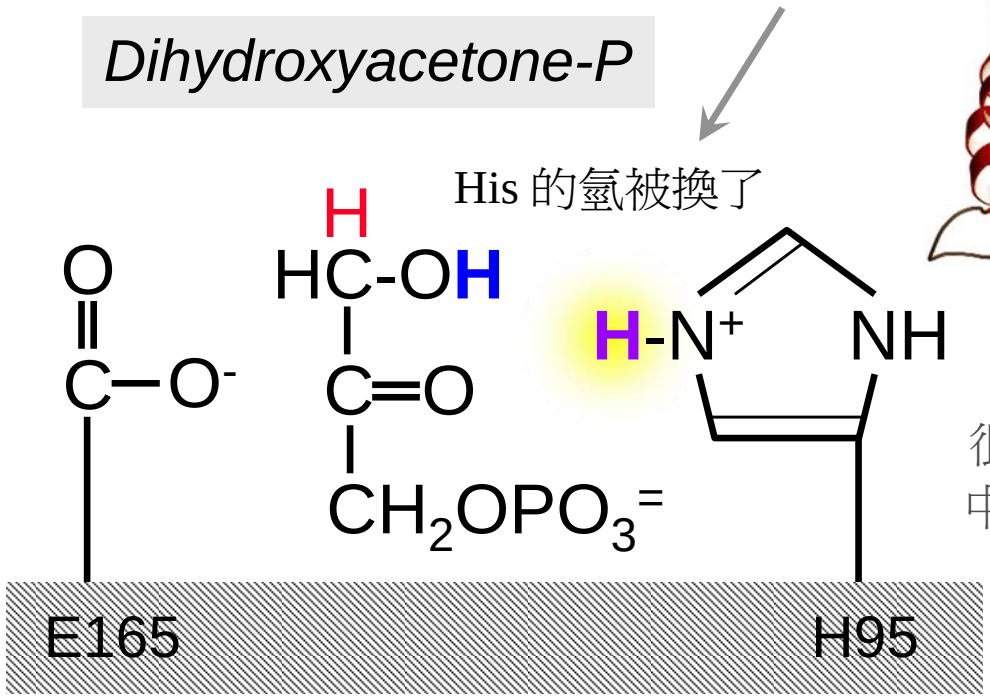


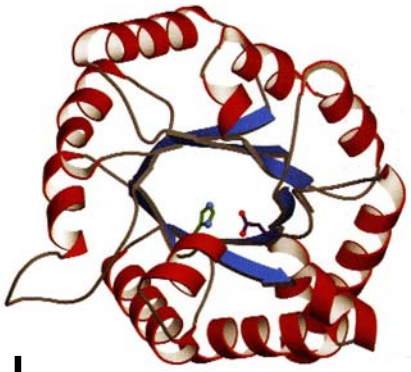
Histidine in an enzyme

(1) Imidazole has neutral p*Ka*
(2) Proton donor & acceptor
(3) Nucleophile & electrophile
(4) Stabilize charged groups
(5) Could be phosphorylated

G3P formula unchanged

此酵素的最佳作用 pH？

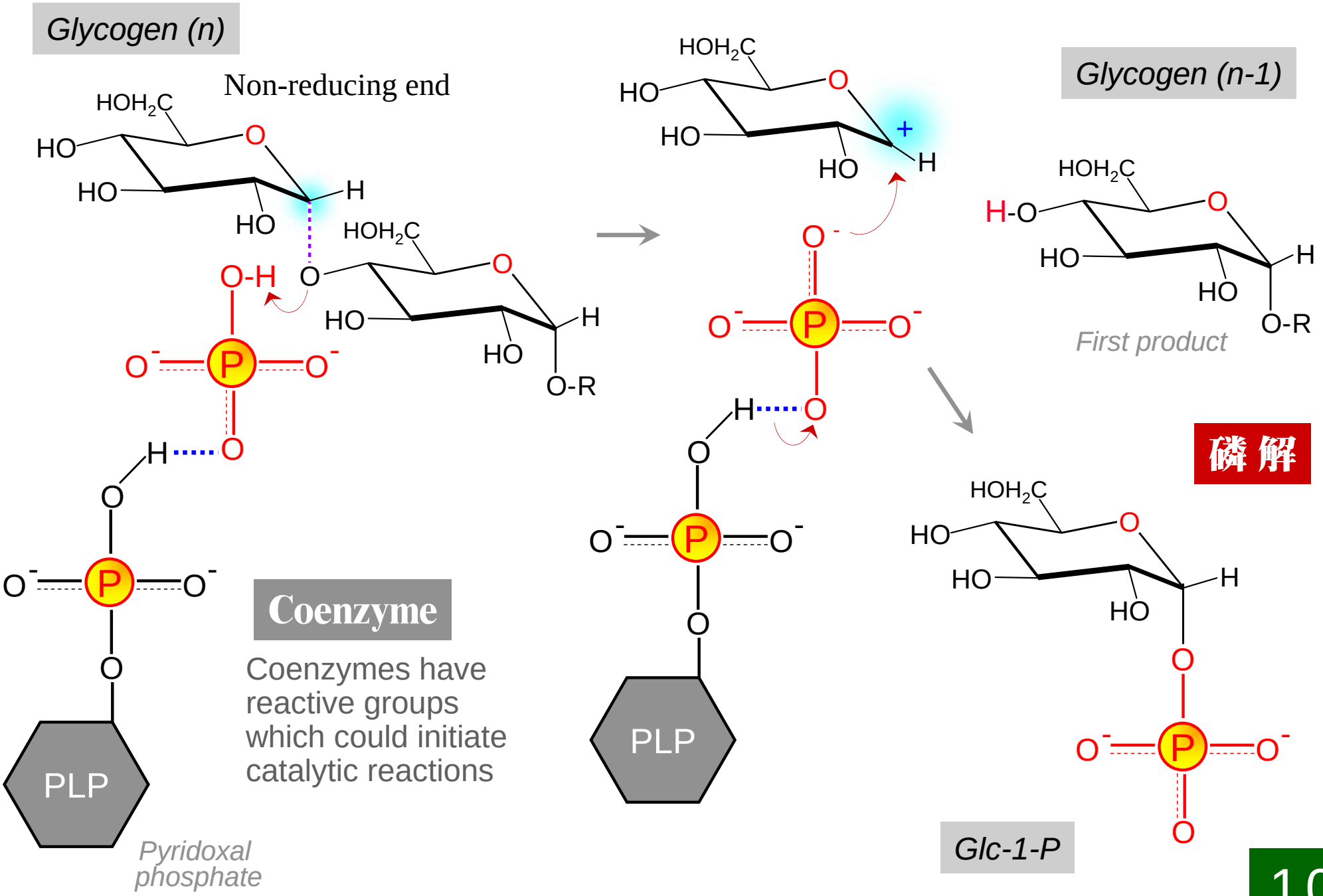




$\alpha_8\beta_8$ barrel
很常見構造 (10%)
中央有非極性口袋

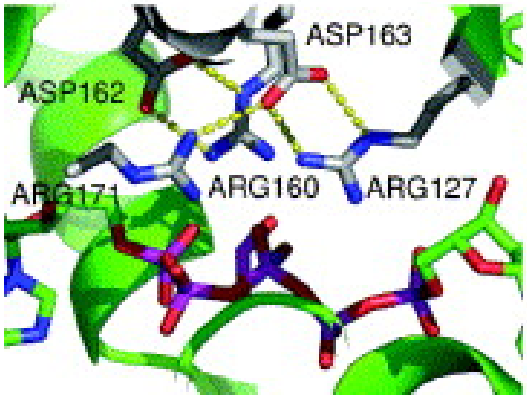
Petsko GA, Ringe D (2004) ***Protein Structure and Function*** F4-37

Glycogen phosphorylase

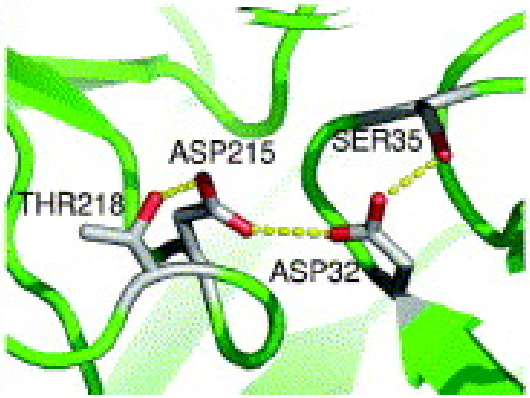


Catalytic toolkit for active sites

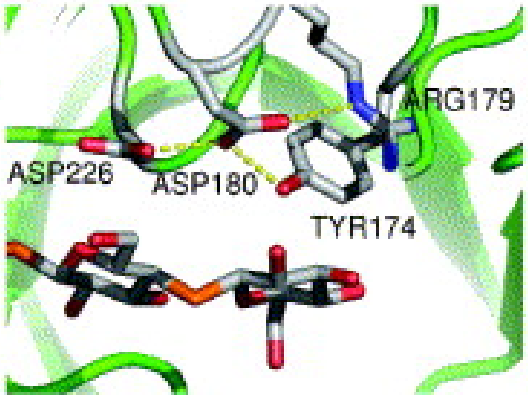
(a) 三連 Arg-Arg-Arg 正電集團



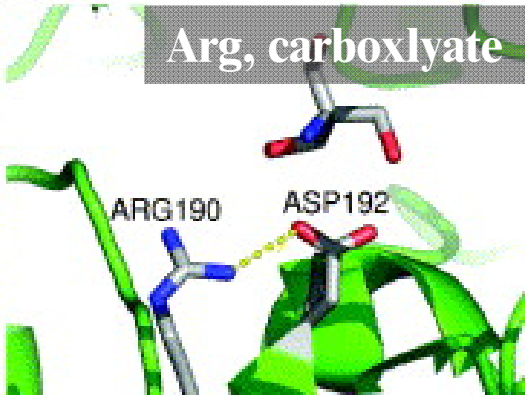
(b) Asp-Asp 酸鹼配對 (Thr 定位)



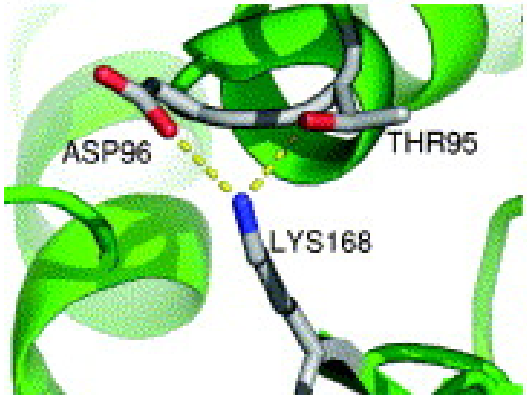
(c) Asp-Arg 離子配對 (影響 pK_a)



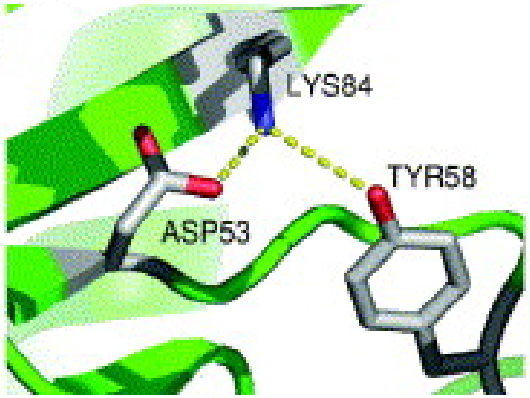
(d) Arg…Asp-COO^- (親核攻擊)



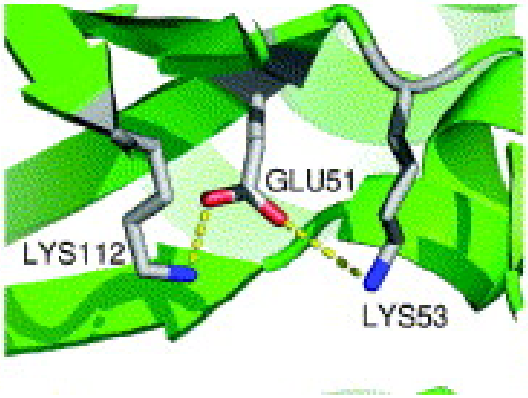
(a) Asp-Lys-Thr triad (reactive -O^-)



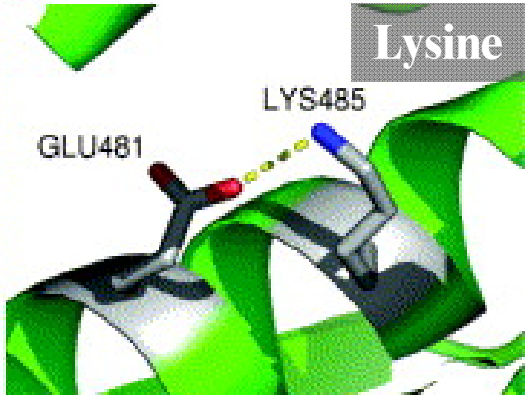
(b) Asp-Lys 配對 (調節 Tyr pK_a)



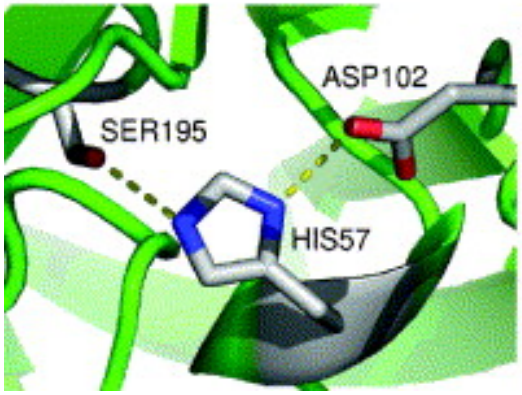
(c) KEK (EK 控制 K112 pK_a)



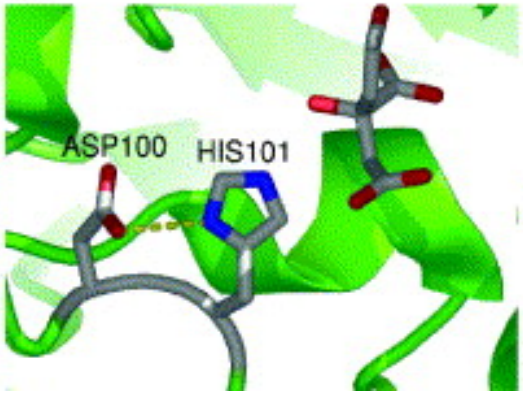
(d) 簡單的酸鹼對 (質子供需)



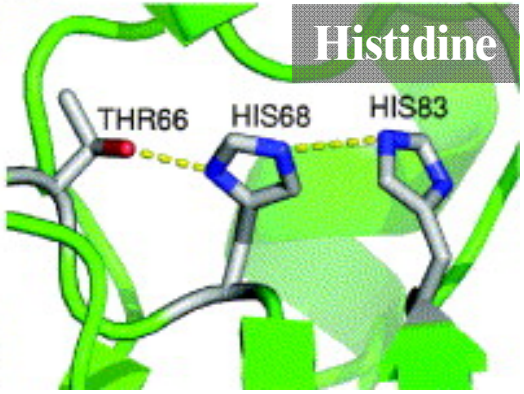
(a) Asp-His-Ser triad (reactive -O^-)



(b) Asp-His 配對 (攻擊基質)



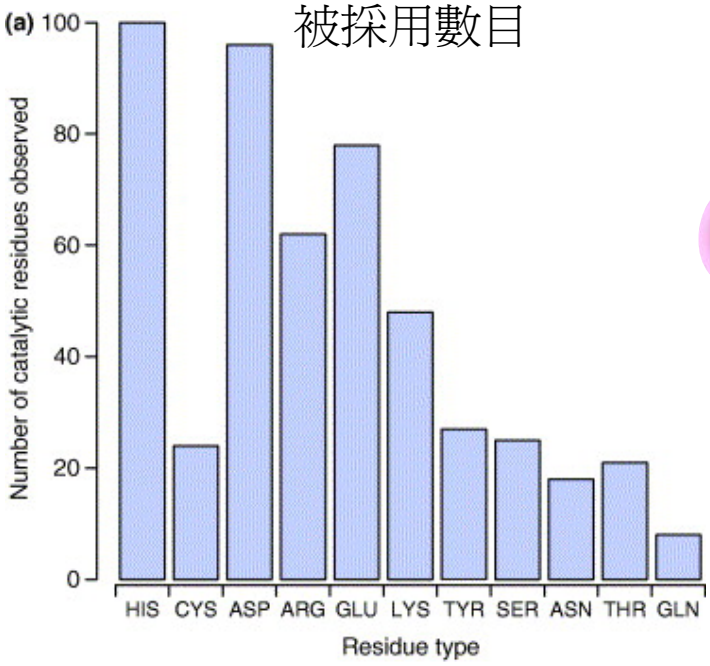
(c) His-His dyad (H83 攻擊磷酸)

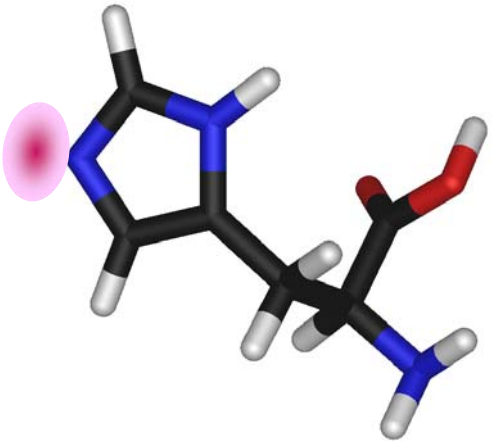


Combinations of different residues
form 'catalytic units' that are found repeatedly in different unrelated enzymes

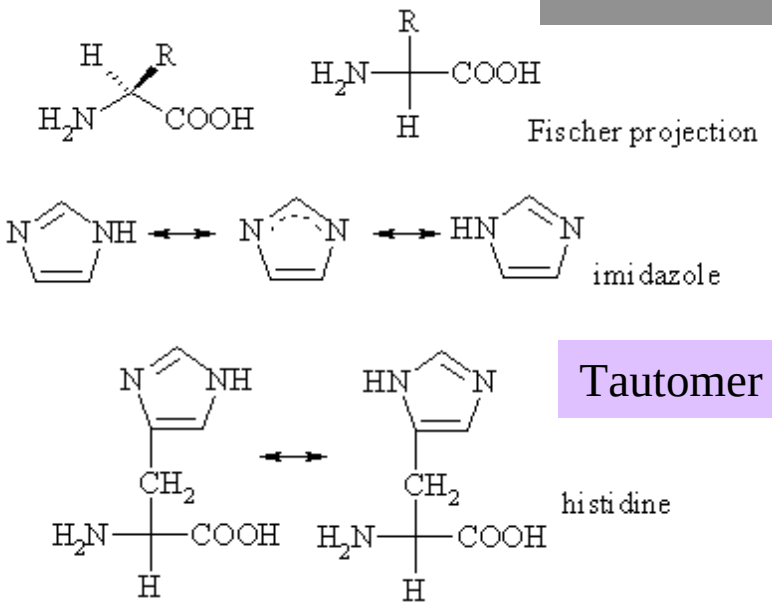
Gutteridge A, Thornton JM (2005) ***Understanding nature's catalytic toolkit.*** TIBS **30**: 622

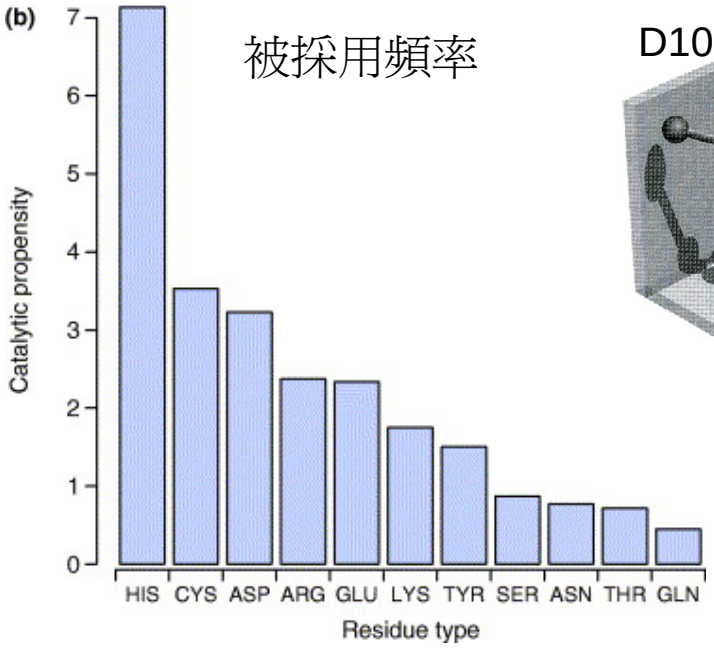
Amazing histidine





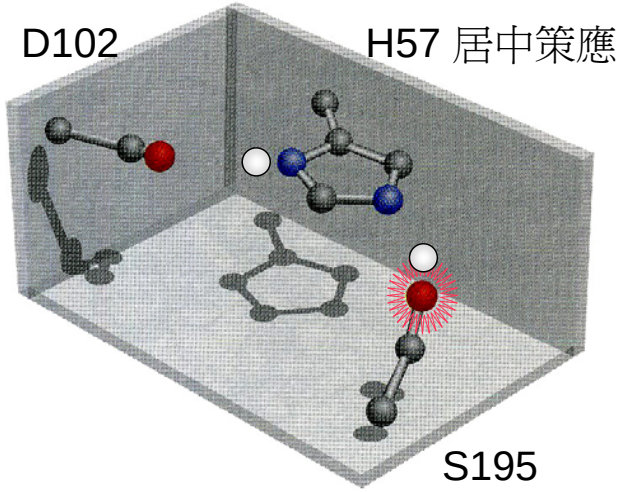
WIKIPEDIA





Gutteridge A, Thornton JM (2005) TIBS **30**: 622

Catalytic triad



Petsko GA, Ringe D (2004)
Protein Structure and Function
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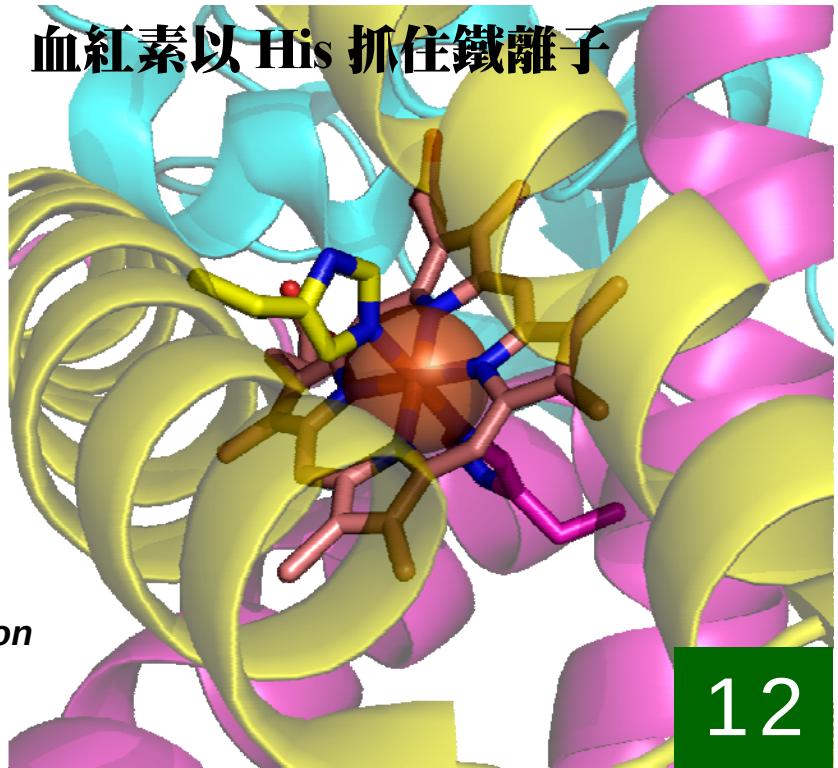


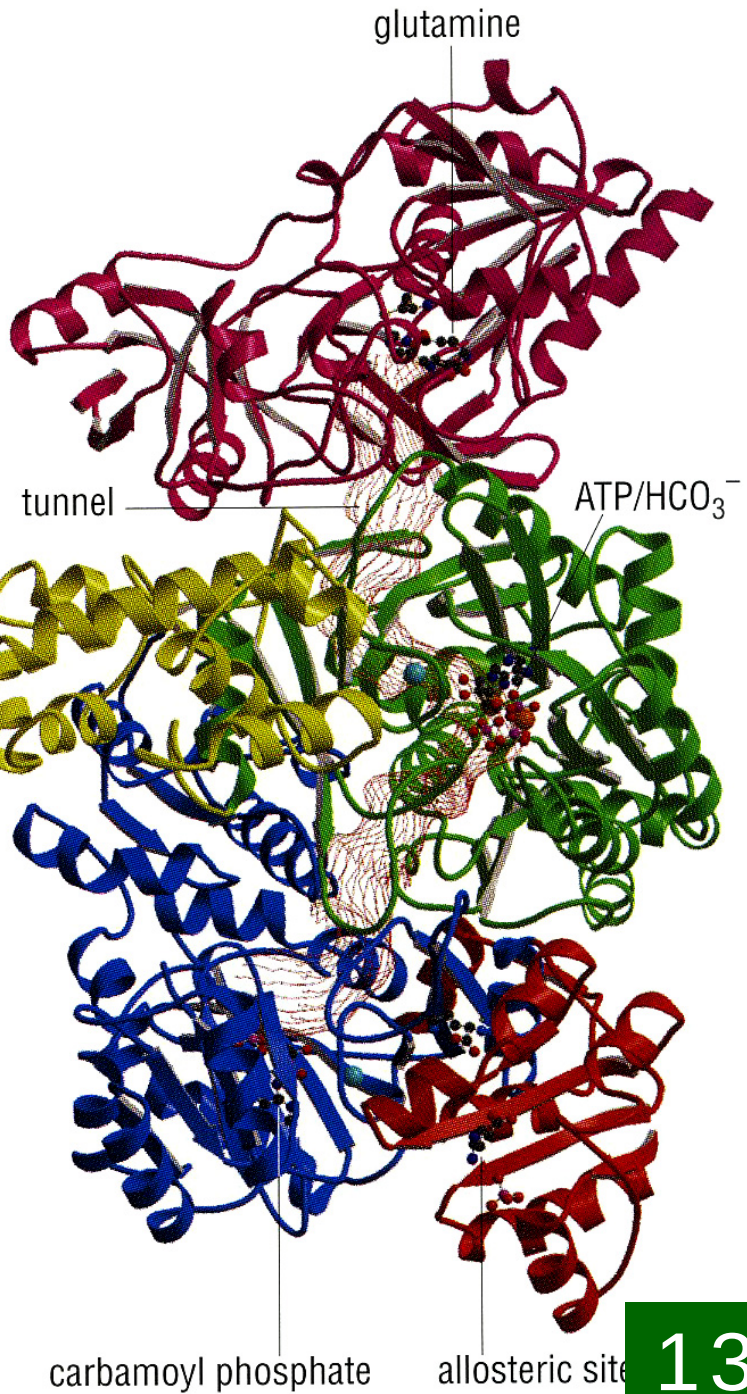
Multifunctional enzymes

(1) One active site, two reactions

(2) Two active sites, two reactions

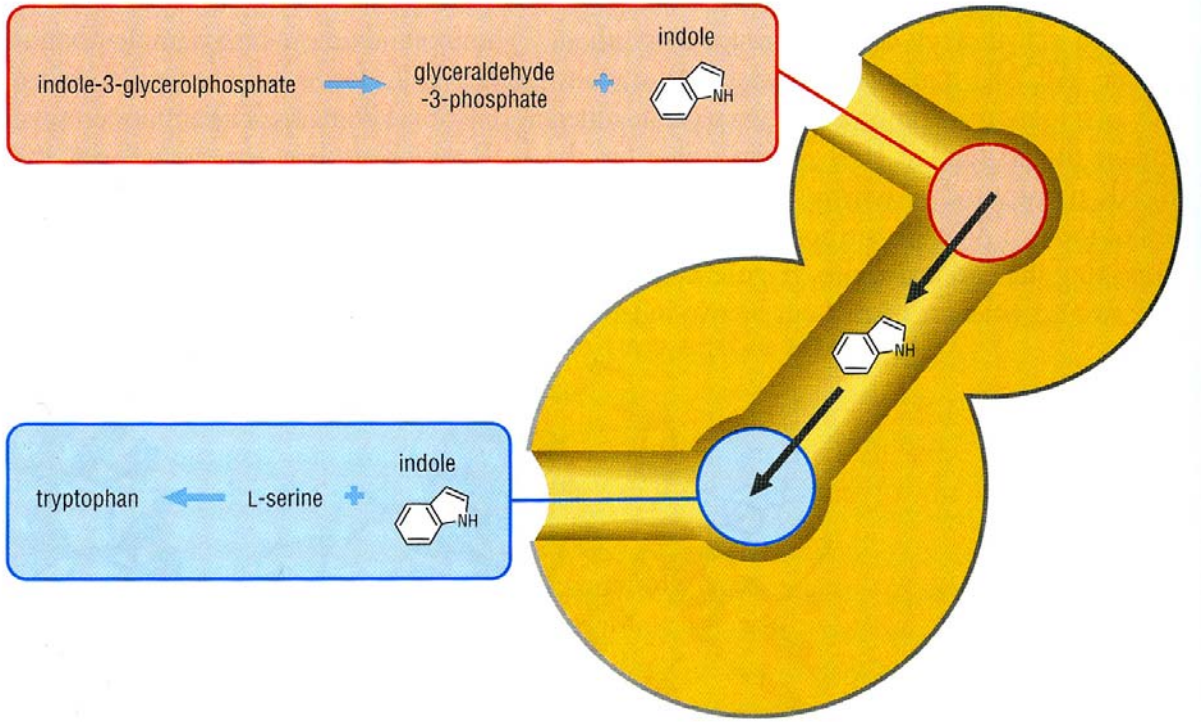
(3) Trifunctional enzyme with tunnel

Carbamoyl phosphate synthetase →



演化真是無限可能

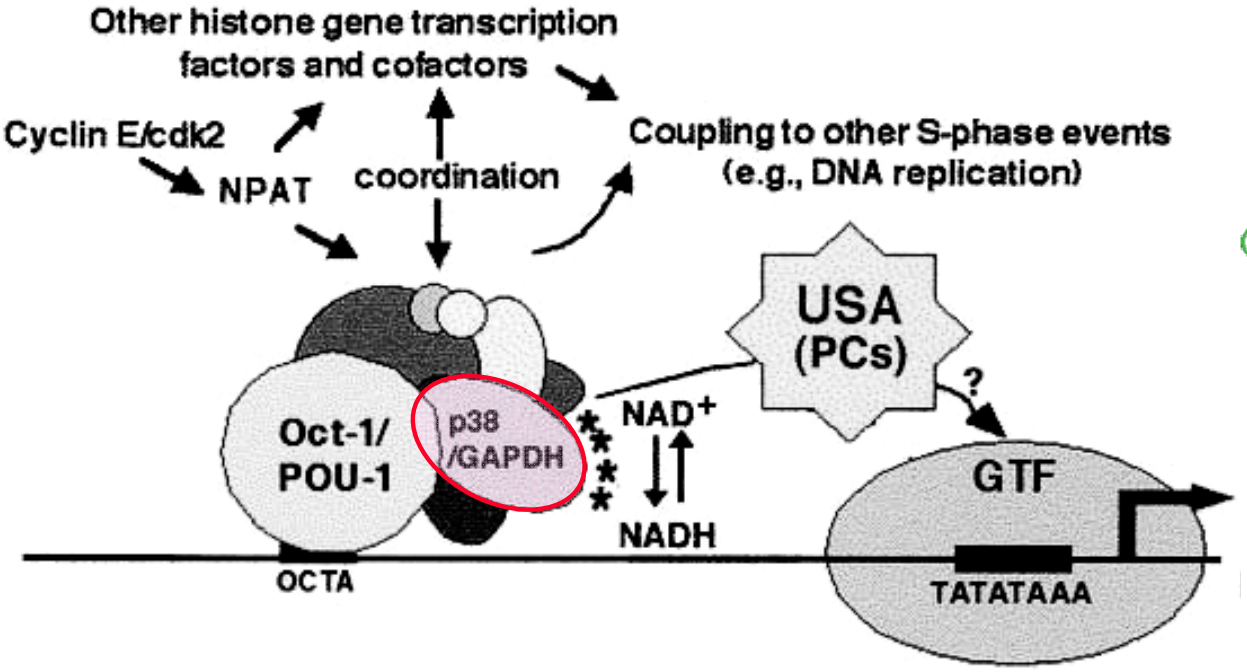
Tryptophan synthase ↓

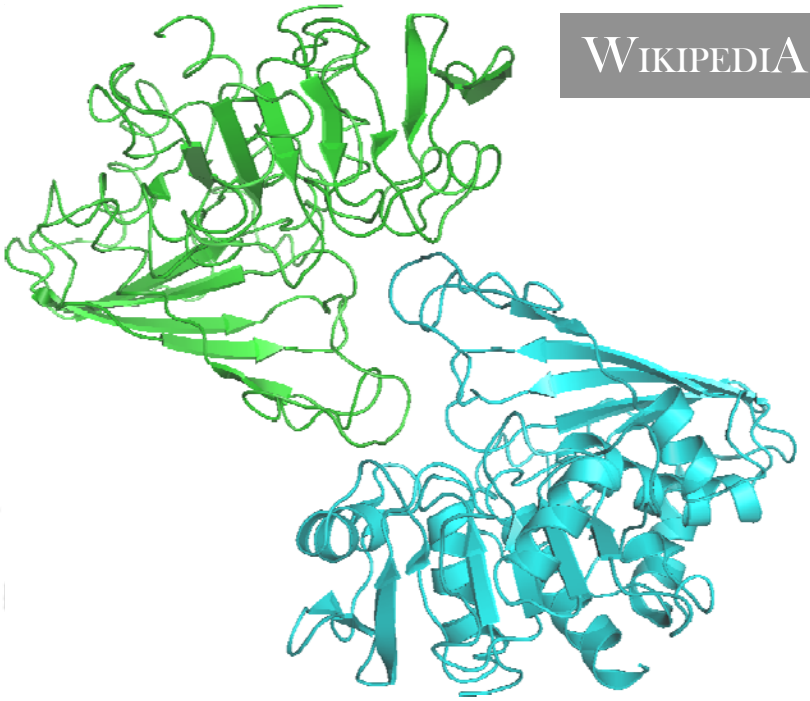


Petsko GA, Ringe D (2004) ***Protein Structure and Function*** F2-44, 45

Enzymes also has *non-catalytic* functions

Zheng, L et al (2003) S phase activation of the histone H2B promoter by OCA-S, a coactivator complex that contains GAPDH as a key component. **Cell** 114: 255~266





GAPDH has several functions:

(0) Glycolysis enzyme

(1) Transcription cofactor

(2) Initiates apoptosis

(3) ER to Golgi transportation

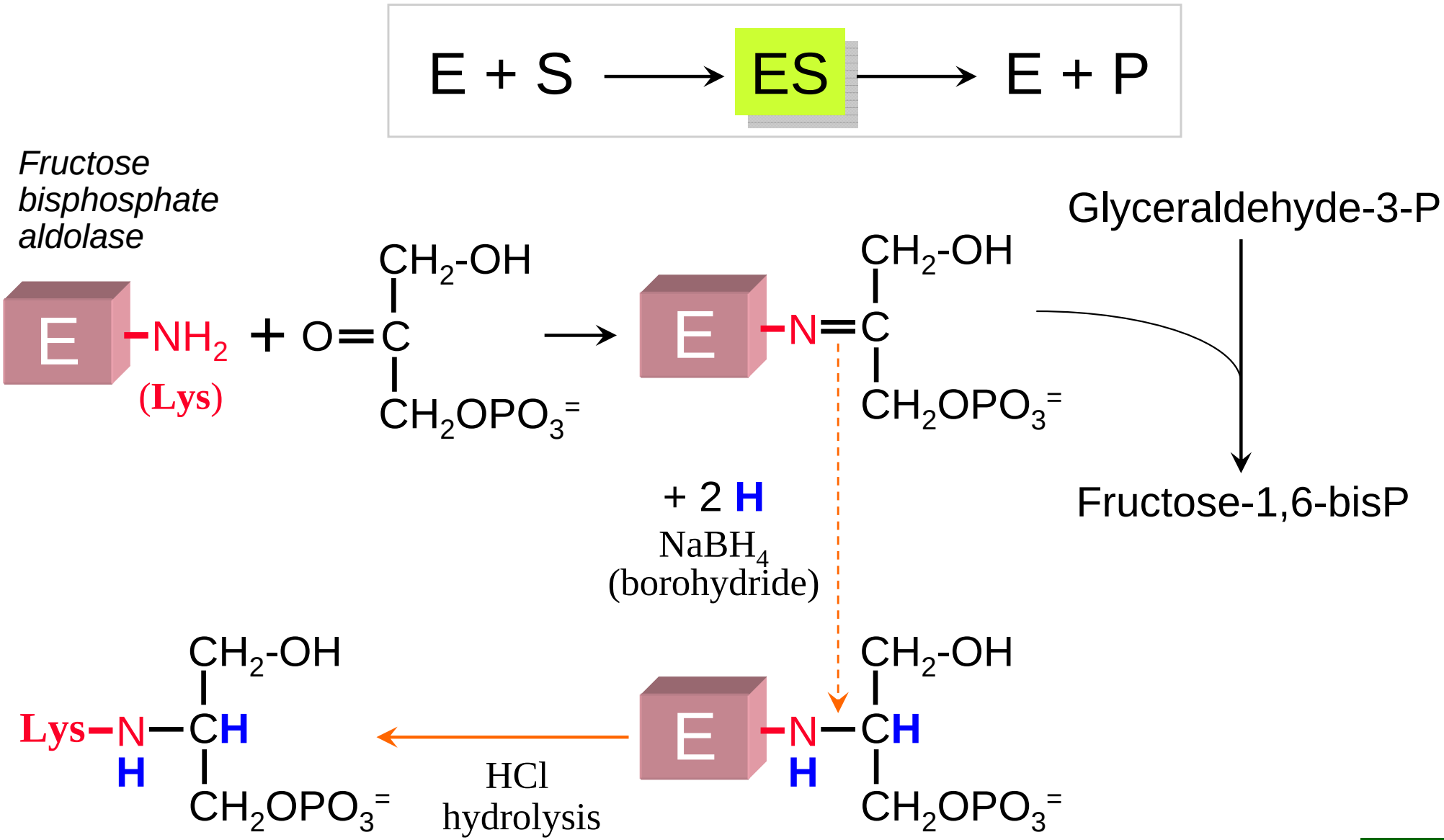
Other examples:

Phosphoglucose isomerase
(Glycolysis & Cytokine)

LON (Mitochondrial protease & Chaperone protein)

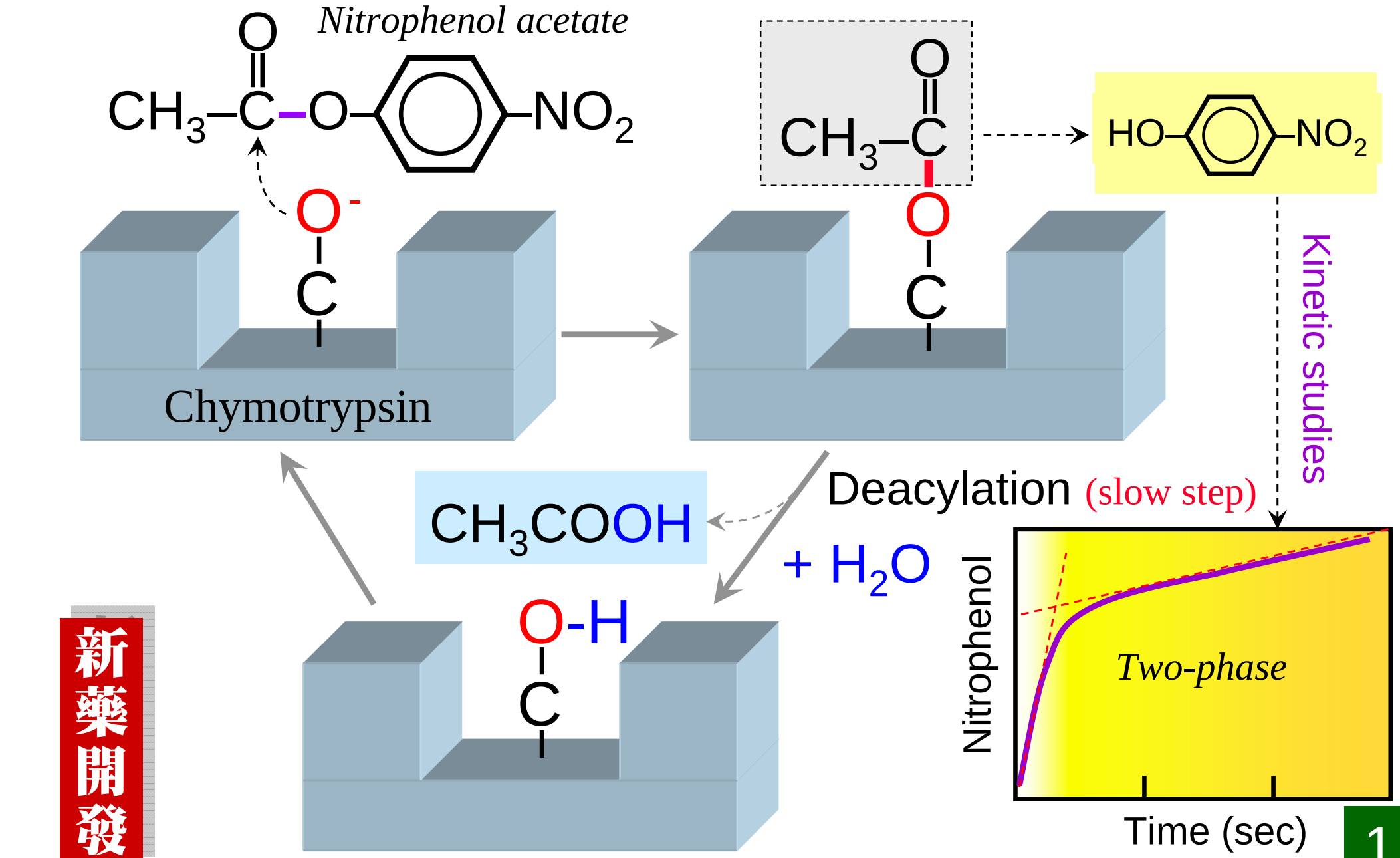
III Identification of enzyme catalytic sites

(1) Trapping the enzyme-substrate complex (classical)



Identification of enzyme catalytic sites

(2) The use of substrate analogues or rapid-reaction tech

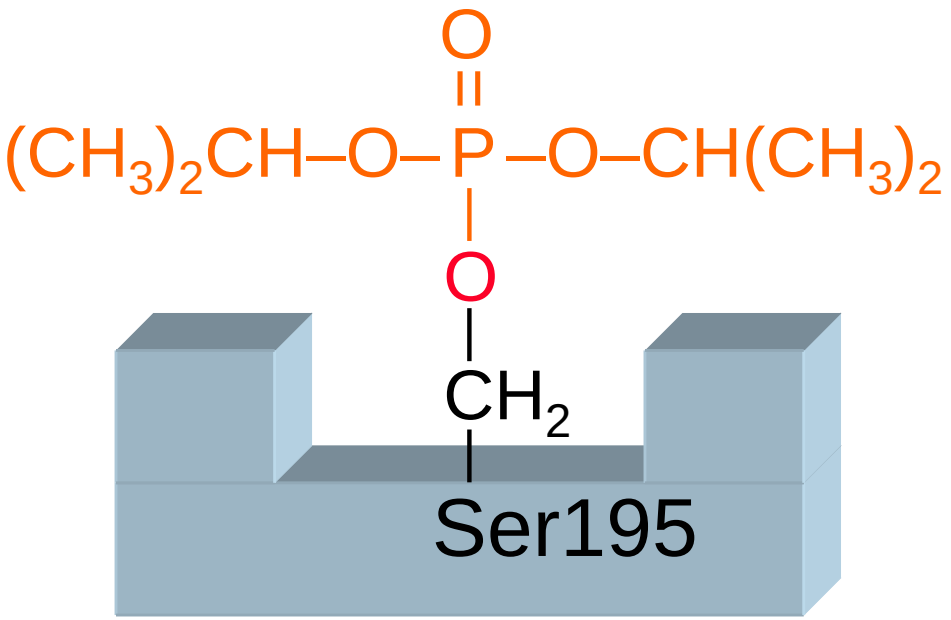


新藥開發

Identification of enzyme catalytic sites

(3) Chemical modification protected by substrate (classical)

DIFP (Diisopropyl-fluorophosphate)

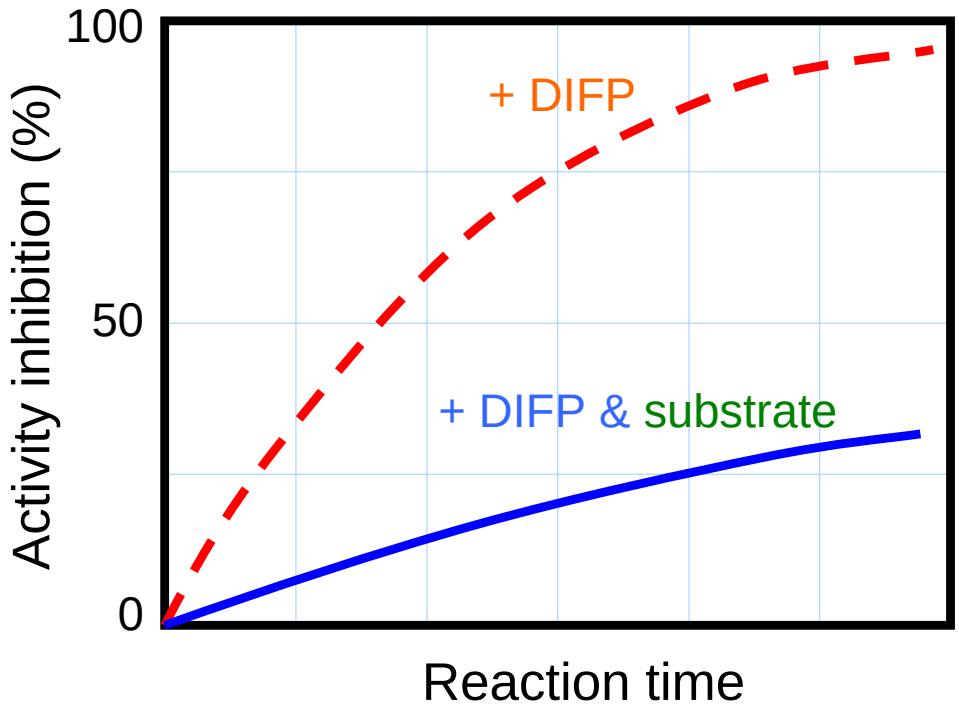


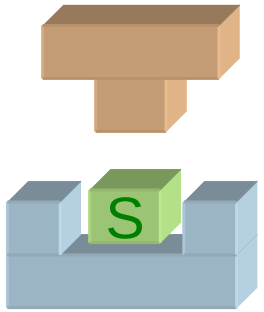
Partial hydrolysis

DIFP

Gly-Asp-Ser-Gly-Gly-Pro

以質譜儀分析片段序列可推知活性區重要資訊





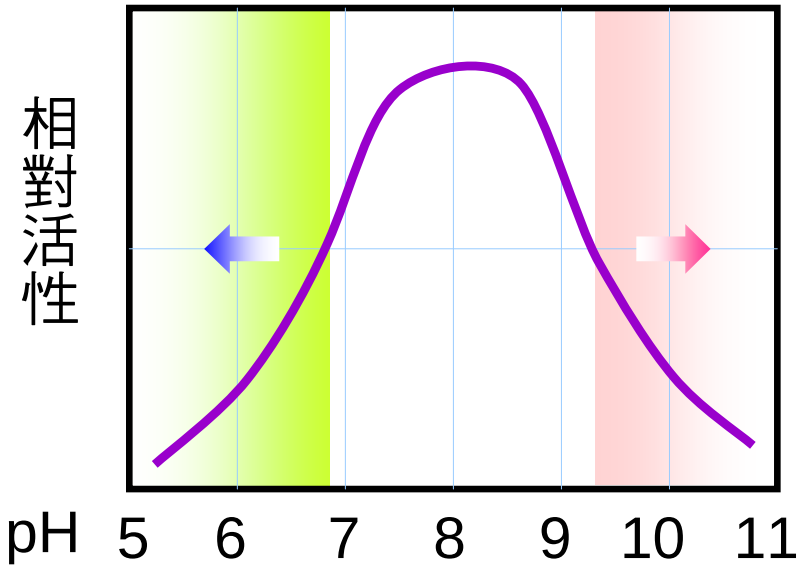
K_m increased

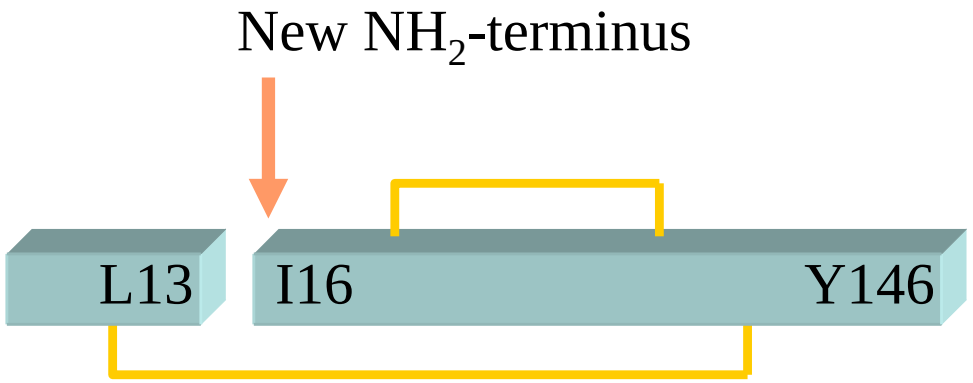
V_{max} unchanged

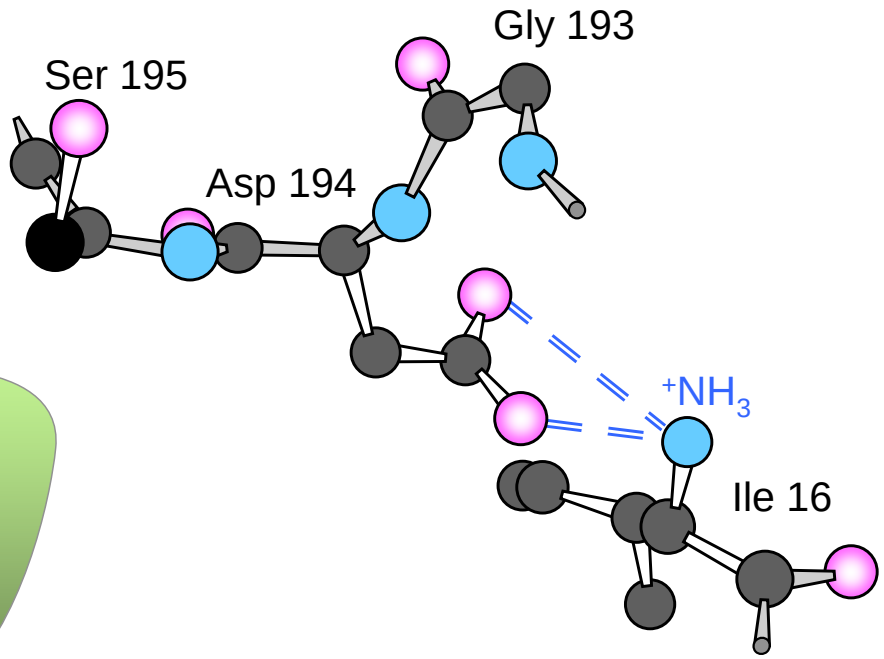
Active site protected by substrate

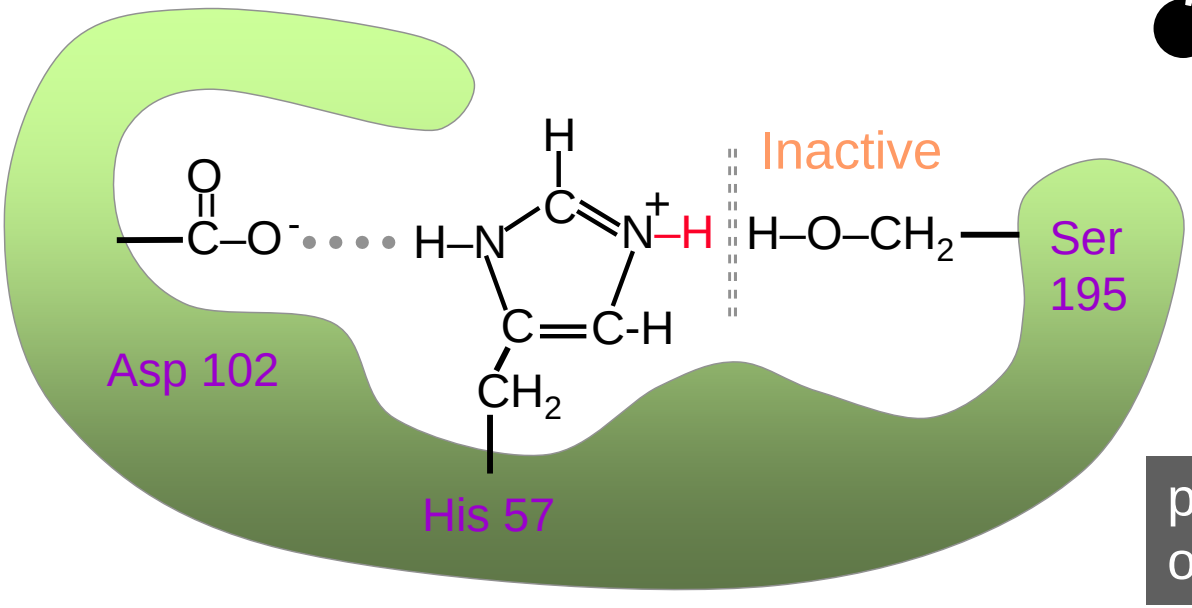
Identification of enzyme catalytic sites

(4) The effect of changing pH (classical but useful)









pK_a changes depending on microenvironment

Identification of enzyme catalytic sites

(5) Enzyme modified by site-directed mutagenesis (hot!)

Mutations	*Triad:*	*Amino acids in active site* Ser	His	Asp	Relative activity
No enzyme					1
Subtilisin		●	●	●	**10,000,000,000**
Ser, His & Asp → Ala		○	○	○	4,000
His & Asp → Ala		●	○	○	37,000
Ser → Ala		○	●	●	5,000
Asp → Ala		●	●	○	330,000
Asn^{155} → Leu		●	●	●	10,000,000

(Asn^{155} stabilizes transition state)

蛋白質表現是關鍵

Adapted from Dressler & Potter (1991) Discovering Enzymes, p.245

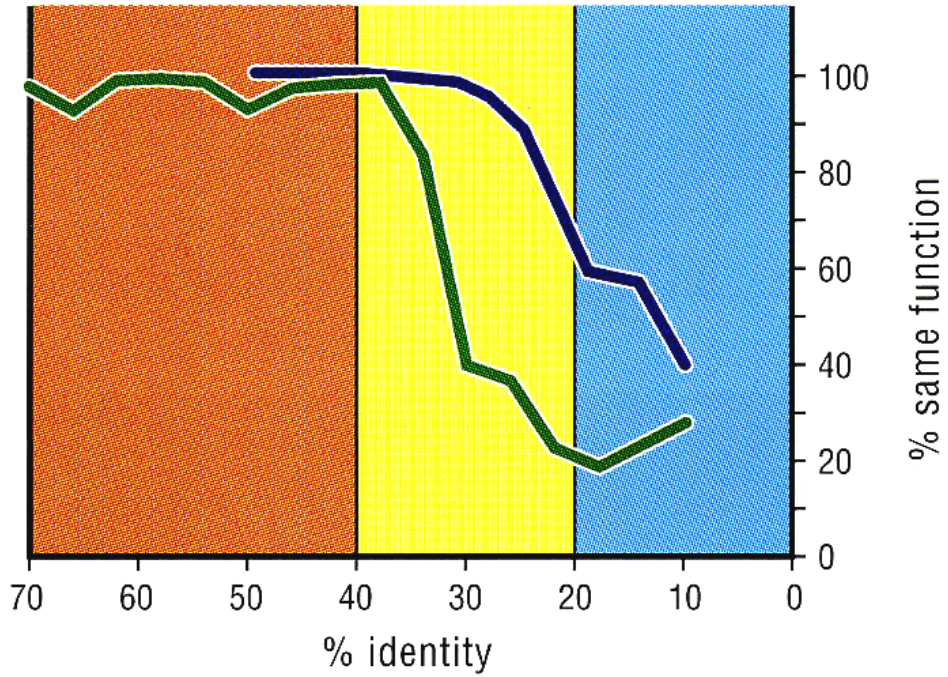
Identification of enzyme catalytic sites

(6) Enzyme kinetics and inhibition kinetics (real useful)

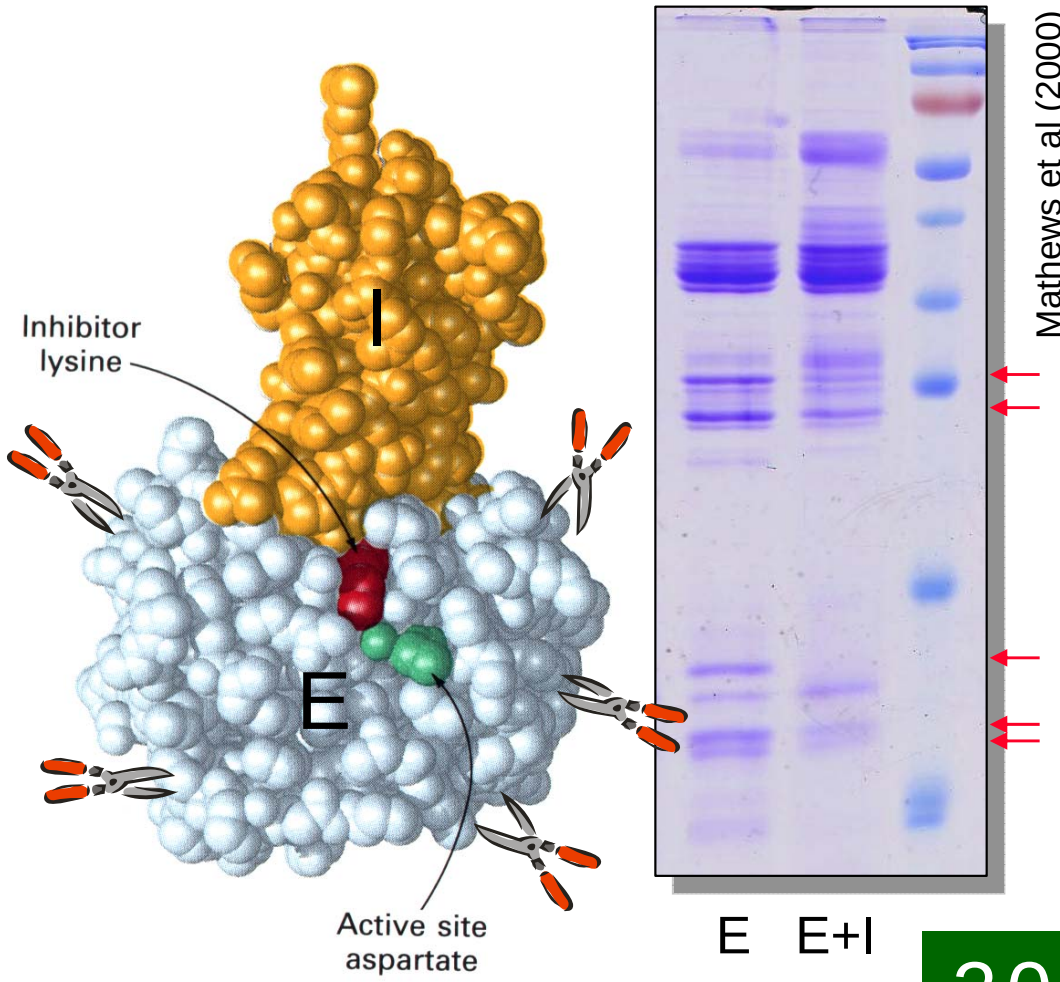
(7) Partial proteolysis protected by binding groups

(8) Prediction from protein sequences (very reliable!)

兩酵素序列若有 30% 相似則功能相同



Relationship of sequence similarity to similarity of function. Enzyme, blue curve; non-enzyme, green curve



Mathews et al (2000) Biochemistry (3e) p.208

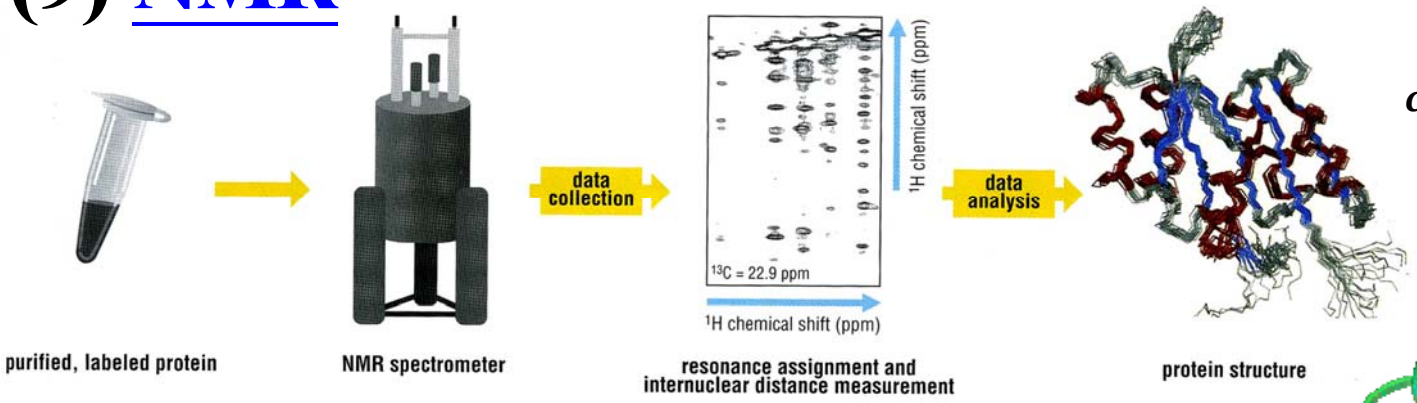
Petsko GA, Ringe D (2004) ***Protein Structure and Function*** F4-12

Stryer (1995) Biochemistry (4e) p.252

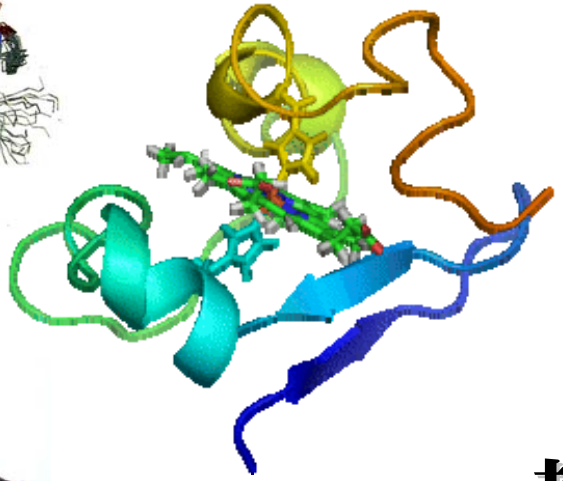
Identification of enzyme catalytic sites

(9) NMR 核磁共振

Petsko GA, Ringe D (2004) ***Protein Structure and Function*** F5-3, 5-4



NMR structures of
cytochrome c in solution

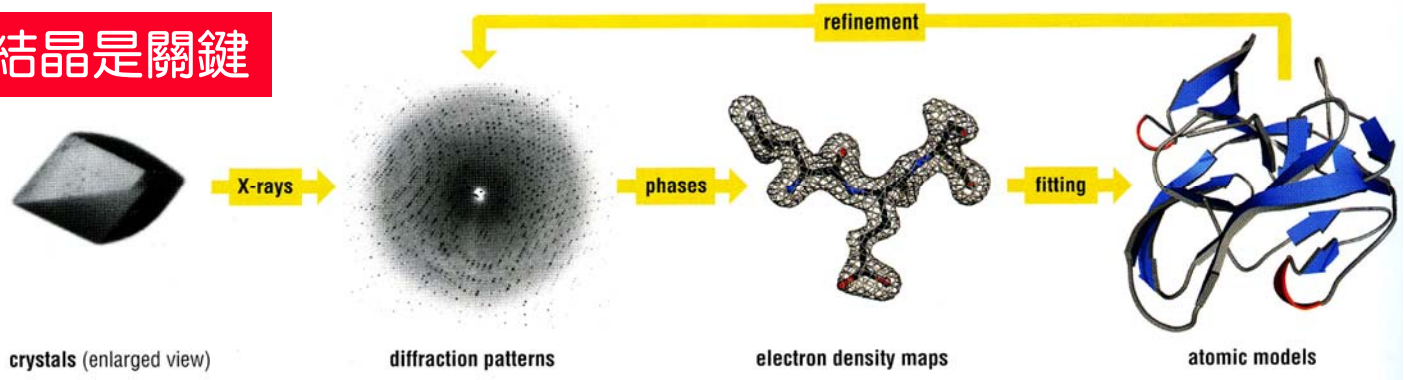


分子量

小 → 大 → 超大

(10) X-ray crystallography X 光結晶學

結晶是關鍵



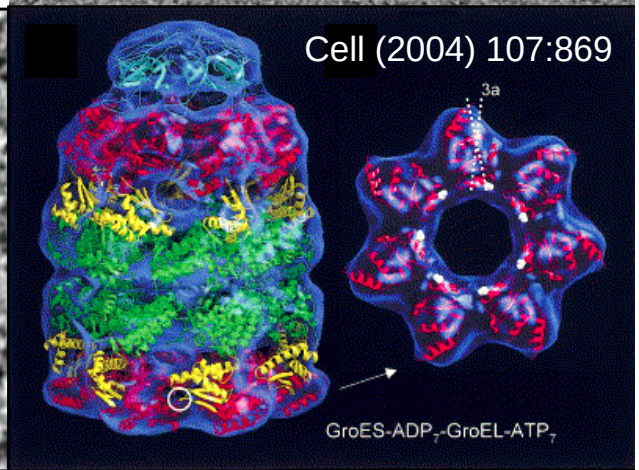
WIKIPEDIA

電腦影像科技之貢獻

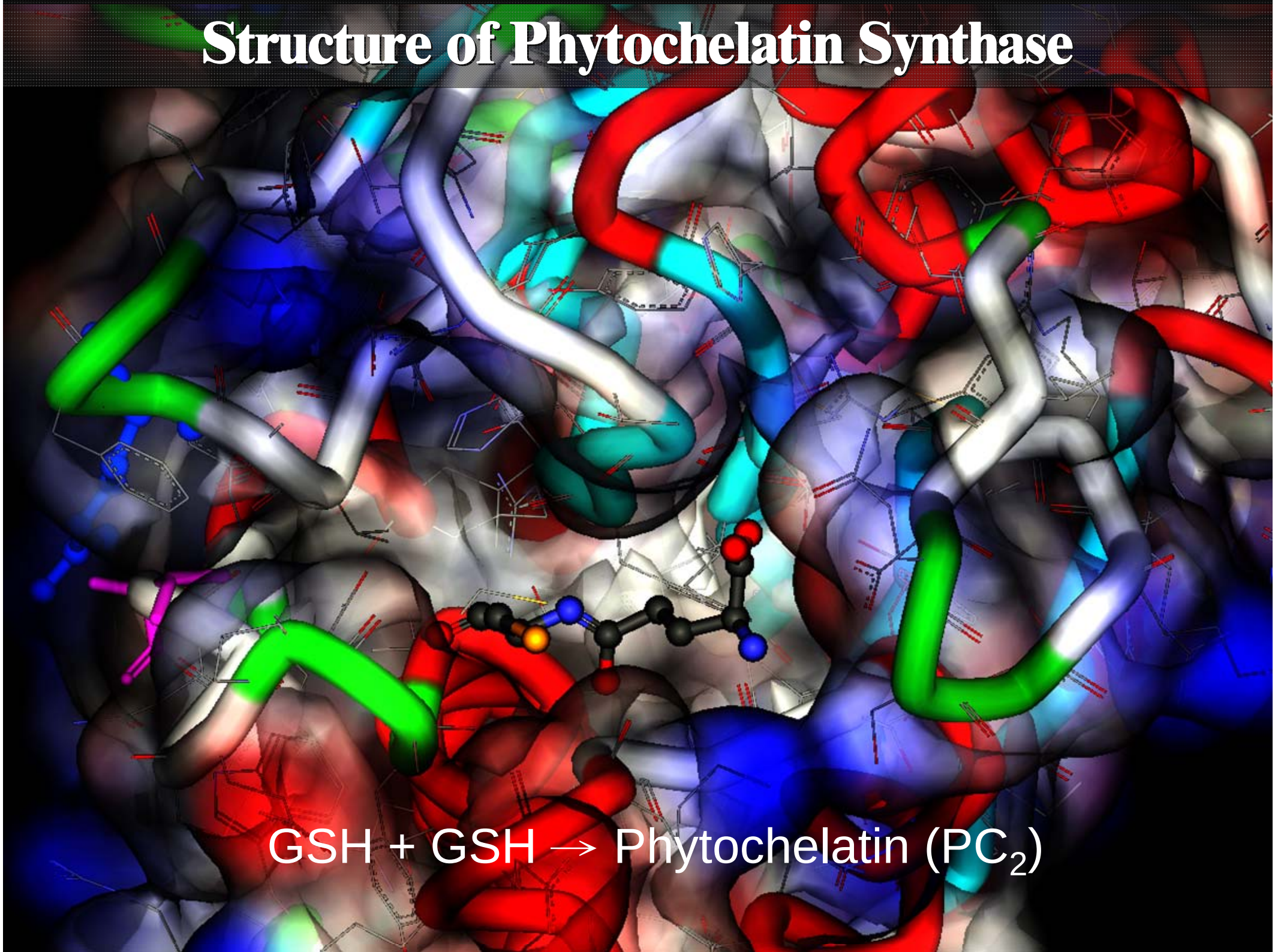
(11) Cryo-EM (new)

超低溫電顯

不必用結晶

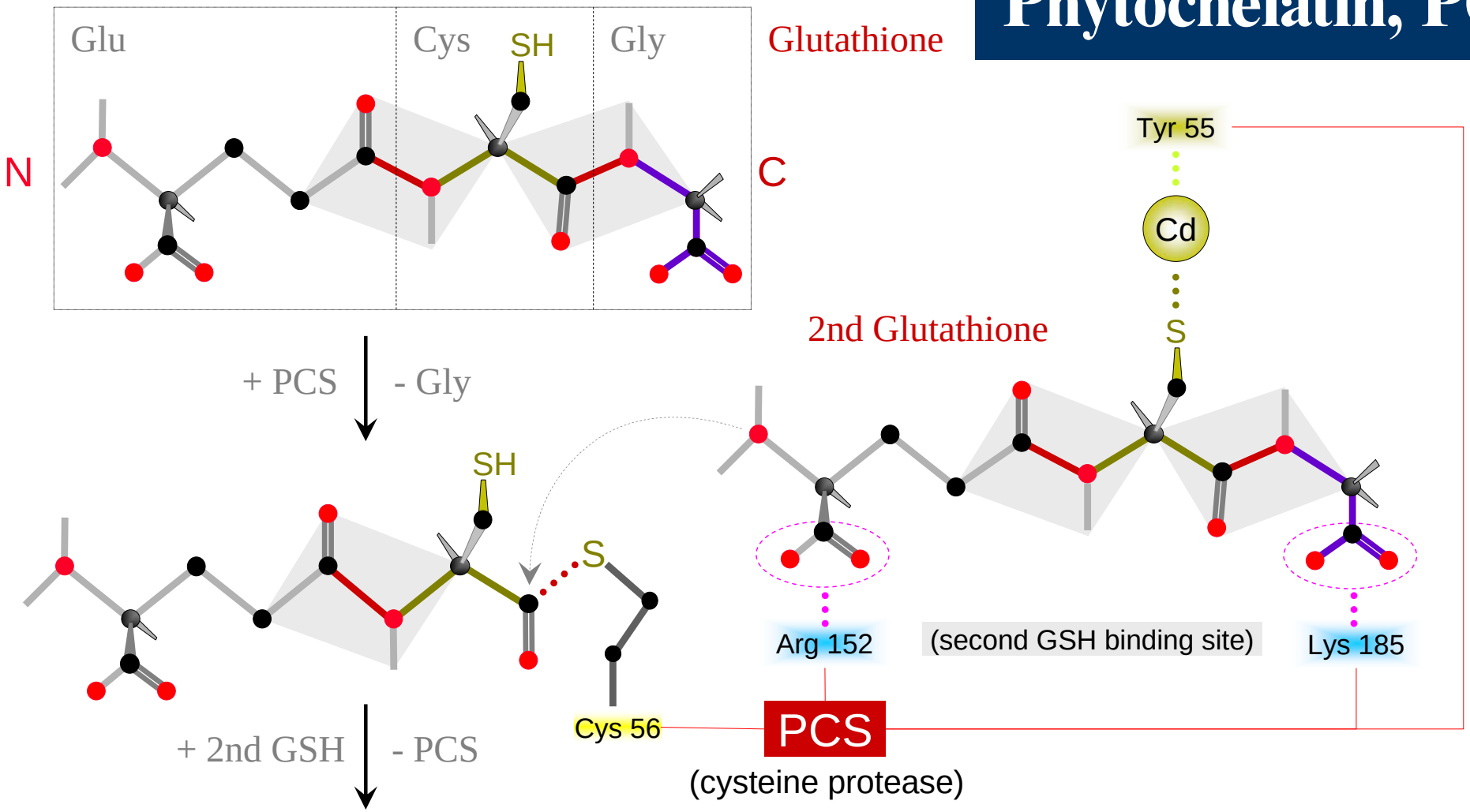


Structure of Phytochelatin Synthase

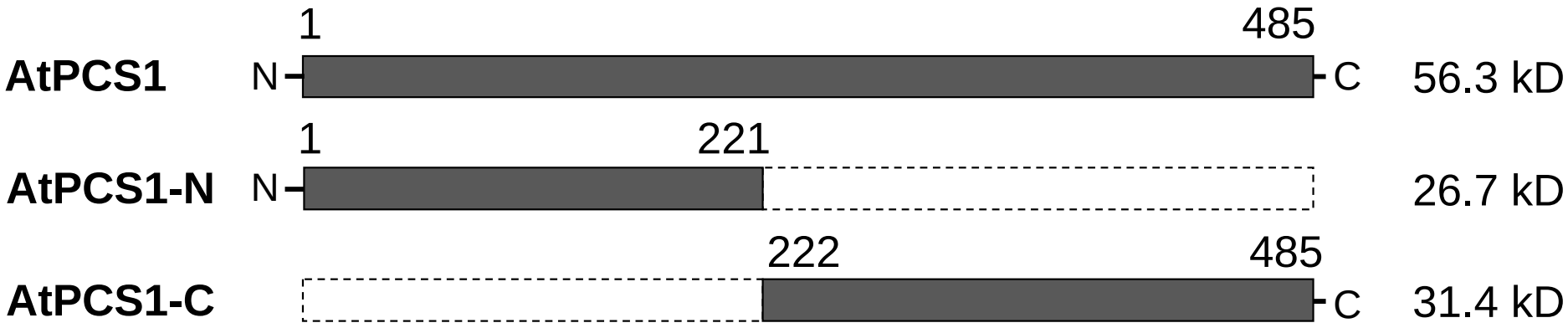


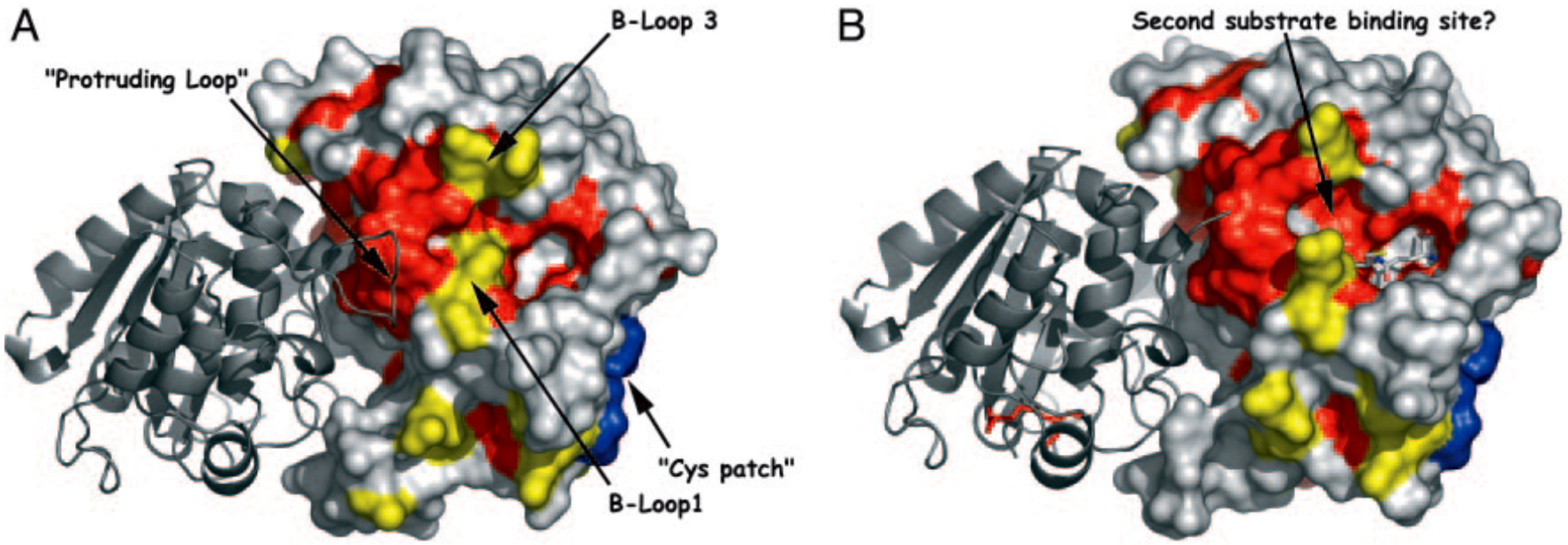
GSH + GSH → Phytochelatin (PC_2)

Phytochelatin, PC



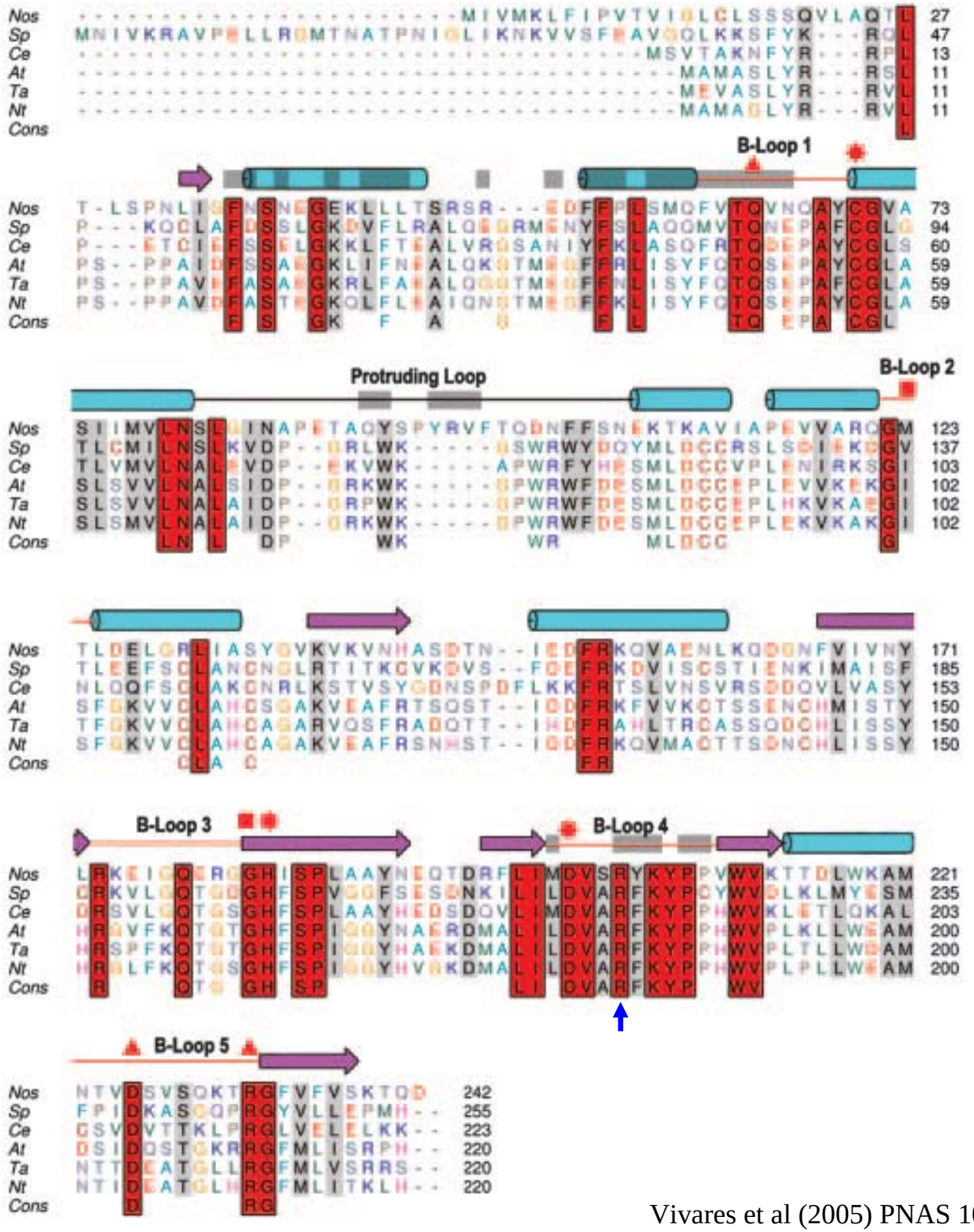
Phytochelatin synthase molecules





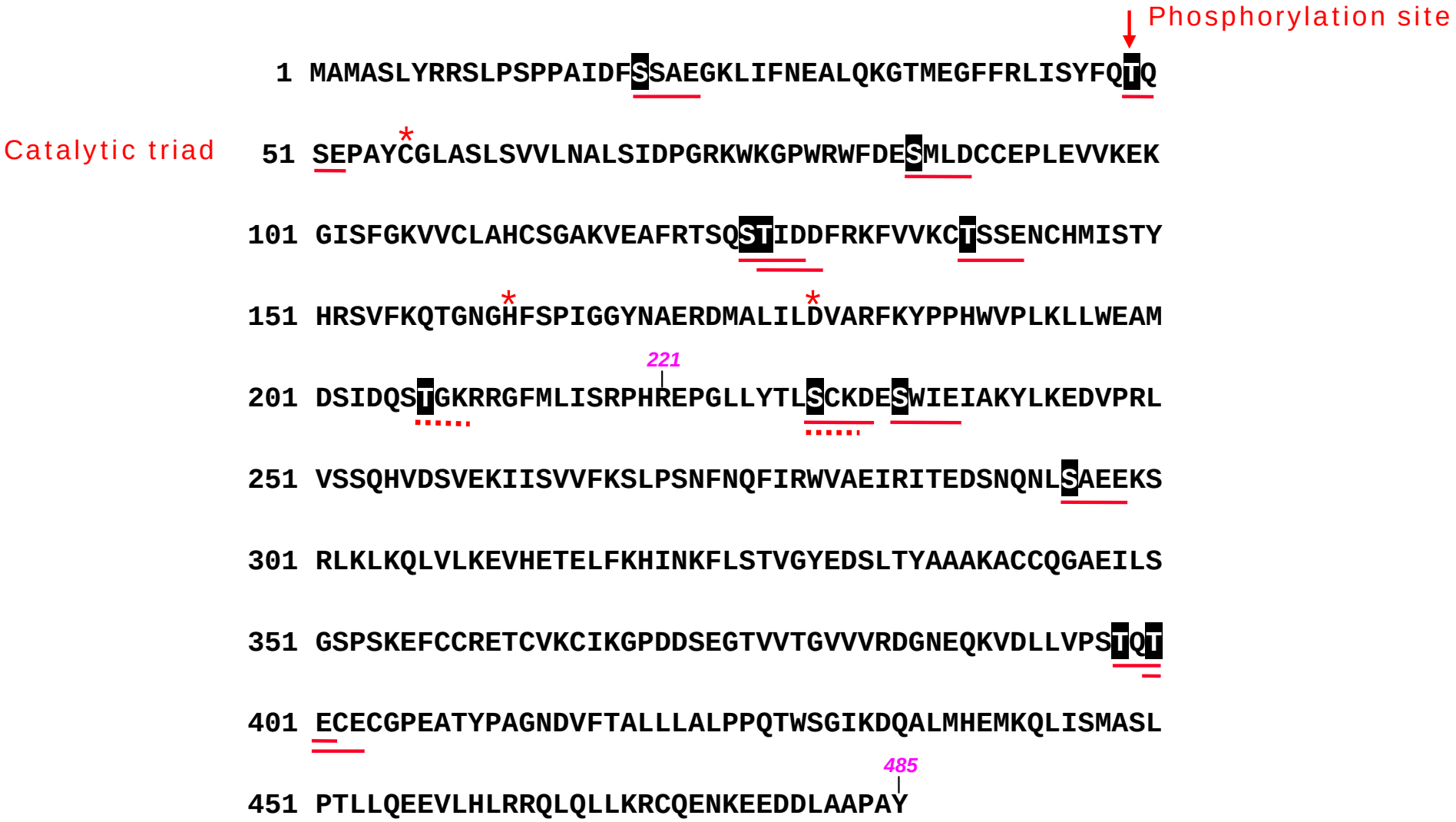
NsPCS is a Cys protease-like enzyme

Vivares et al (2005) PNAS 102:18848-18853



Vivares et al (2005) PNAS 102:18848-18853

Motif prediction for PCS phosphorylation



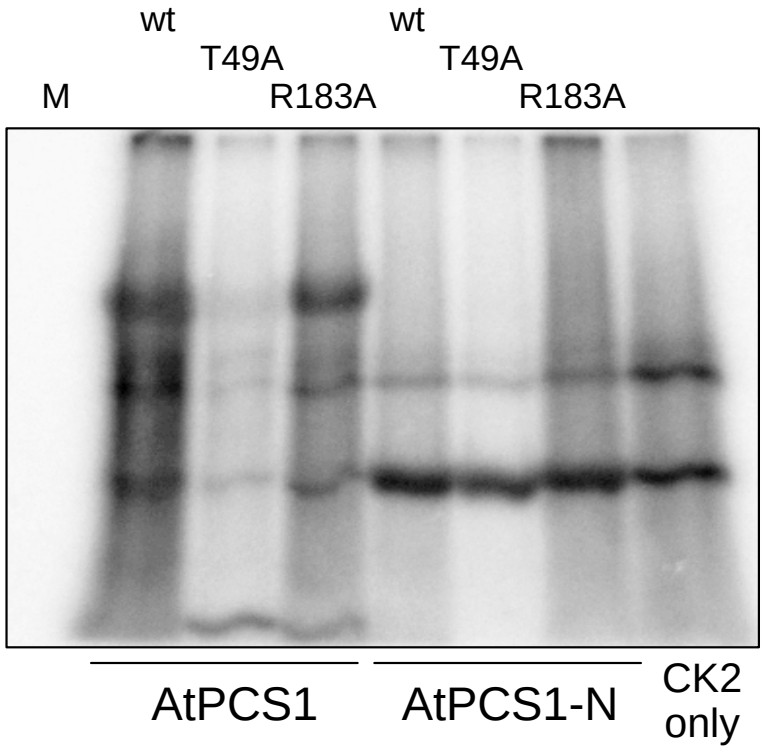
CKII_PHOSPHO_SITE
[ST]-x(2)-[DE]

PKC_PHOSPHO_SITE
[ST]-x-[RK]

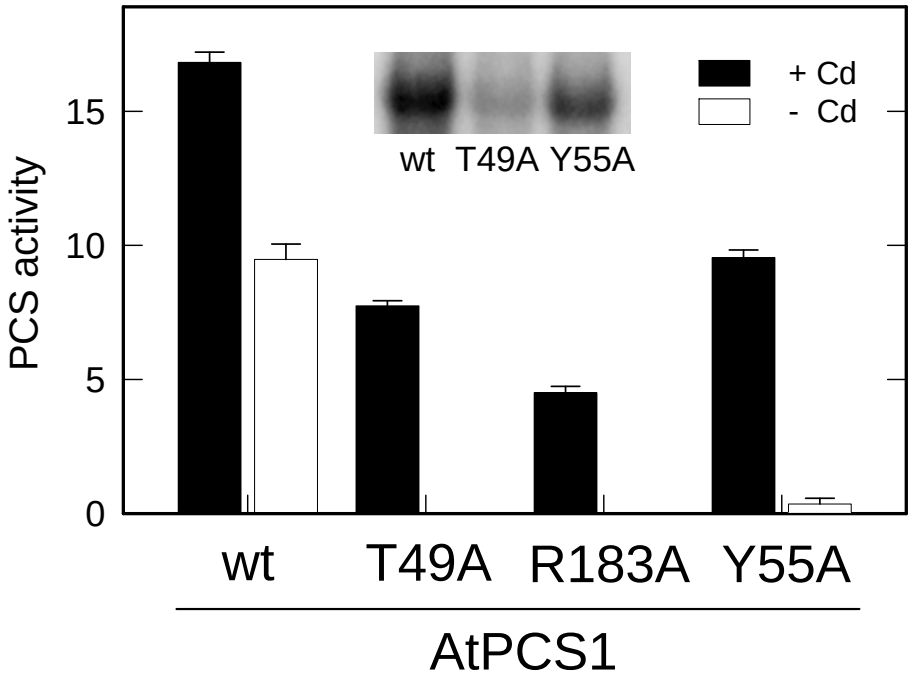
TQSE

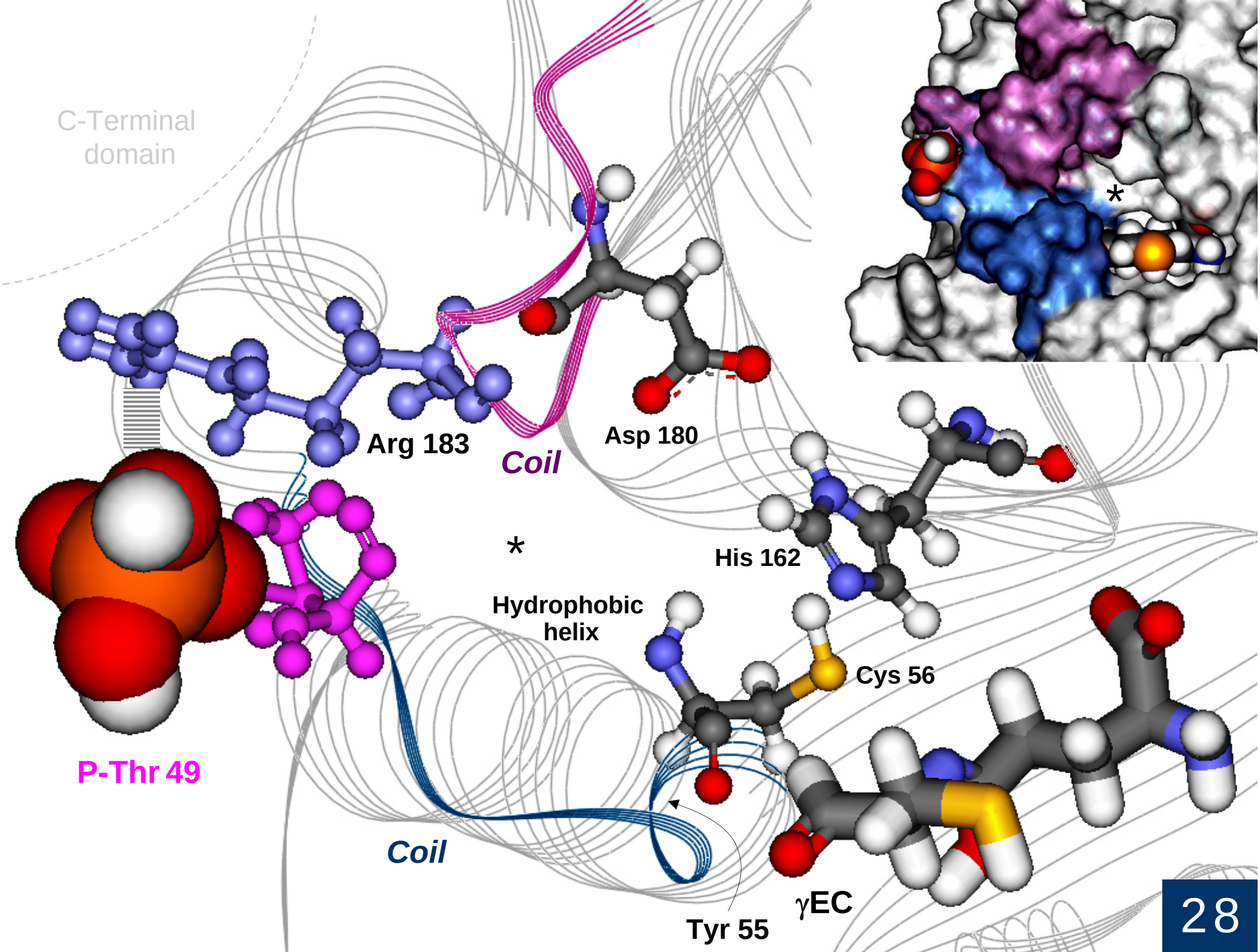
PCS is phosphorylated on Thr 49

Autoradiograph

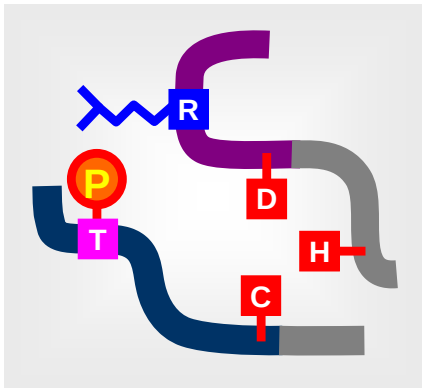


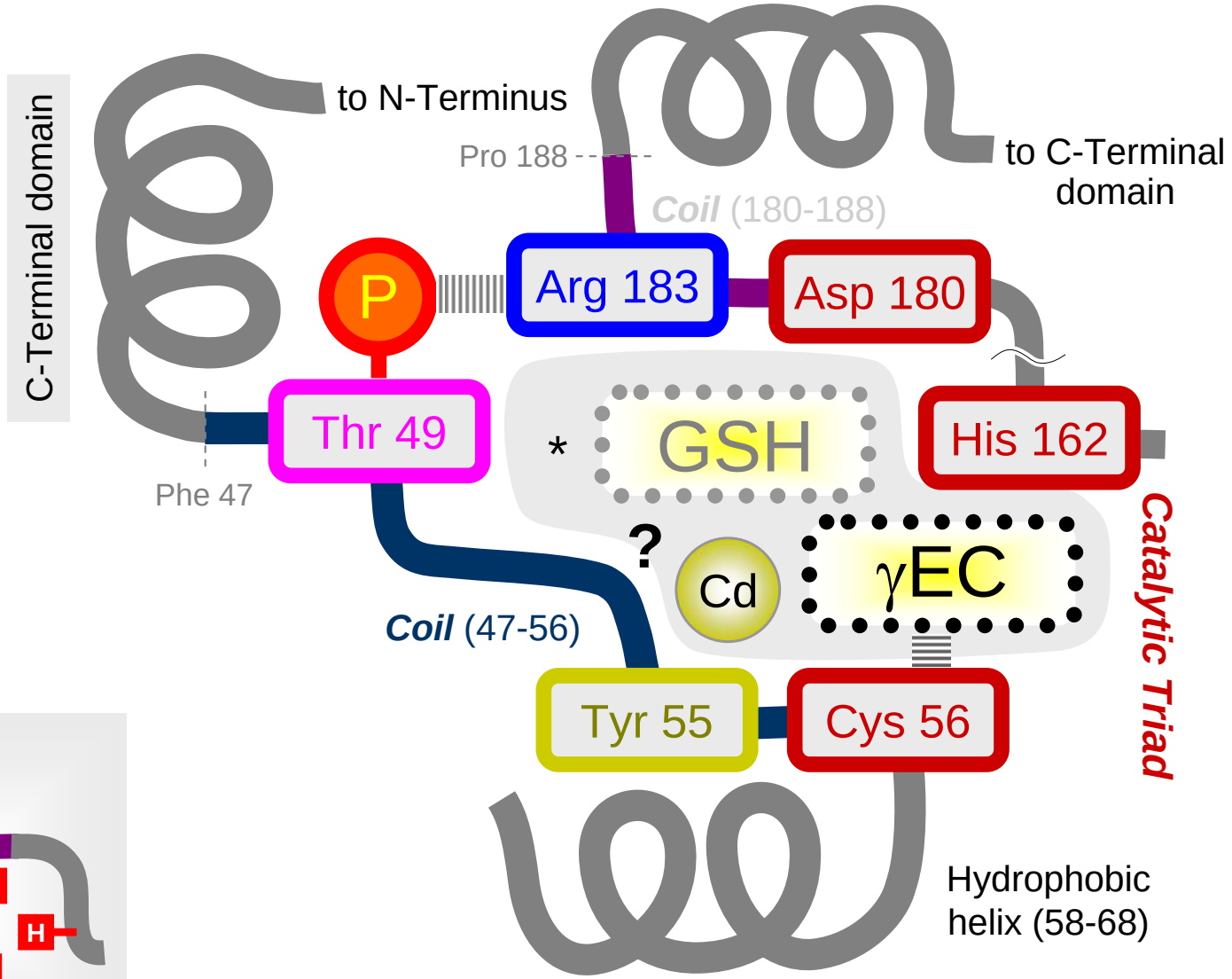
GSH + GSH → Phytochelatin (PC_2)




C-Terminal
domain
Arg 183
Coil
Asp 180
*
His 162
Hydrophobic
helix
Cys 56
P-Thr 49
Coil
γEC
Tyr 55
*

The active site of PCS





3-Dimensional structure prediction

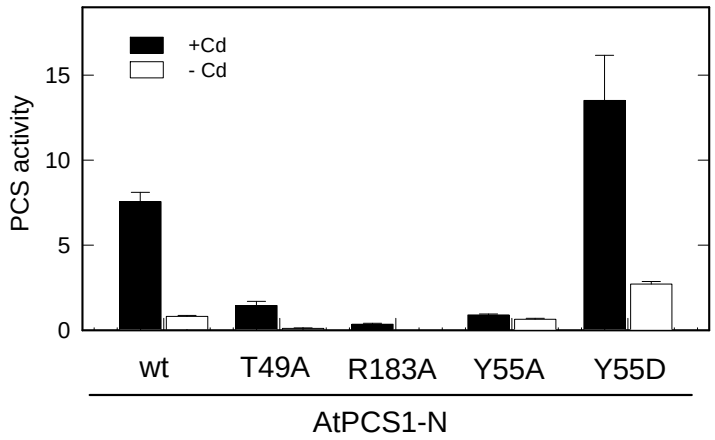
Phytochelatin synthase 螯合素合成脢

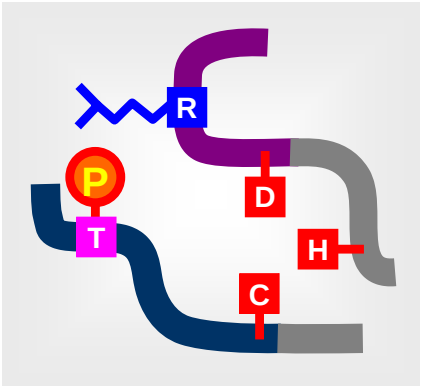
GSH + GSH → Phytochelatin (PC_2)

Phosphorylation site

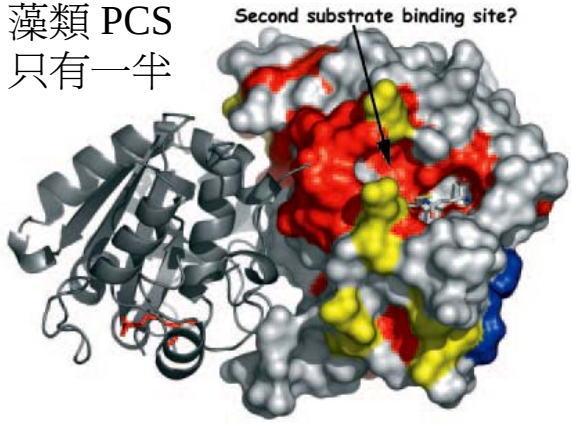
*Catalytic triad

```
  1 MAMASLYRRSLPSPPAIDFSSAEGKLIFNEALQKGTMEGFFRLISYFQTQ
 51 SEPAYCGLASLSVVLNALSIDPGRKWKGPWRWFDESMLDCCEPLEVVKEK
101 GISFGKVVCLAHCSGAKVEAFRTSQSTIDDFRKFVVKCTSSENCHMISTY
151 HRSVFKQTGNGHFSPIGGYNAERDMALILDVARFKYPPHWVPLKLLWEAM
                                  221
201 DSIDQSTGKRRGFMLISRPHREPGLLYTLSCKDESWIEIAKYLKEDVPRL
301 RLKLKQLVLKEVHETELFKHINKFLSTVGYEDSLTYAAAKACCQGAEILS
351 GSPSKEFCCRETCVKCIKGPDDSEGTVVTGVVVRDGNEQKVDLLVPSTQT
                                                  485
401 ECECGPEATYPAGNDVFTALLLALPPQTWSGIKDQALMHEMKQLISMASL
451 PTLLQEEVLHLRRQLQLLKRCQENKEEDDLAAPAY
```





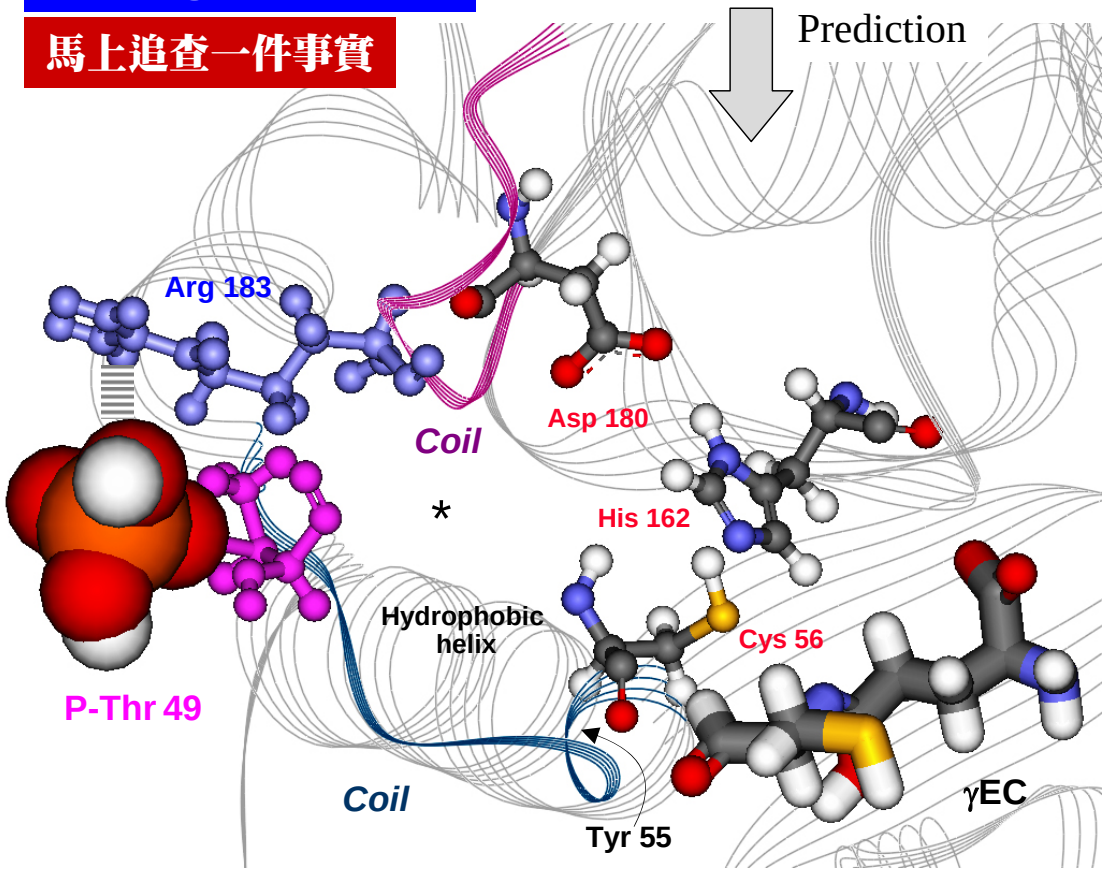
藻類 PCS
只有一半



預測 Arg 183 可能角色

馬上追查一件事實

Prediction



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『**上課演講**』搜尋
即可在首行找到

講義部份

指定閱讀

參考資料