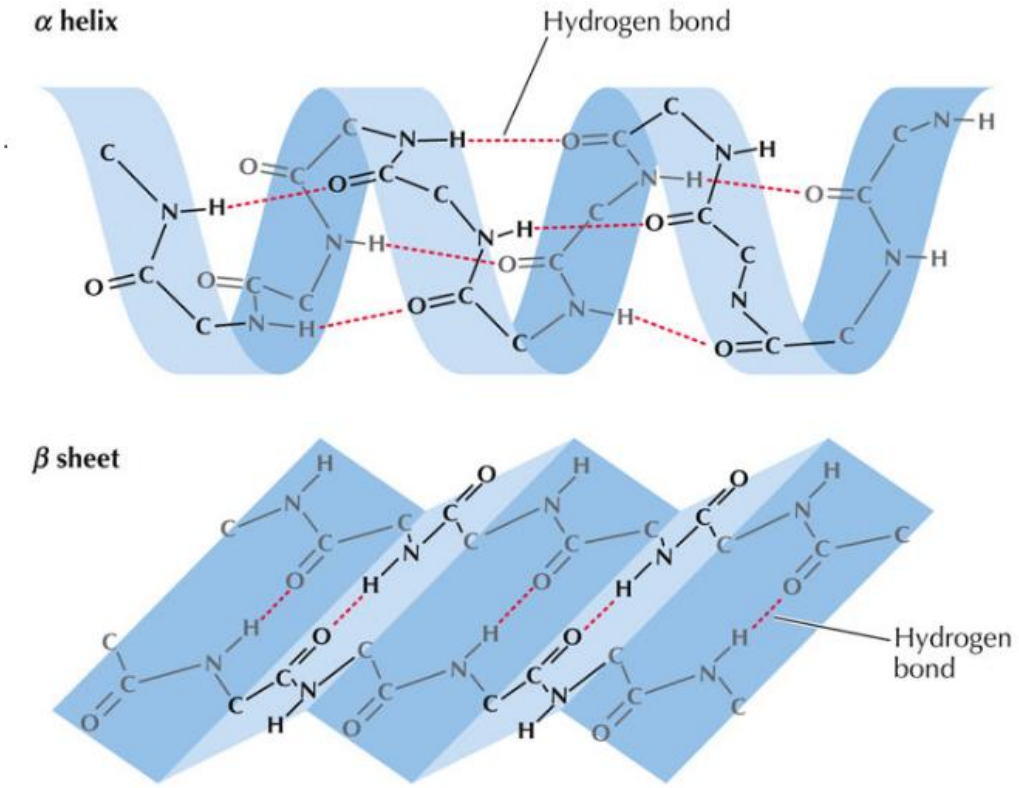
生命的化學工業：化學反應與酵素

10-5-2020

解釋形成 alpha helix and beta pleated sheet 作用力之異同

Intramolecular
vs
Intermolecular

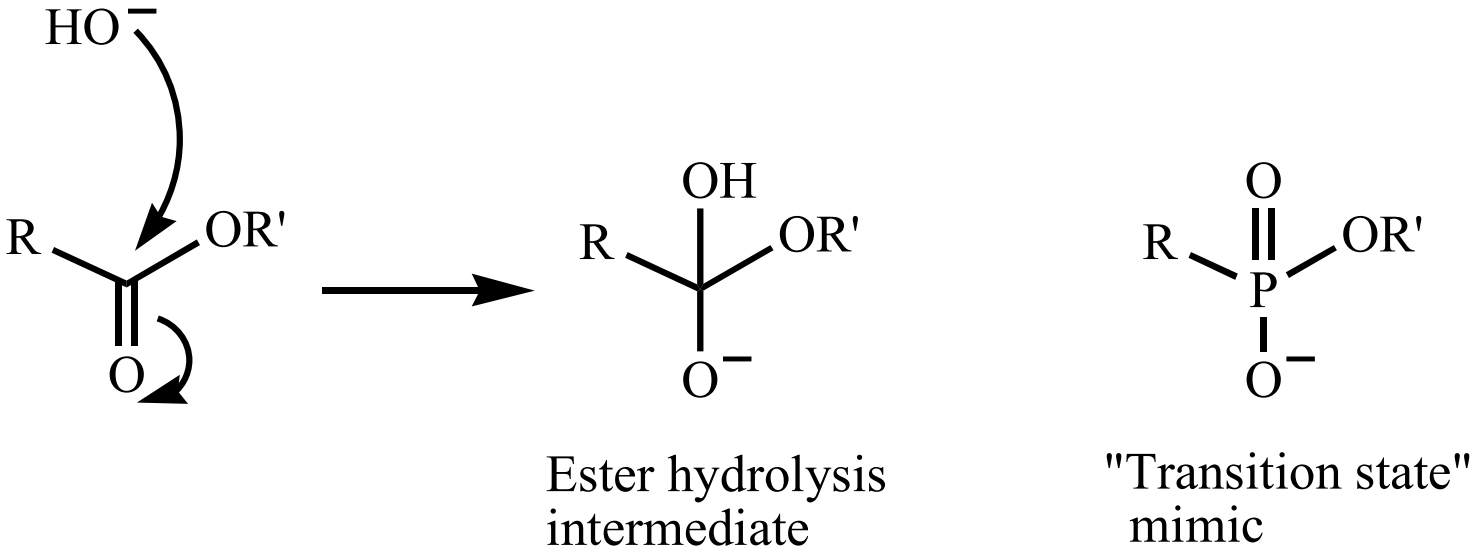


Antibody can catalyze specific chemical reaction we call abzyme. 它的原理是什麼？

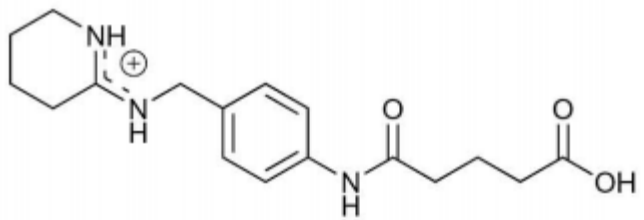
Can antibody bind to substrate and play as an enzyme (catalysis power) to convert substrate to product?

Development of Catalytic Antibodies

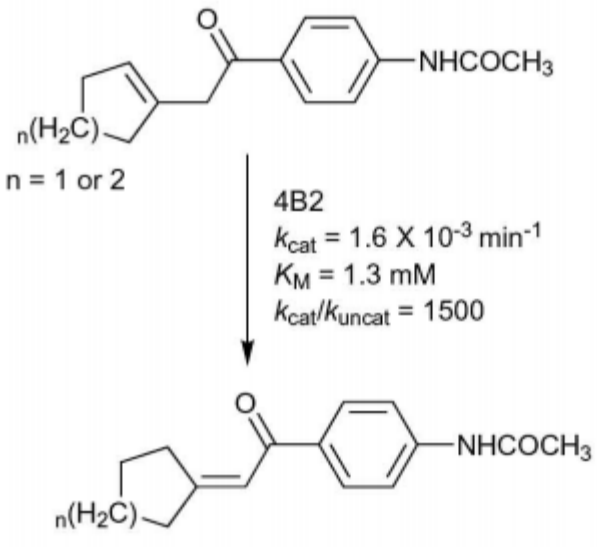
Comparison of an ester hydrolysis tetrahedral intermediate and a phosphonate “transition state” mimic



Antibody-catalyzed Double-bond Isomerization



Hapten



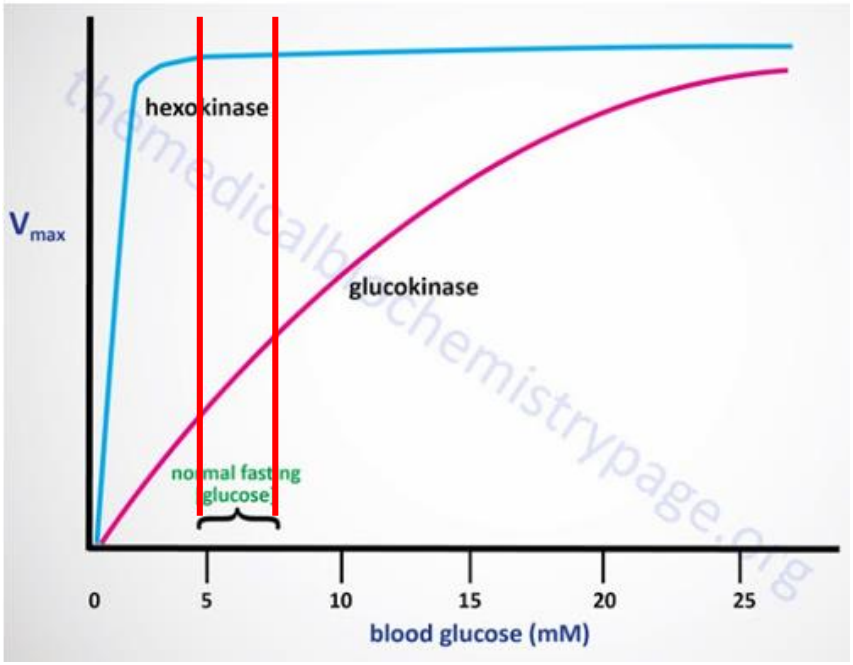
- The hapten induced an acidic residue in the active site (Bait-and-switch strategy).
- A dienol intermediate was proposed.

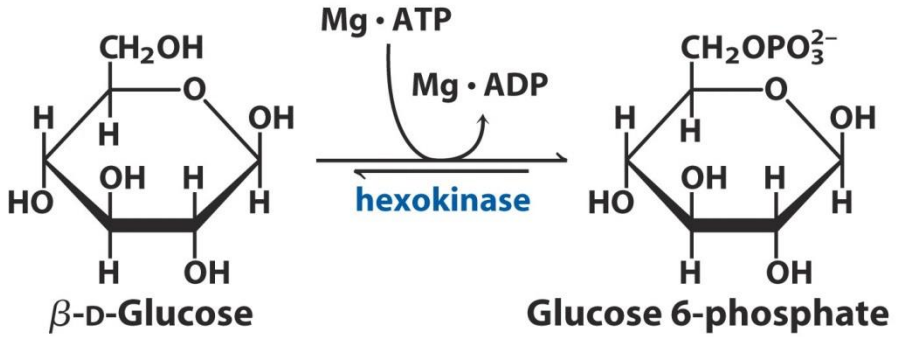
Goncalves, O.;Dintinger, T.;Lebreton, J.;Blanchard, D.;Tellier, C. *Biochem. J.* **2000**, *346*, 691.

4, 解釋為什麼肌肉中 hexokinase 的Km 為0.5 mM而肝臟中 glucokinase 的Km為10Mm? 重要的參考是血中 glucose 濃度約在5~10Mm 之間。

1, Both hexokinase and glucokinase catalyze the same chemical reaction: phosphorylate glucose to become glucose-6-phosphate.
2, Km is the concentration of substrate which make enzyme reaches 50% of its maximal velocity.
3, Another important point is the concentration of glucose in blood is between 5-10 mM.
4, Is hexokinase activity glucose concentration dependent?
5, How about glucokinase?

By substrate concentration!





Glucokinase: Km = 10 mM,
Present in liver and in pancreas β cells.

Hexokinase: Km= 0.2 mM,
Present in most cells.

Pepsin 活性最佳pH 為2如何推論出pepsin 活性中心是那一類的胺基酸在負責催化反應? (能不能不查google自己想出可能的答案?)

活性中心主要是由鹼性的胺基酸(lysine、arginine、histidine)在負責催化反應，在酸性的環境中可以釋放電子去和 subtrate結合。

能在酸性的環境催化的蛋白質其反應中心亦為酸性才能穩定存在 ，所以天門冬胺酸和麩胺酸較有可能。 why?

- Why enzyme activity is pH dependent?
- You can only consider the chemical property of the side chain of amino acid but not amino acid itself!
- At that **pH range**, which side chain of amino acid may **change** it structure or function?
- Considering pKa of side chain of amino acid.
- Is it possible lysine?
- No, it is aspartic acid!

One mistake in my lecture

- 放射性同位素的衰變是零級反應（zero order kinetics）嗎?
- It follows first order kinetics!
- Giving one example of reaction with zero order kinetics.
- Limited amount of enzyme with much more excess substrate！
- If protein degradation follows the first order kinetics, how to estimate rate constant of protein degradation?

One protein degradation enzyme has been observed to be degraded gradually. This enzyme may be degraded by itself or be degraded by other enzyme. How to distinguish these two possibility by experiment?

從动力學的观点，這兩种反應的差别是什麼？

This enzyme is degraded by itself or is degraded by other enzyme.

This enzyme is degraded by itself (first order kinetics) or is degraded by other enzyme (second order kinetics).

一級反應$R=k[A]$可推導成$\ln[A]=-kt+\ln[A]_0$。因此，可在t=0時測量蛋白質濃度，一段時間t後，再測量剩餘蛋白質的濃度，代入公式可得出速率常數k。

How do you know it is first order kinetics?

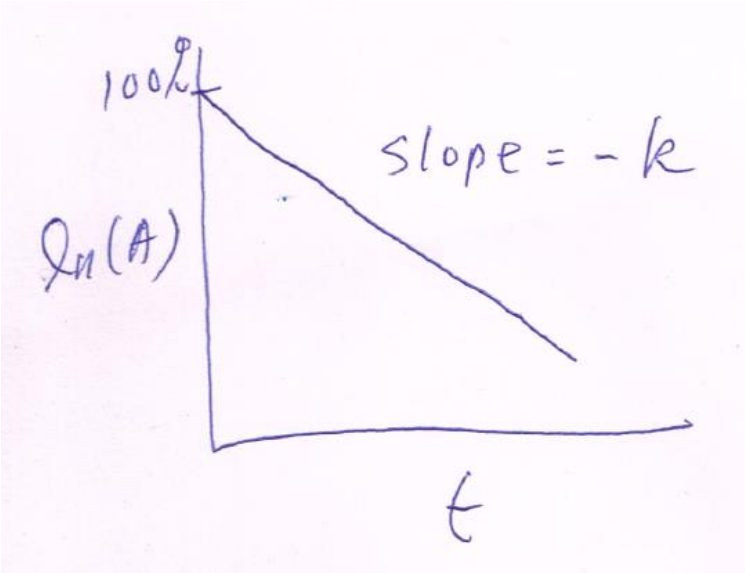
First-Order

$$-\frac{d[A]}{dt} = k[A]$$

$$\frac{d(A)}{(A)} = -kt$$

$$\int \frac{d(A)}{(A)} = -k\int t$$

$$\ln(A) = -kt$$



Semi-log plot

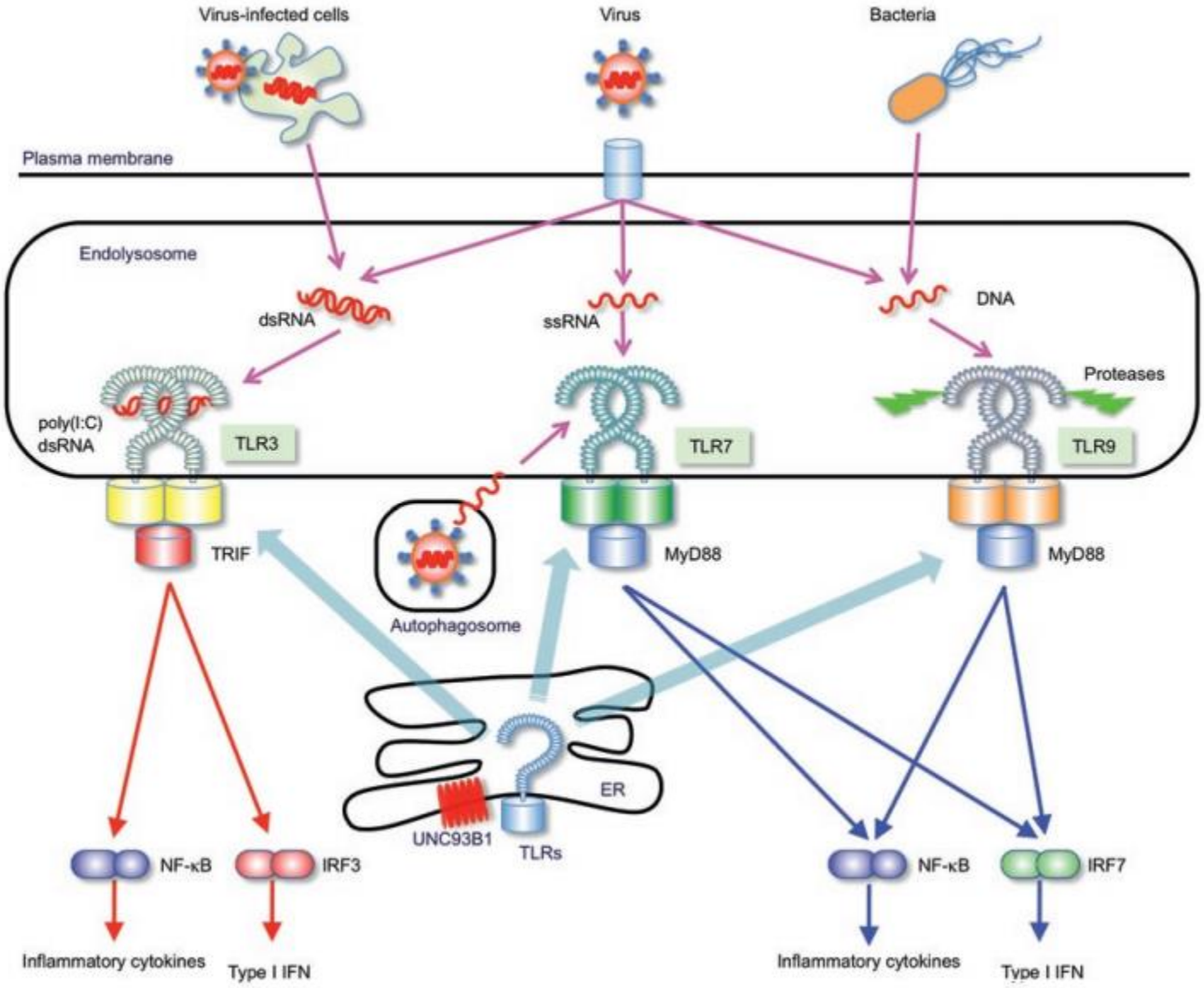
DNA能不能扮演enzyme的角色？

Is there any DNA with catalytic activity in cell?

Is any free DNA or RNA in the cytosol?

沒有單獨DNA存在(both single & double stranded), RNA也一樣. 它們會合蛋白質結合形成complex.

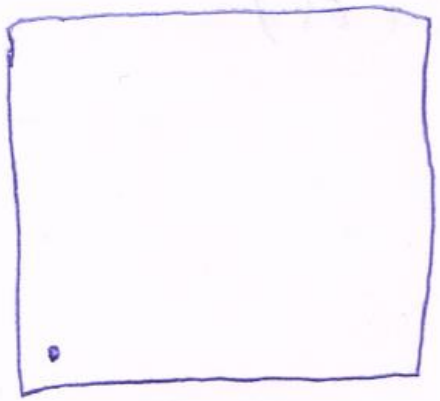
單獨的DNA or RNA如果出現在cytosol, it must be 外來的, i.e. 病毒, 細菌.



The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors Nature Immunology 11: 373 ; 2010

We have used $\triangle S = q/T$ to derive Gibbs free energy. However, in the first week we learned $S = k \log W$. How these two equation related?

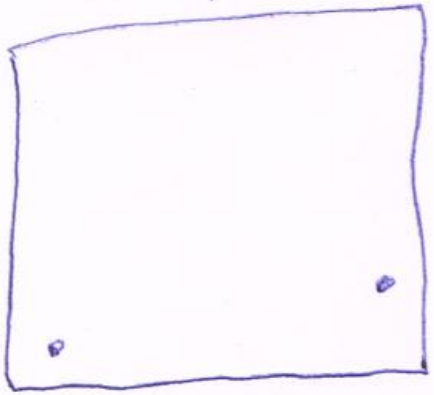
one particle



X positions

possible state X

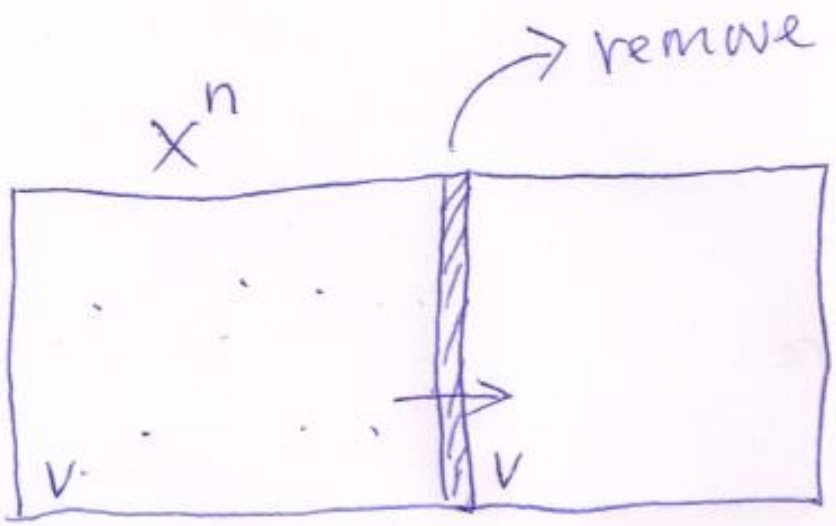
two particles



X positions

possible state $X \cdot X = X^2$

N particles possible state $X \cdot X \cdots = X^n$



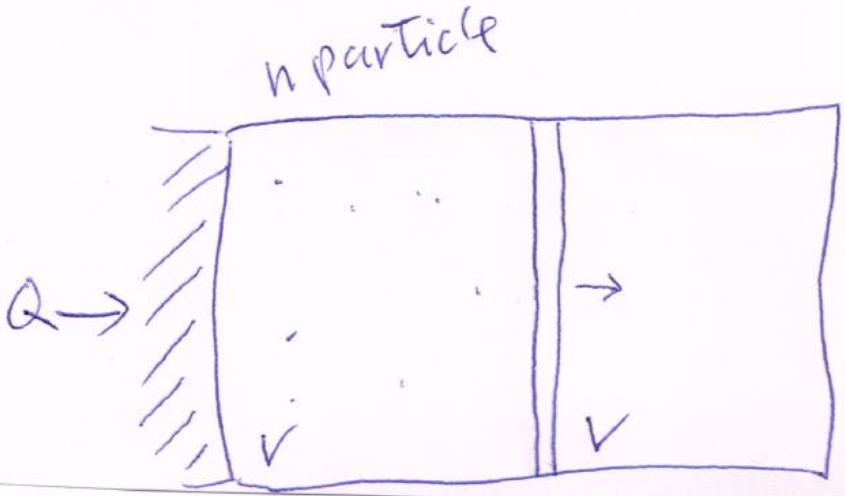
$(2X)\cdot(2X)\cdots = (2X)^n$

Befor the barrier is removed $S = k\log X^n$

After " $S = k\log (2X)^n$

$\Delta S = k\log (2X)^n - k\log X^n$

$= k\log \dfrac{(2X)^n}{X^n} = k\log 2^n$



系统温度会不会改变？

$PV=nRT \quad P=\frac{nRT}{V}$

$\Delta U=\Delta Q-\Delta W$ T不变 ΔU不变为0 $\therefore \Delta Q=\Delta$

$$dW=P\cdot dV=\frac{nRT}{V}\cdot dV=\frac{N}{6.02\times10^{23}}\cdot R\cdot T\cdot\frac{dV}{V}$$

$$\int dW=\frac{R}{6.02\times10^{23}}\cdot T\cdot N\int_{V_1}^{V_2}\frac{dV}{V}$$

$$\Delta Q=k\cdot T\cdot N\cdot \ln 2$$

$$\Delta Q=T\cdot k\ln 2^{N}$$

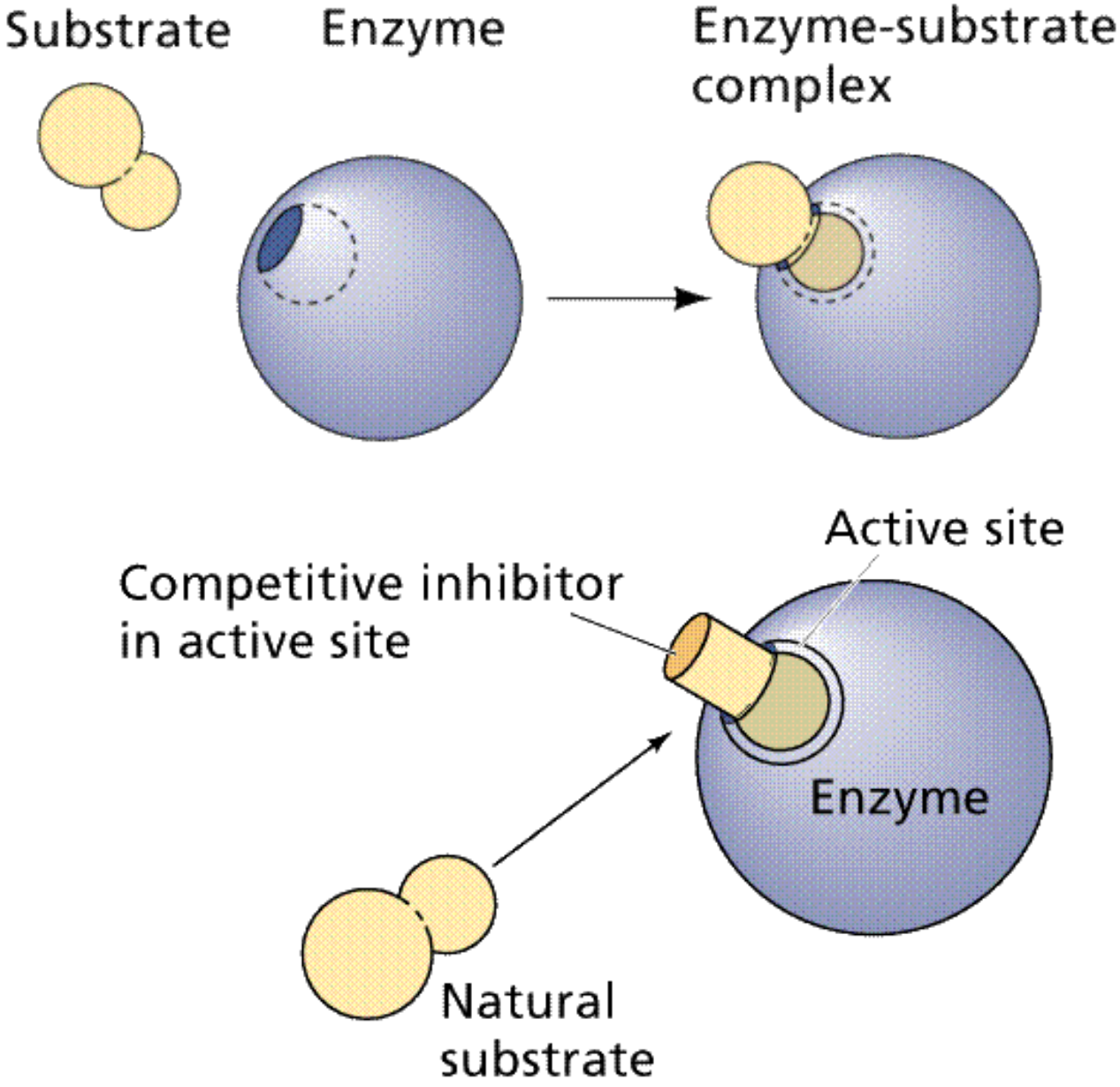
$$=T\cdot\Delta S$$

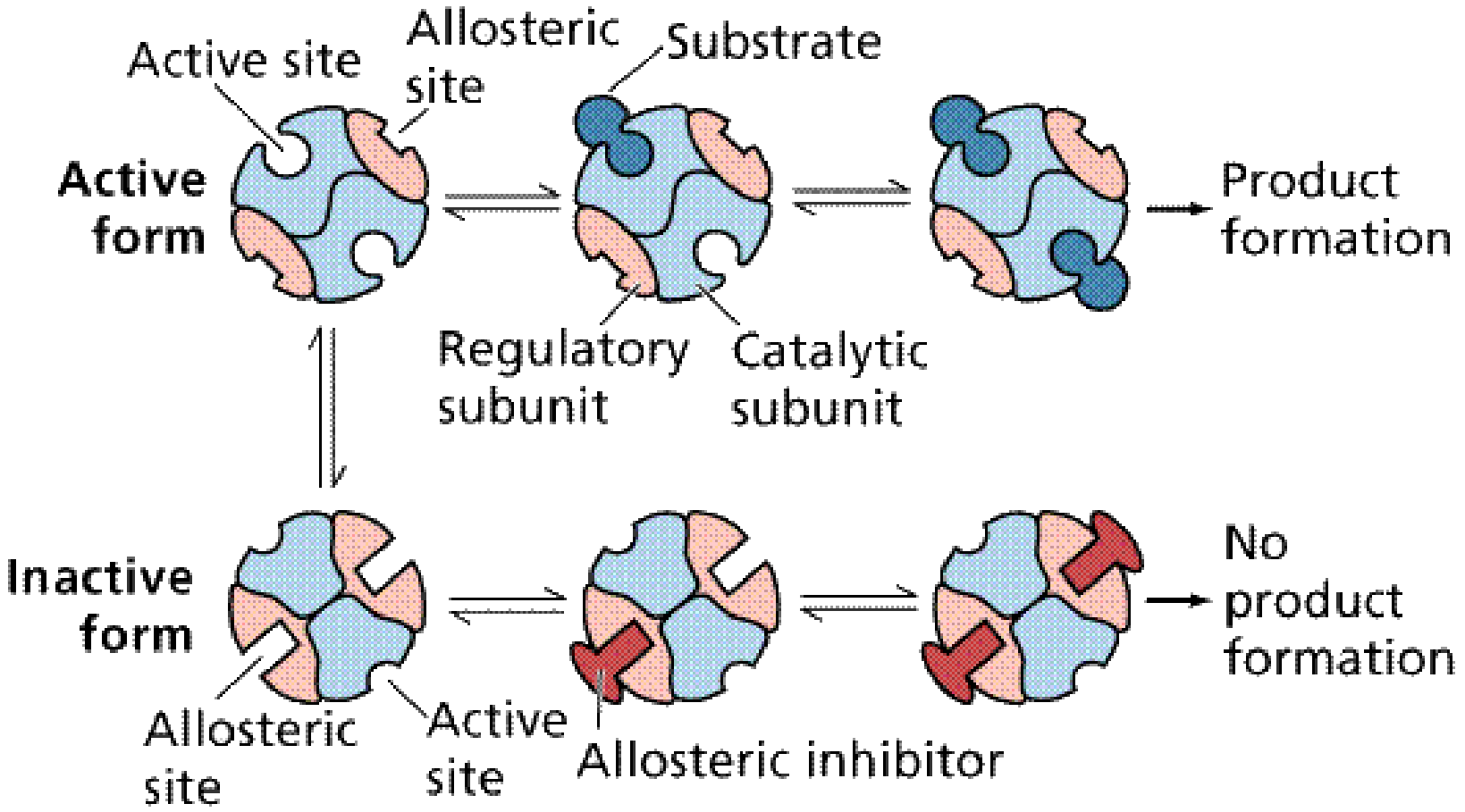
Explaining your understanding of the following statement!

Allosteric inhibitors decrease V_{max} but do not alter K_m
Competitive inhibitors increase K_m but do not alter V_{max}

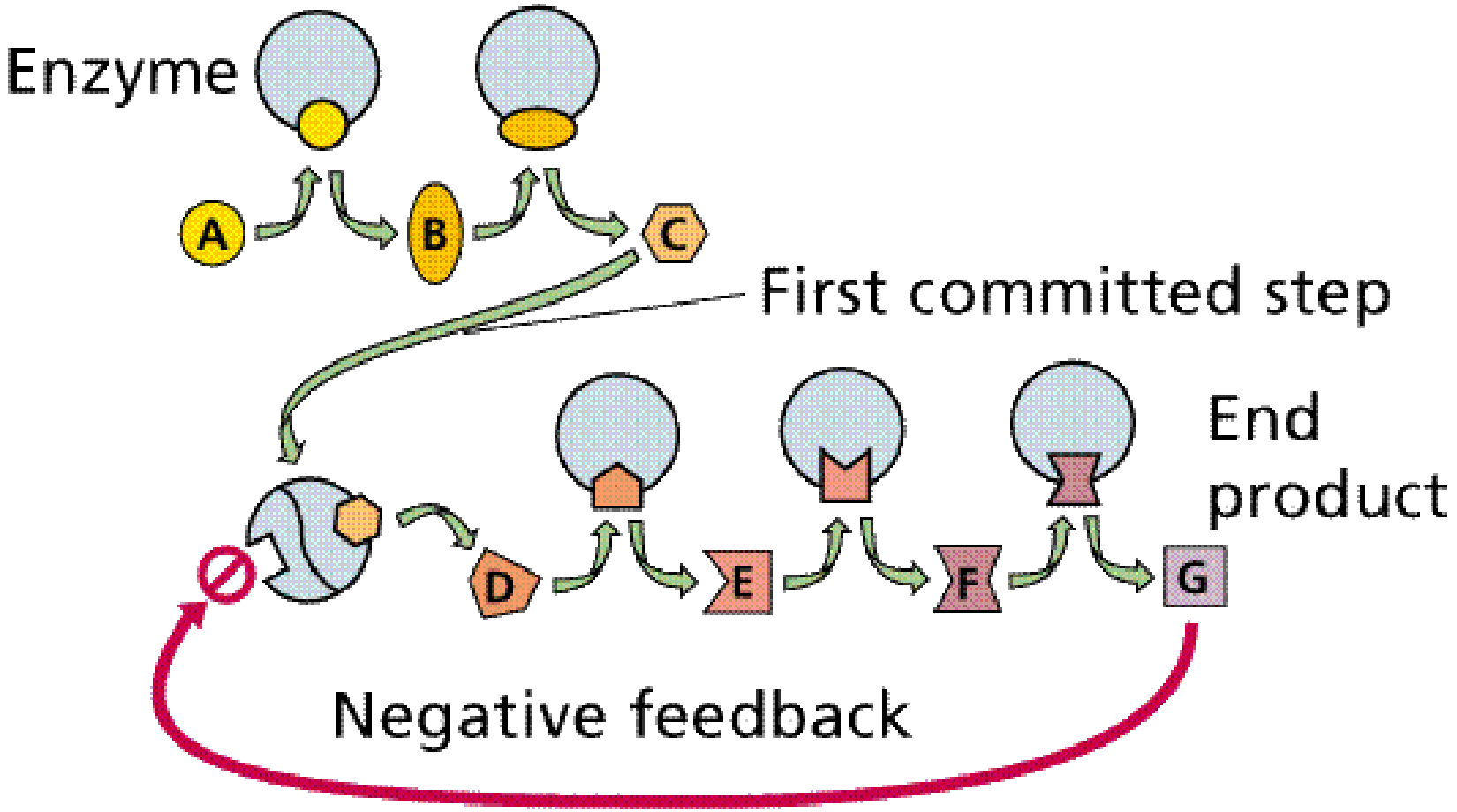
Vmax是基於酵素濃度所產生的速率限制???

Turn over number of the enzyme

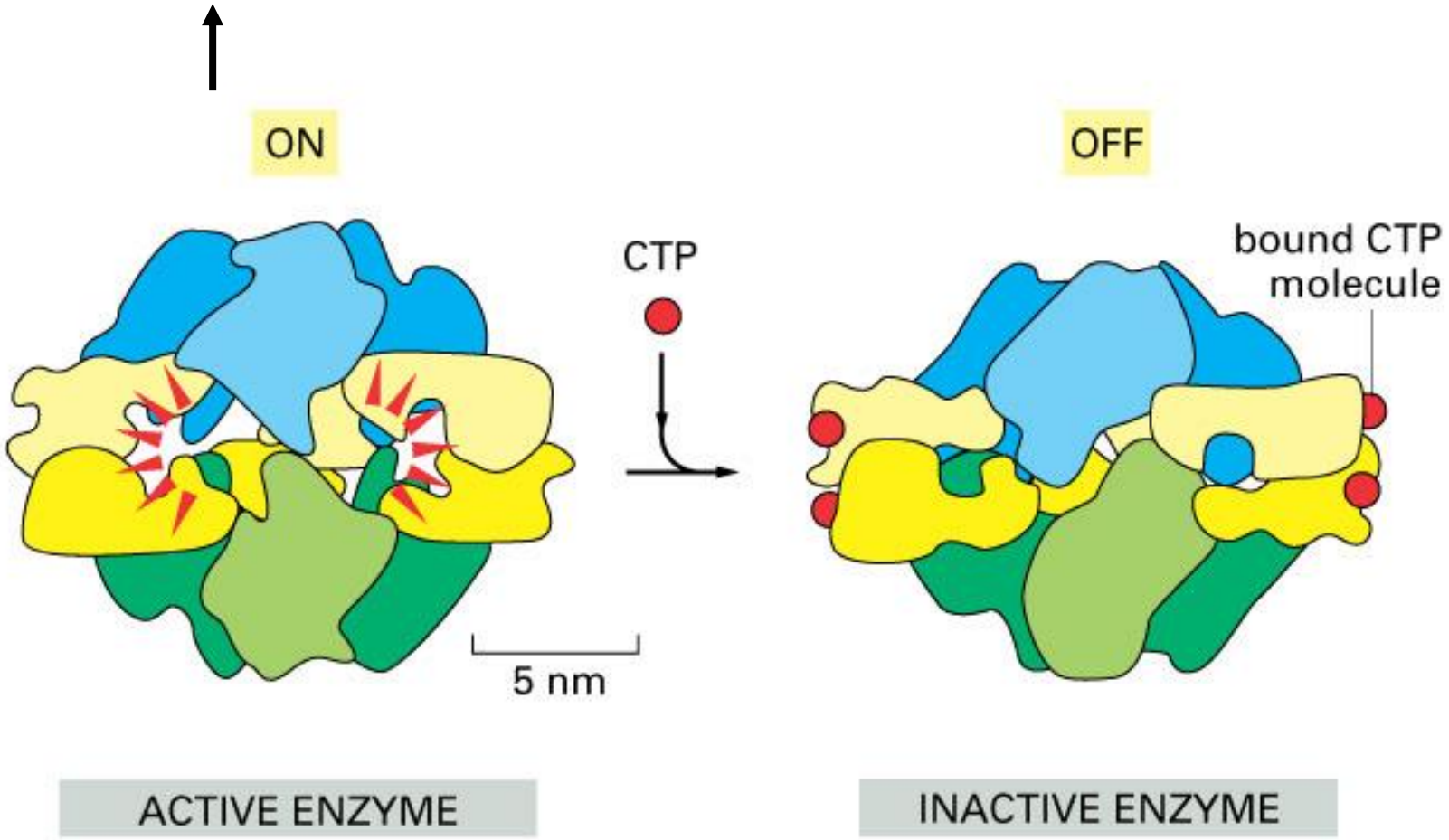

Substrate
Enzyme
Enzyme-substrate complex
Active site
Competitive inhibitor in active site
Enzyme
Natural substrate



Allosteric inhibitor or activator shares similar working mechanism.


Enzyme
A
B
C
First committed step
End
product
e
D
E
F
G
Negative feedback

Synthesize C, U and T



Figur Figure 4-39 Essential Cell Biology, 2/e. (© 2004 Garland Science)

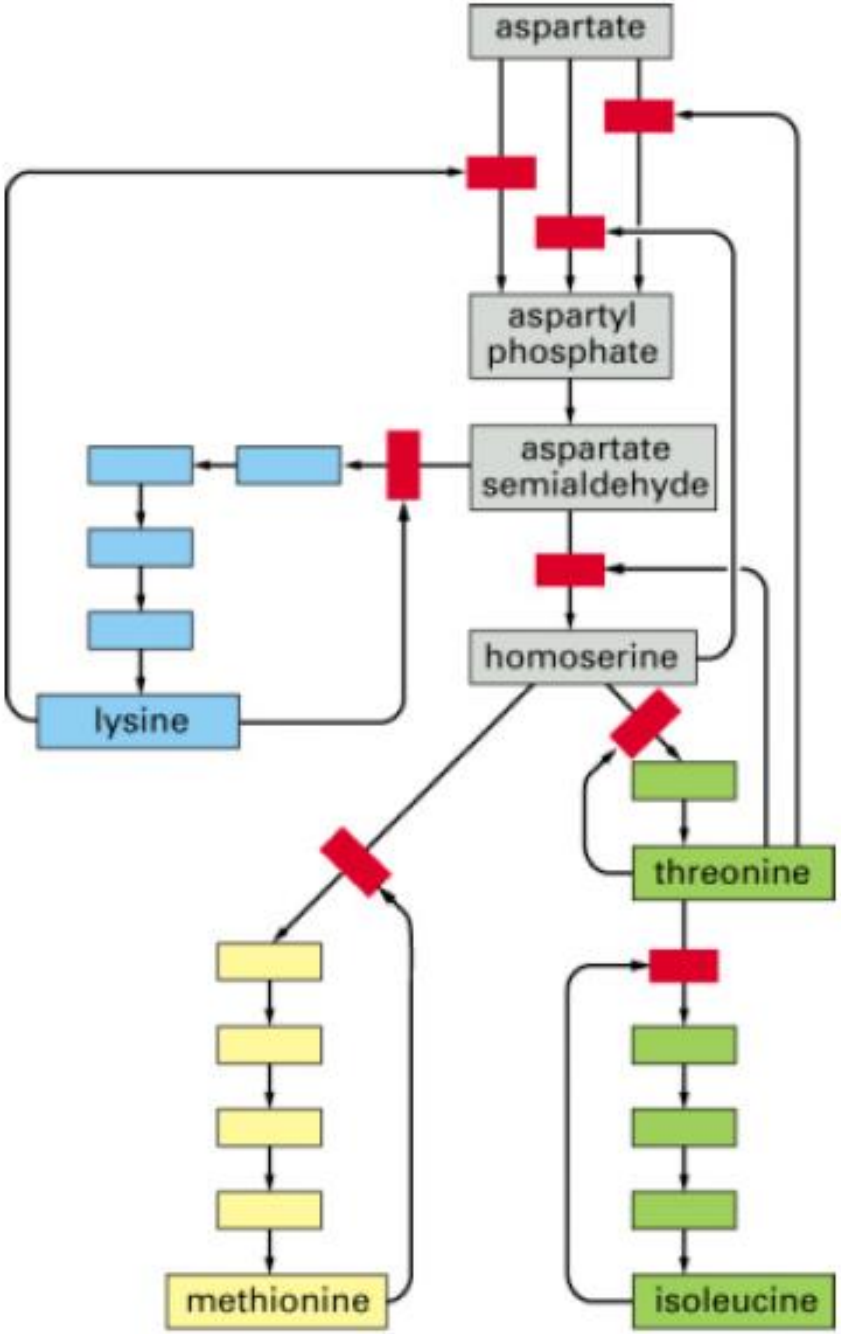
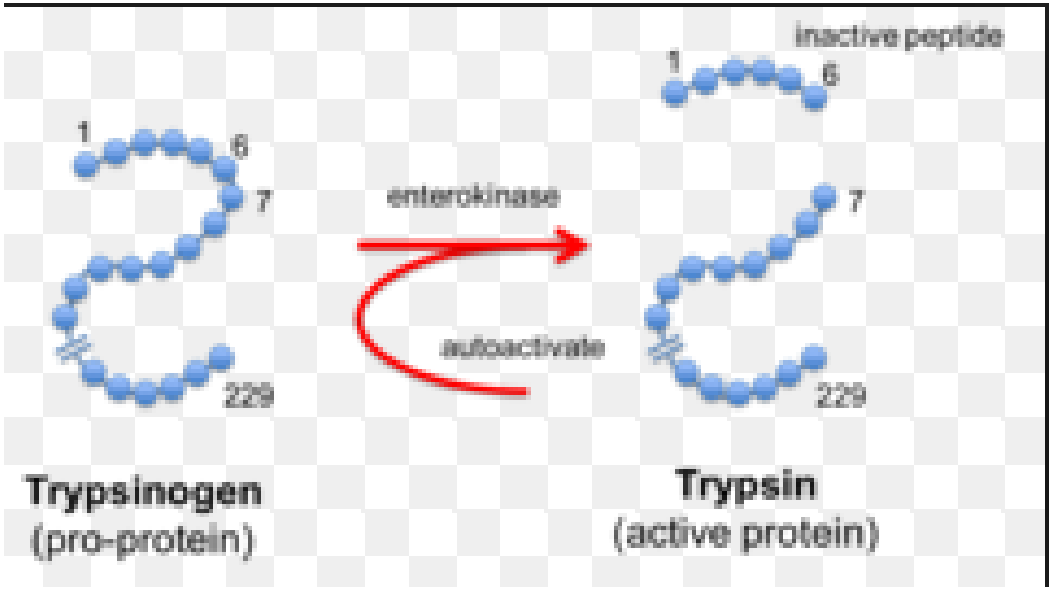


Figure 3–56. Molecular Biology of the Cell, 4th Edition.



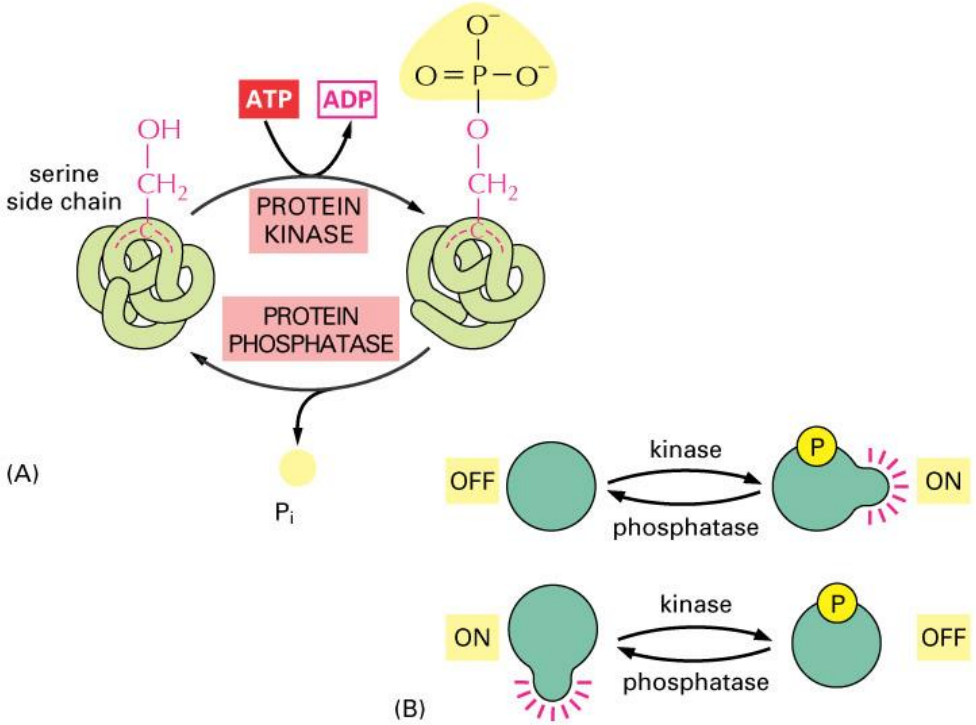
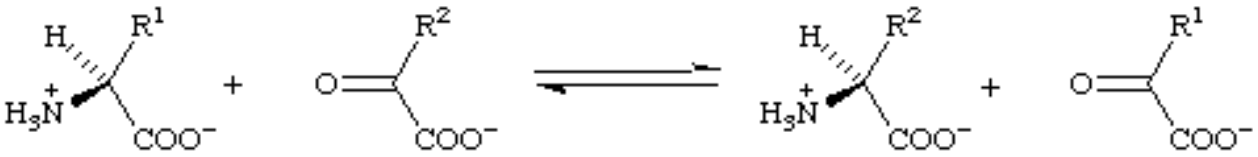
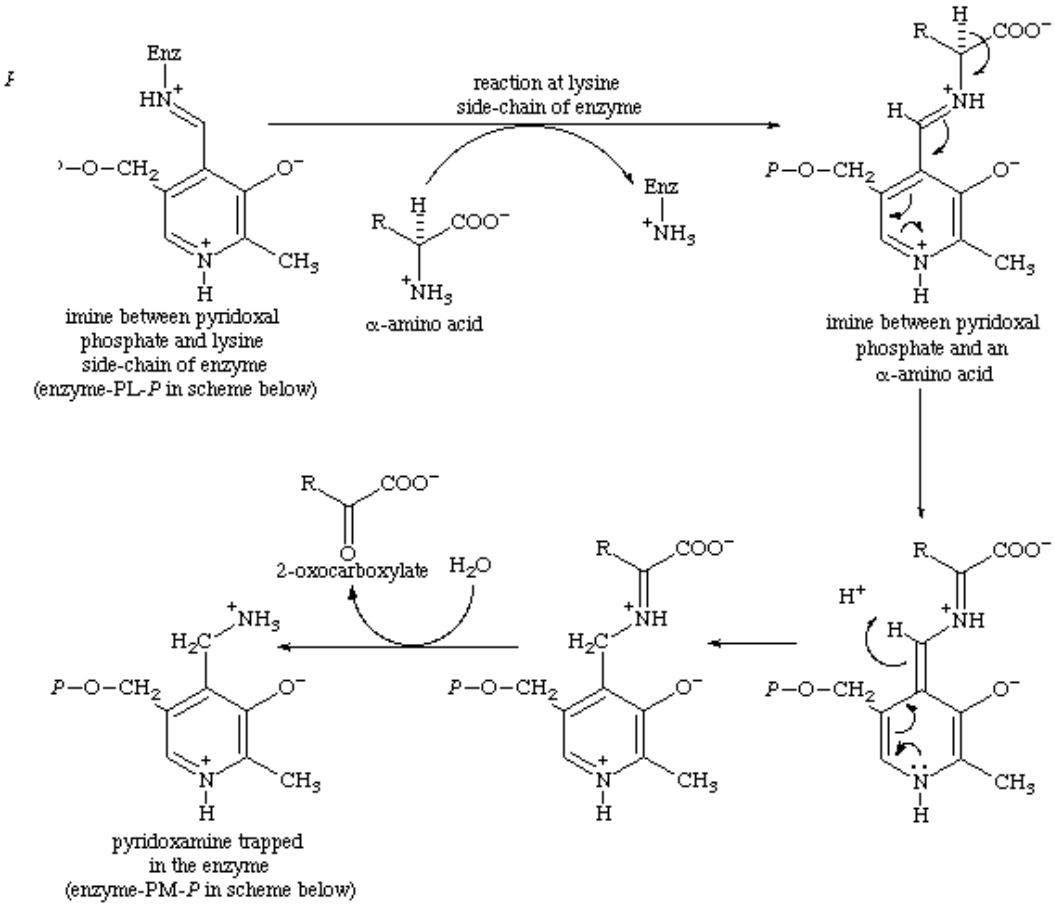


Figure 4-41 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Why enzyme need coenzyme?




reaction at lysine
side-chain of enzyme
imine between pyridoxal
phosphate and lysine
side-chain of enzyme
(enzyme-PL-P in scheme below)
α-amino acid
imine between pyridoxal
phosphate and an
α-amino acid
2-oxocarboxylate
pyridoxamine trapped
in the enzyme
(enzyme-PM-P in scheme below)

What catalytic factor was missing from the lecture?

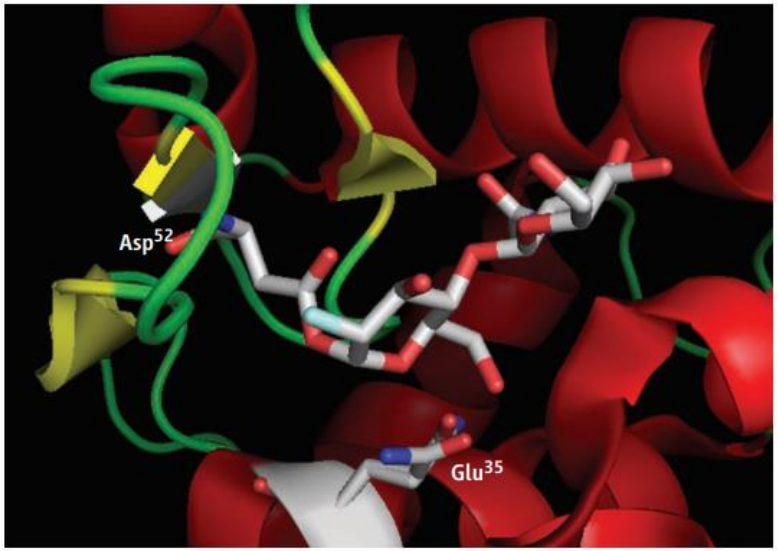
BIOCHEMISTRY

How Enzymes Work

Fifty years of research have led to a detailed understanding of the mechanisms of enzymatic catalysis.

Dagmar Ringe and Gregory A. Petsko

Gazing at the three-dimensional structures of enzymes that regularly grace the covers of scientific publications, it is hard to imagine that there are still people alive who remember when many biochemists thought that enzymes had no ordered structure. But that was the case until James Sumner crystallized urease in 1926 (*1*)—a development so revolutionary that he was taken into custody as a dangerous lunatic when he tried to explain what he had done to a famous European scientist. When biochemists realized that enzymes had persistent structure and that destruction of that structure could abolish enzyme activity, they rapidly adopted the view that enzymes were rigid scaffolds whose specificity and catalytic power came

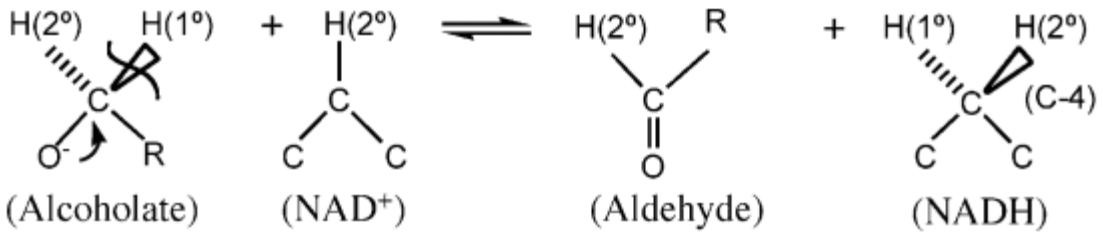


Elucidating the active site. In the crystal structure of a lysozyme mutant bound to a synthetic sugar substrate, the sugar ring in the active site is distorted, and the scissile bond is close to the acid-base residues Asp^{52} (left) and Glu^{35} (lower right; mutated to Gln in this structure) (*5*). All these features were deduced by Phillips and co-workers more than 40 years ago (*4*). Unexpectedly, the structure also shows that lysozyme can form a covalent intermediate with its substrates (*5*).

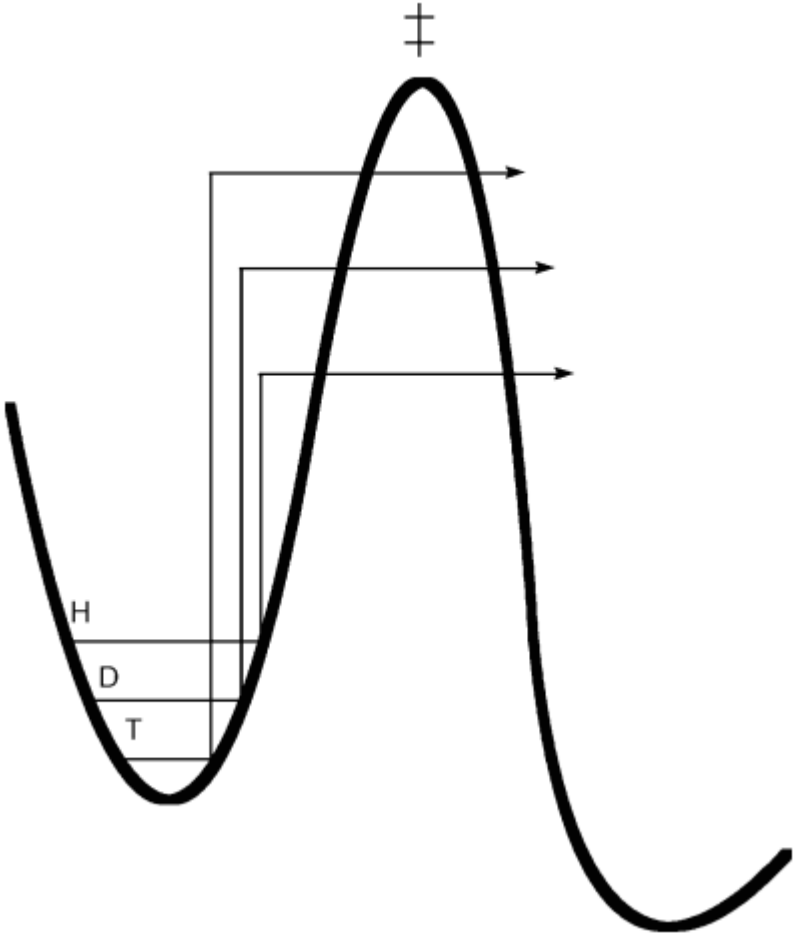
even if almost every other factor were eliminated by mutating the enzyme, the protein would still be a respectable catalyst. Second, Koshland was right: The active-site residues usually adjust to permit the binding of the specific substrate. Induced-fit changes involving the movement of entire protein domains by several nanometers have been observed (*6*). Third, the protein structure can create specialized microenvironments that dramatically alter the reactivity of key catalytic groups, in some cases by shielding the catalytic site from contact with bulk solvent. Fourth, enzymes can distort the substrate, causing it to adopt a high-energy conformation with increased reactivity (*7*). Finally, enzymes

What was missing in this picture? Three relatively recent discoveries stand out. ?

- the contribution of quantum mechanical tunneling to the rates of enzyme-catalyzed transfer of hydrogen ions reactions.



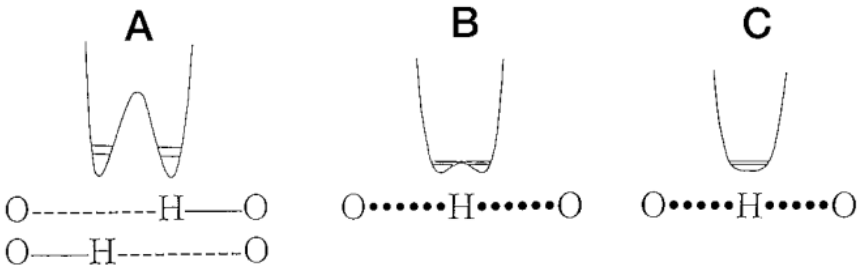
1, hydrogen has a de Broglie wavelength on the order of the distances over which it is expected to be transferred.
2, the heavier isotopes will have the same chemical properties but significantly smaller de Broglie wavelengths
3, the hydrogen donor and acceptor wave function overlap will be a very sensitive function of the donor-acceptor distance

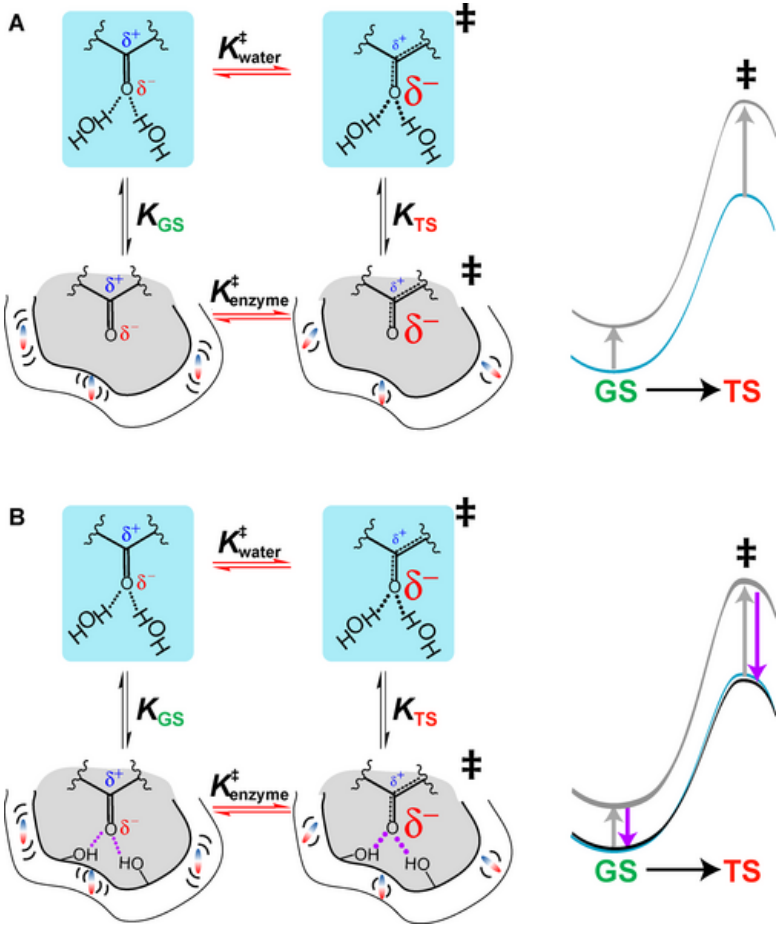


What was missing in this picture? Three relatively recent discoveries stand out. ?

- the precise matching of the pKa's of the donor and acceptor atoms in hydrogen bonds that stabilize the transition state. Such matching can lead to short, symmetrical hydrogen bonds of greater-than-normal strength.

The Low Barrier Hydrogen Bond in Enzymatic Catalysis
THE JOURNAL OF BIOLOGICAL CHEMISTRY 273, pp. 25529–25532, 1998





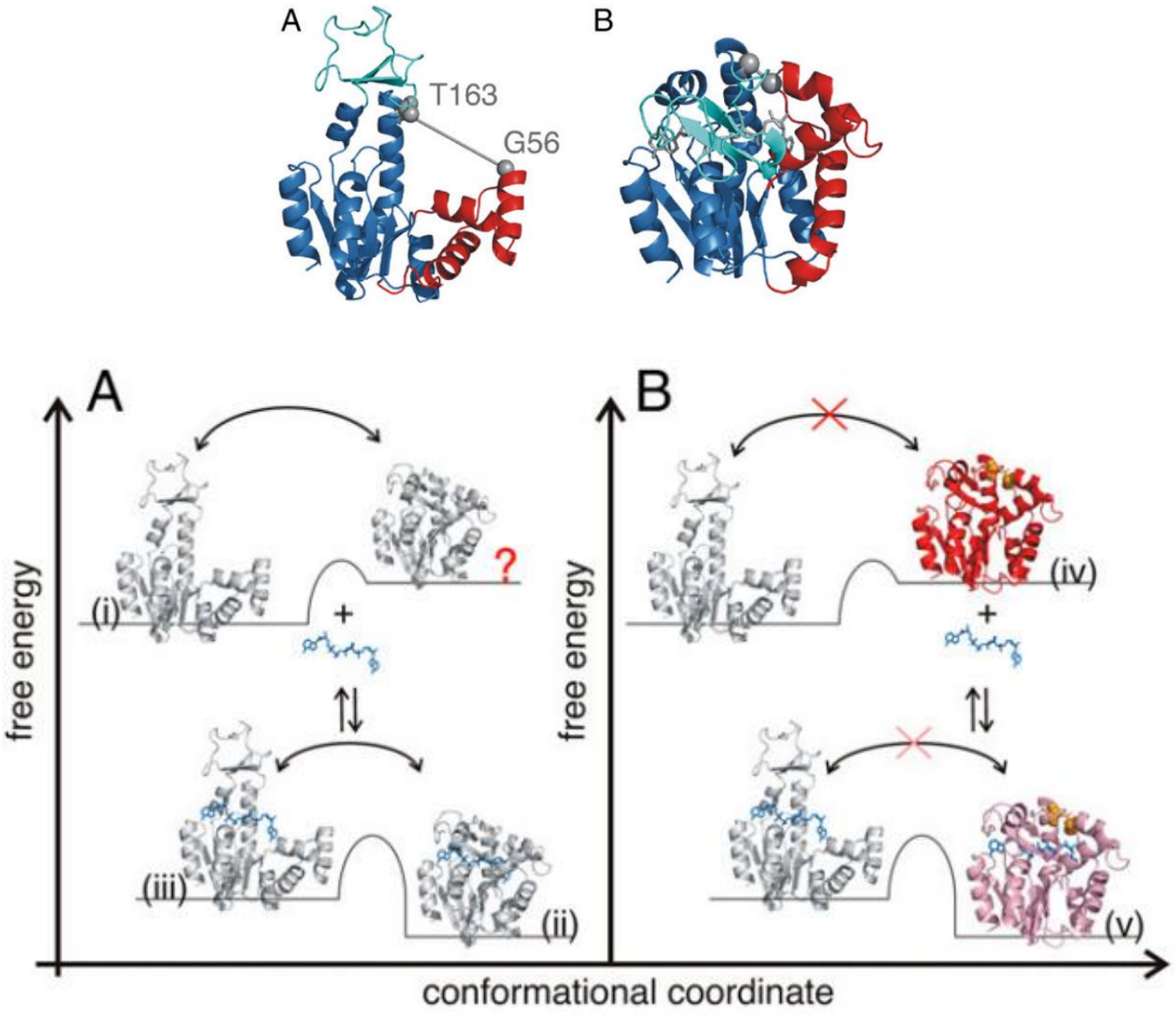
Testing Electrostatic Complementarity in Enzyme Catalysis: Hydrogen Bonding in the Ketosteroid Isomerase Oxyanion Hole. PLoS Biol. 4, e99, (2006)

What was missing in this picture? Three relatively recent discoveries stand out. ?

- the role of protein dynamics in aiding the reacting species in crossing the transition-state barrier to the reaction.

Proteins can bind target molecules through either induced fit or conformational selection pathways.

- In the conformational selection model, a protein samples a scarcely populated high-energy state that resembles a target-bound conformation.
- In enzymatic catalysis, such high-energy states have been identified as crucial entities for activity and the dynamic interconversion between ground states and high-energy states can constitute the rate limiting step for catalytic turnover.
- The catalytically active state of adenylate kinase, the enzyme is a closed conformation, for which the structure (with bound ligand) has been determined by X-ray crystallography.
- Sampling of a closed conformation in a ligand-free “apo enzyme” is one of the prerequisites for the conformational selection model.
- It is inherently difficult to address the function of high-energy states directly because they are, first, transient and, second, in a dynamic equilibrium with more stable ground states.



Structural basis for ligand binding to an enzyme by a conformational selection pathway. PNAS 114: 6298–6303; 2017

Recent advancements in enzyme research

How to improve enzyme activity and its specificity?

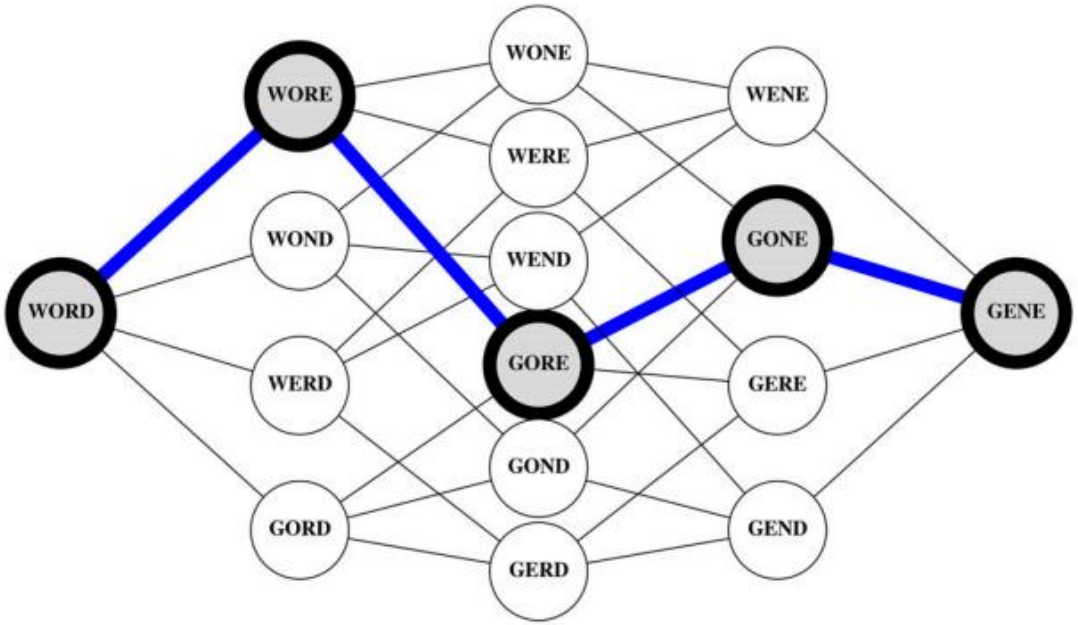
John Maynard Smith Natural Selection and the Concept of a Protein Space
Nature 225, 563–564; 1970

A Reflection on 50 Years of John Maynard Smith's "Protein Space"

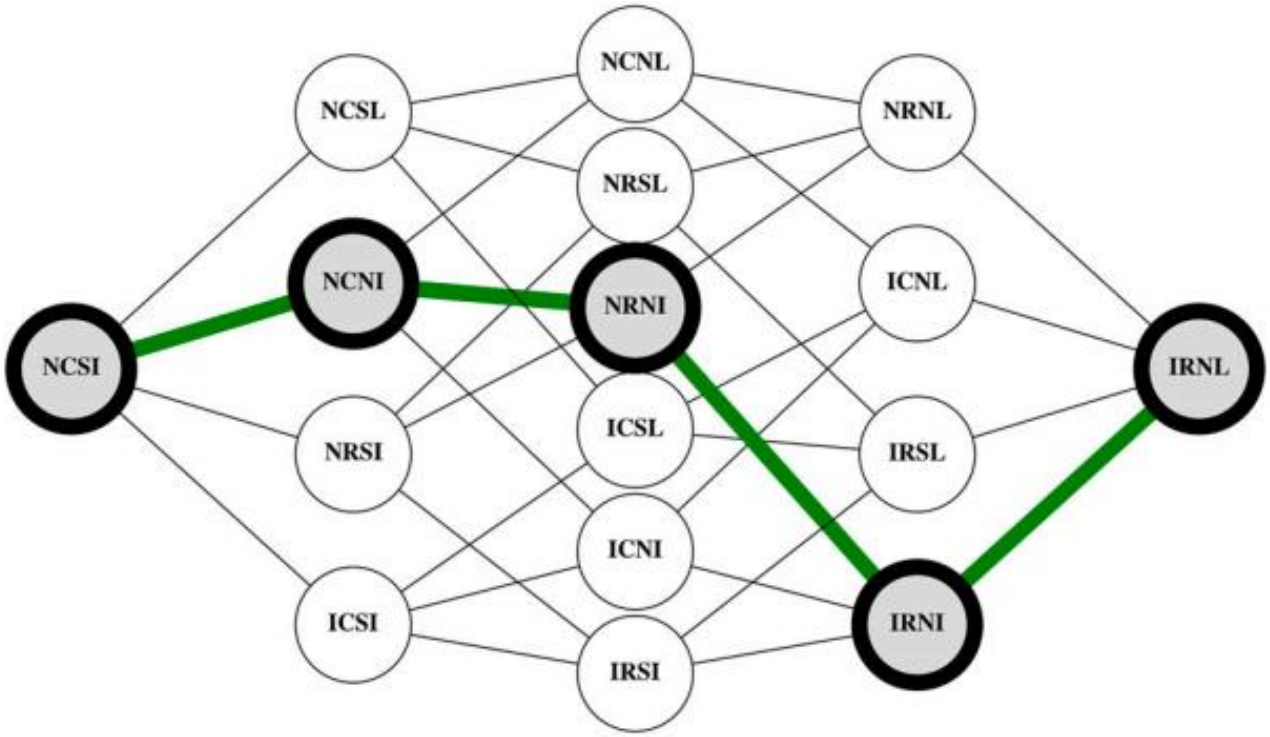
C. Brandon Ogbunugafor[1]
Department of Ecology and Evolutionary Biology, Brown University, Providence, Rhode Island 02912
ORCID ID: 0000-0002-1581-8345 (C.B.O.)

WORD → WORE → GORE → GONE → GENE

WORD → GORD → GOND → GEND → GENE
WORD → WOND → WEND → WENE → GENE
WORD → WERD → GERD → GERE → GENE



A hypercube representation of an empirical fitness landscape corresponding to mutations in dihydrofolate reductase, an enzyme target of drugs in many microbial diseases. Specifically, the mutations are associated with resistance to pyrimethamine, an antimicrobial drug



The Nobel Prize in Chemistry 2018

© Nobel Media AB. Photo: A. Mahmoud

Frances H. Arnold

Prize share: 1/2

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George P. Smith

Prize share: 1/4

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Sir Gregory P. Winter

Prize share: 1/4

Frances H. Arnold "for the directed evolution of enzymes"

Innovation by Evolution: Bringing New Chemistry to Life (2018 Nobel Lecture in Chemistry)

Angew.Chem. Int. Ed. 2019, 58,14420 –14426

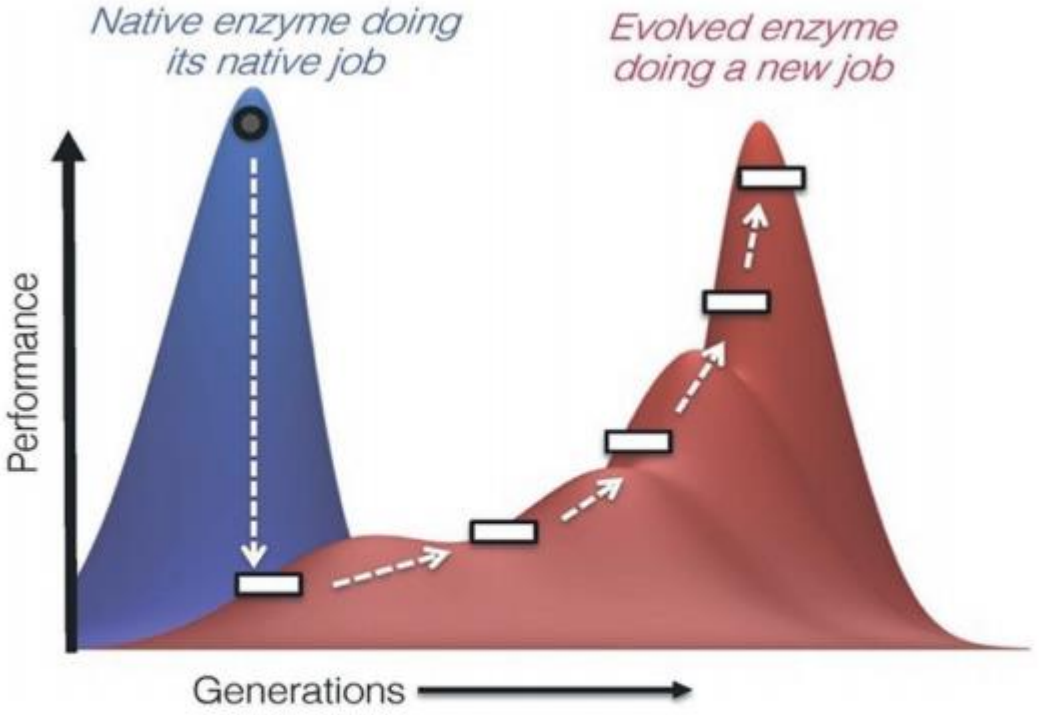


Figure 1. An enzyme whose function is optimized for its native job generally performs poorly in a new role. Directed evolution through rounds of mutation and screening can discover changes in sequence that improve performance, climbing a new fitness peak.

Figure 2: Overview of directed evolution.

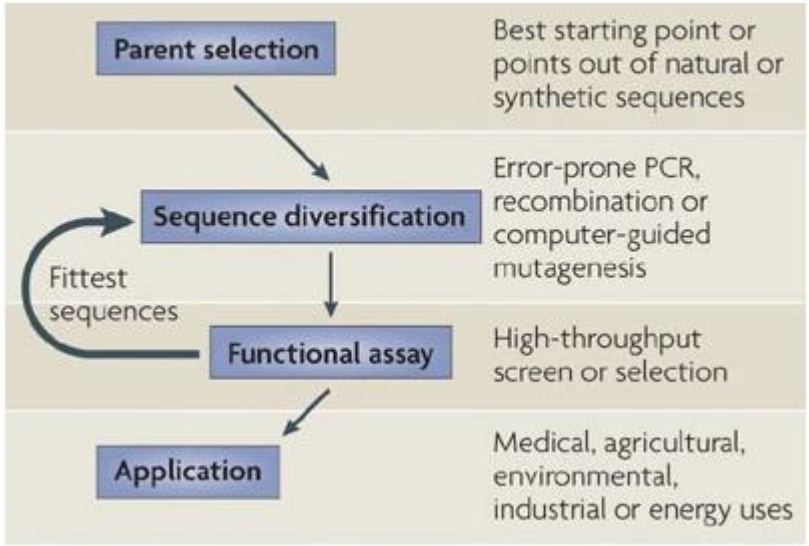
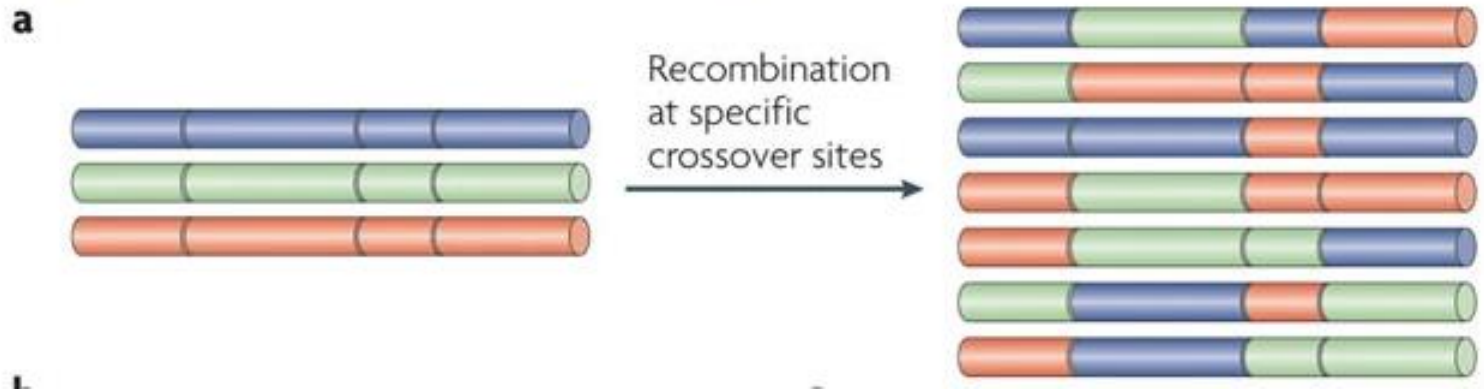
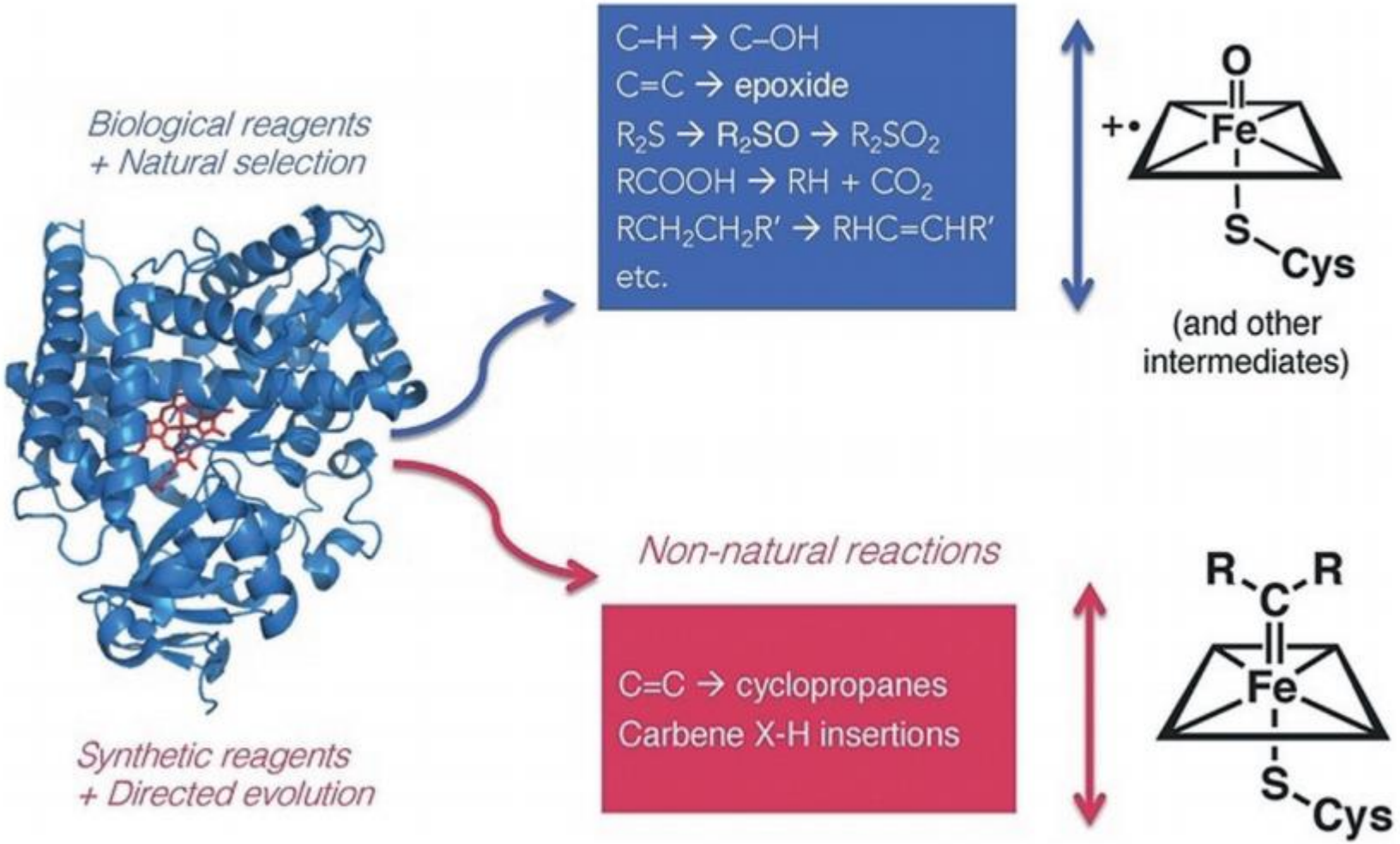


Figure 3: Recombination of homologous sequences.





"Olefin Cyclopropanation via Carbene Transfer Catalyzed by Engineered Cytochrome P450 Enzymes": Science 2013, 339, 307 – 310.

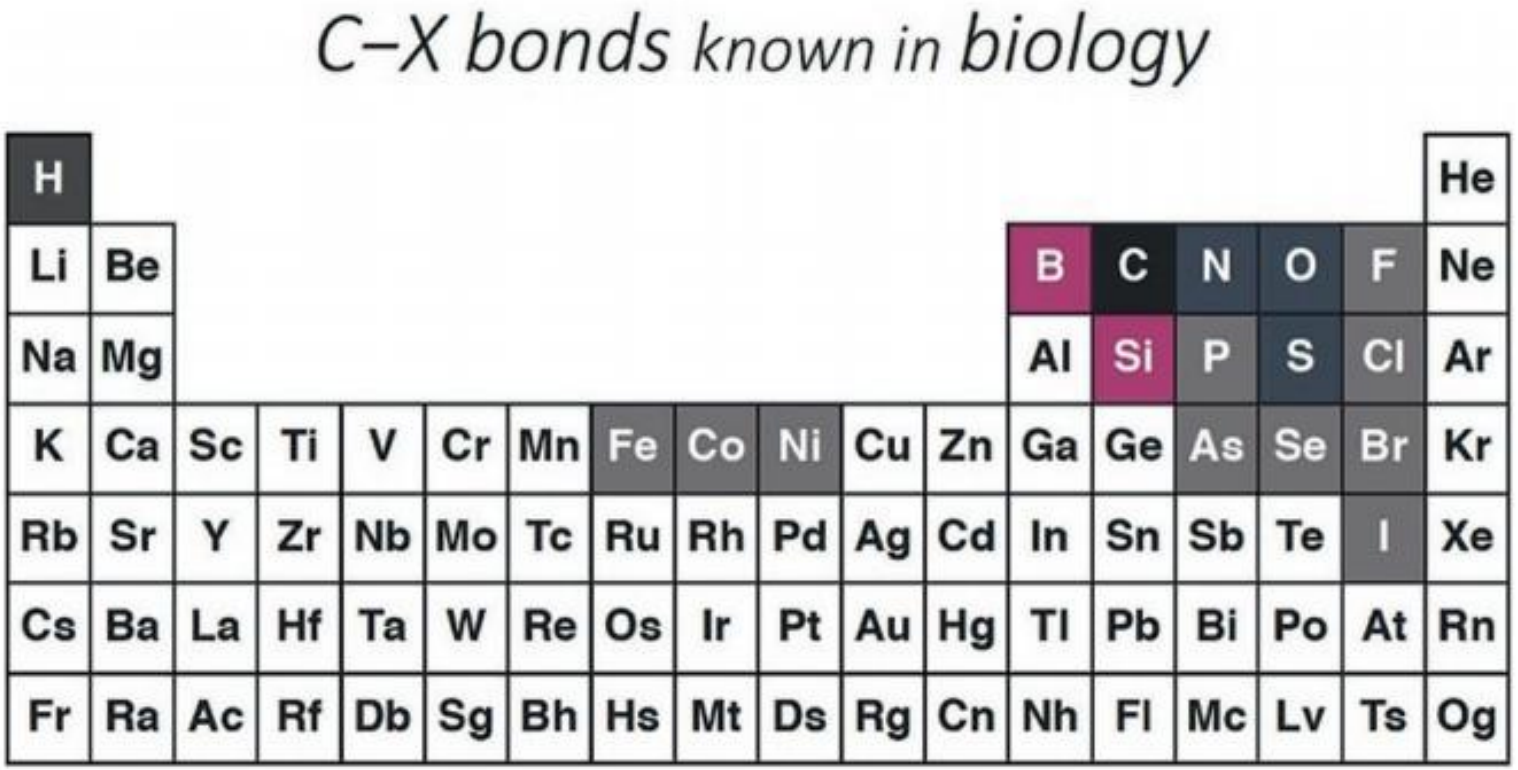


Figure 4. New "carbene transferases" made by directed evolution have added C–Si and C–B bonds to biology's DNA-encoded synthetic repertoire.

LETTER

13 JUNE 2019 | VOL 570 | NATURE | 219

https://doi.org/10.1038/s41586-019-1262-8

Design and evolution of an enzyme with a non-canonical organocatalytic mechanism

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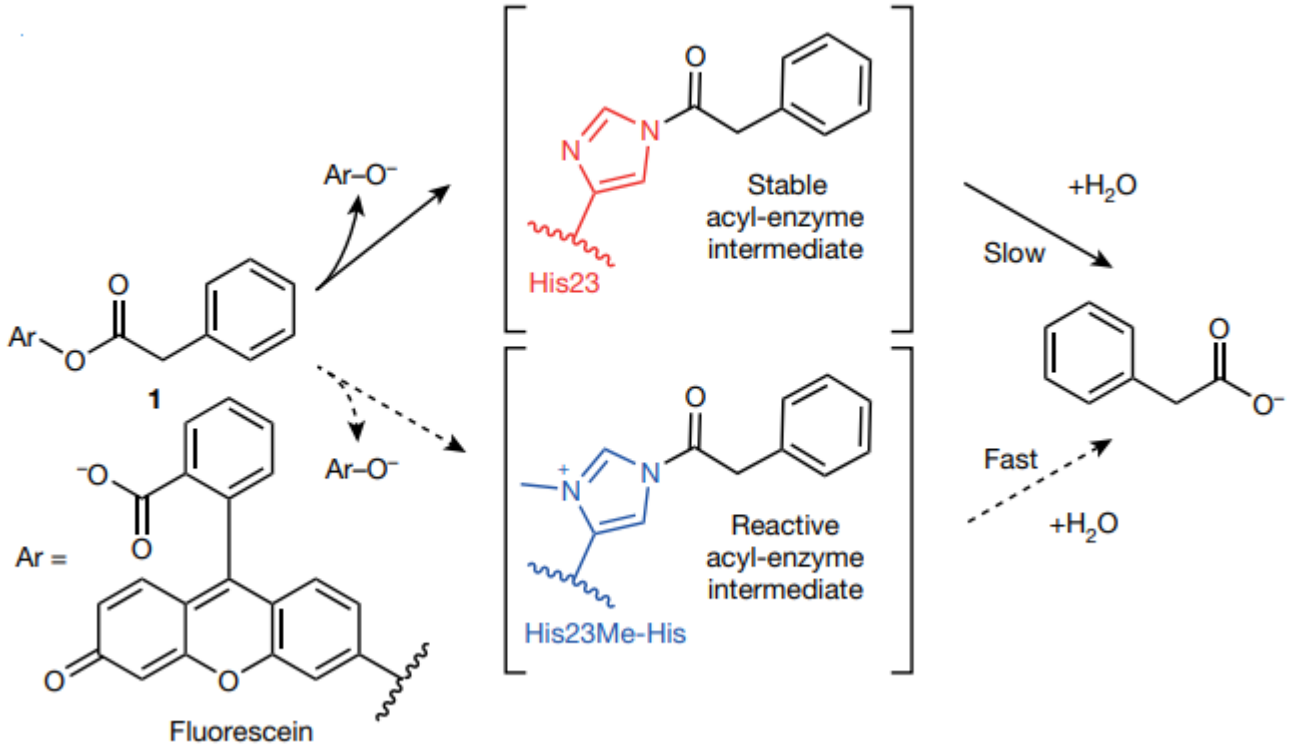
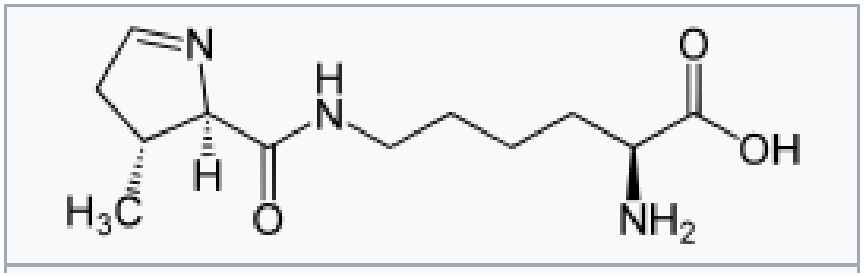
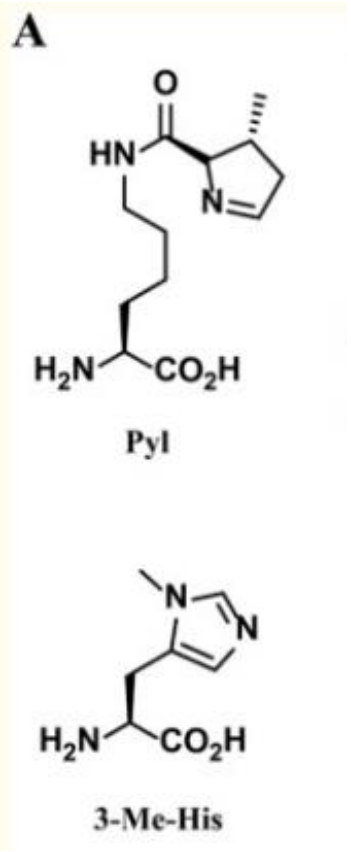


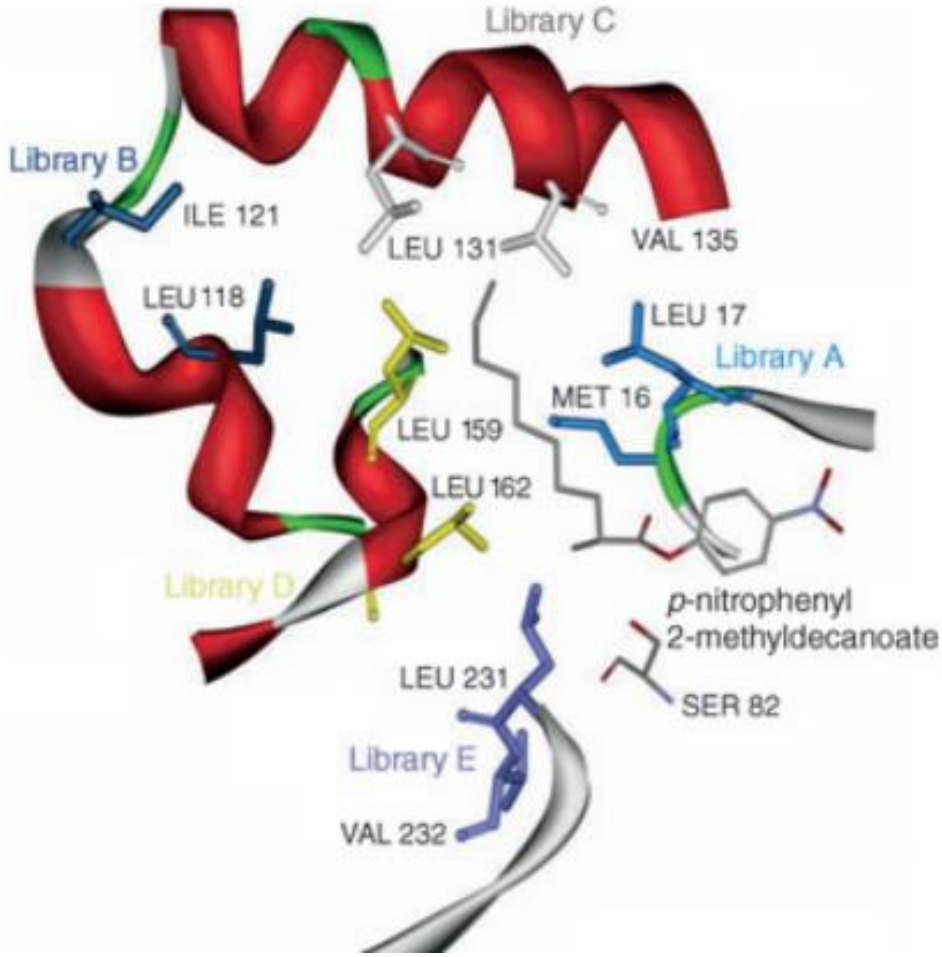
Can we incorporate his23-Me to replace his23 in the active site ?

An engineered pyrrolysyl-tRNA synthetase/pyrrolysyl-tRNA pair was used to introduce a Me-His23 residue in response to a UAG stop codon

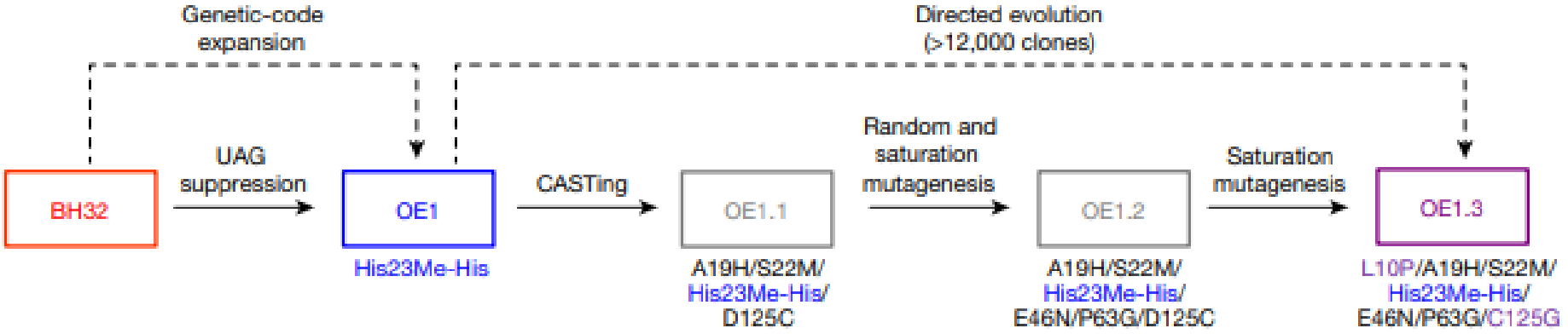
Pyrrolysine

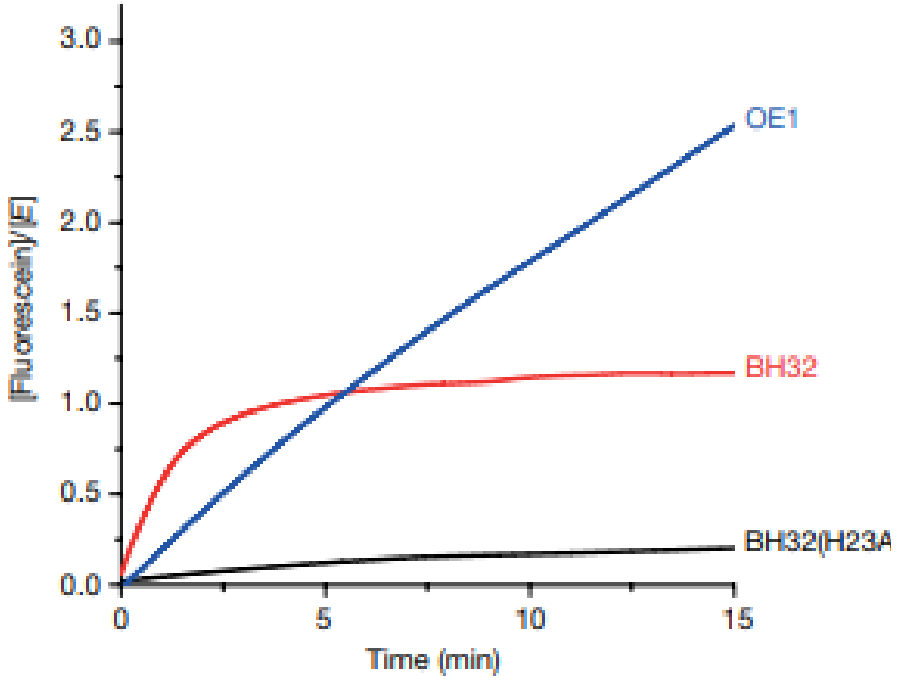


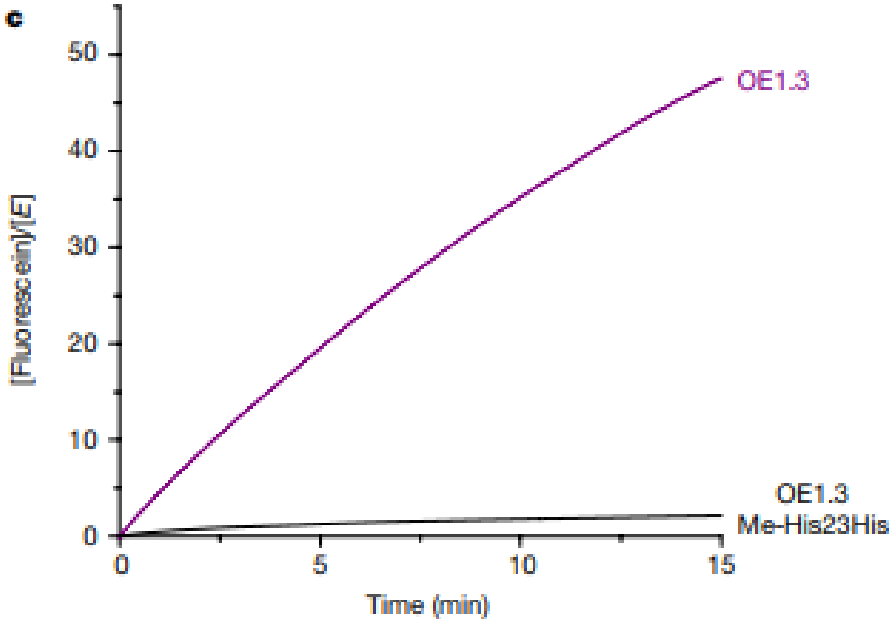




Five CASTing libraries (Ala19/Ser22, Tyr45/Glu46, Tyr87/ Trp88, Met94/Ser95 and Asp125/Gln128) were prepared by overlap extension PCR using pBbE8k_OE1 as a template and degenerate primer pairs.


Genetic-code expansion
Directed evolution (>12,000 clones)
BH32
UAG suppression
OE1
His23Me-His
CASTing
OE1.1
A19H/S22M/ His23Me-His/ D125C
Random and saturation mutagenesis
OE1.2
A19H/S22M/ His23Me-His/ E46N/P63G/D125C
Saturation mutagenesis
OE1.3
L10P/A19H/S22M/ His23Me-His/ E46N/P63G/C125G


[Fluorescein]/[E]
Time (min)
OE1
BH32
BH32(H23A


c
[Fluorescein]/[E]
Time (min)
OE1.3
OE1.3 Me-His23His

Quiz for lecture 5

- 1, 細胞中 glucose氧化燃烧會釋放能量，為什麼這個能量一定要以 proton gradient 的型式儲存?
- 2, Complex 1 的結構 suggest the piston mechanism 請解釋。
- 3, Rubisco 的Turnover number 這麼低，那為甚麼沒有演化或被取代？
- 4, Please make comment on: “植物的光系統II吸收光子的能量而將水分子分解”.
- 5, how glucose suppress gene expression of some gene cluster?
- 6, Cancer cell expresses PKM2 instead of PKM1. Can this mechanism explain Warburg Effect of cancer?
- 7, Cancer cell 真的不需要有氧呼吸嗎?
- 8, Why brain tumor patients with IDH mutations have a better survival?