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DE FRANCE
—1530—

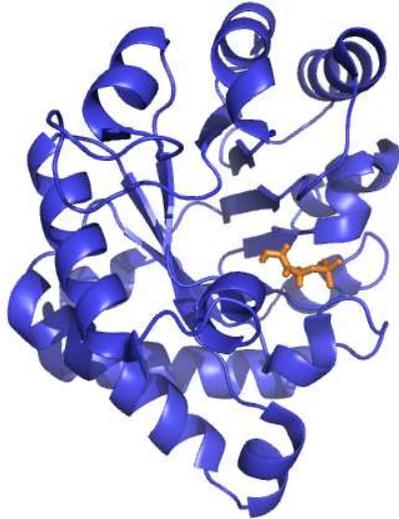
L'âme des enzymes: le mystère de la biocatalyse

Marc Fontecave

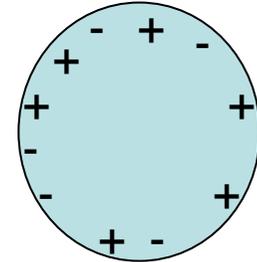
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Protéines

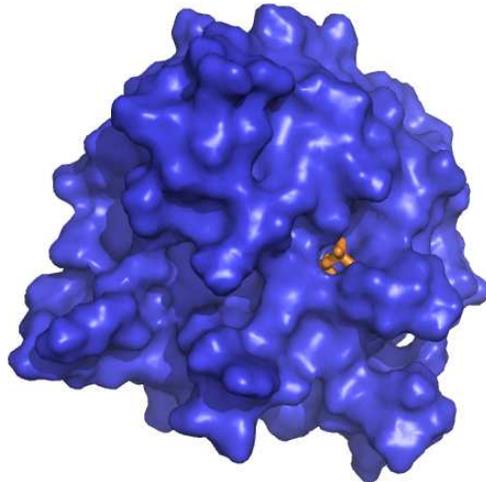


- Densité de remplissage: 0.7-0.8
- pénétration des substrats ??
- Interaction substrats/acides aminés ??



- Milieu interne hydrophobe: $\epsilon_r = 4-10$
- Attention!! (Warshel)

- Respiration 1: « quenching » de fluorescence de W par O_2
- Respiration 2: mouvements protéiques (échelles de temps)



Triose phosphate isomerase

- Vibration de liaisons: subfemtoseconde (10^{-14} - 10^{-13} s)
- Fluctuations locales (déplacements d'atomes de 0.1 nm): picoseconde (10^{-12} s)
- Torsion de méthyles (Leu, Val, Ile, Ala): picoseconde à nanoseconde
- Rotation de chaînes latérales en surface: picoseconde à nanoseconde
- Mouvements de boucles: nanoseconde à microseconde
- Flexibilité de domaines autonomes: milliseconde
- Rotation de chaînes latérales internes: milliseconde à seconde
- Transitions allostériques: milliseconde à seconde
- Isomérisation cis/trans proline: seconde

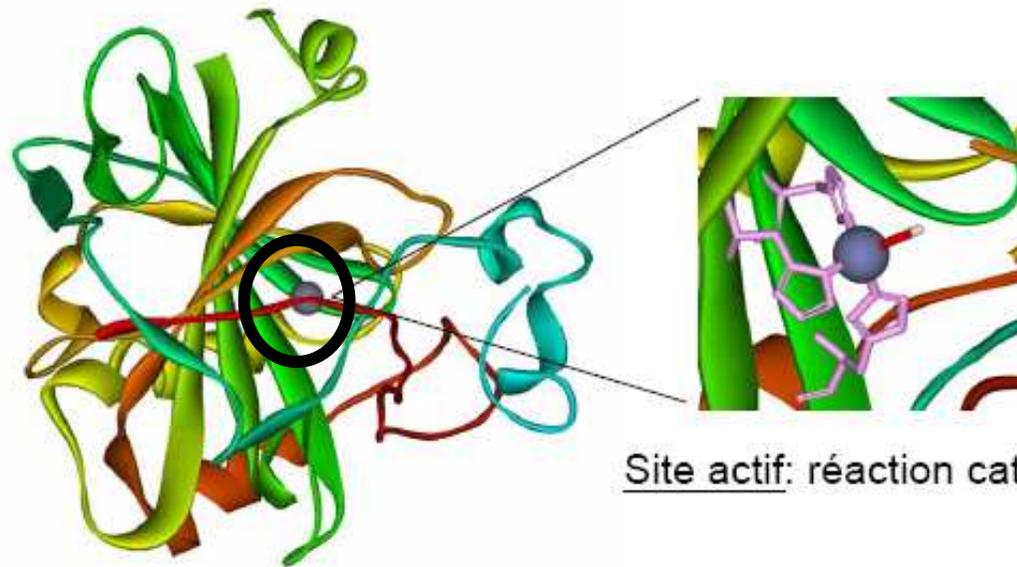


CATALYSE

- Accès et fixation des substrats
- Complémentarité sites réactifs/substrats
- Départ des produits
- Transferts d'électrons

Protéine et site actif

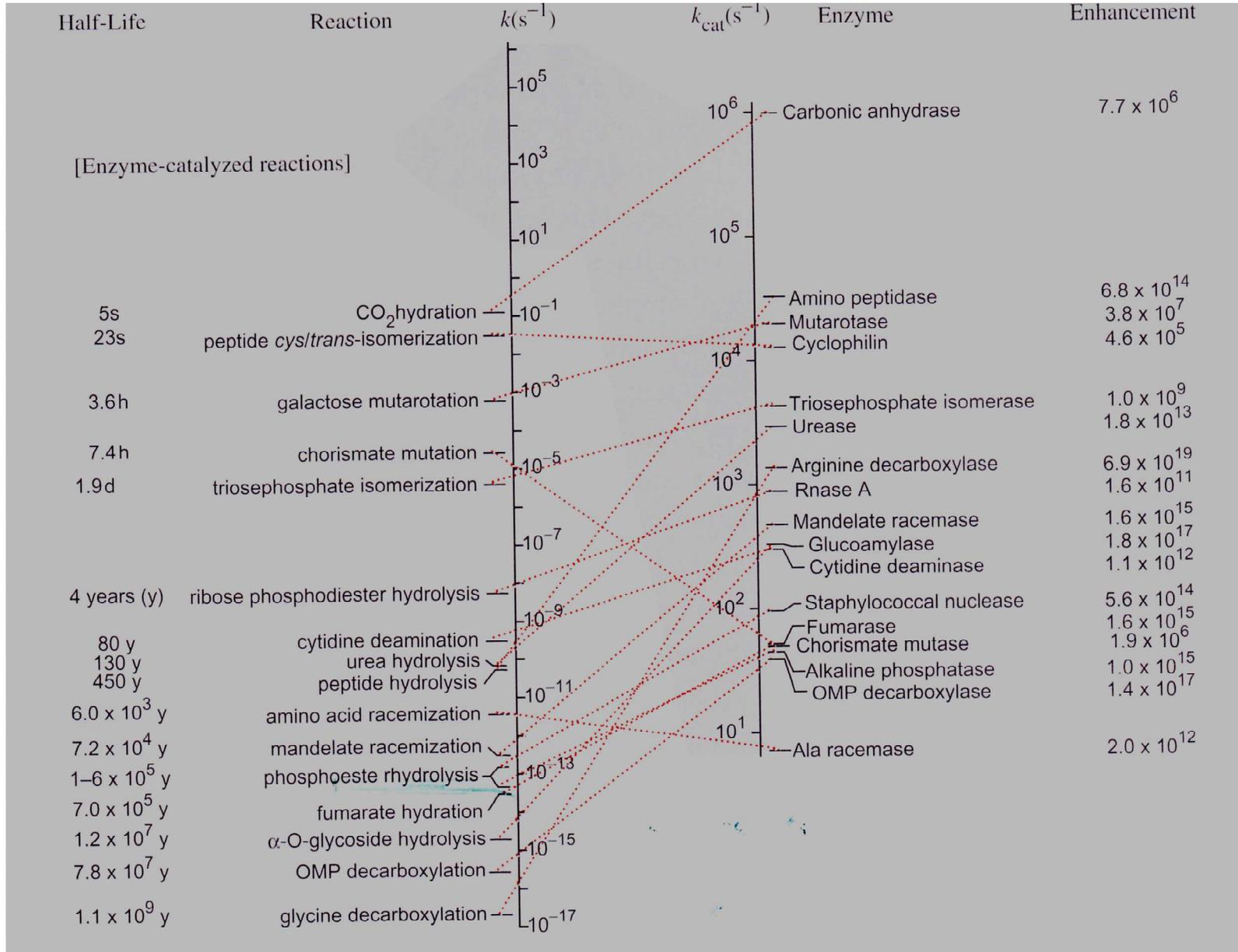
- Là où la réaction catalytique a lieu
- Domaine très restreint de la protéine



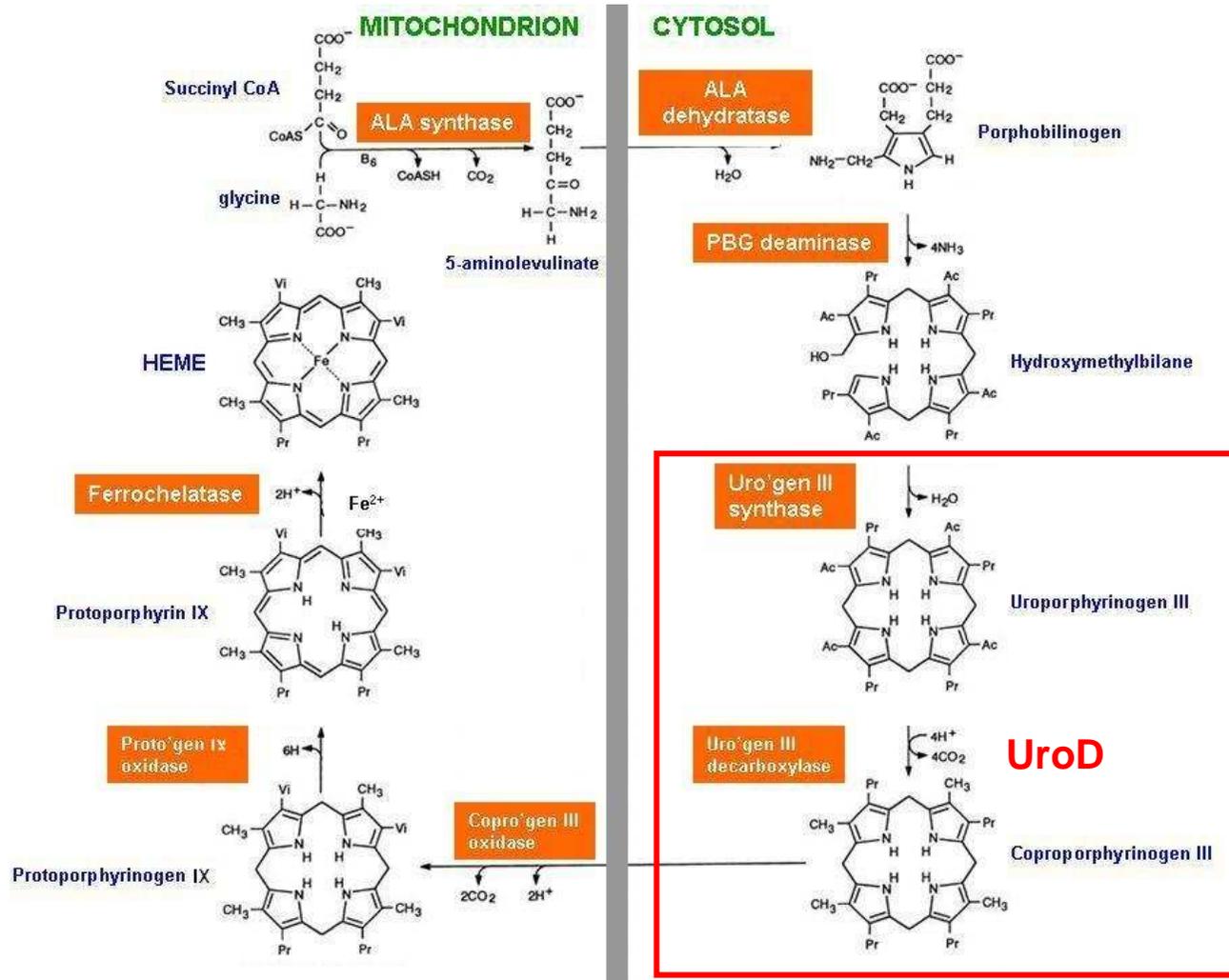
Site actif: réaction catalytique

Enveloppe protéique: Stabilité, régulation, allostérie, fixation de cofacteurs, interaction avec d'autres biomolécules

Catalyse enzymatique: des accélérations fantastiques !!



Décarboxylation de l'Uroporphyrinogen



UroD:

-Synthèse de l'hème, de la chlorophylle et des cytochromes

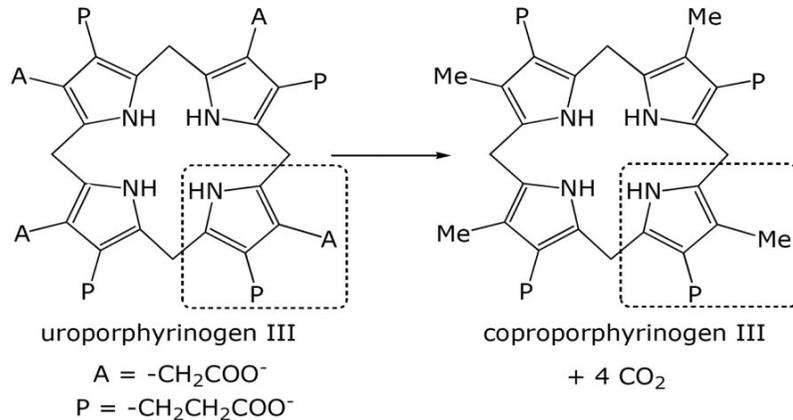
-uroD⁻: maladie génétique (porphyria cutanea tarda)



Une réaction modèle

uroporphyrinogen decarboxylase

$k_{cat} = 0.16 \text{ s}^{-1}$; $K_m = 7.10^{-8} \text{ M}$



pKa = 4.95

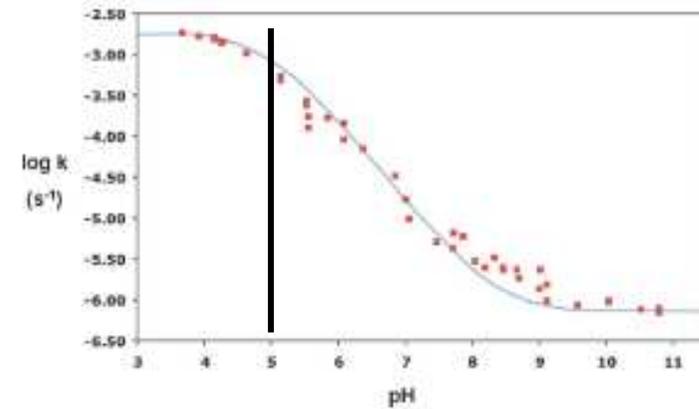
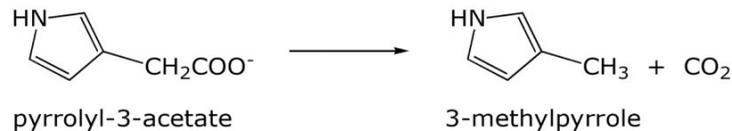


Fig. 2. First-order rate constants for the decarboxylation of PAA at 200 °C, in acetate, borate, phosphate, and carbonate buffers (0.1 M).

+ mesures de k en fonction de T (180-250°C)

model reaction

$\text{pH } 10, 25^\circ\text{C}: k = 9.5 \cdot 10^{-18} \text{ s}^{-1}; t_{1/2} = 2.3 \cdot 10^9 \text{ ans}$



Facteur d'accélération: $1.2 \cdot 10^{17} !!$
(état de transition $K_d = 4 \cdot 10^{-25} \text{ M} !!$)

Les valeurs les plus élevées pour une enzyme sans cofacteur

Quelle est l'origine de cette accélération ? (mystère)

→ **Accélération de $10^{17} = 25 \text{ kcal.mol}^{-1}$**

-Emil Fischer (1894)

Le concept « clé-serrure » (ne dit rien sur l'accélération)

-JBS Haldane (1930)

Le concept de « déstabilisation de l'état fondamental »: l'enzyme contraint le substrat, le « poussant » à se transformer

-Linus Pauling (1948)

Le concept de « stabilisation de l'état de transition »

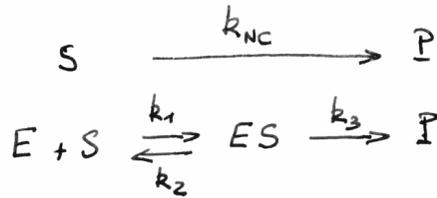
« I think that enzymes are molecules that are complementary in structure to the activated complexes of the reactions that they catalyze, that is, to the molecular configuration that is intermediate between the reacting substances and the products of reaction for these catalyzed processes. The attraction of the enzyme for the activated complex would thus lead to a decrease in the energy of activation of the reaction and to an increase in the rate of reaction »

Nature (1948)161 707

-première structure cristallographique d'une enzyme (lysozyme) et son substrat

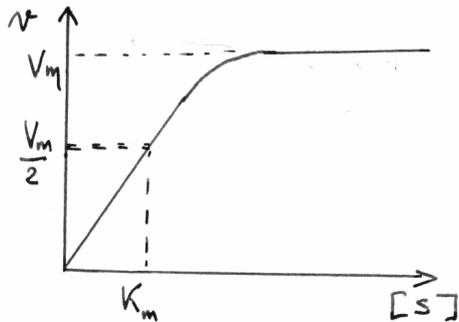
Nature (1965) 206 757

Théorie de l'état de transition



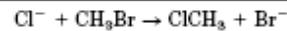
$$v = \frac{k_3 [S][E]_0}{k_2 + k_3 + [S]} = \frac{V_m [S]}{K_m + [S]}$$

$$k_3 = k_{cat} ; K_m = \frac{k_2 + k_3}{k_1} ; K_d = \frac{k_2}{k_1} \neq K_m$$



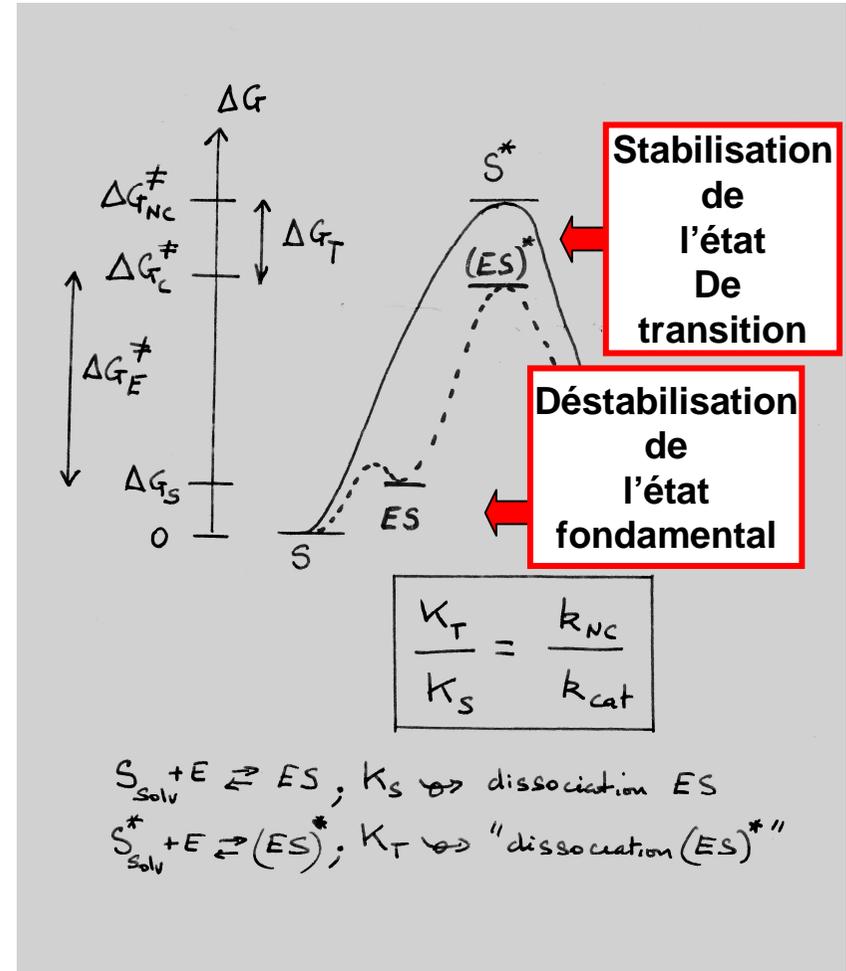
Effet de solvant

Reaction rates for an S_N2 reaction in various solvents of differing dielectric response and polarity, including the gas phase reaction (13)

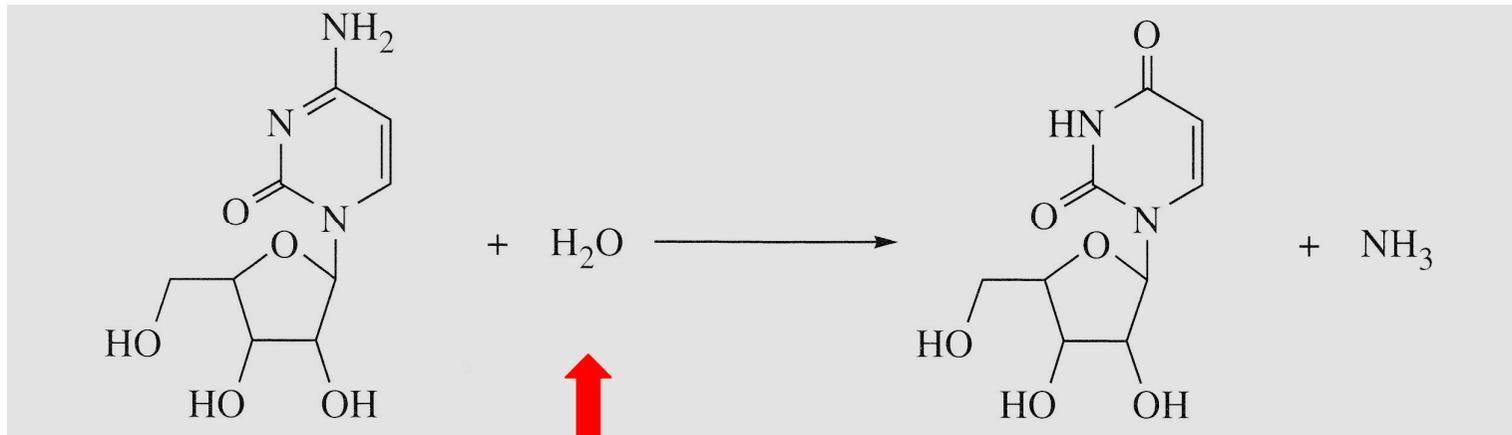


Solvent	Relative rate
	$cm^3 \text{ molecule}^{-1} s^{-1}$
Gas phase	1
MeCO	10^{-10}
DMF ^a	10^{-11}
CH ₃ OH	10^{-15}
H ₂ O	10^{-16}

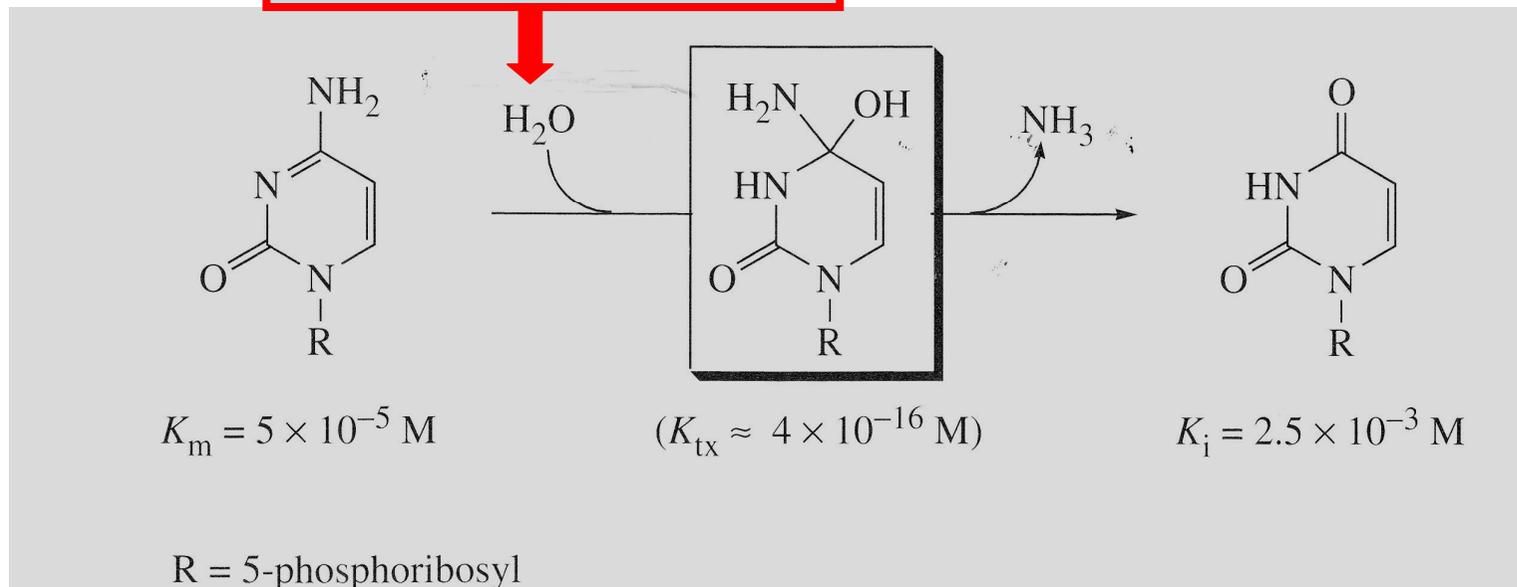
^a Dimethylformamide.

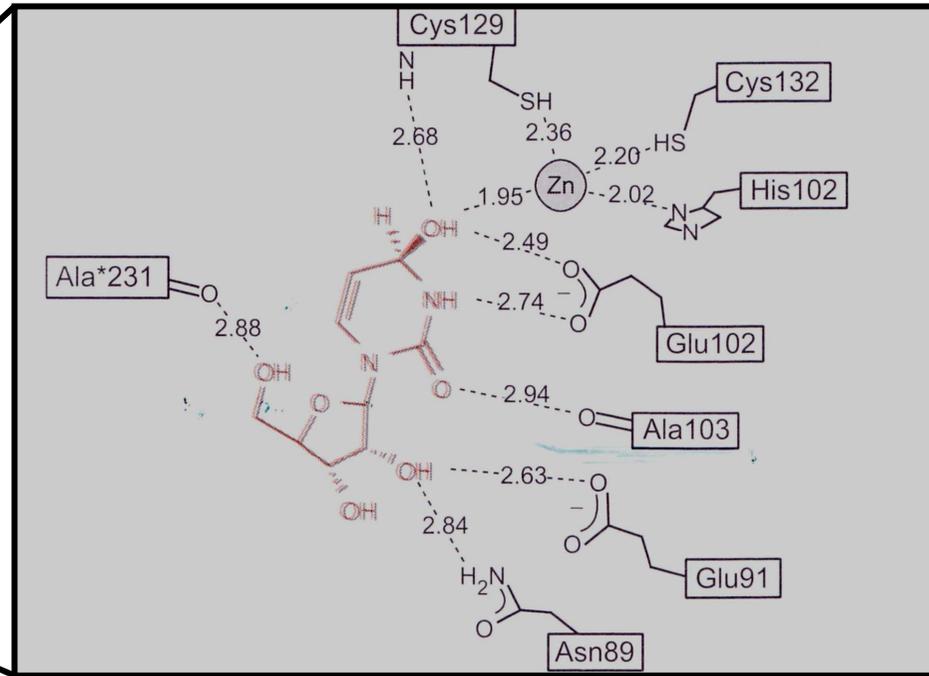
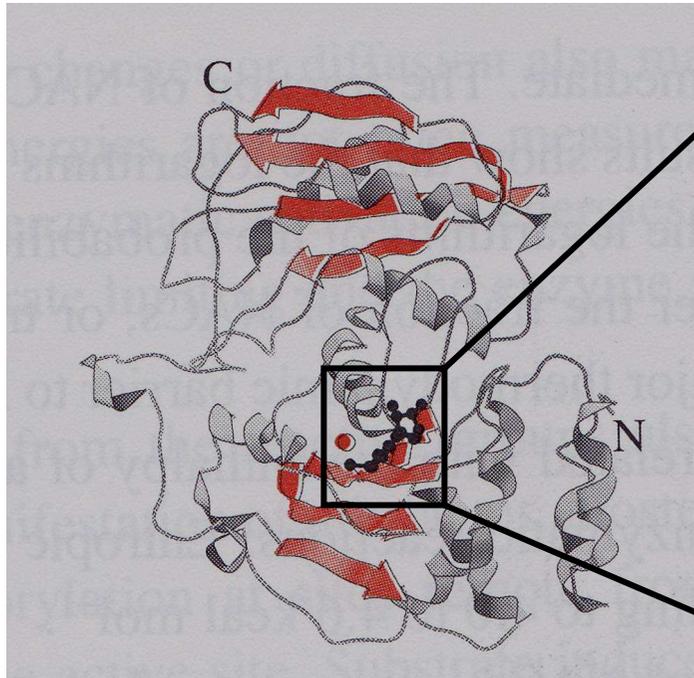


Exemple: cytidine deaminase

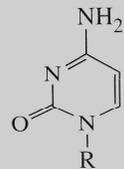


Activation H₂O par Zn²⁺
Zn-OH₂

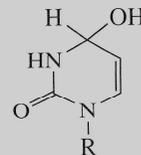




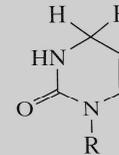
Inhibiteurs: analogues de « l'état de transition »



Cytidine
 $K_m = 5 \times 10^{-5} \text{ M}$

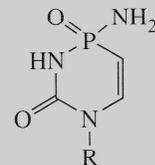


A
 $K_i = 1.2 \times 10^{-12} \text{ M}$



B
 $K_i = 2.5 \times 10^{-3} \text{ M}$

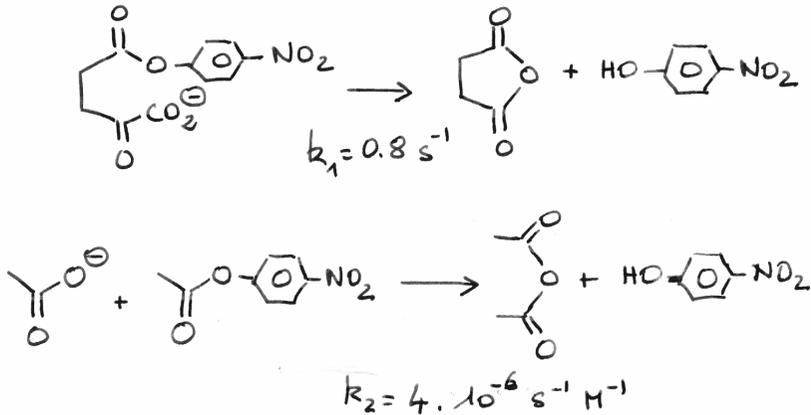
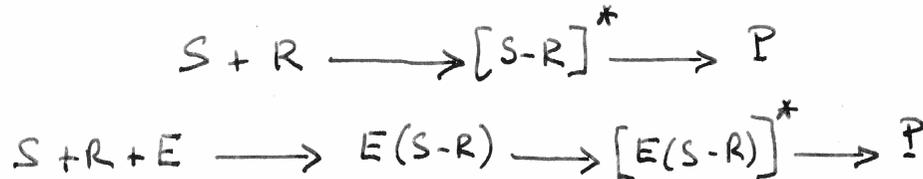
R = 5-phosphoribosyl



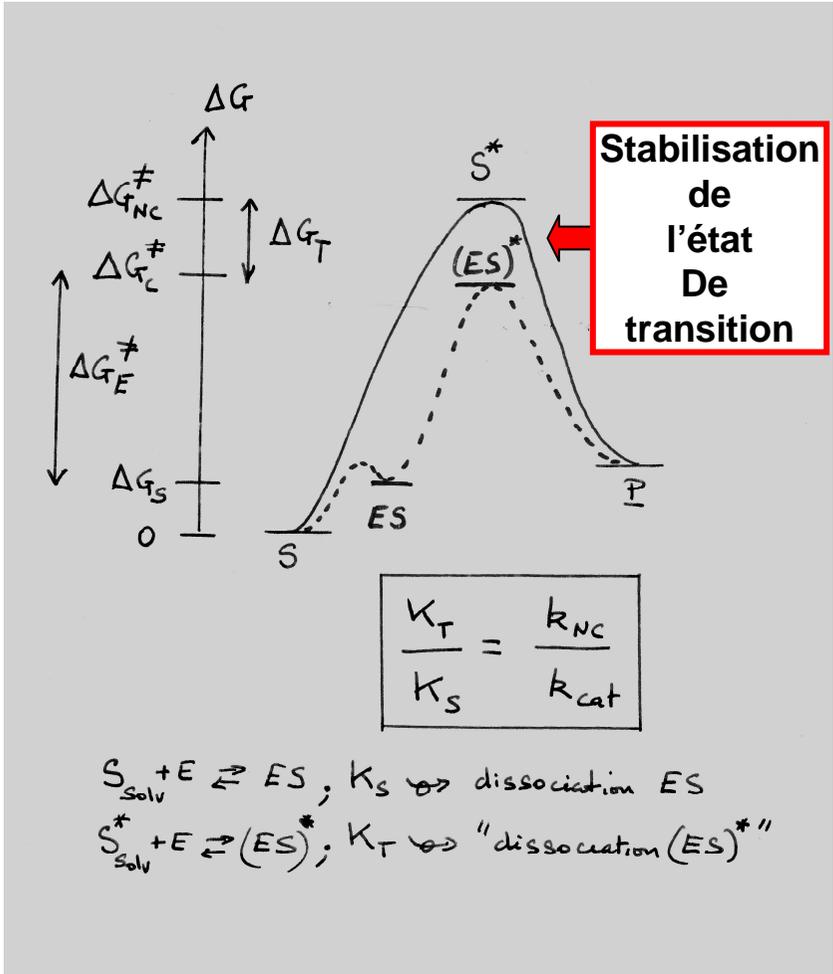
C
 $K_i = 9 \times 10^{-10} \text{ M}$

$$v = \frac{k_{cat}}{K_m} (E)_0 (S) = \frac{k_0}{K_m} e^{-\frac{\Delta H^\ddagger}{RT}} e^{\frac{\Delta S^\ddagger}{R}} (E)_0 (S)$$

Effets entropiques:



$$[-\text{CO}_2^\ominus]_{\text{eff}} = 2.10^5 \text{ M}$$



→ 4 - 6 kcal.mol⁻¹

$$v = \frac{k_{cat}}{K_m} (E)_0(S) = \frac{k_0}{K_m} e^{-\frac{\Delta H^\ddagger}{RT}} \cdot e^{\frac{\Delta S^\ddagger}{R}} (E)_0(S)$$

Effets enthalpiques:

➔ **Préorganisation des « réactifs »**

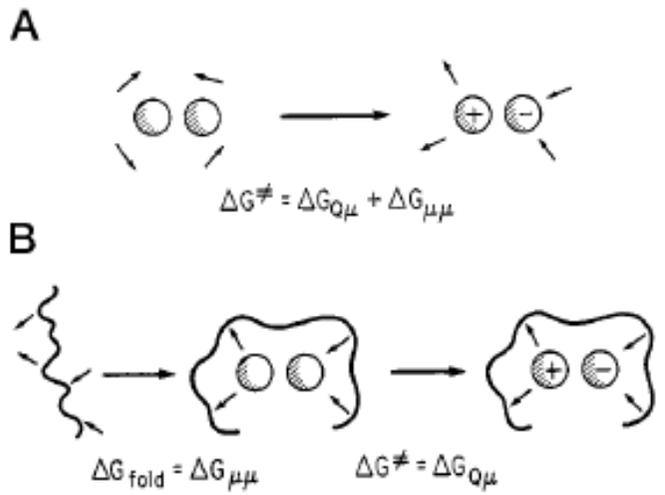
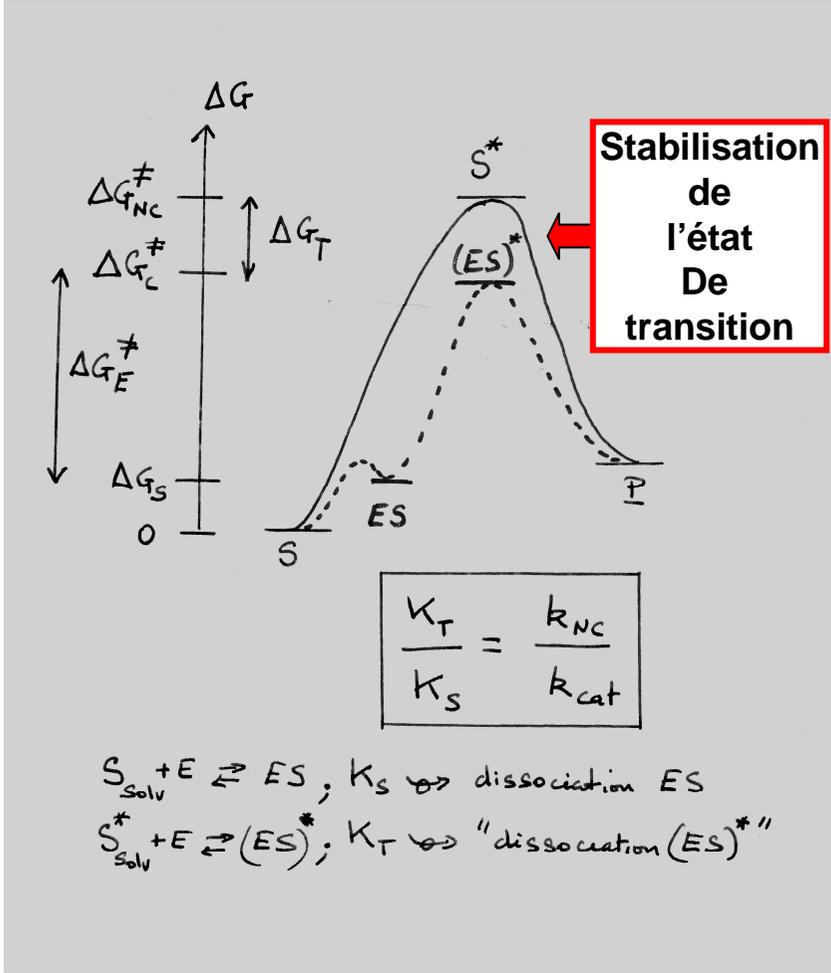
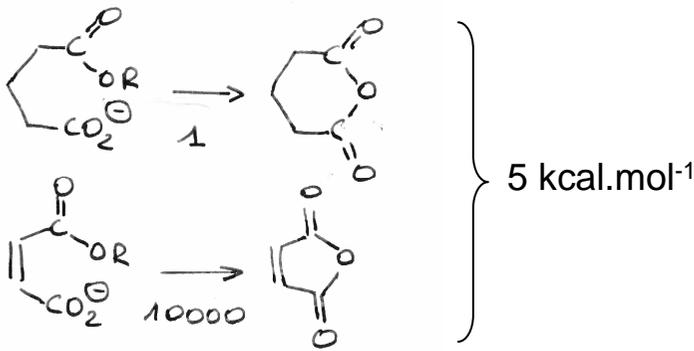
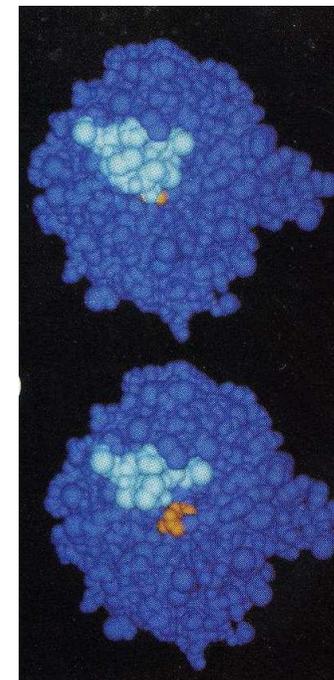
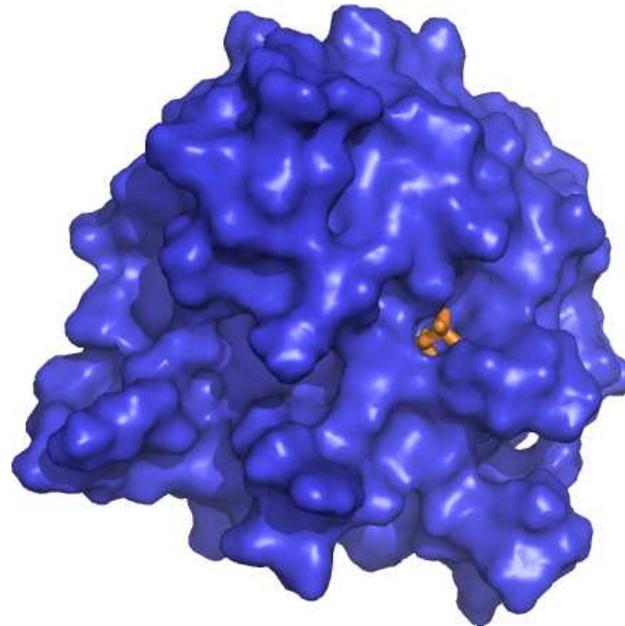
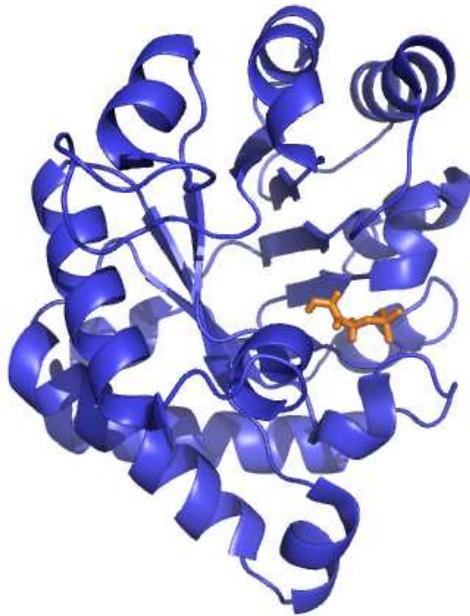
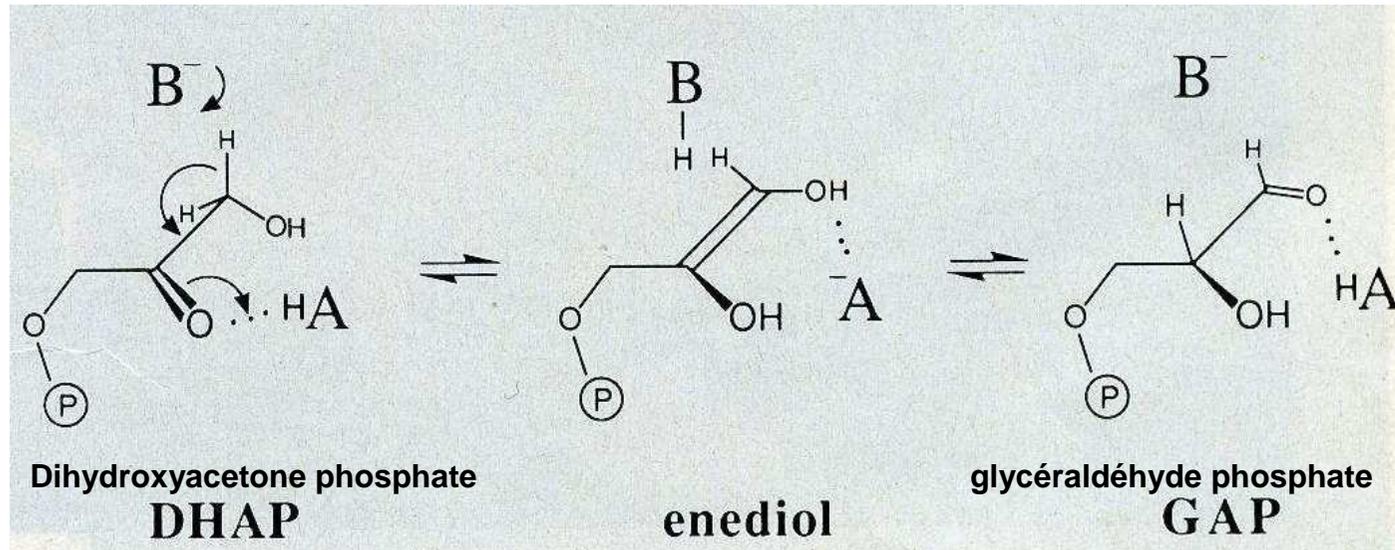
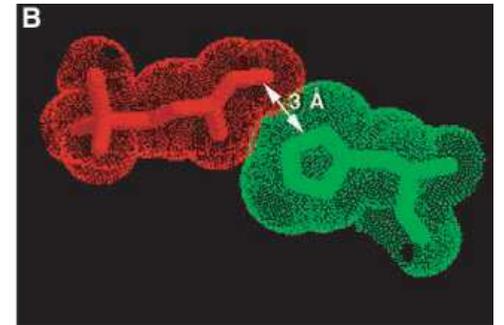
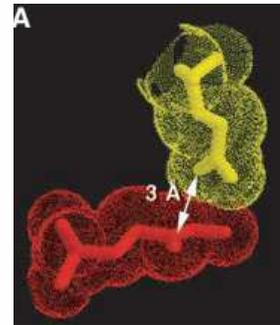
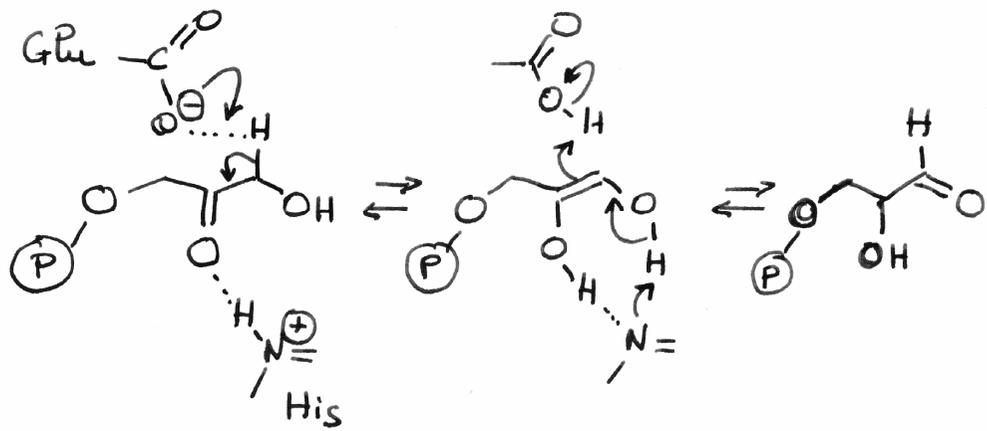
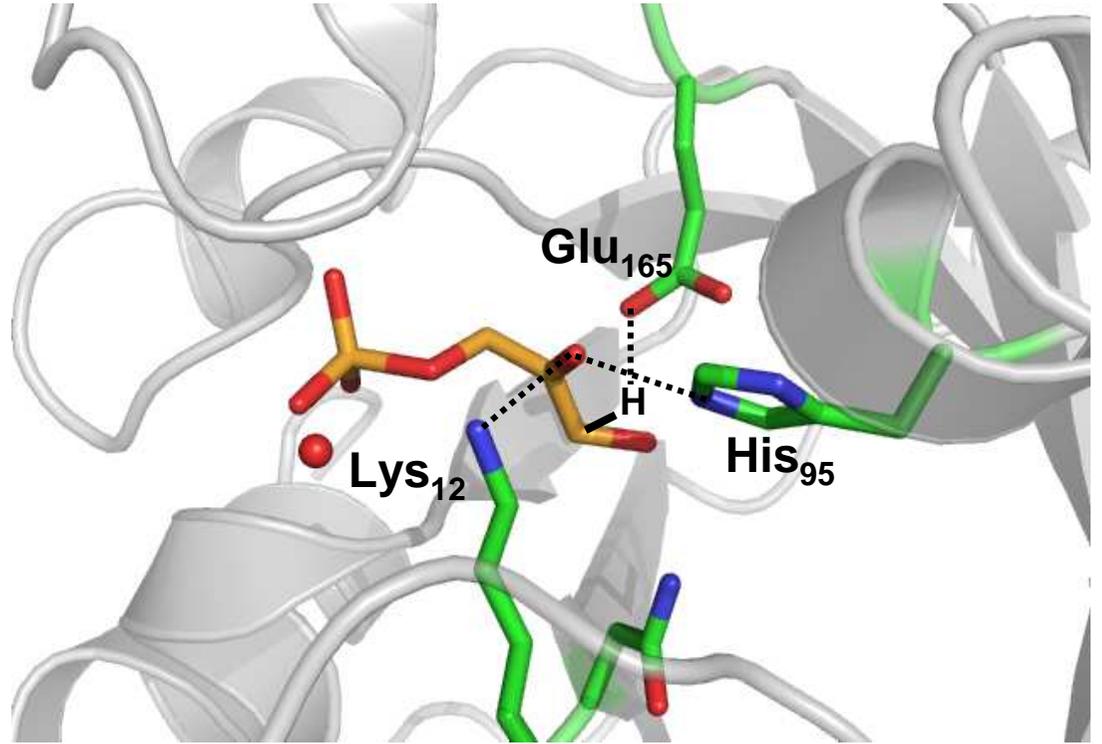
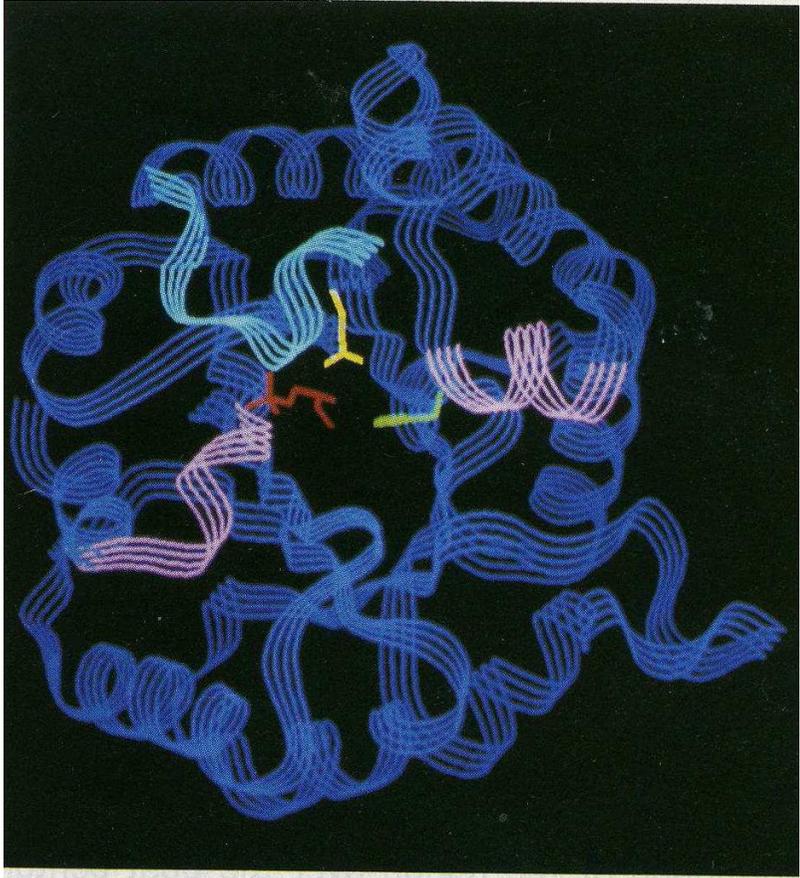


FIG. 3. Illustrating the catalytic origin of enzyme catalysis. In water (A) we have to pay for orienting the solvent, and this reduces the solvation free energy. In enzymes (B) we already have preorientation dipoles so we do not have to pay so much for the increase in dipole-dipole repulsion ($\Delta G_{\mu\mu}$).

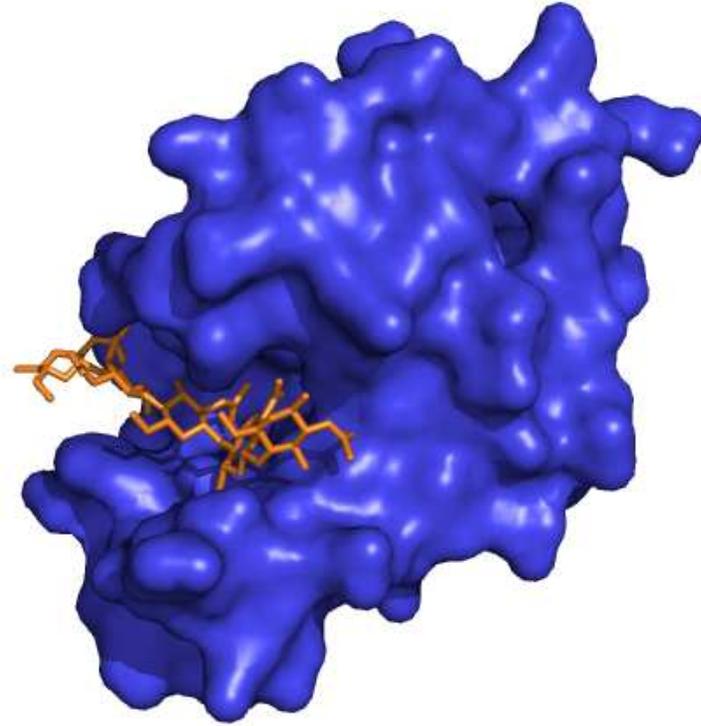
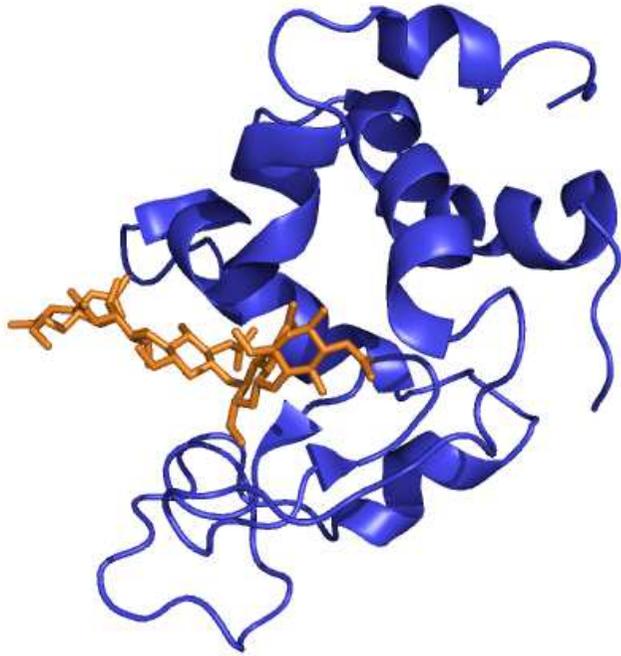
➔ **15 - 18 kcal.mol⁻¹**

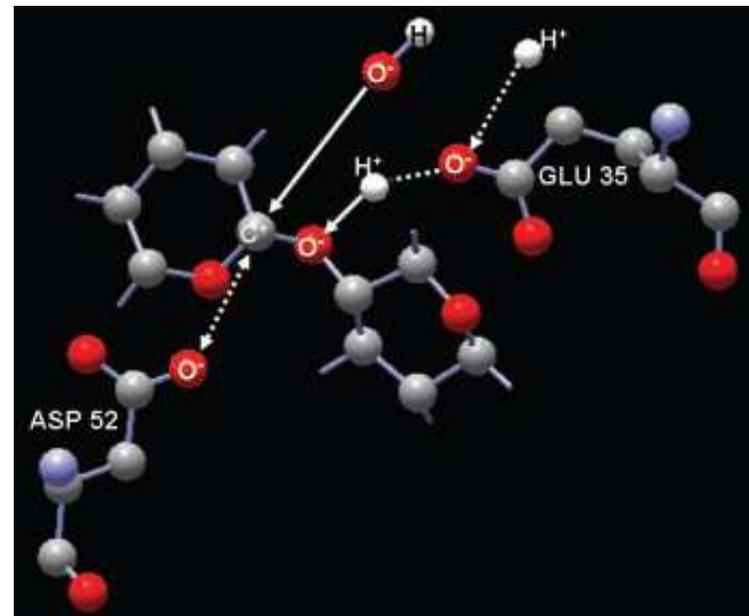
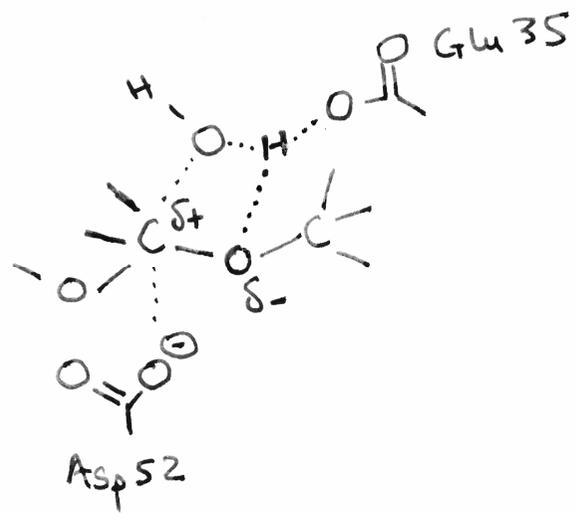
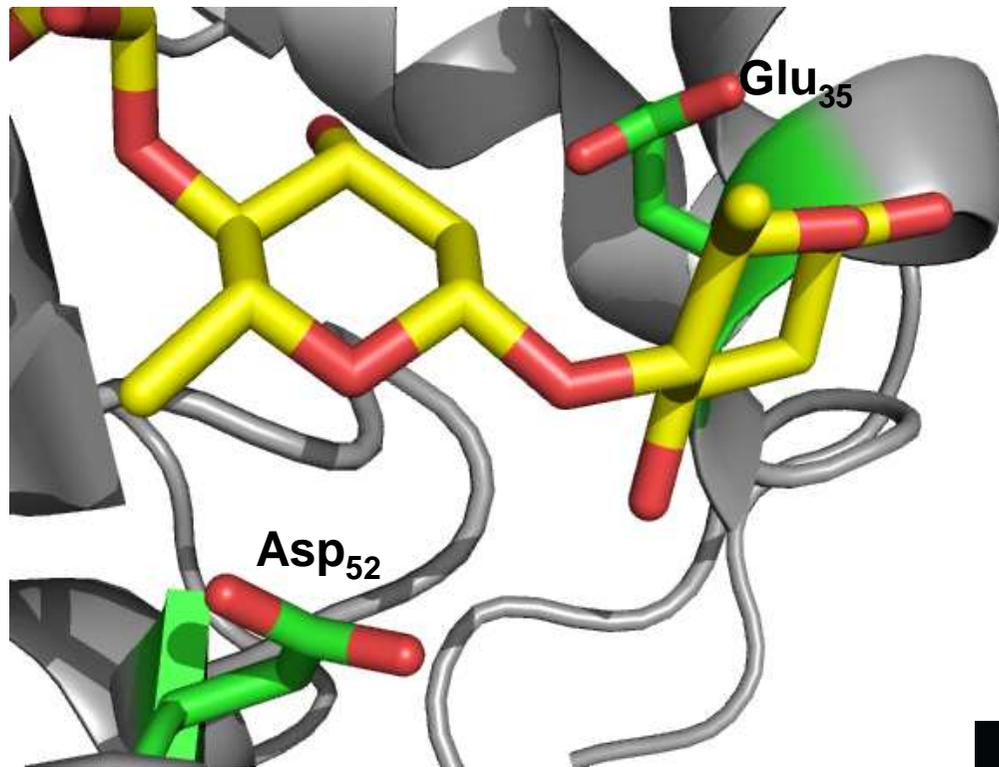
Triose phosphate isomérise



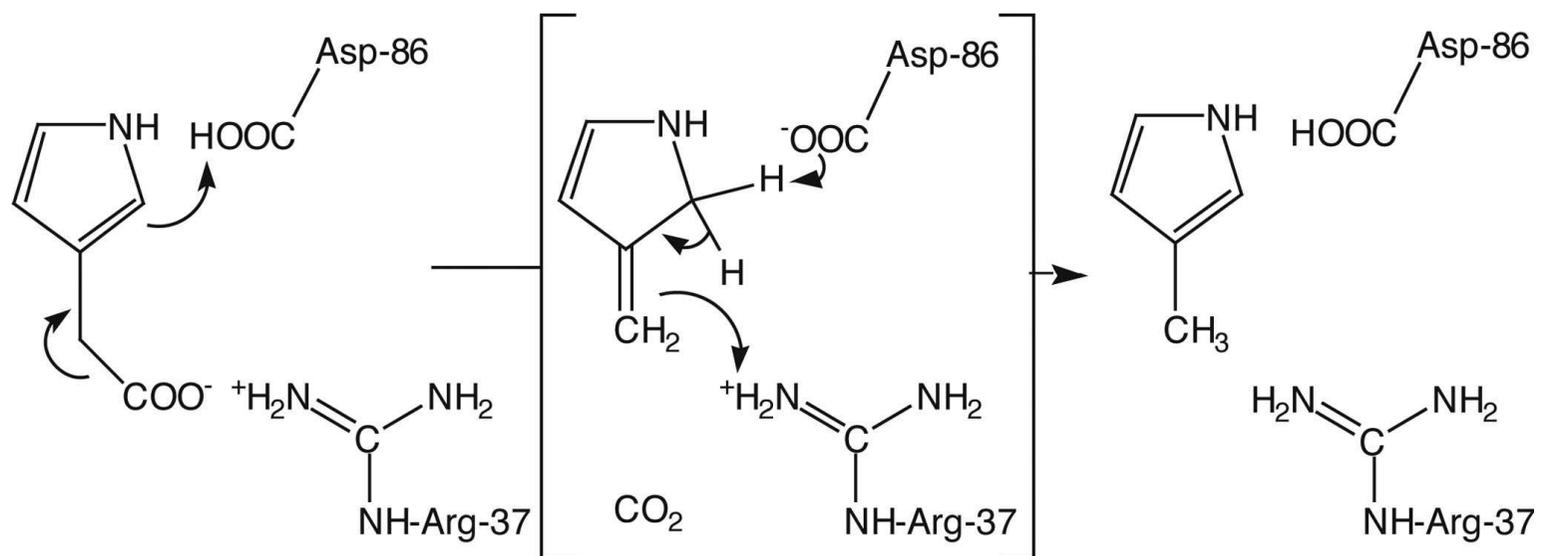
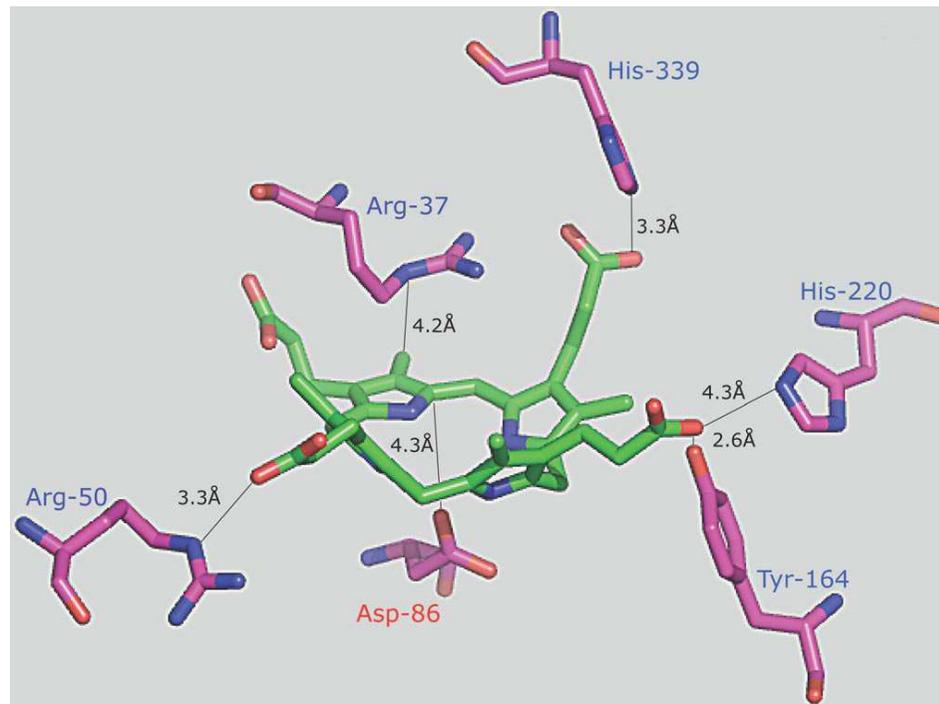
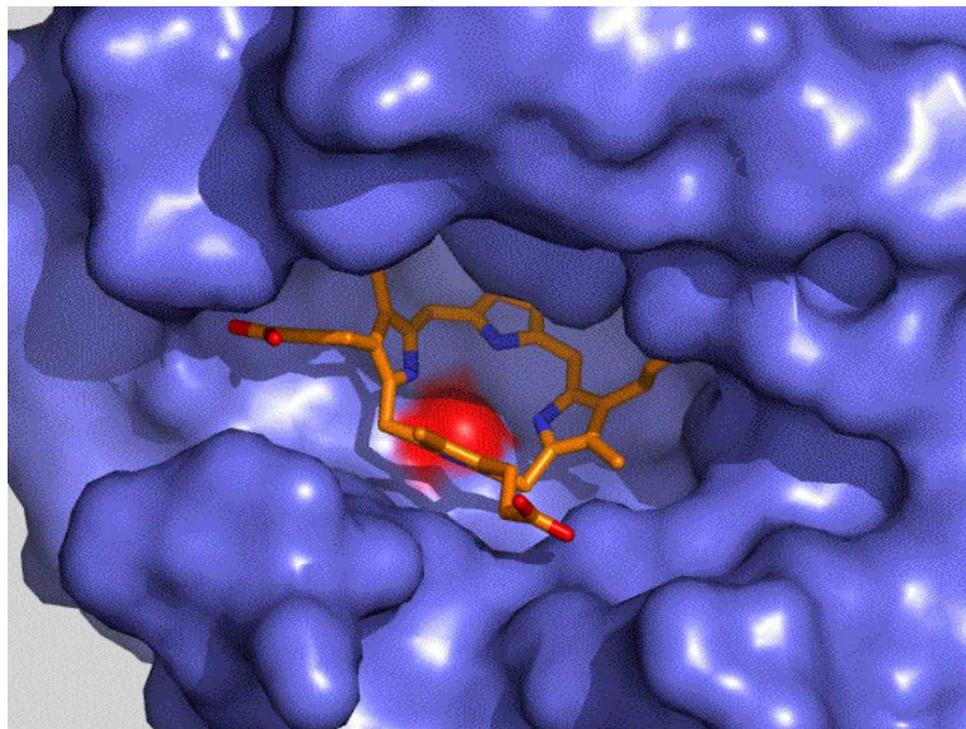


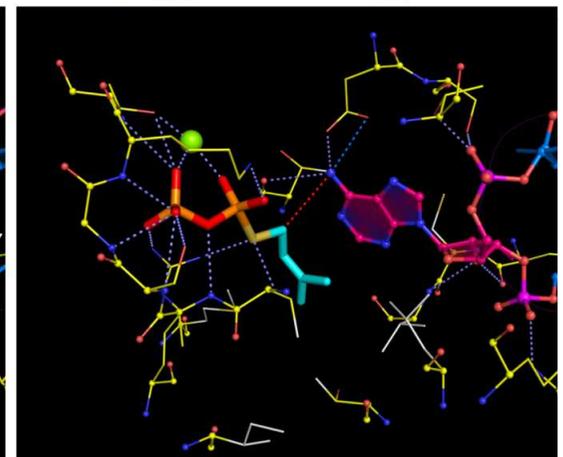
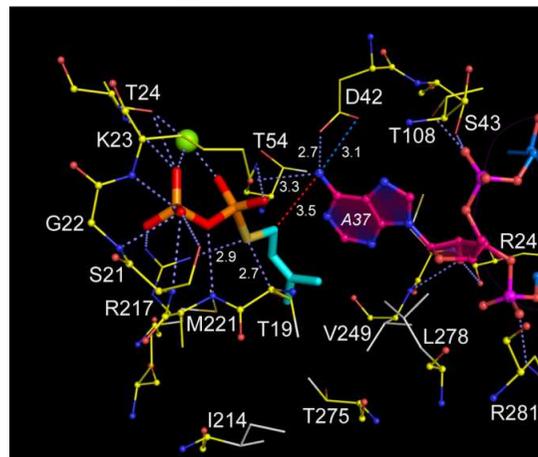
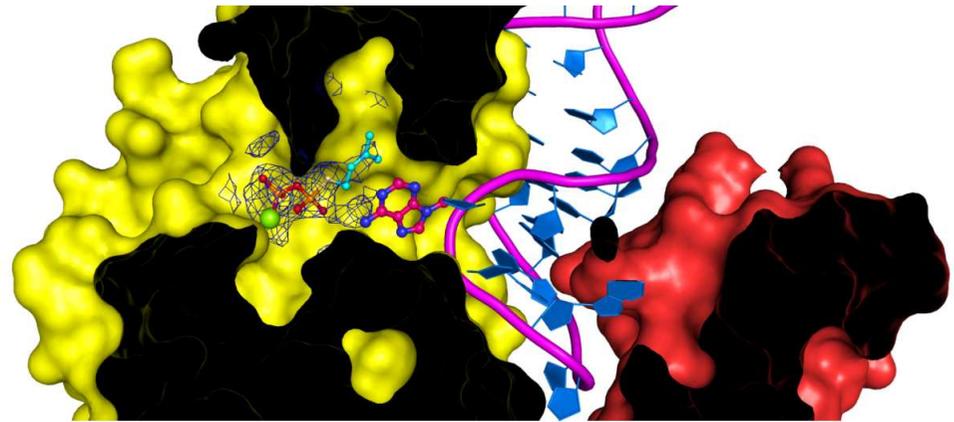
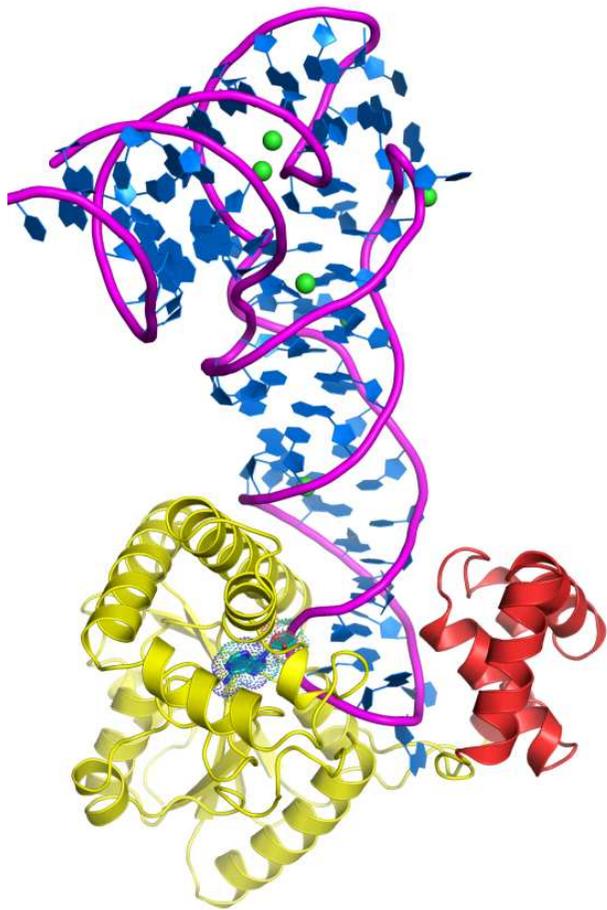
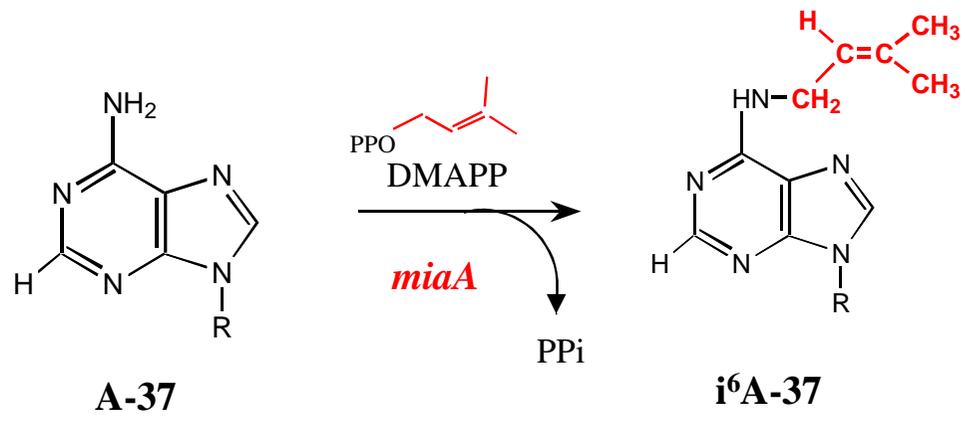
Lysosyme





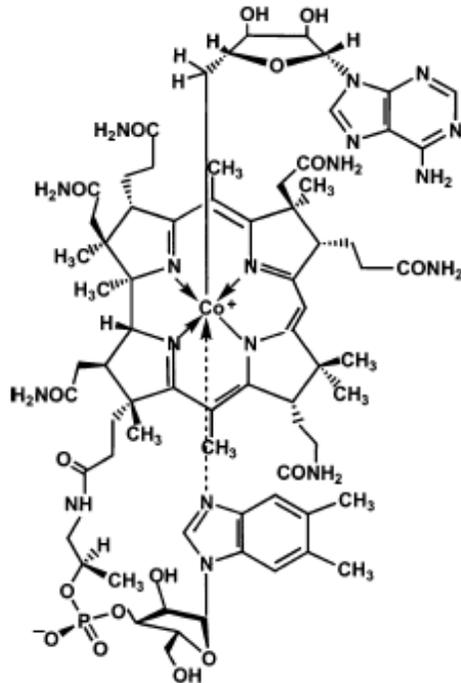
Retour à UroD



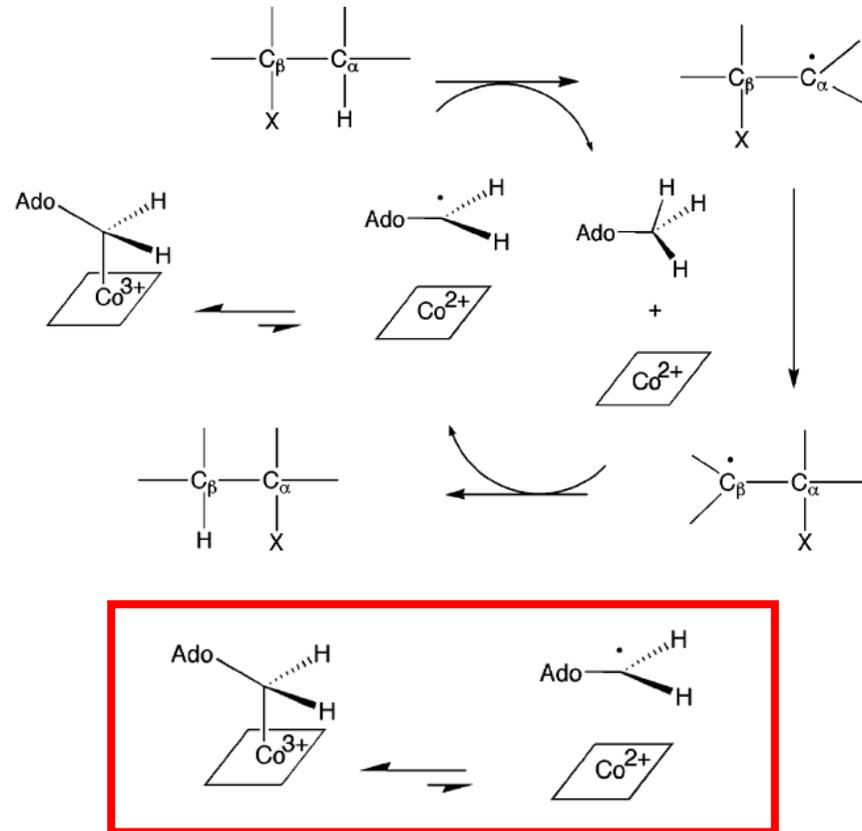


Abaissement de la barrière d'activation par addition du substrat/stabilisation du produit ?

Adenosylcobalamine (vitamine B12)



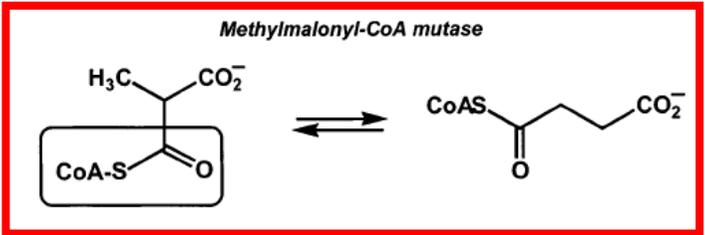
Découverte en 1958 (Barker)
Structure en 1961 (Hodgkin)



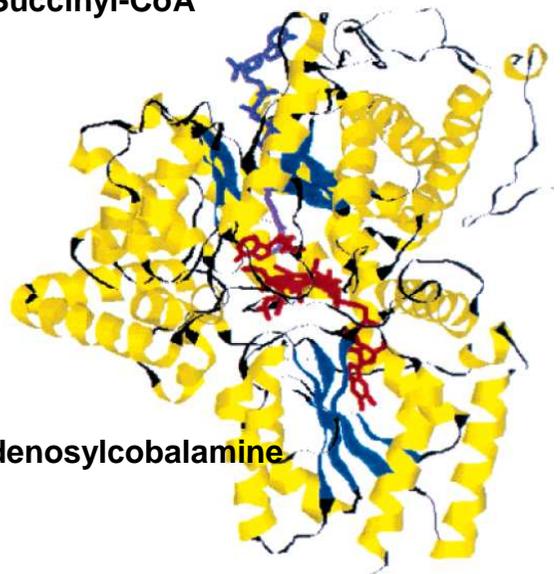
$E_{\text{Co-C}} = 30 \text{ kcal.mol}^{-1}$; $k = 3,8 \cdot 10^{-9} \text{ s}^{-1}$; $t_{1/2} = 6 \text{ ans}$

Enzymes (isomérases): $k = 100\text{-}500 \text{ s}^{-1}$; $t_{1/2} = 1 \text{ ms}$

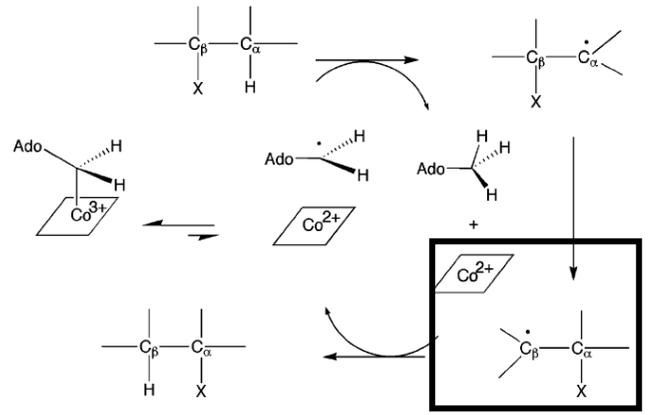
↳ **Accélération: 10^{12}**
Déstabilisation de Co-C: 17 kcal.mol^{-1}



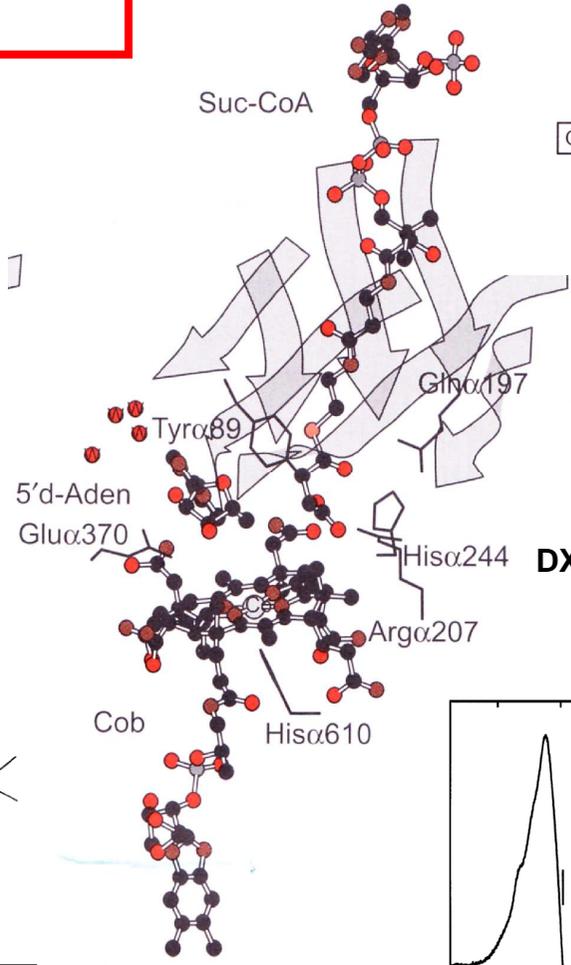
Succinyl-CoA



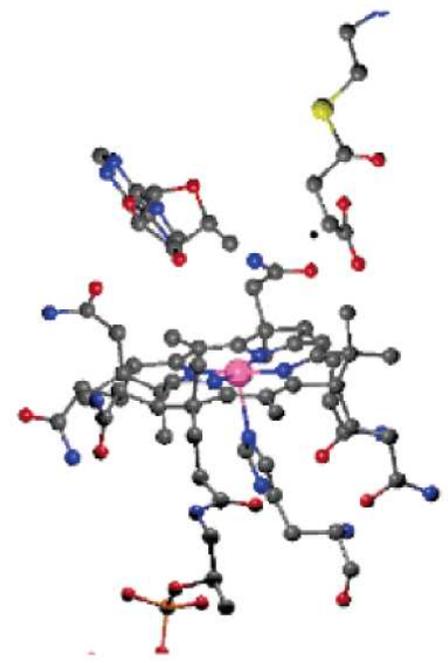
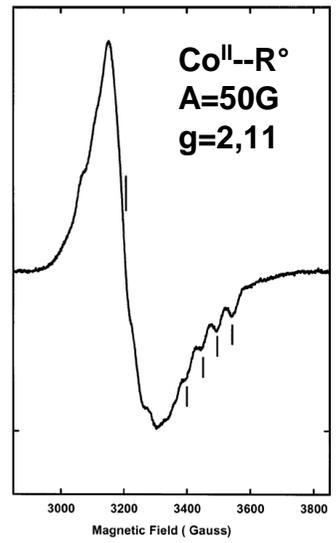
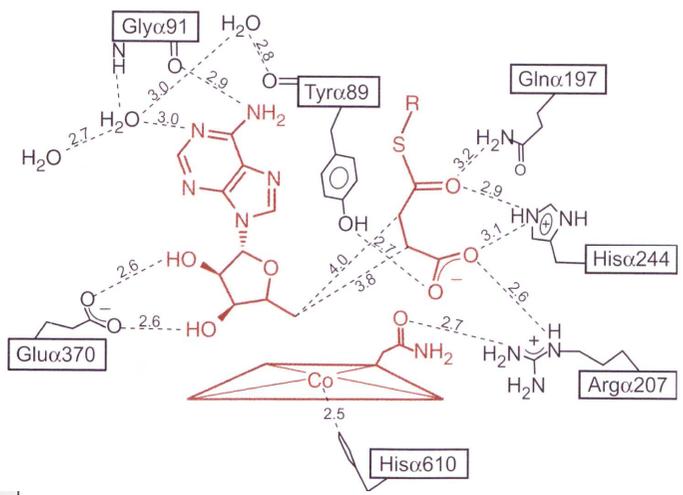
adenosylcobalamine

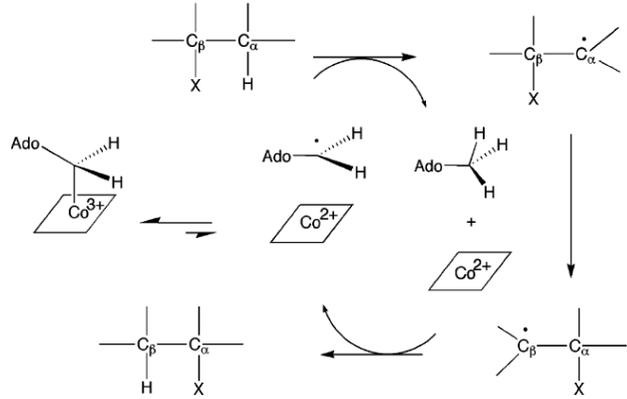
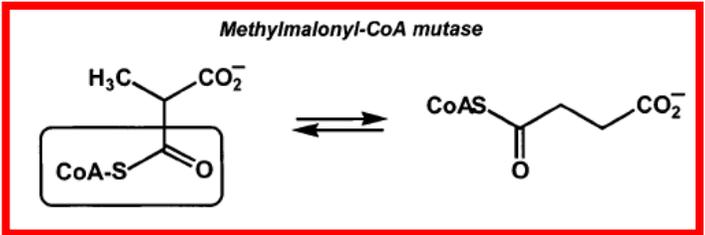


Suc-CoA

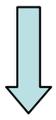


DXHXXG

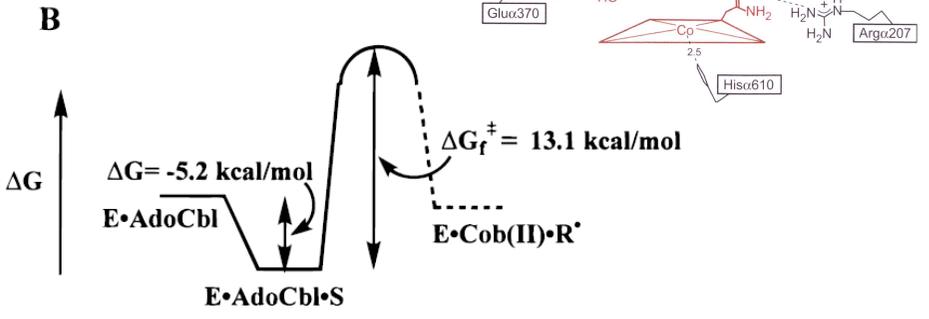
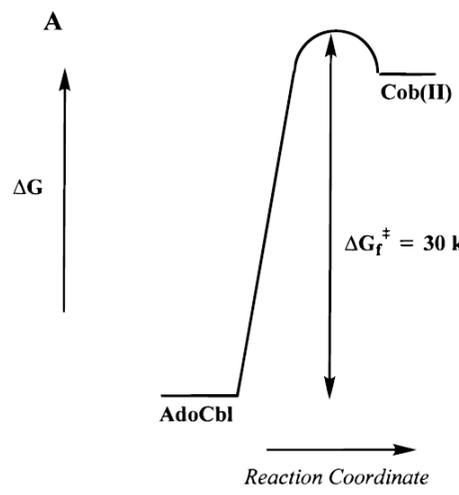
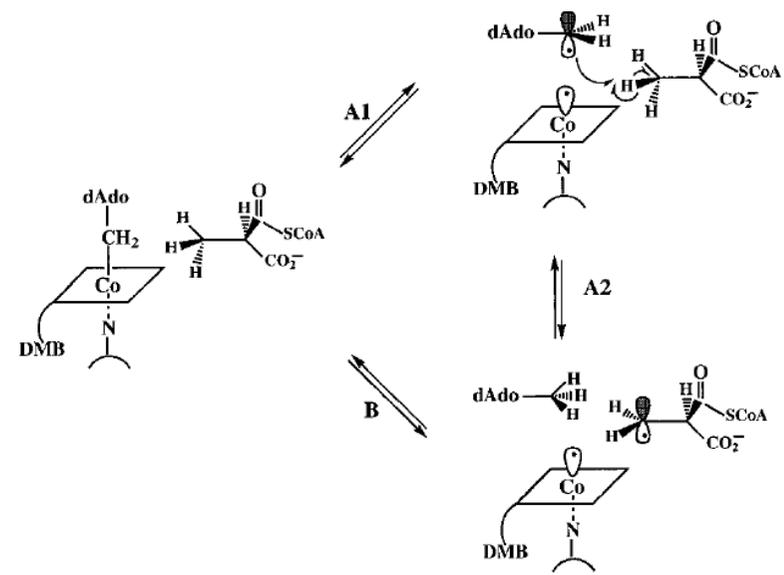




$k_H/k_D > 20$
Couplage cinétique
Cassure Co-C et arrachement H

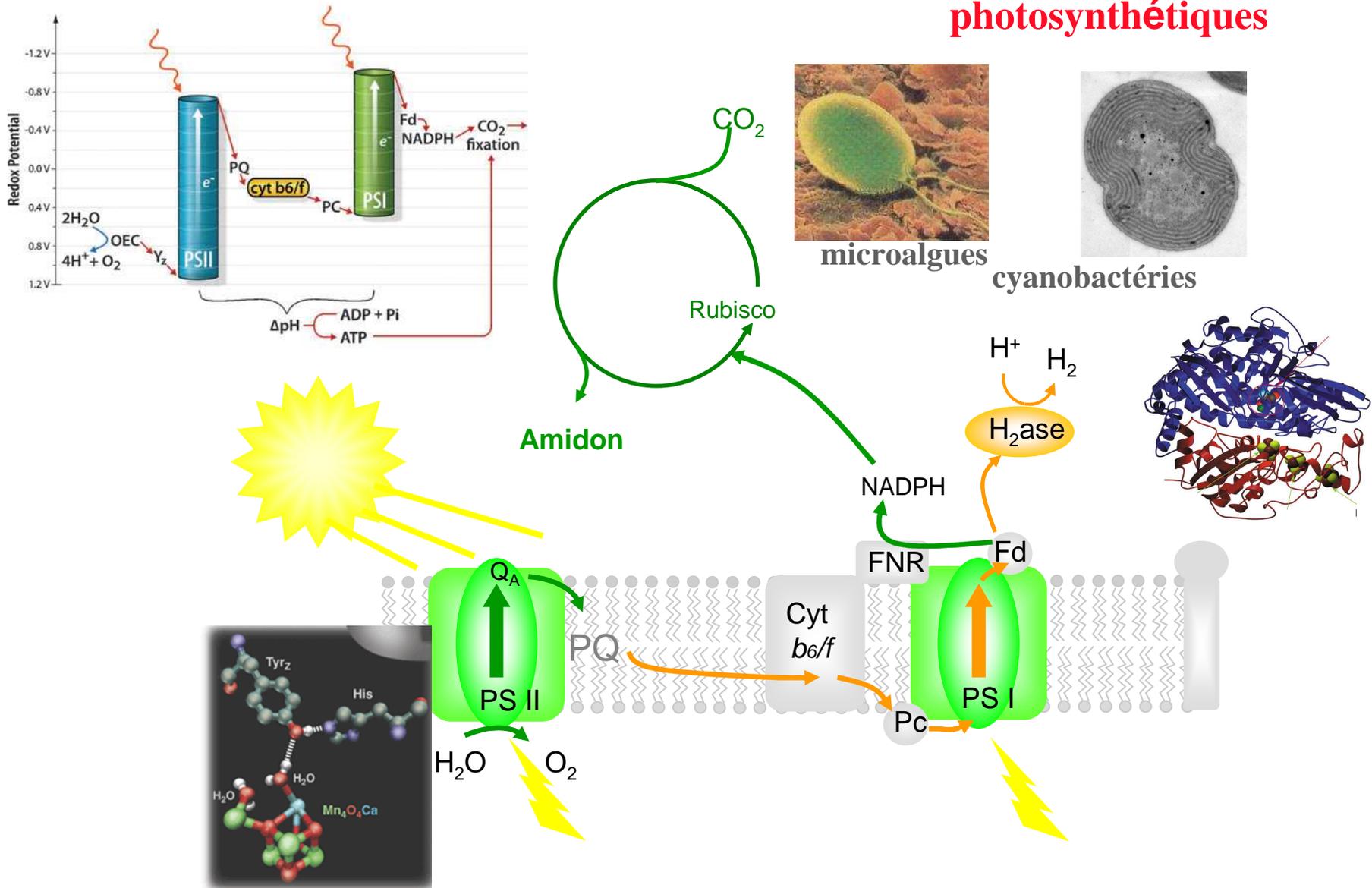


Mécanisme concerté ?

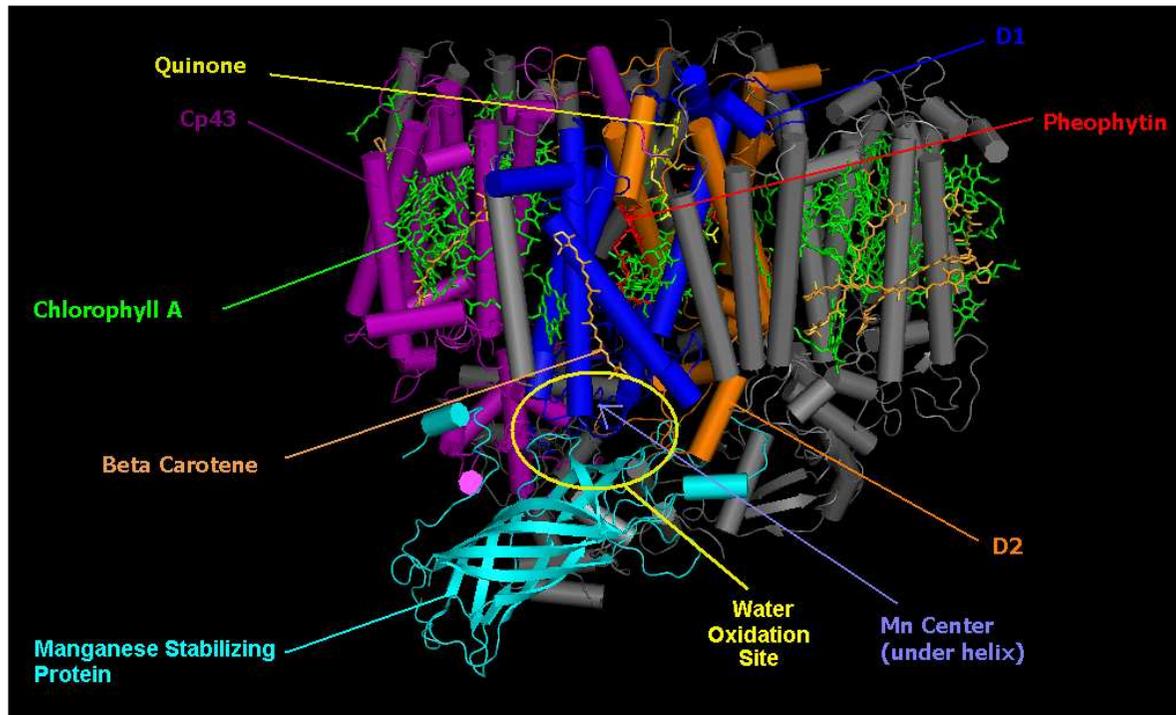


Protéines et transport des électrons: Quelques exemples

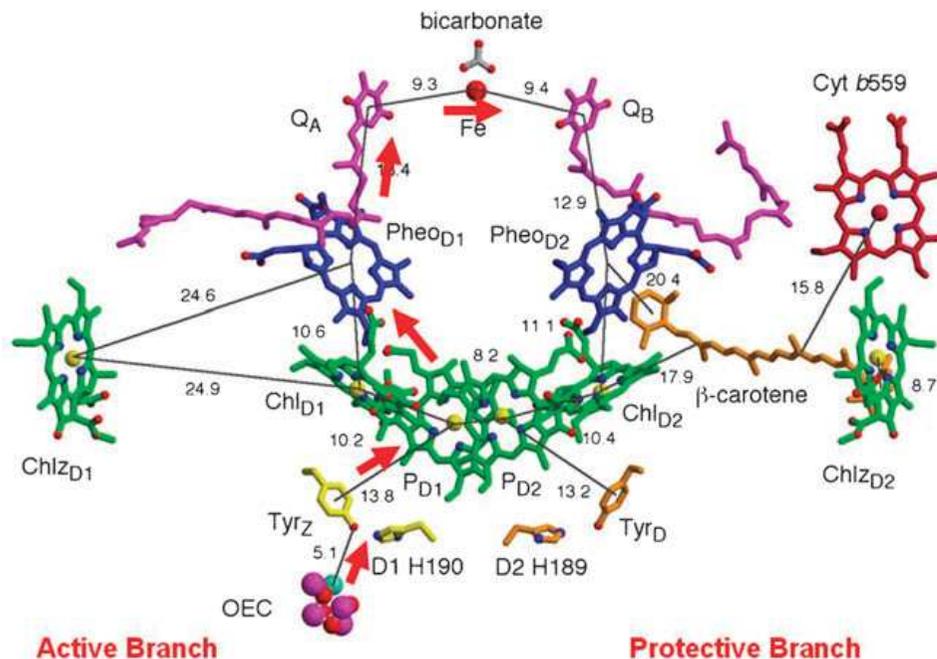
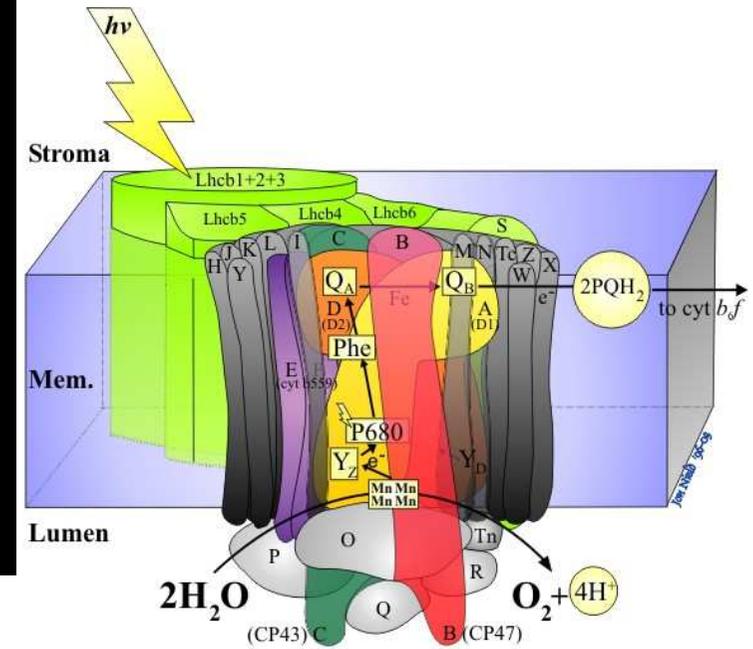
**Microorganismes
photosynthétiques**



Photosystème II



Electron transport cofactors



- Collecte l'énergie du soleil (« Chl », « Car »)
- Convertit en paires électron-trou (P680⁺, Pheo⁻)
- Capture les trous avec un catalyseur (Mn)

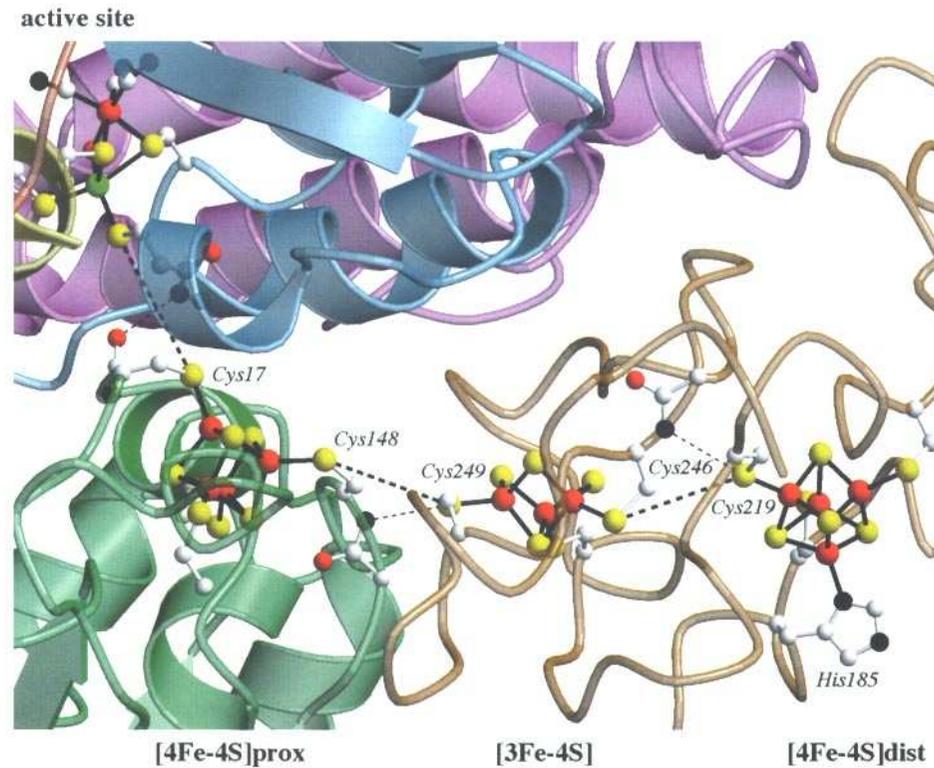
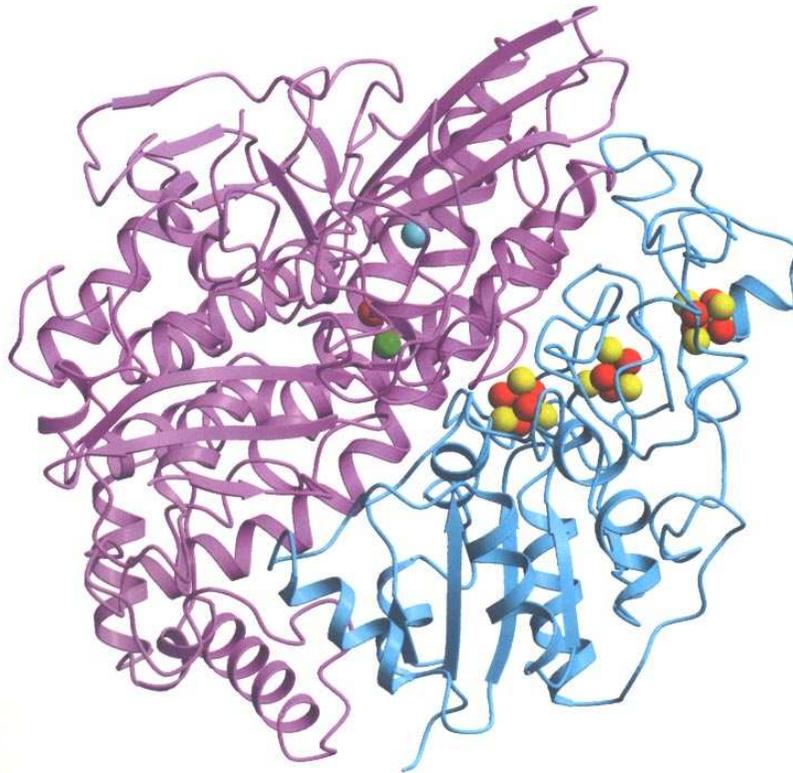
$$4 \oplus + 2H_2O \longrightarrow O_2 + 4H^+$$
- Capture les électrons avec une quinone

$$4 \ominus + 2Q + 4H^+ \longrightarrow 2 QH_2$$

Protéines et transport des électrons

Exemple de transfert intra-moléculaire

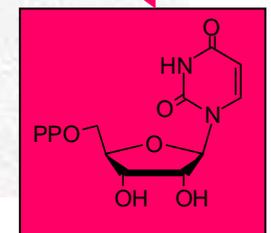
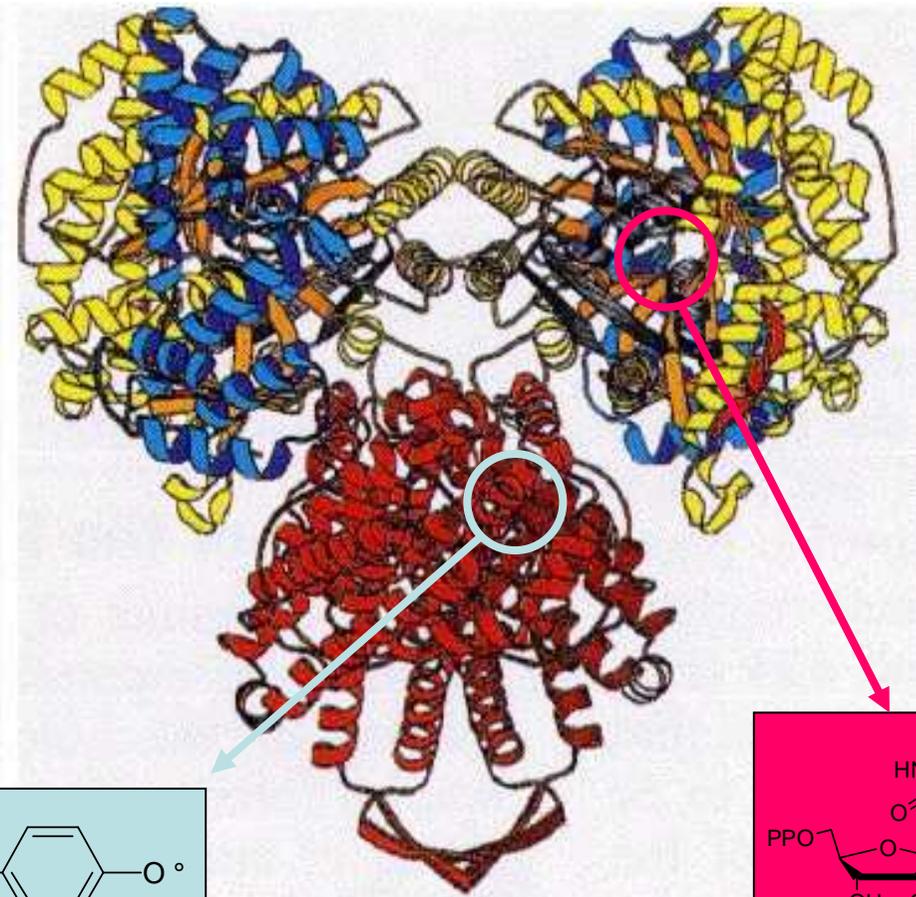
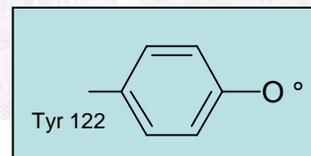
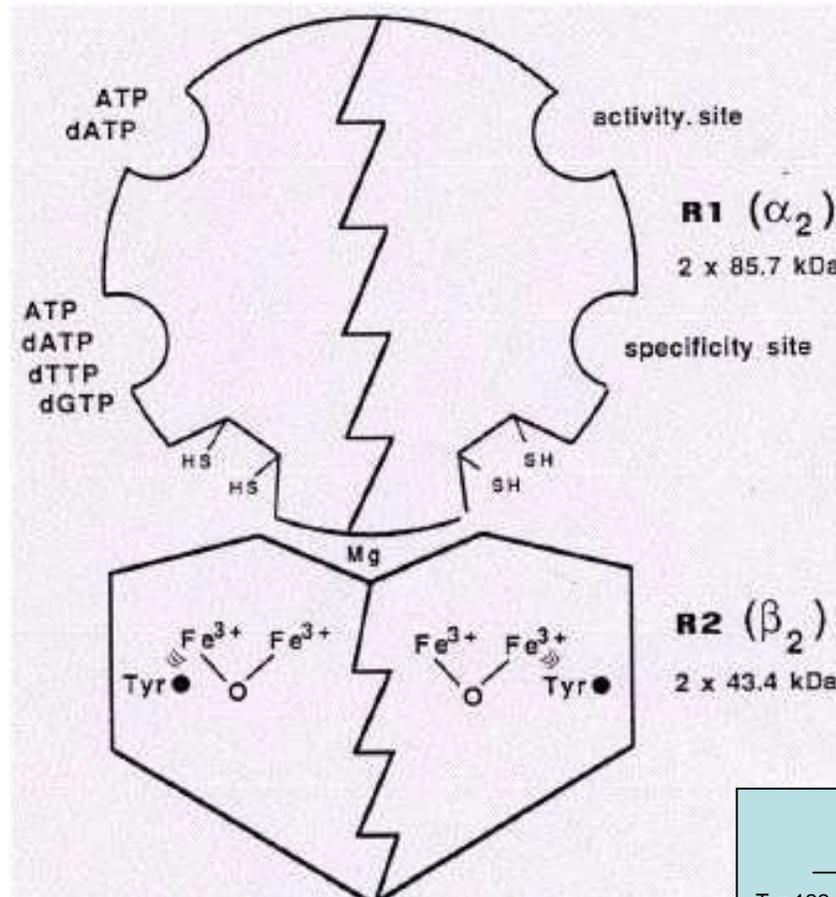
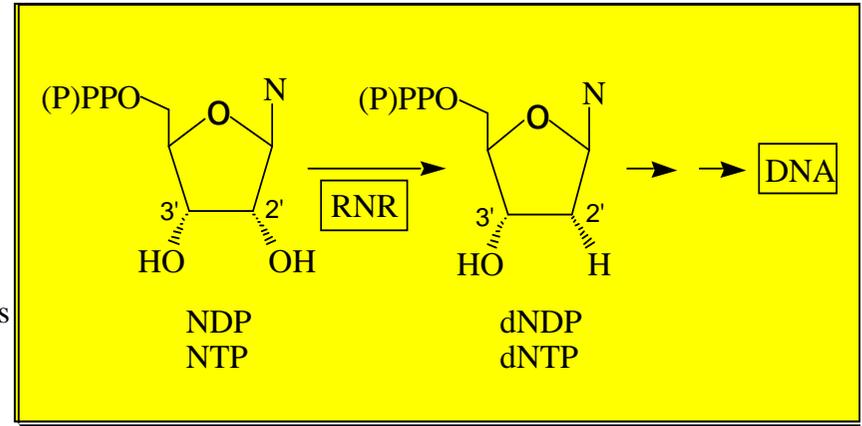
Hydrogénases

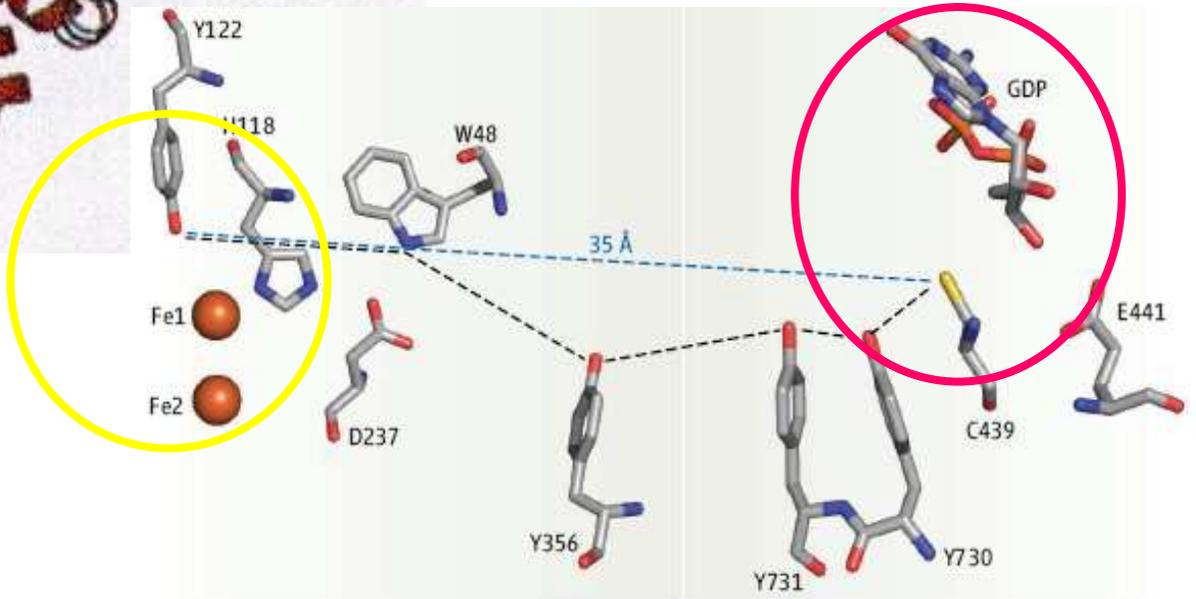
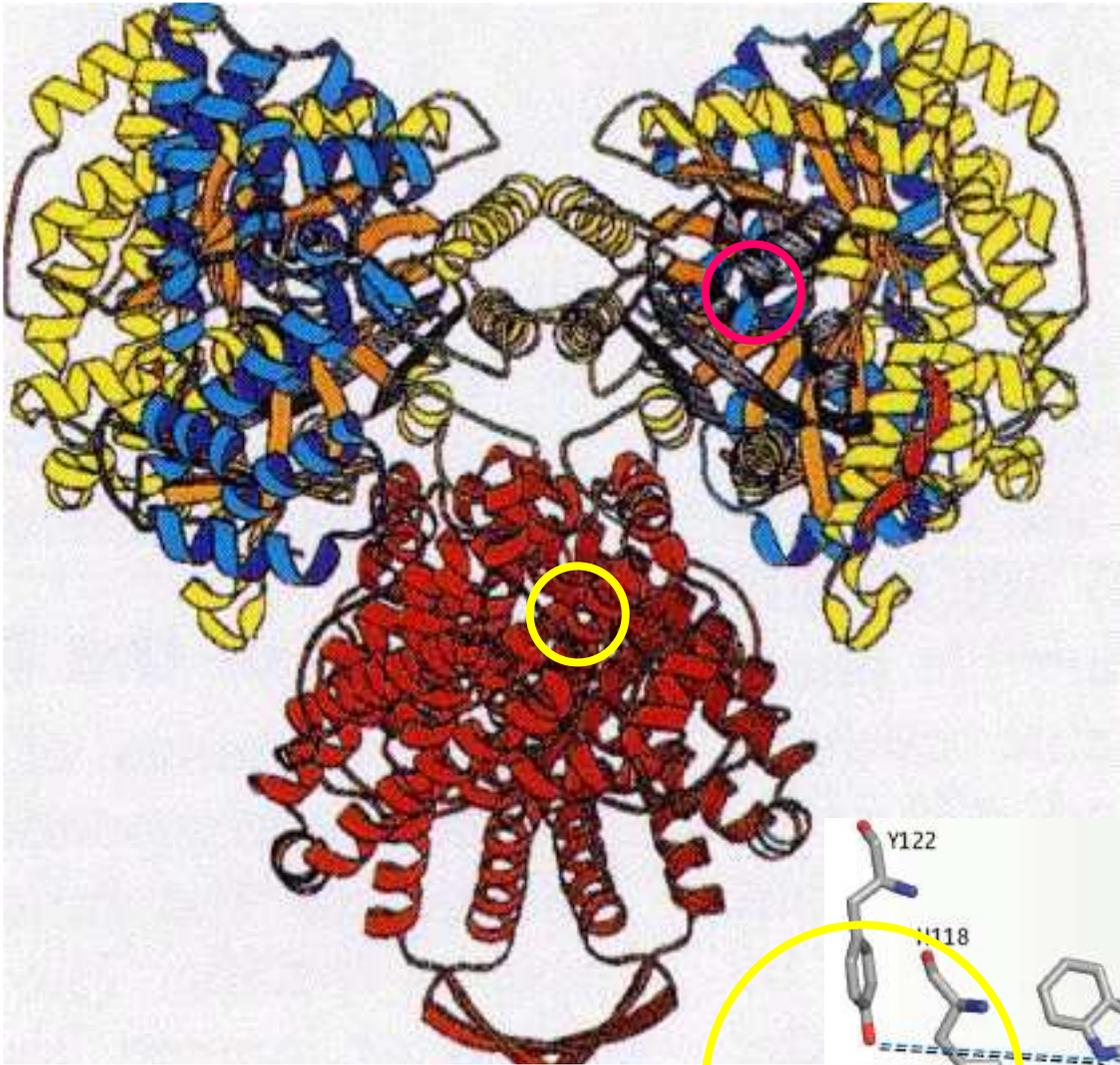


Ribonucléotide réductase

The redox centers of ribonucleotide reductase from *Escherichia Coli*.
 M. Fontecave, P. Nordlund, H. Eklund, P. Reichard
Advances in Enzymology, 1992, 65, 147-183.

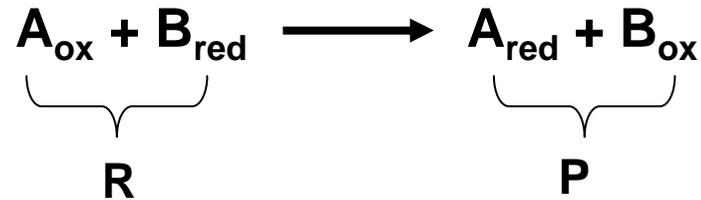
Ribonucléotide réductase et régulation de la biosynthèse des désoxyribonucléotides
 M. Fontecave, V. Nivière, E. Mulliez
Annales de l'Institut Pasteur, 1992, 3, 159-165.





Transfert des électrons: principes

Théorie de Marcus



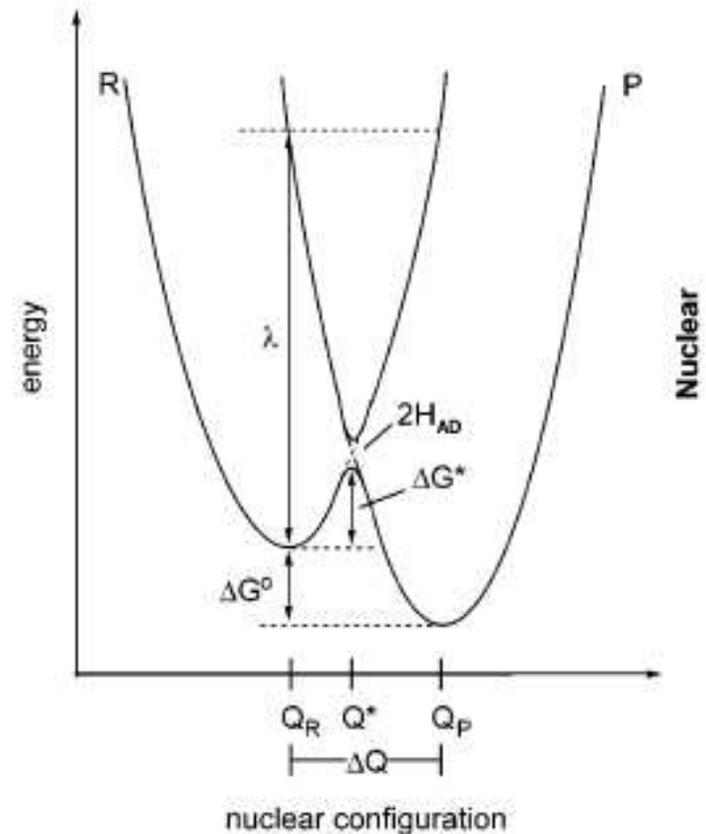
$$k_{\text{ET}} = A \exp\left[\frac{-\Delta G^*}{RT}\right]$$

$$\Delta G^* = \frac{(\lambda + \Delta G^\circ)^2}{4\lambda}$$

$$k_{\text{ET}} = k_{\text{ET}}(0) \exp\left[\frac{-(\lambda + \Delta G^\circ)^2}{4\lambda RT}\right]$$

ΔG° = enthalpie libre de la réaction
 λ = énergie de réorganisation

Pas de dépendance vs distance !!



Transfert des électrons: méthodes

- Complexes protéine-protéine
- Marquage rédox de métalloprotéines
Ex: cytochromes, protéines à Cu,.
- Systèmes naturels (modifiés)
Photosystèmes (non oxygéniques) bactériens

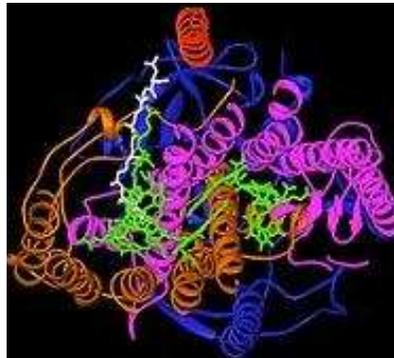
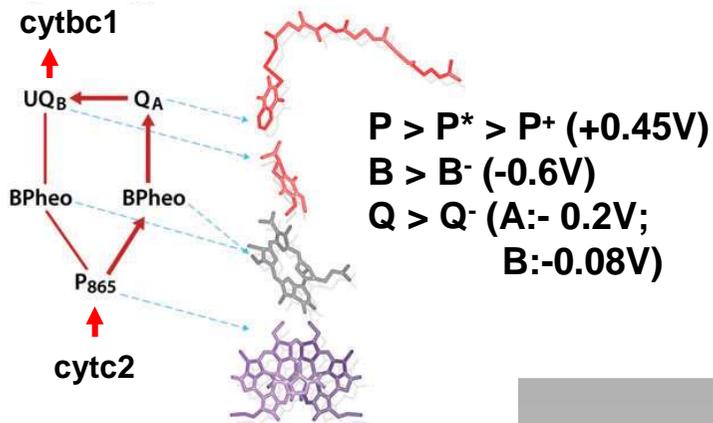
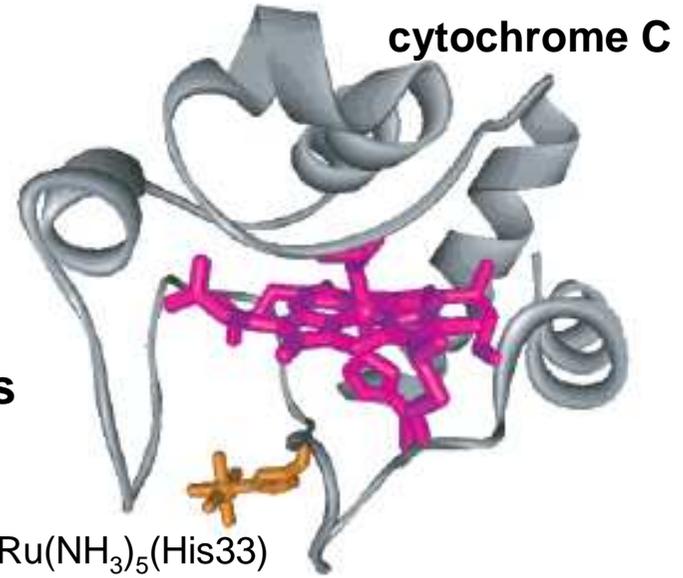
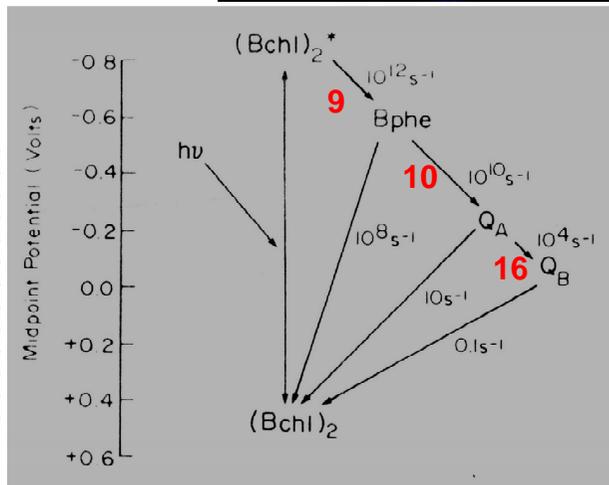
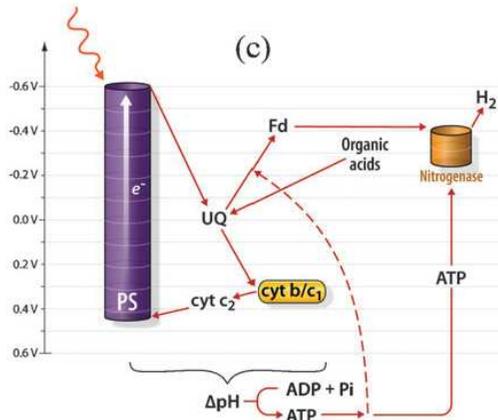


Table 1. Reorganization Energy of Different Classes of Enzymes and Proteins

protein/enzyme	λ /mV	ref
cytochrome <i>c</i>	700	326
$\text{Ru}(\text{LL})_2(\text{im})(\text{His}33)$ -cytochrome <i>c</i> (LL = polypyridine ligands)	740	67, 327
blue copper proteins		
$\text{Ru}(\text{bpy})_2(\text{im})(\text{His}83)$ -azurin	700	328, 329
$\text{Ru}(\text{trpy})(\text{LL})(\text{His}59)$ -plastocyanin	640-700	330
HiPIPs		
$\text{Ru}(\text{LL})_2(\text{im})(\text{HisX})$ -HiPIP	600-800	331
bacterial reaction center	700	99
cytochrome <i>d</i> cytochrome <i>b</i> ₅	700	332



➡ $\lambda = 0.7 \text{ eV} (16 \text{ kcal.mol}^{-1})$

➡ Transferts sur de longues distances

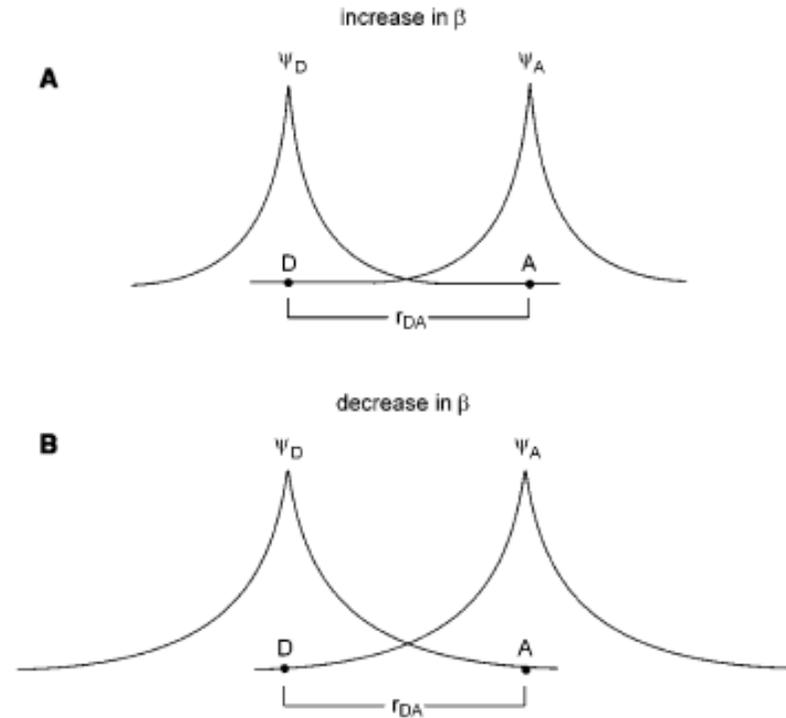
Transfert des électrons: effet de la distance

$$k_{\text{ET}} = \sqrt{\frac{4\pi^3}{h^2 \lambda k_B T}} H_{\text{AD}}^2 \exp\left[\frac{-(\lambda + \Delta G^\circ)^2}{4\lambda RT}\right]$$

$$H_{\text{AD}} = H_{\text{AD}}^\circ e^{-\beta(r_{\text{DA}} - r_D)}$$

H_{AD} = couplage des états électroniques
du donneur et de l'accepteur

β = contribution du milieu dans la
propagation des fonctions d'onde

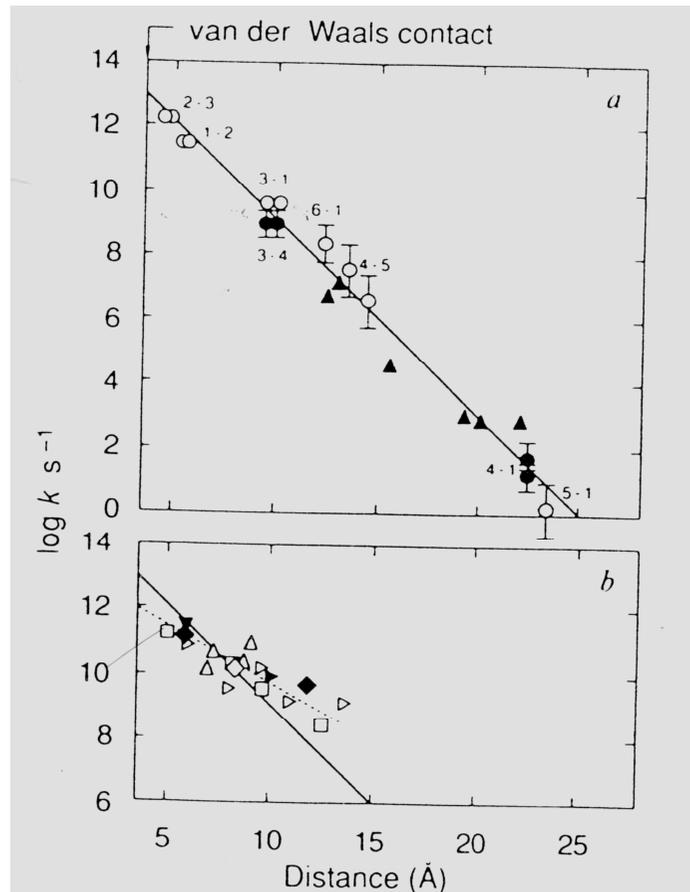


Vide: $\beta = 3,4 \text{ \AA}^{-1}$ 10 \AA parcouru en 100 ms

Transfert des électrons: modèles

➤ **Modèle « tunneling pathways »**: pas de valeur uniforme de β ; il existe des chemins préférentiels (liaisons covalentes, liaisons H, contacts à travers l'espace,...)

➤ **Modèle « uniform barrier »**: il y a une valeur uniforme de β ; La protéine est un milieu conducteur particulier et homogène



$$\beta = 1.2 \pm 0.2 \text{ \AA}^{-1}$$

Vide: 3.4

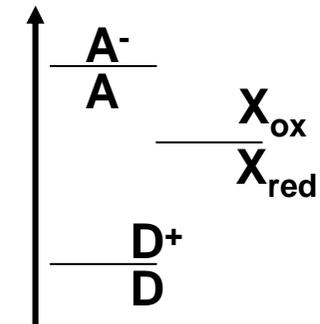
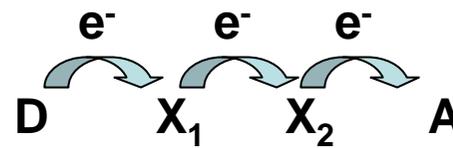
D-A covalent: 0.7

Verre organique (THF): 1.2

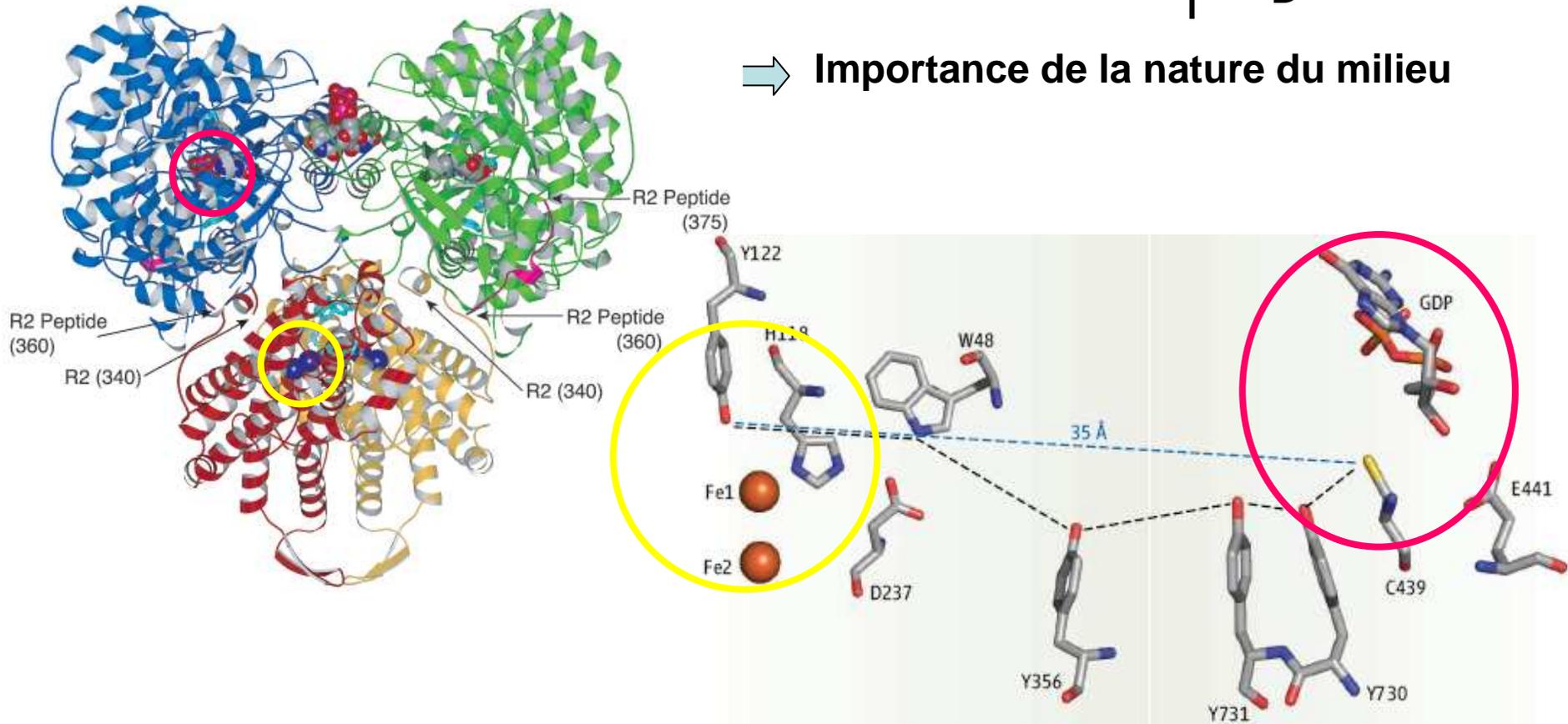
a: protéines (photosystème, Ru-cytc, Ru-Mb)
b: systèmes liés de façon covalente

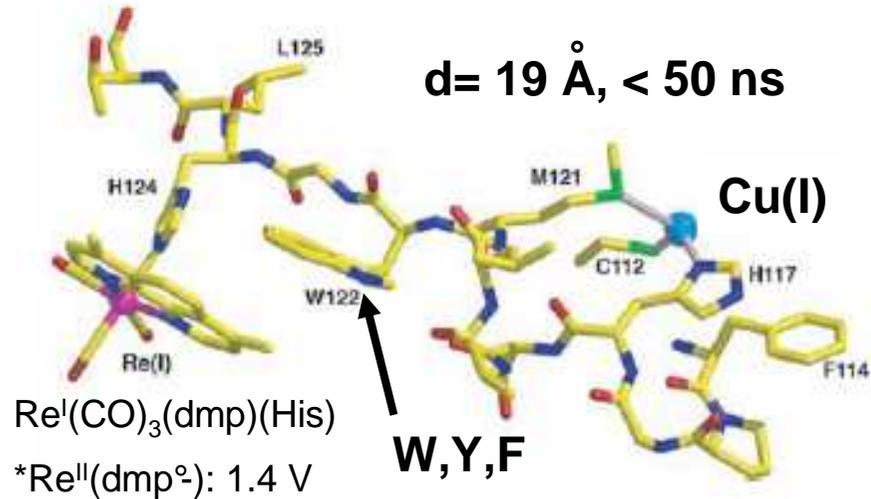
Au-delà de 2 nm ? : cofacteurs intermédiaires « multistep tunneling »

Cofacteurs rédox: ions métalliques, quinones,....,
chaines latérales d'AA

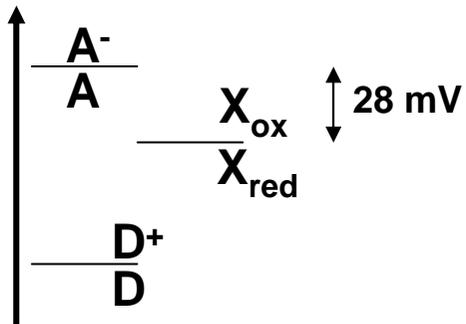


➔ Importance de la nature du milieu

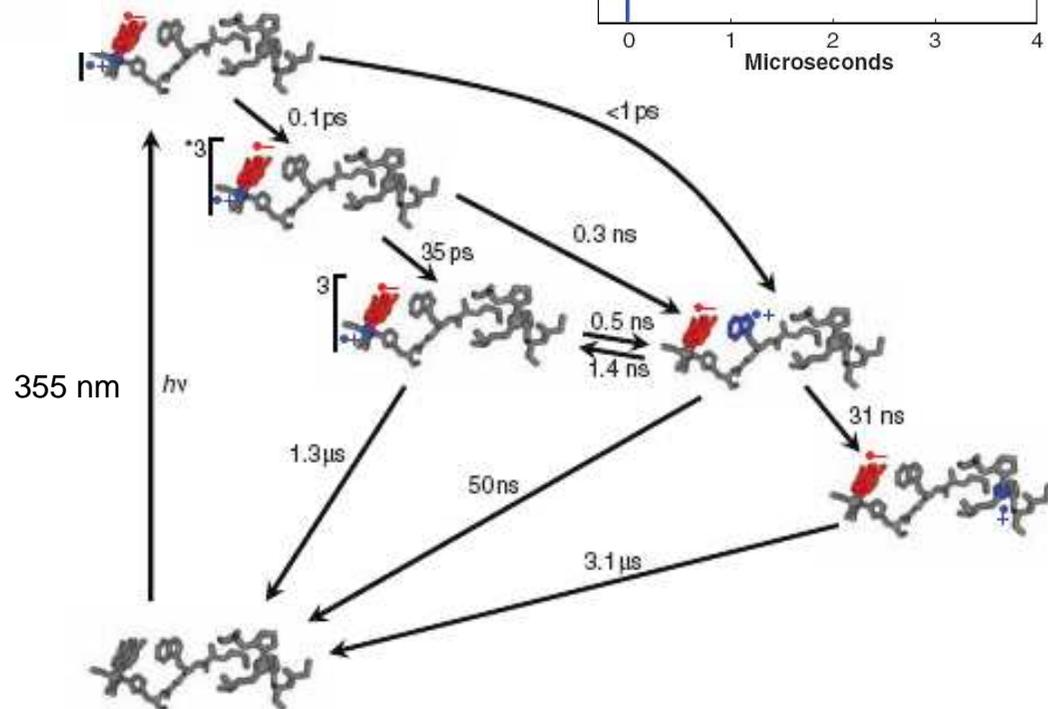
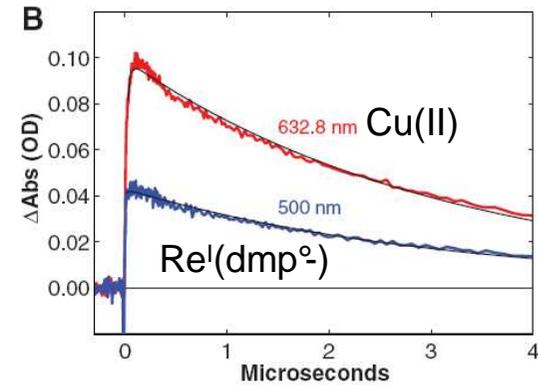




Formation de Cu(II)
uniquement avec W



Modèle: Re-azurin(Cu)



Couplage transfert d'électron/transfert de proton Régulation/switch

