



COLLÈGE
DE FRANCE
—1530—

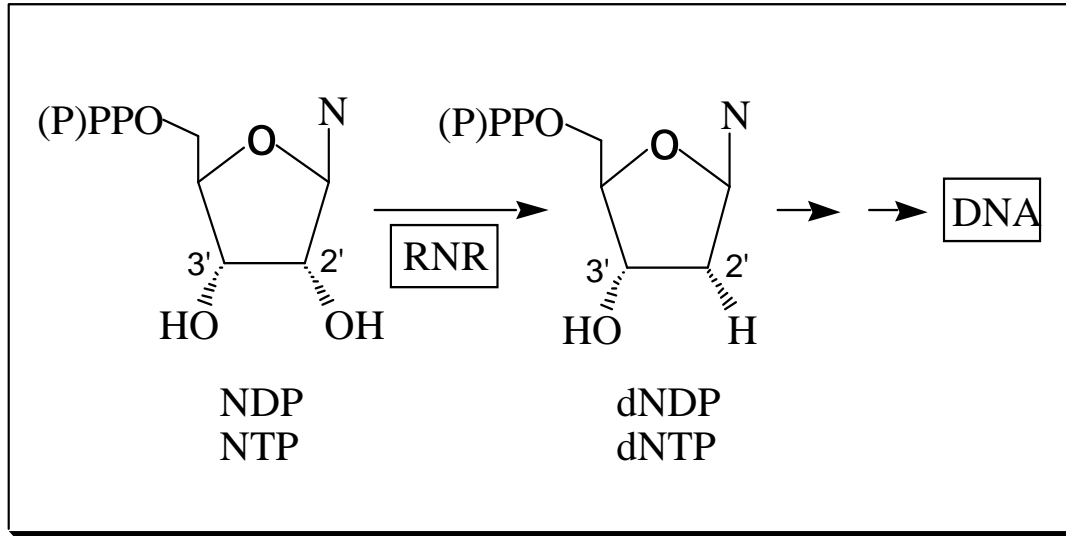
Biosynthèse des précurseurs de l'ADN chez les organismes aérobies: du fer, de l'oxygène et des radicaux libres

Marc Fontecave

*Laboratoire de Chimie et Biologie des Métaux, Université Joseph Fourier, CNRS, CEA/DSV/iRTSV
CEA-Grenoble 17 rue des martyrs 38054 Grenoble cedex 9, France
mfontecave@cea.fr; Phone: (0033)438789103 ; Fax: (0033)438789124*

Collège de France, 11 Place Marcelin Berthelot, 75231 Paris Cedex 05

La découverte de la ribonucléotide réductase Peter Reichard



Peter Reichard
(1925-)

1. Evidence d'une activité ribonucléotide réductase

Reichard P, Rutberg L. **1960**. Biochim. Biophys. Acta 37:5.54-55

2. Régulation allostérique (ATP, dTTP,...)

Brown NC, Reichard P. **1969**. 3. Mol. Biol. 46:39-55

3. Purification de la RNR de *E. coli*

Brown NC, Canellakis ZN, Lundin B, Reichard P, Thelander L. **1969**. Eur. J. Biochem

4. La RNR est une enzyme radicalaire. Radical tyrosinyle

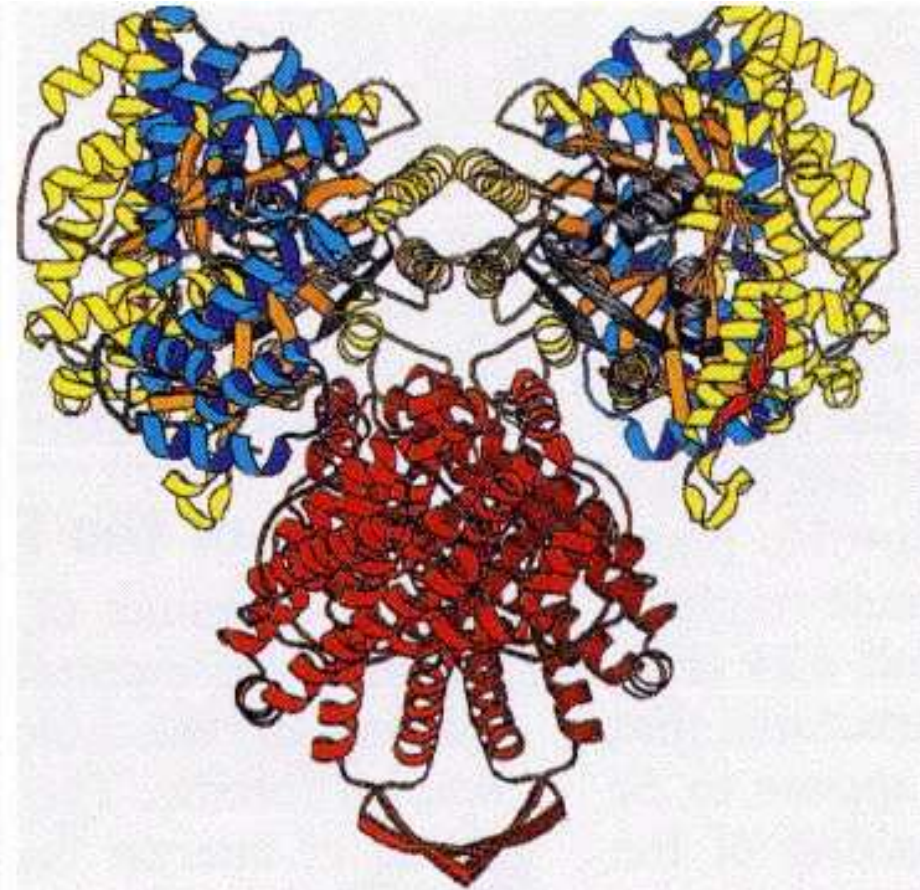
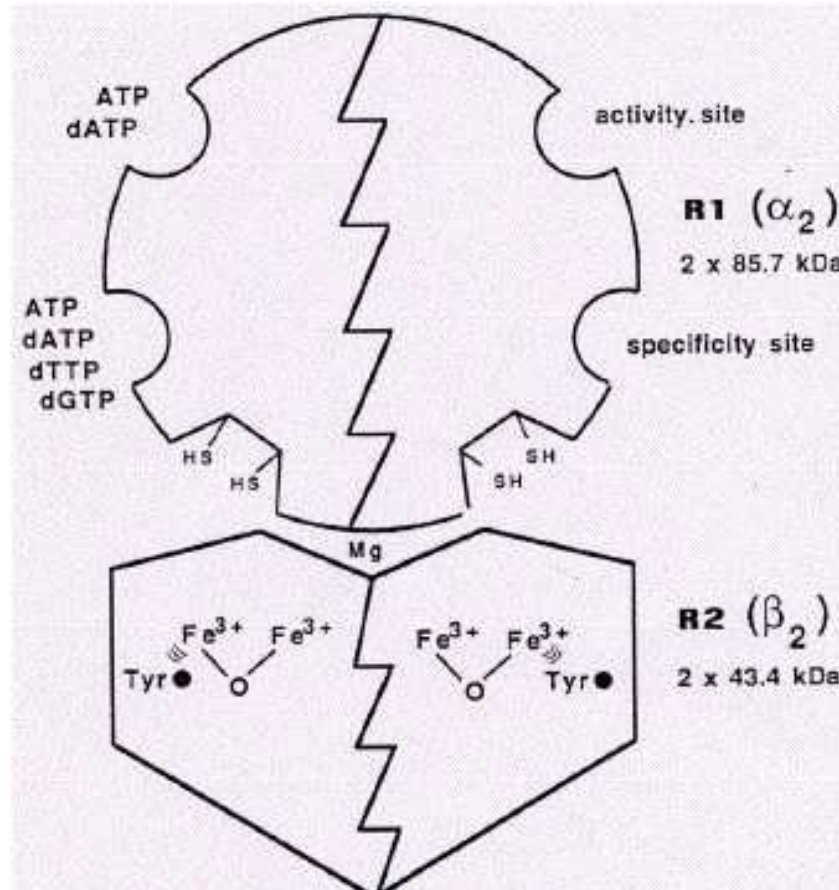
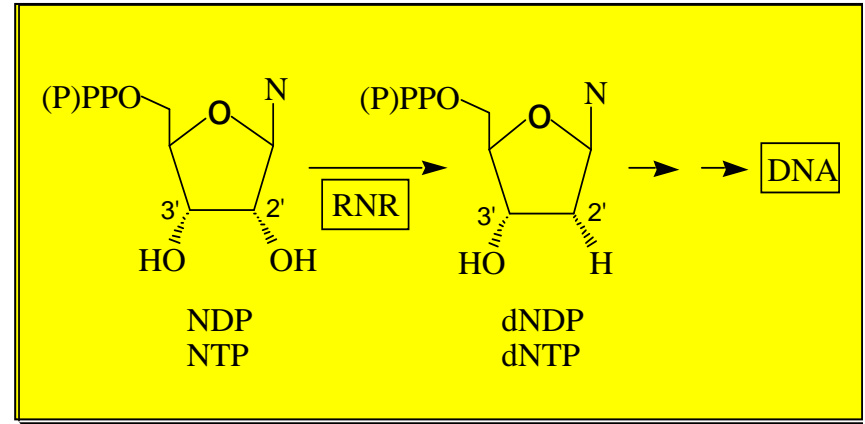
Ehrenberg A, Reichard P. **1972**. J. Biol. Chem. 247:..M.85-88

Larsson A, Sjöberg B-M. **1986**. EMBO J. 5:2031-36

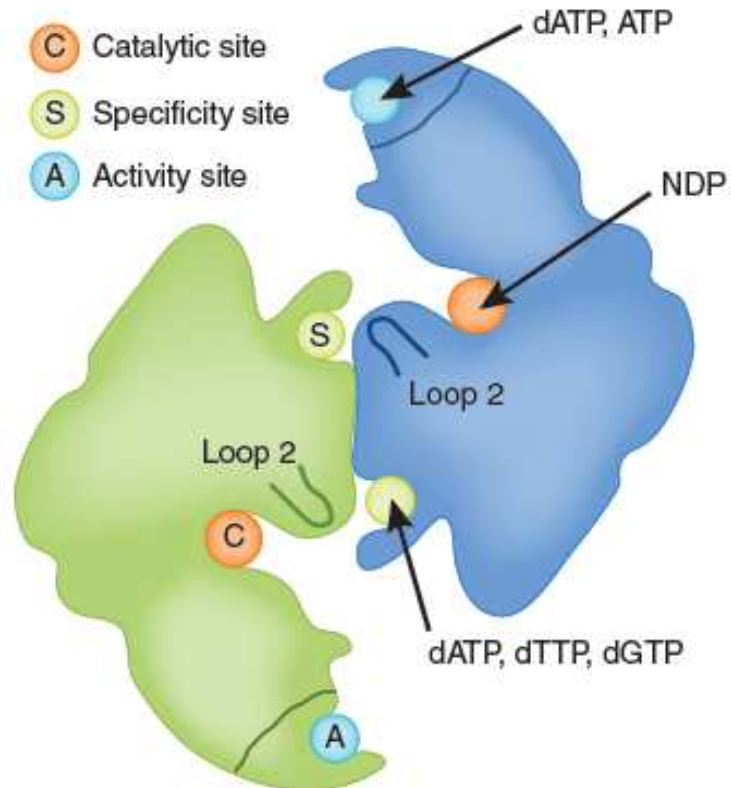
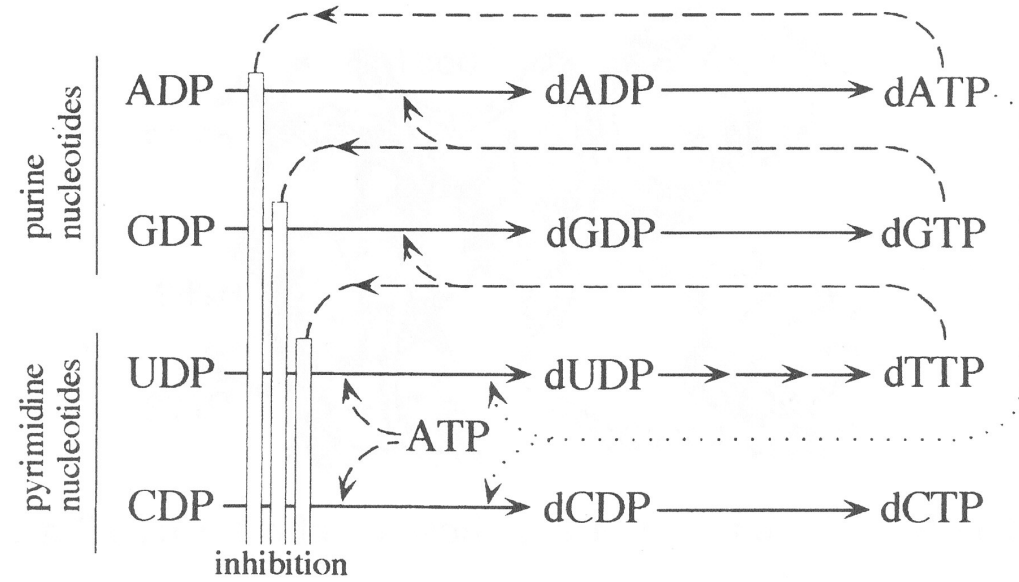
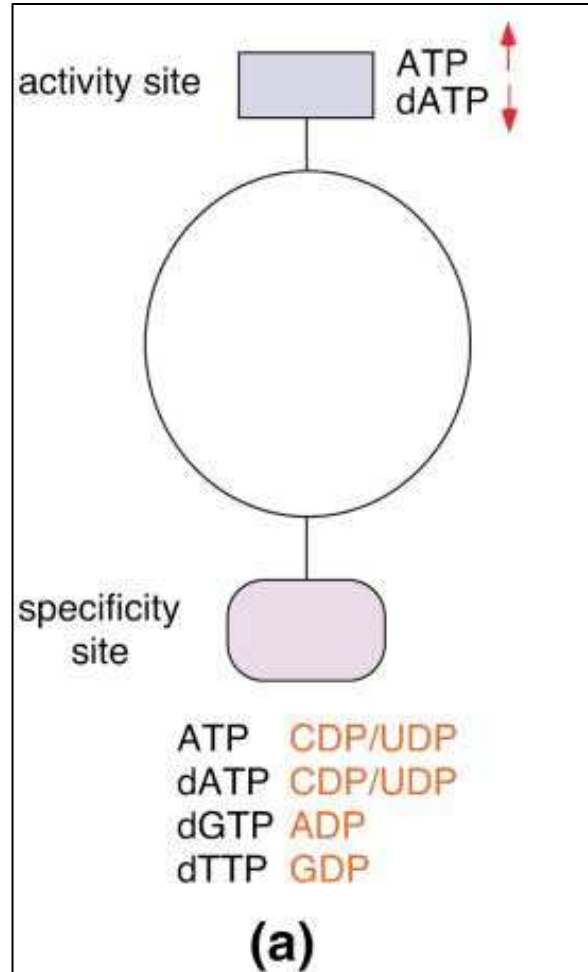
Ribonucléotide réductase

Structure et mécanismes

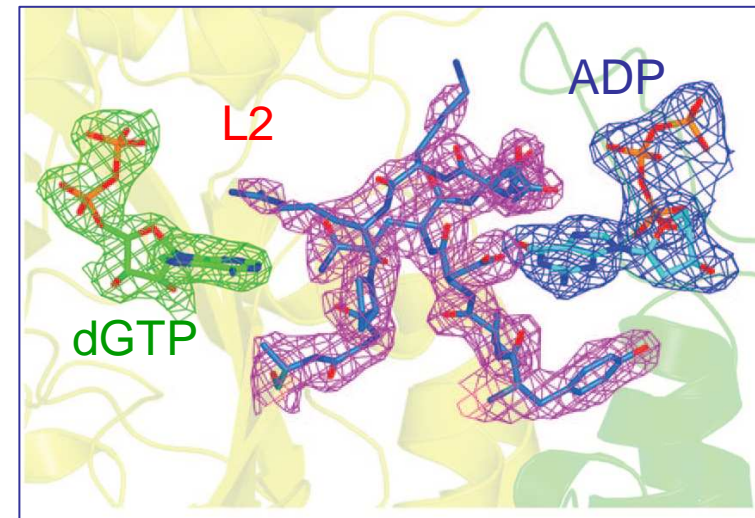
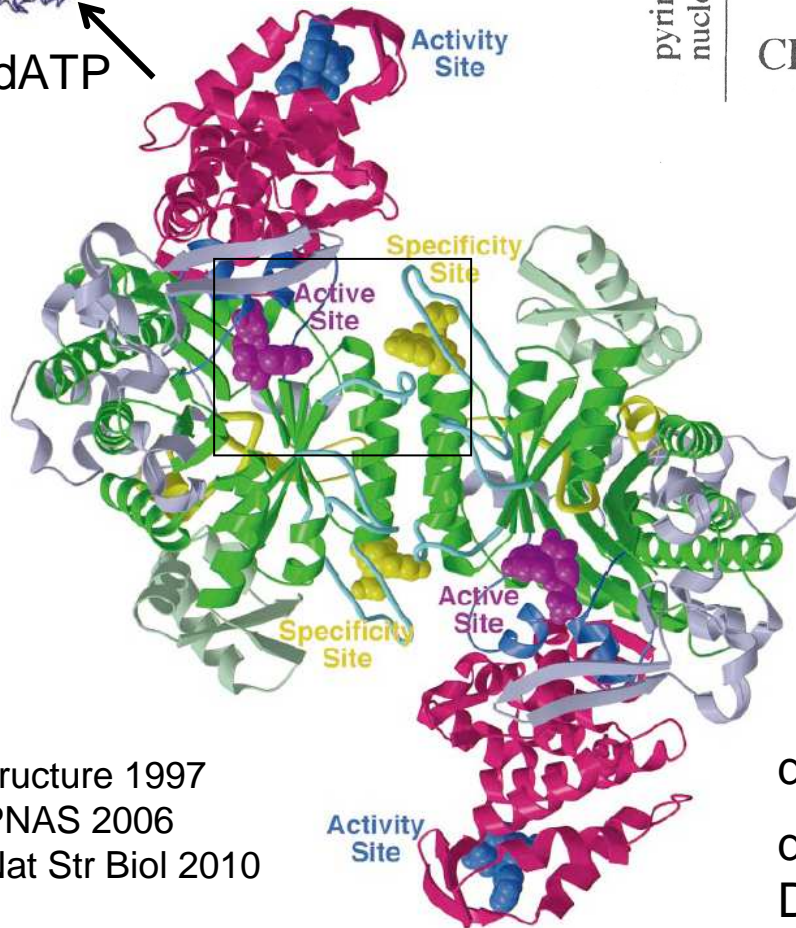
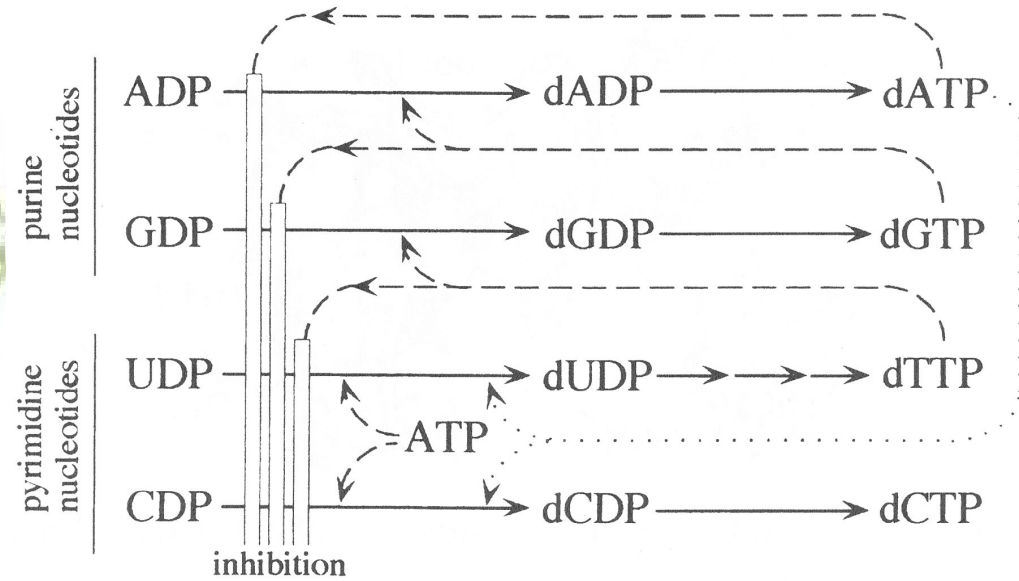
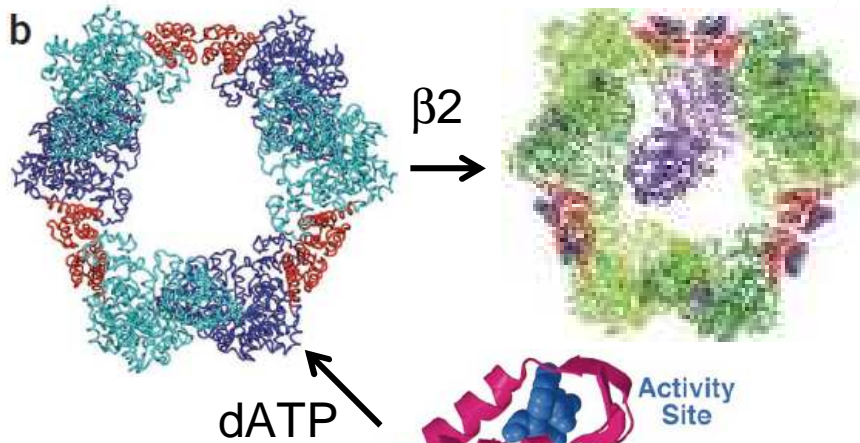
P. Reichard Annu. Rev. Biochem (2006)
 H. Eklund Nature (1990); Nature (1994)
 J. Stubbe Chem Rev (1998); Chem Rev (2003)



Régulation allostérique:
1 enzyme pour 4 substrats!!



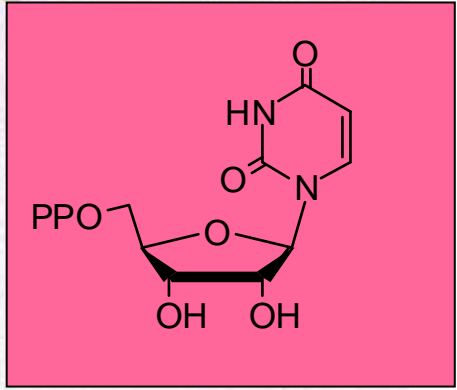
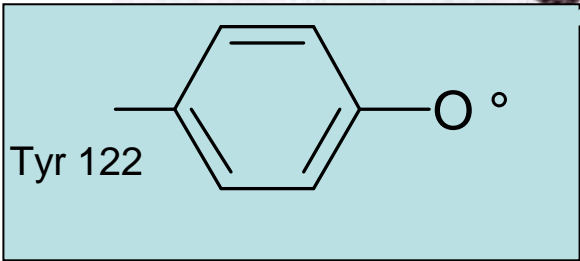
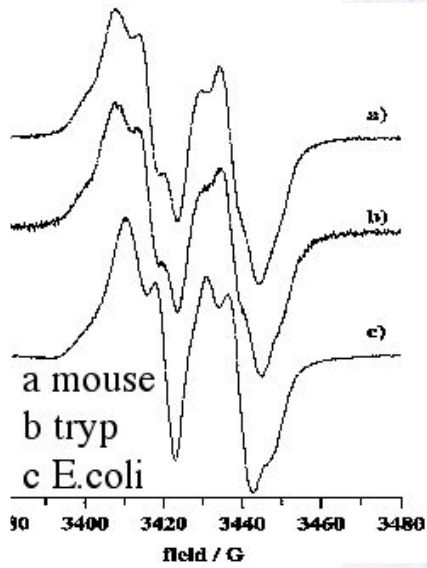
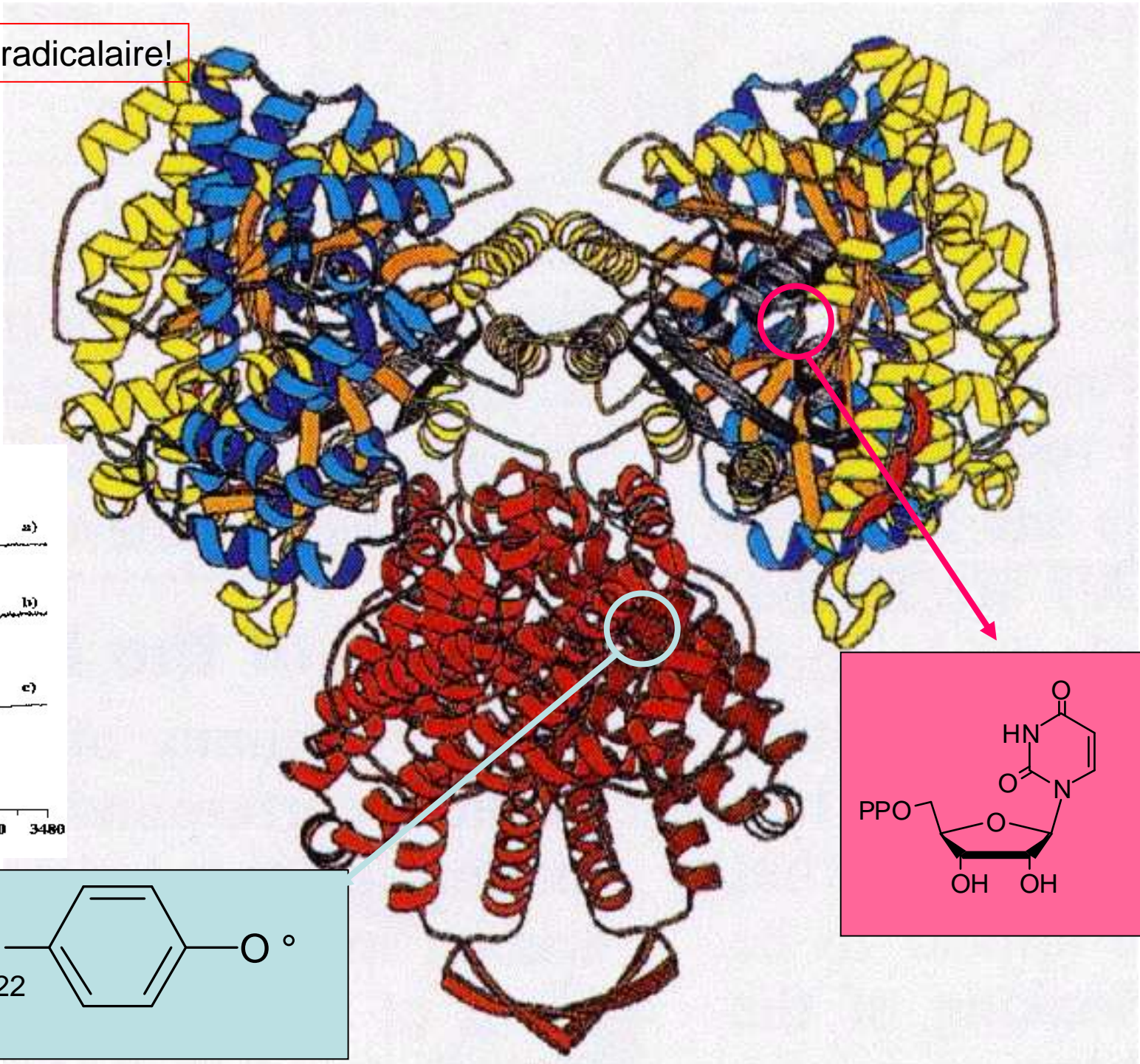
Régulation allostérique:
1 enzyme pour 4 substrats!!



Eklund H, Structure 1997
Dealwis C, PNAS 2006
Dealwis C, Nat Str Biol 2010

dGTP → shift de L2 → espace pour ADP
dATP → hexamérisation of R1 → inhibition?
Différence ATP/dATP?

Une protéine radicalaire!



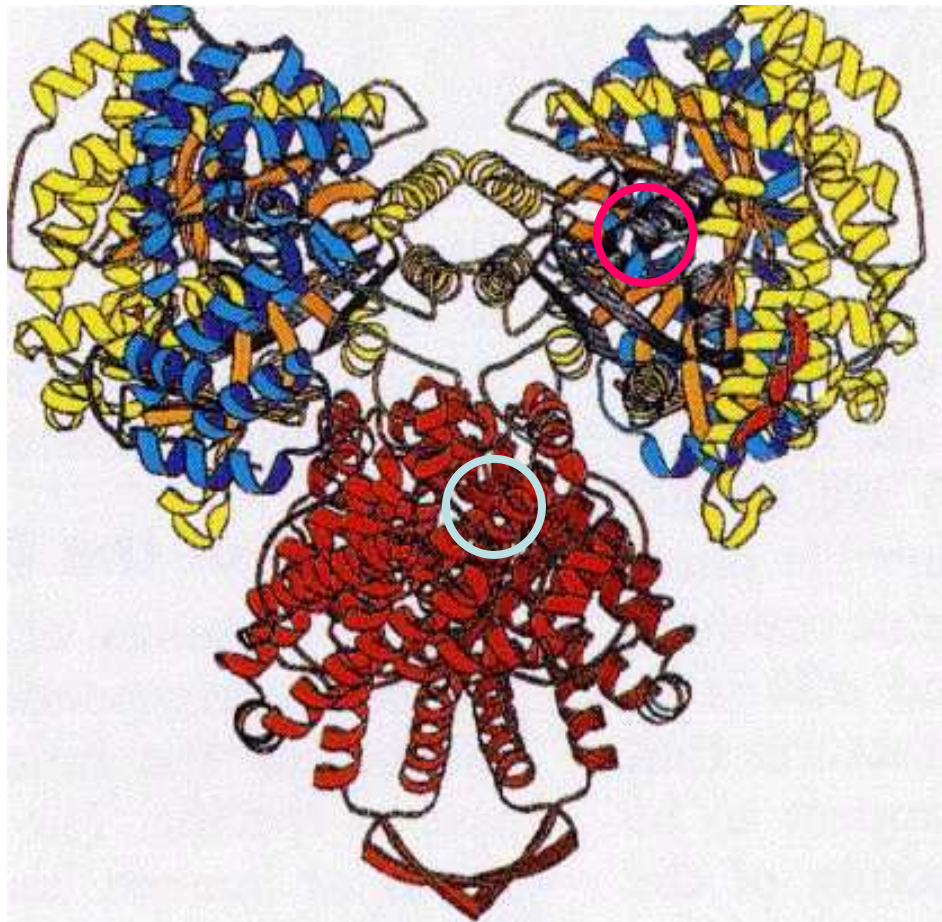
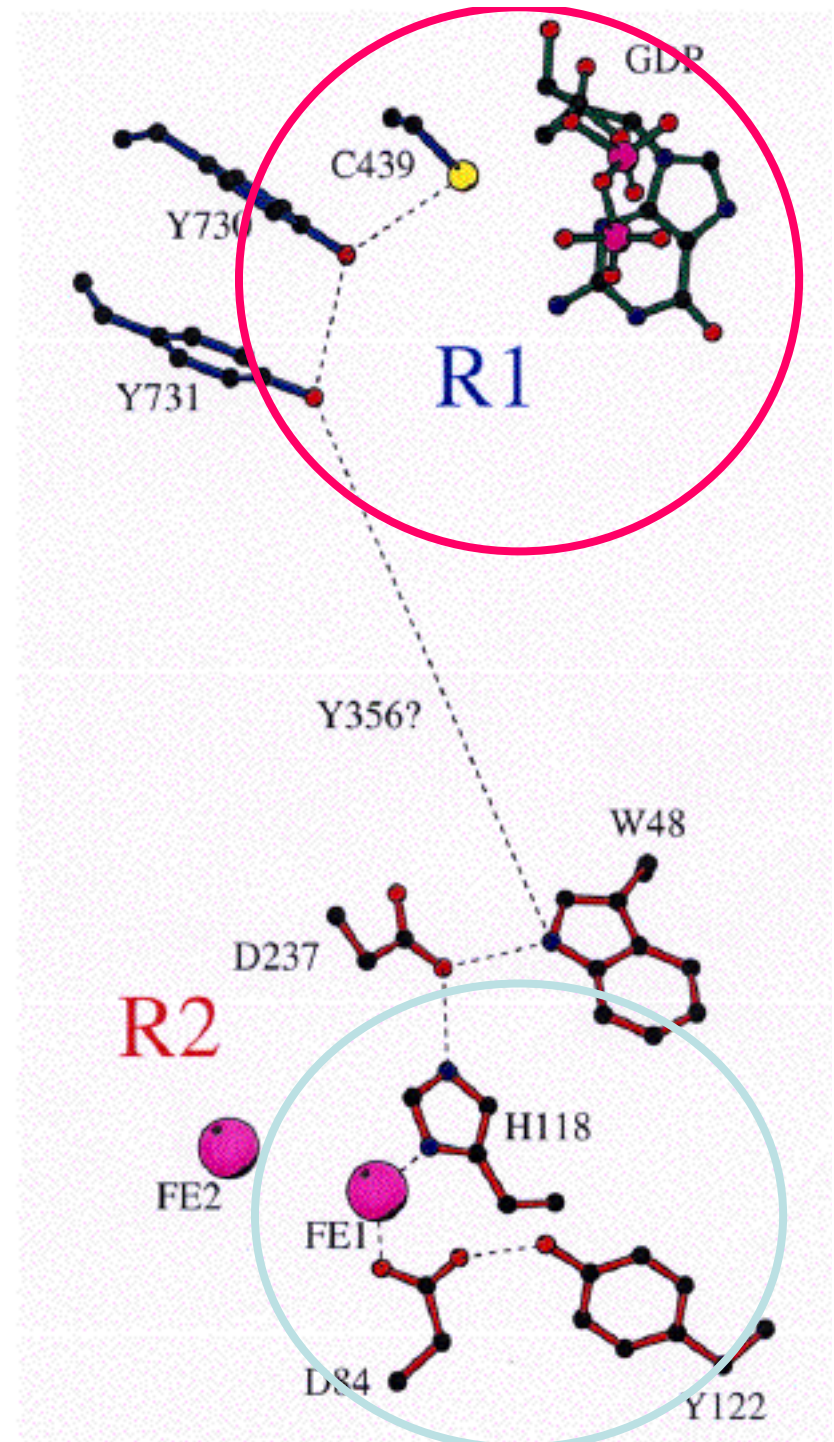
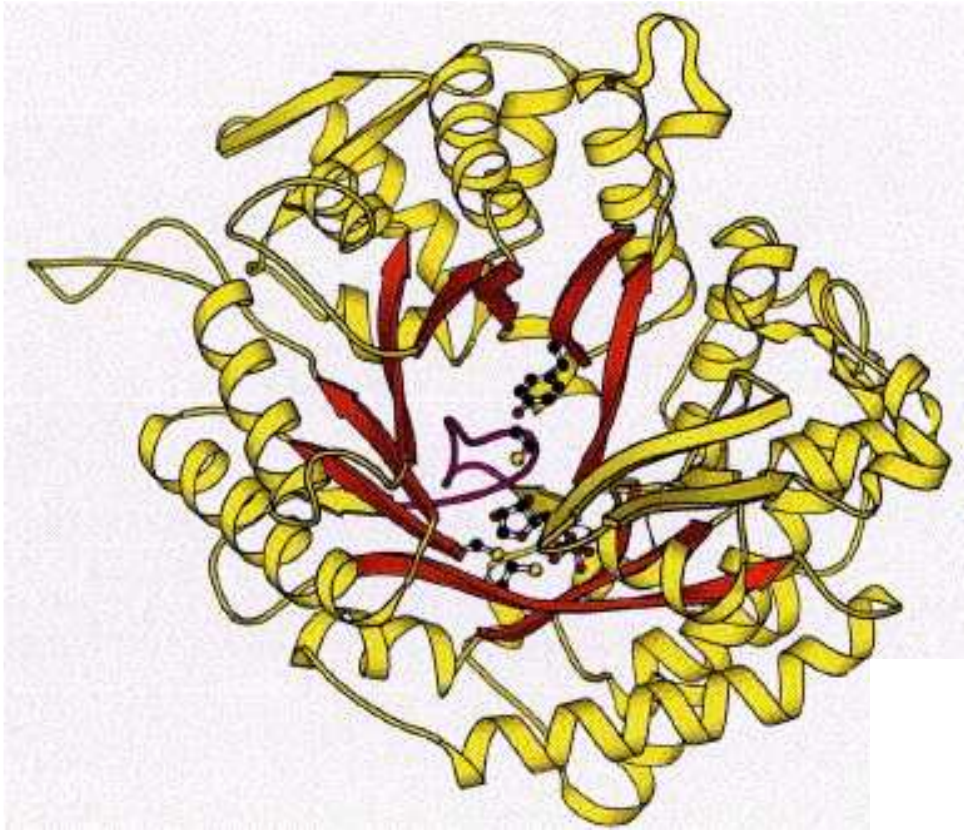


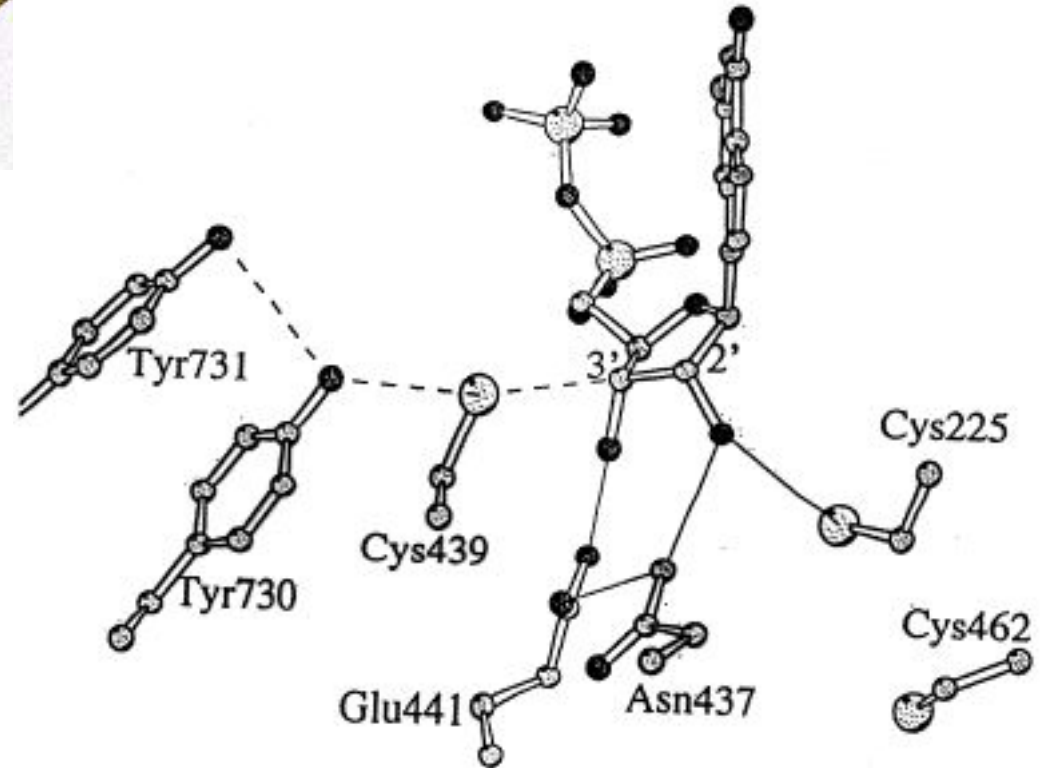
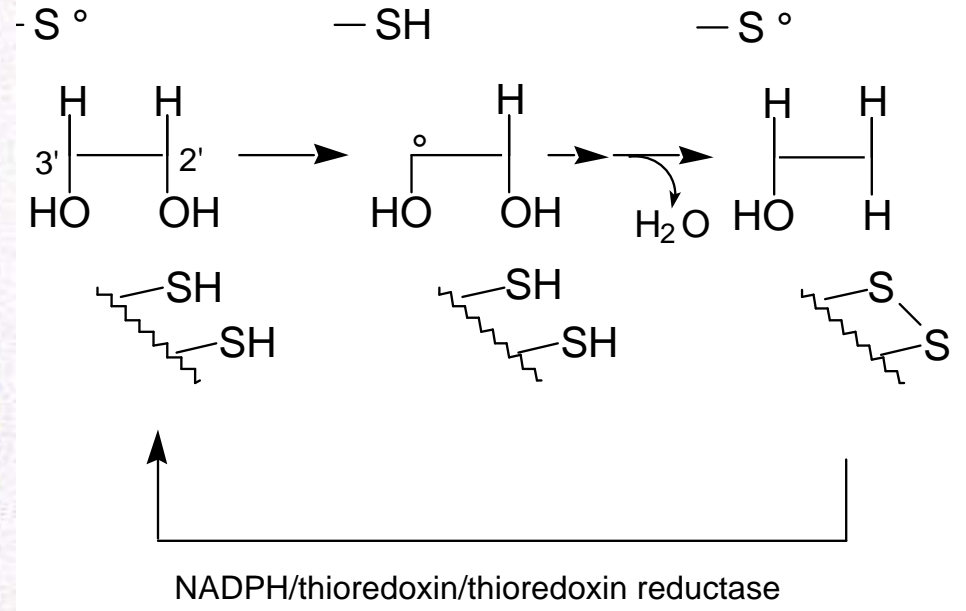
Table 3. Activity of Mutants on the Putative PCET Pathway in *E. coli* and Mouse R2

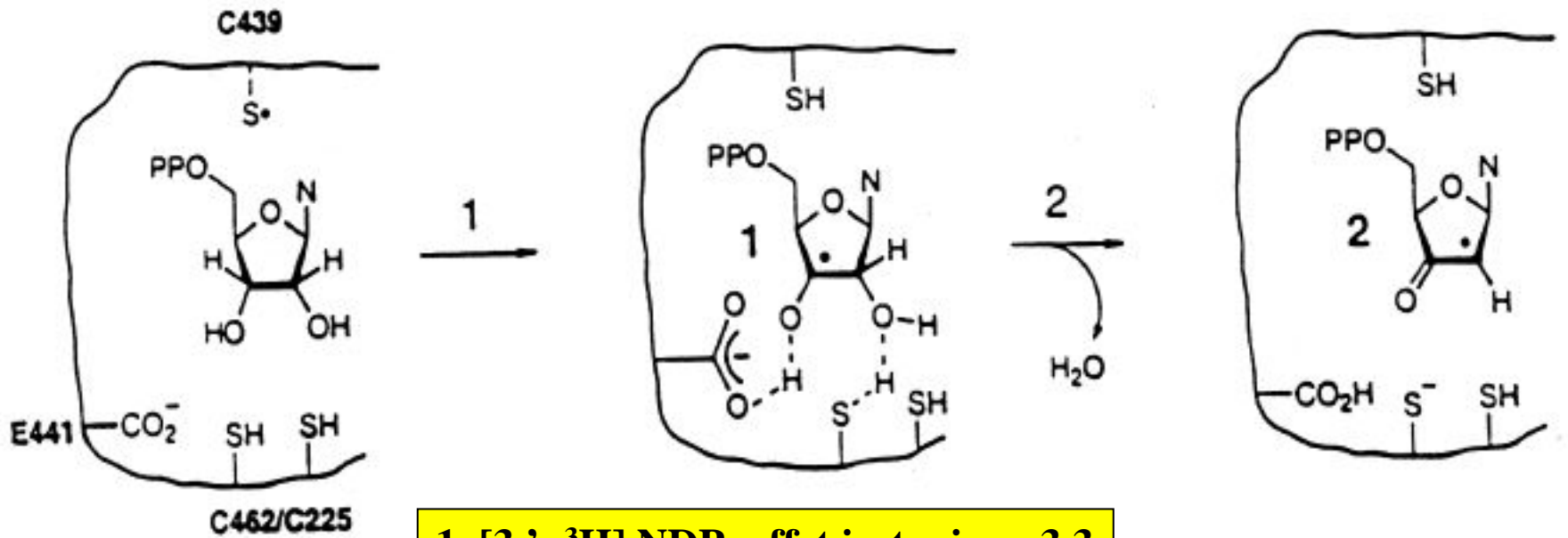
protein	iron/ R2	Y's/R2	SA (nmol/ min mg)	N [*] formation ^c (s ⁻¹)	Y [*] loss ^c (s ⁻¹)
<i>E. coli</i> ^a					
wt-R2	2.9	0.8	5000	0.68	0.64
D237E	2.7	1.0	340 (7%)	0.084	0.044
D237N	4.4	0.6 (unstable)	13 (0.3%)		
Mouse R2					
wt-R1			1650		
Y730F			26 (1.6%)		no loss
Y731F			26 (1.6%)		no loss



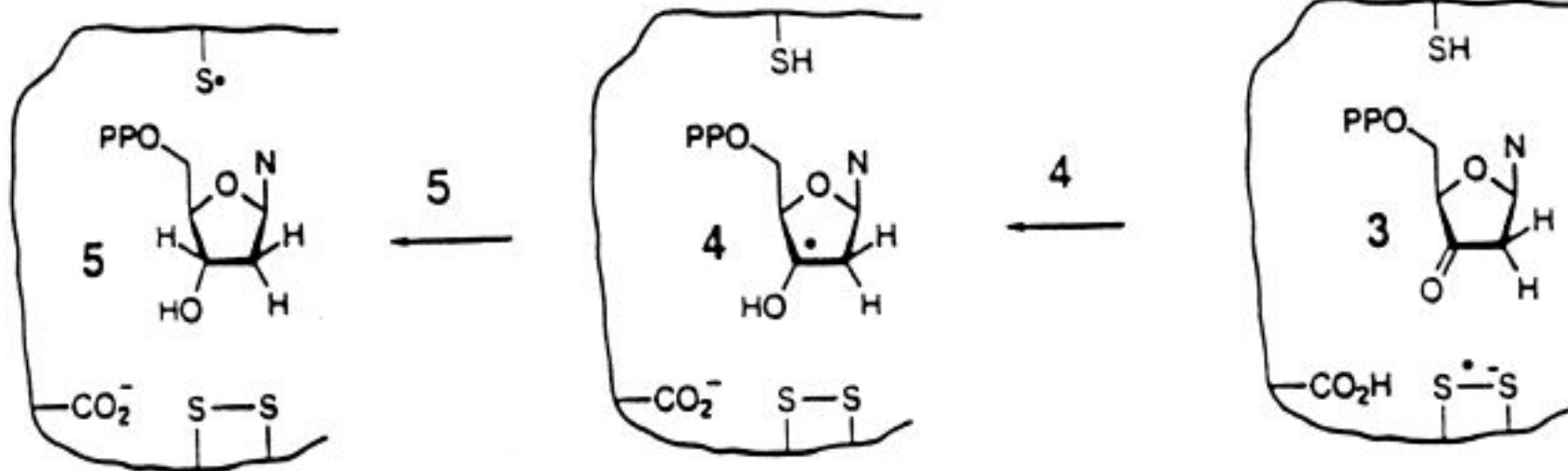


Protéine R1



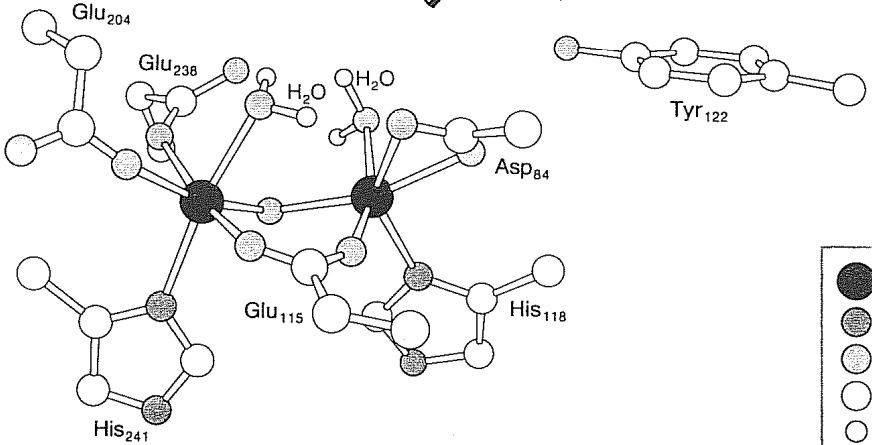
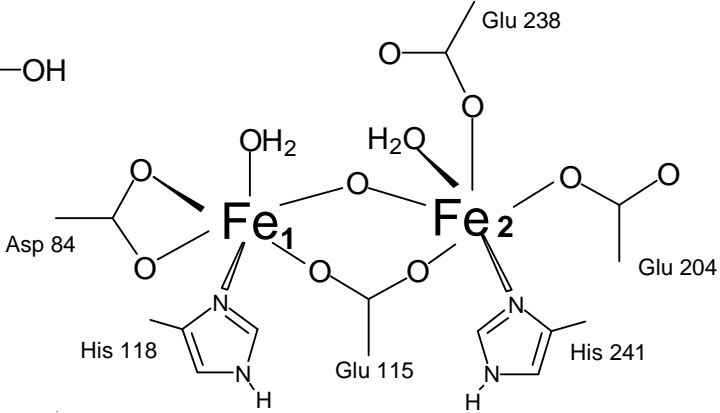
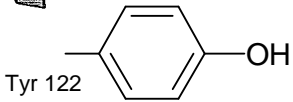


1. [3'-³H] NDP: effet isotopique 3.3 et radioactivité dans H₂O
 2. [3'-²H] NDP: marquage en 3' dans le produit (RMN)

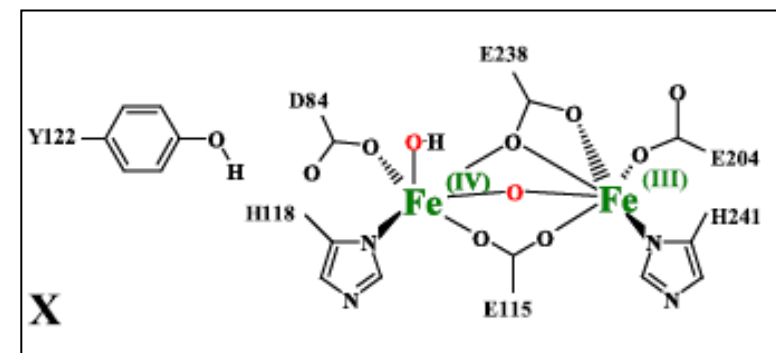
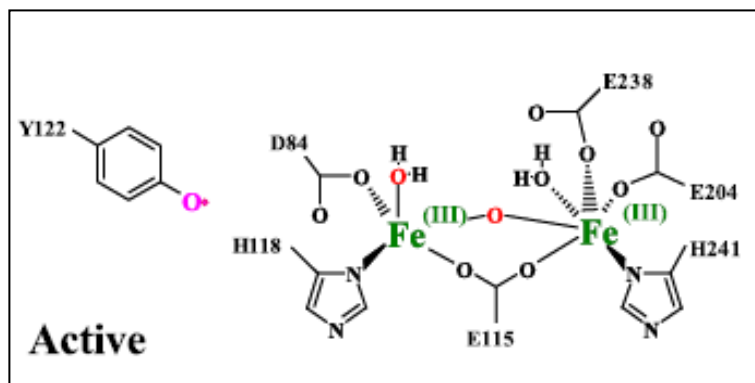
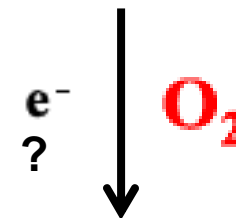
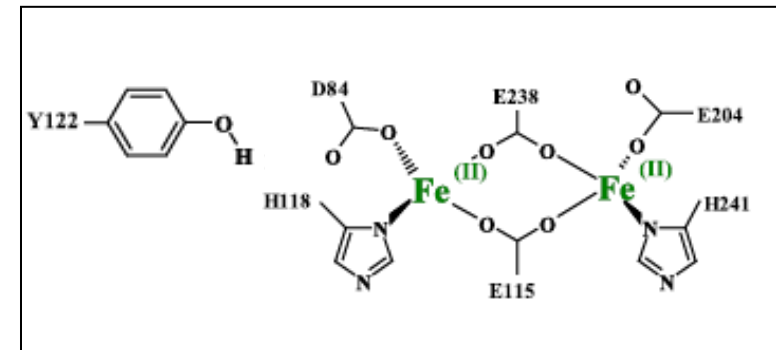
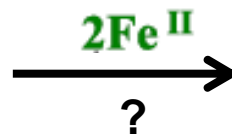
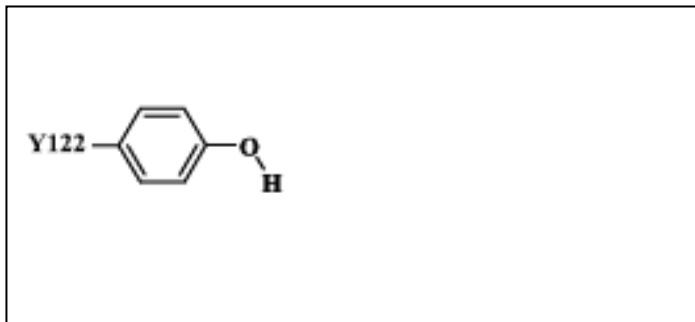
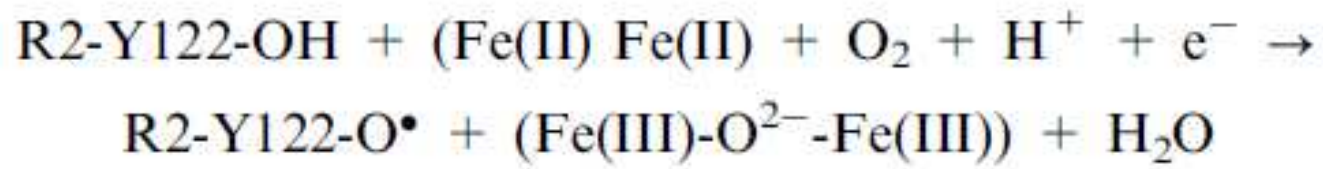


Protéine R2

Formation du radical ?



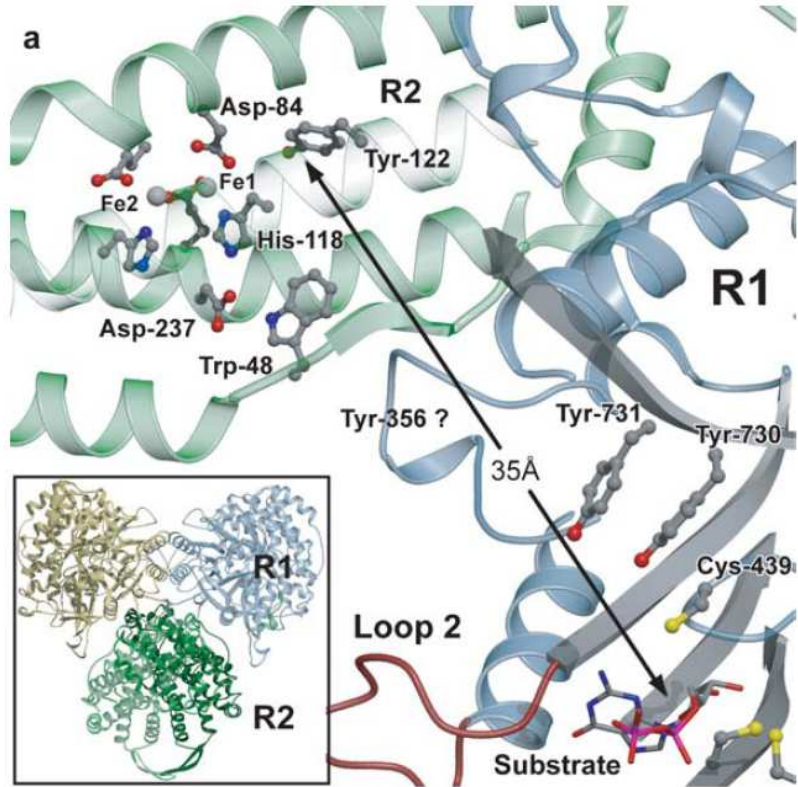
- Iron
- Nitrogen
- Oxygen
- Carbon
- Hydrogen



Etude du transfert de radicaux dans la RNR



J Stubbe (MIT)



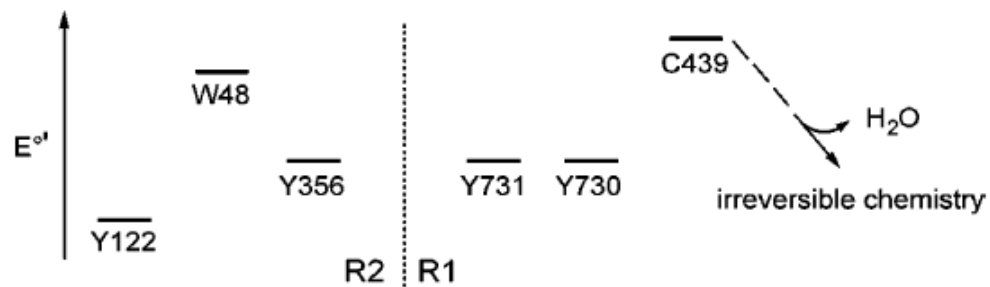
A

$35 \text{ \AA} \leftrightarrow k_{ET} = 10^{-6} \text{ s}^{-1}$
(théorie ET)

En réalité $k_{ET} = 2-10 \text{ s}^{-1}$
!!!

B

Contrainte thermodynamique
Transfert des électrons
couplé
à des transferts de protons



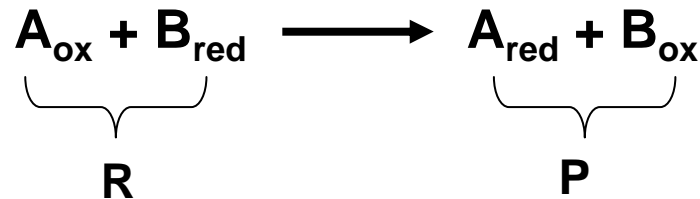
La déprotonation facilite l'oxydation en radical ←
mais rend le radical moins oxydant

Table 2. Reduction Potential for Blocked Amino Acids

reaction	$E^{\circ'} \text{ (NHE)/V}$
$RS^{\bullet} \rightarrow RSH$	1.33 ^a
$RS^{\bullet} \rightarrow RS^{-}$	0.77 ^b
$WH^{+\bullet} \rightarrow WH$	1.15 ^c
$W^{\bullet} \rightarrow WH$	0.9–1.05 ^d
$Y^{\bullet} \rightarrow YOH$	0.83–0.94 ^e
$Y^{\bullet} \rightarrow YO^{-}$	0.65 ^f

Transfert des électrons: principes

Théorie de Marcus



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

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

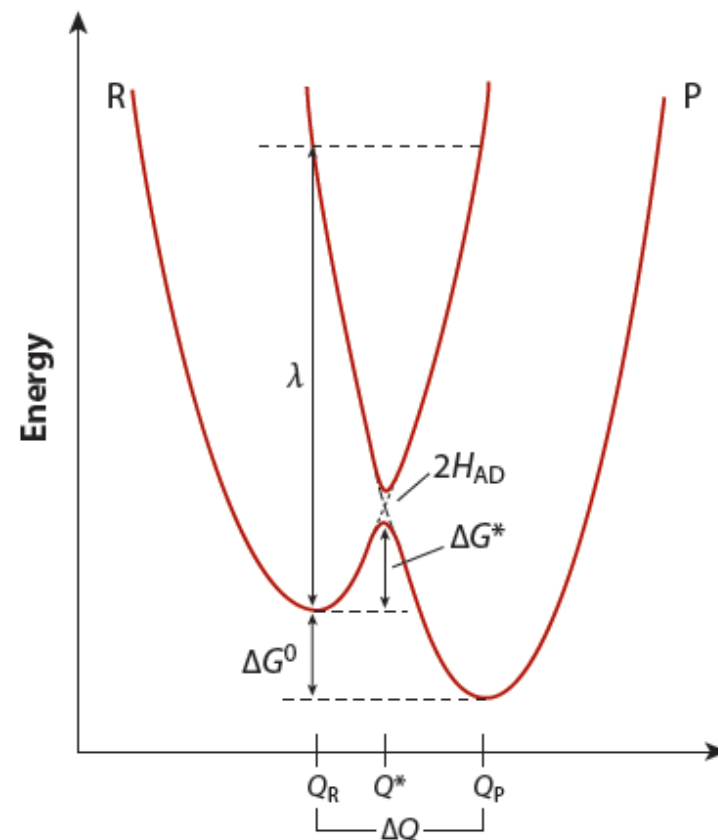
$$k_{\text{ET}} = k_{\text{ET}}(0) \exp\left[\frac{-(\lambda + \Delta G^{\circ})^2}{4\lambda RT}\right]$$

ΔG^* = énergie d'activation

ΔG° = enthalpie libre de la réaction

λ = énergie de réorganisation

La dépendance vs distance n'est pas traitée dans la théorie de Marcus!!



Nocera D, Annu. Rev. Biochem. 2009.

Stubbe J, Nocera D, Chem rev 2009.

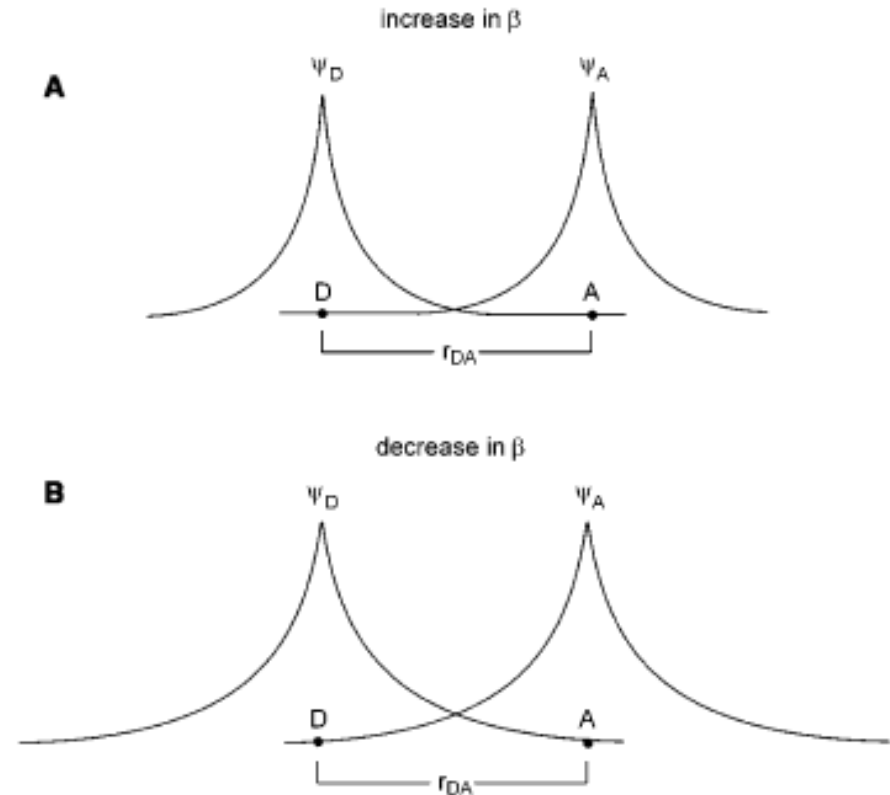
Transfert des électrons: effet de la distance

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

$$H_{\text{AD}} = H_{\text{AD}}^\circ e^{-\beta(r_{\text{DA}} - r_0)}$$

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

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



L'efficacité d'un électron de passer de D à A
dépend du recouvrement des fonctions d'onde

Transfert des électrons: méthodes

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

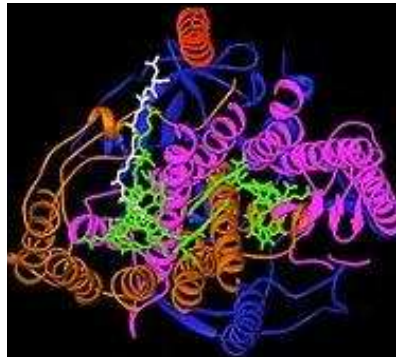
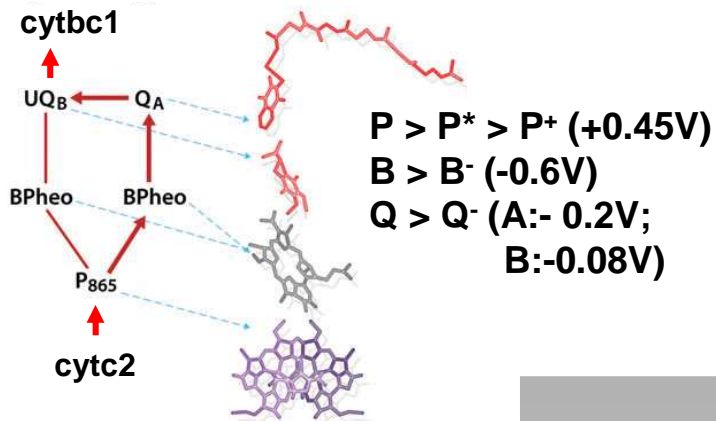
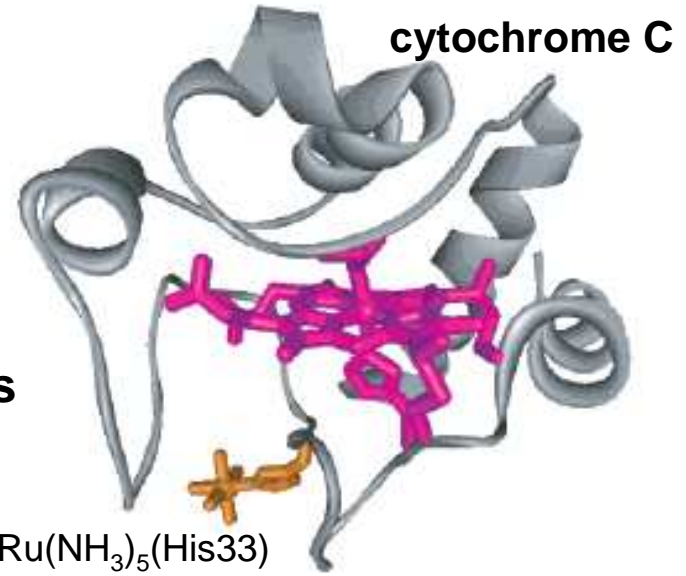
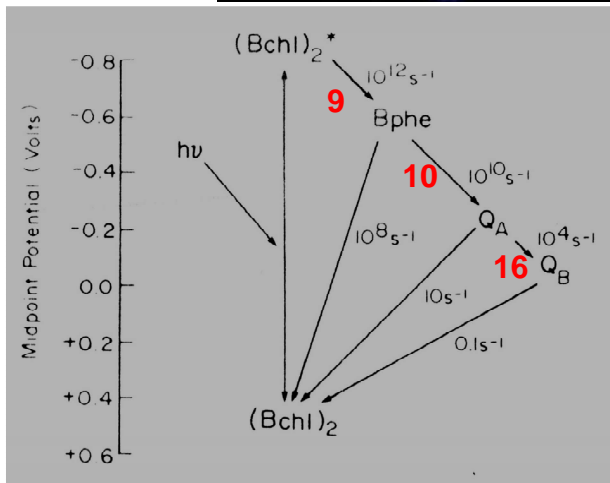
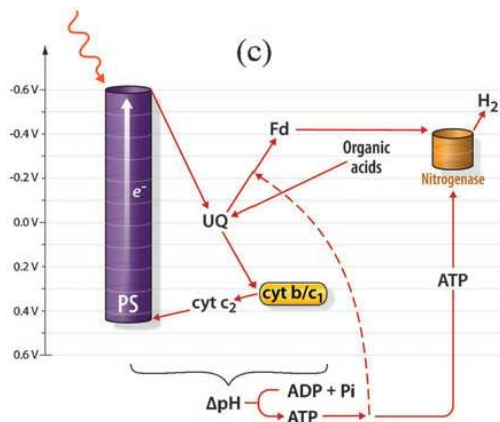


Table 1. Reorganization Energy of Different Classes of Enzymes and Proteins

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>c</i> / cytochrome <i>b</i> ₅	700	332

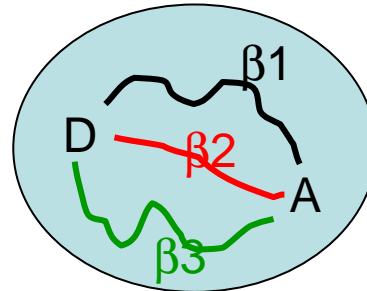


➡ $\lambda = 0.7 \text{ eV} (16 \text{ kcal.mol}^{-1})$

➡ Transferts sur de longues distances

Transfert des électrons: 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 (Dutton $\beta = 1.4 \text{ \AA}^{-1}$)

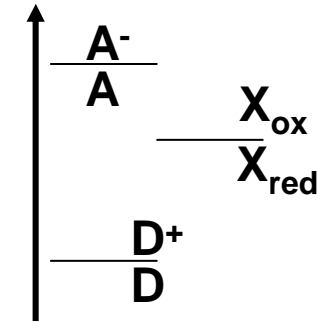
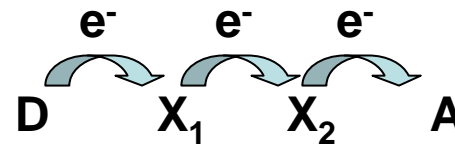
$\beta = 1.2 \pm 0.2 \text{ \AA}^{-1}$ → « Les deux théories convergent »
 $\Delta k = 10^3 - 10^4$
Vide: 3.4

D-A covalent: 0.7

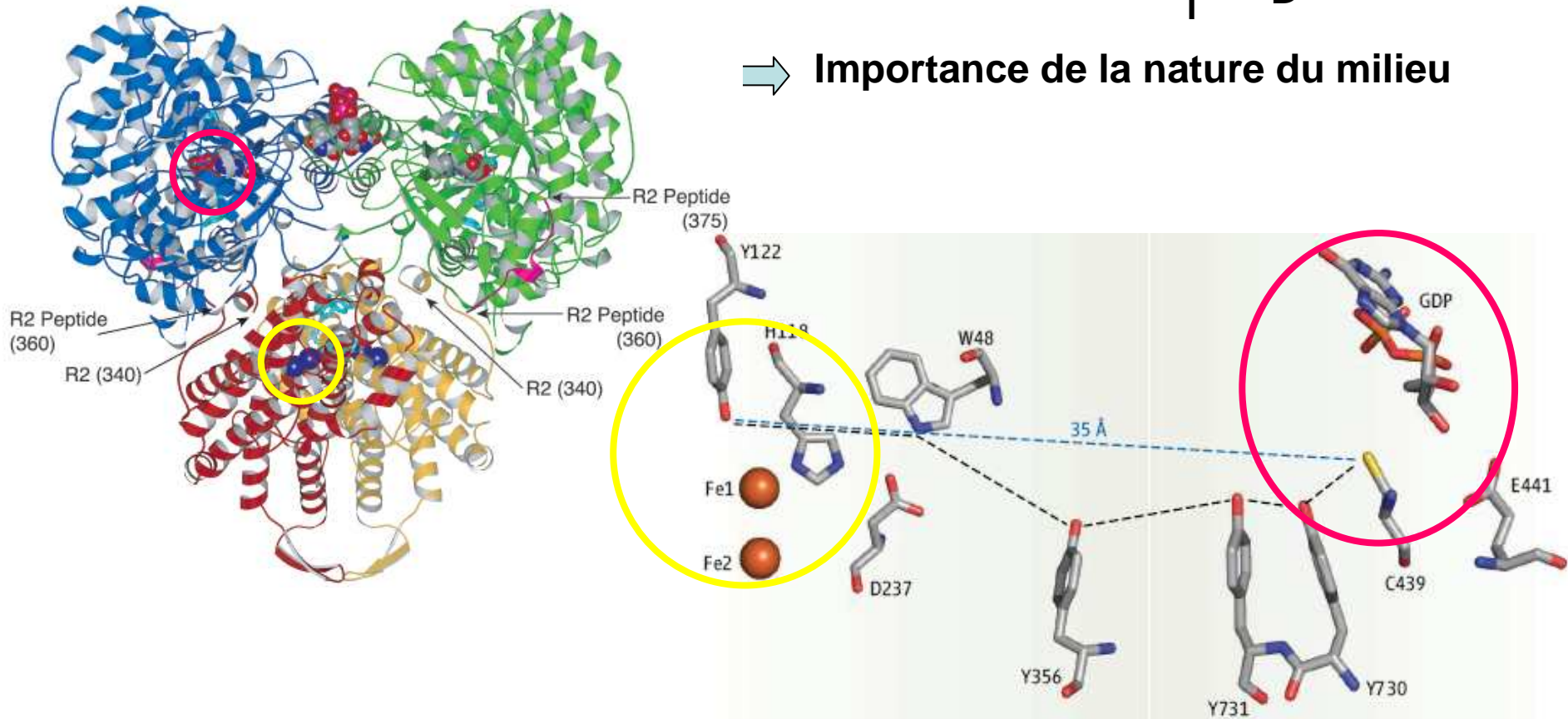
Verre organique (THF): 1.2

Au-delà de 25 Å ? : cofacteurs intermédiaires « multistep tunneling »

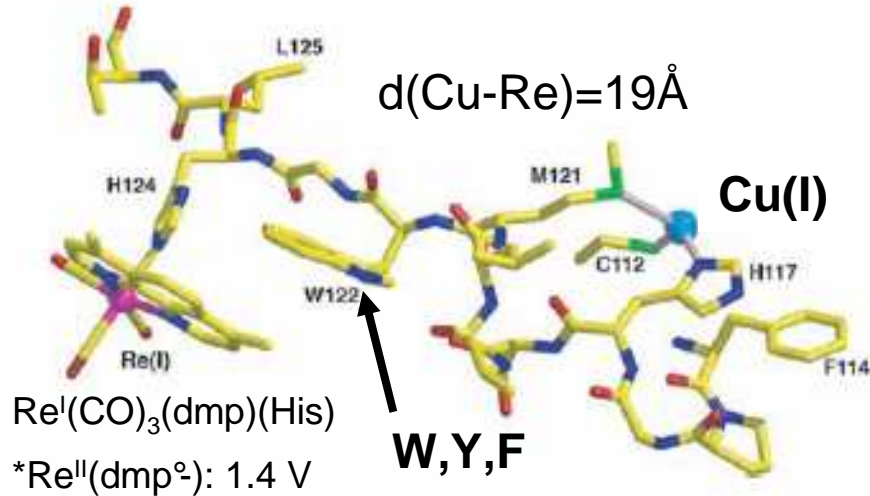
Cofacteurs rédox: ions métalliques, quinones,....,
chaines latérales d'AA



➔ Importance de la nature du milieu

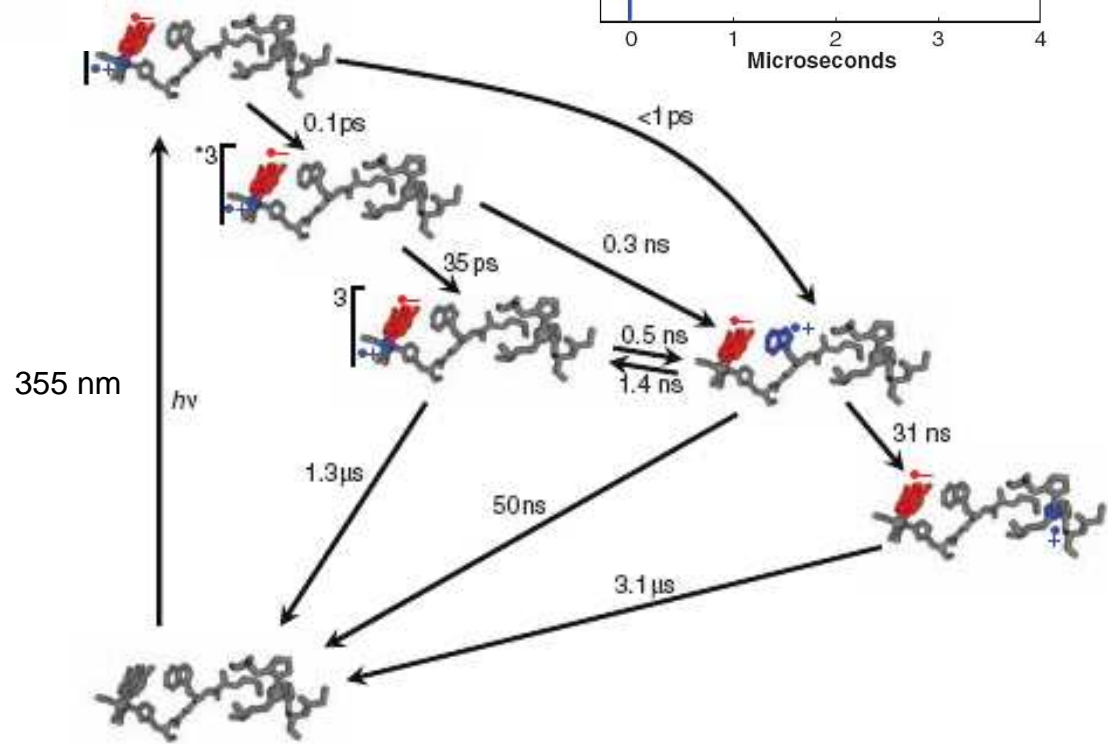
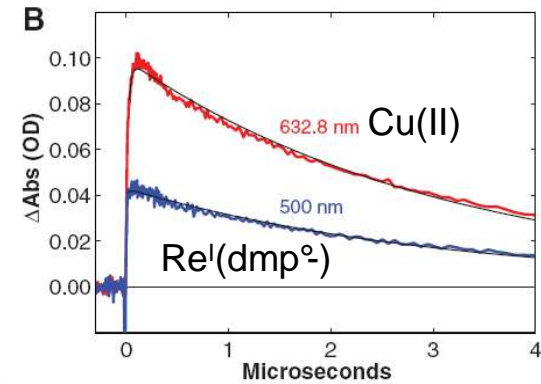
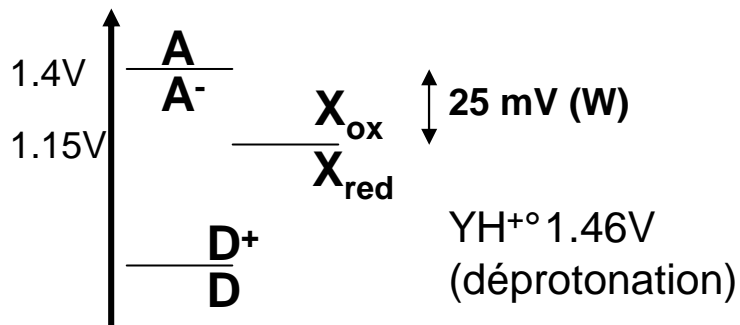


Modèle: Re-azurin(Cu)



Formation de Cu(II) uniquement avec W!

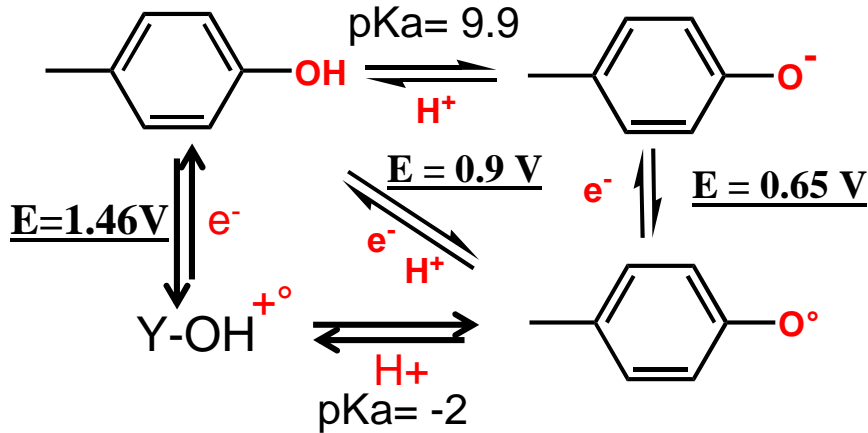
« Cu oxidation is more than two orders of magnitude faster than expected for electron tunneling over 19 Å ».



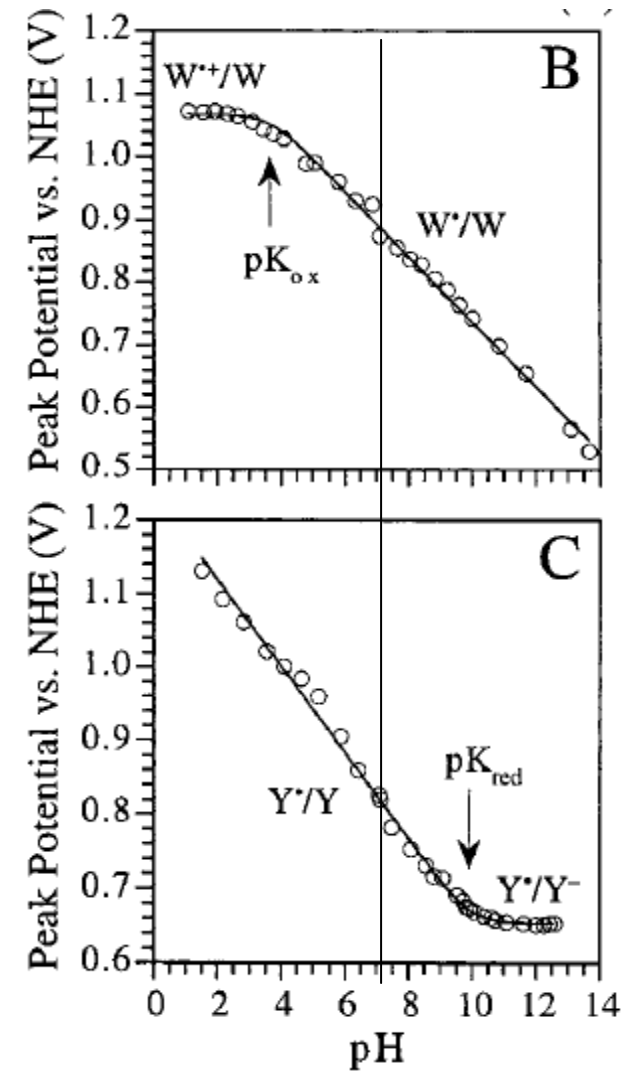
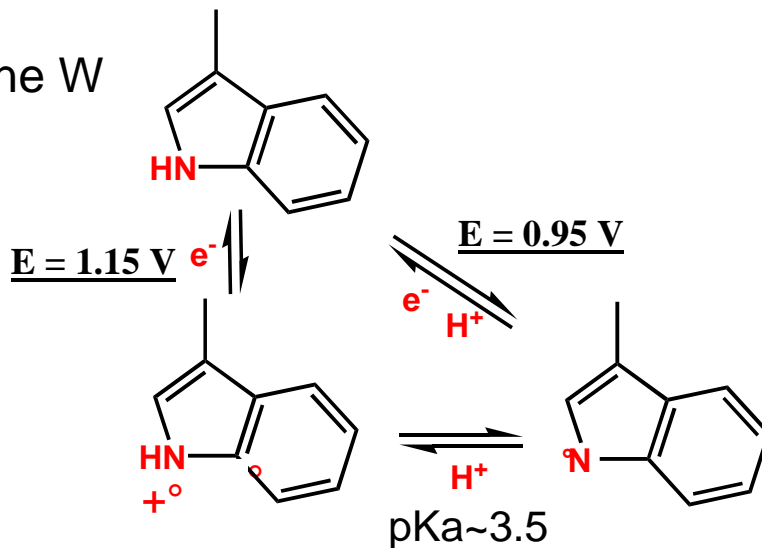
Couplage transfert d'électron/transfert de proton

Tyrosine= Y-OH

La déprotonation de la tyrosine facilite son oxydation !

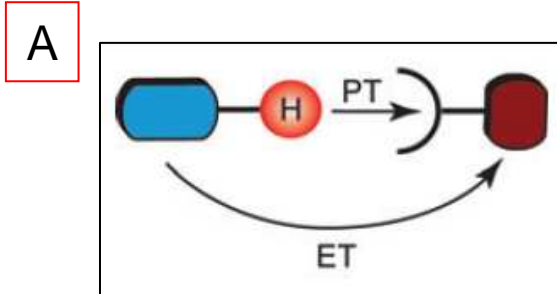


Tryptophane W



Tommos C Biochemistry 1999

« Proton-coupled electron transfer »



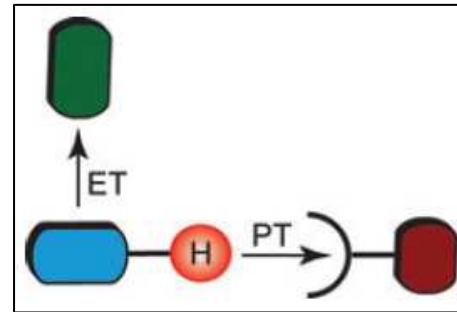
« colinéaire »

-Sans cassure de liaisons

ET à longue distance
à travers liaisons H

-avec cassure de liaisons

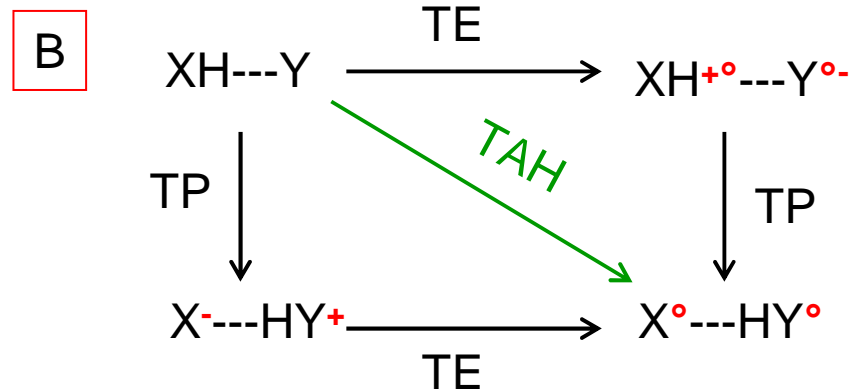
Transfert de radicaux



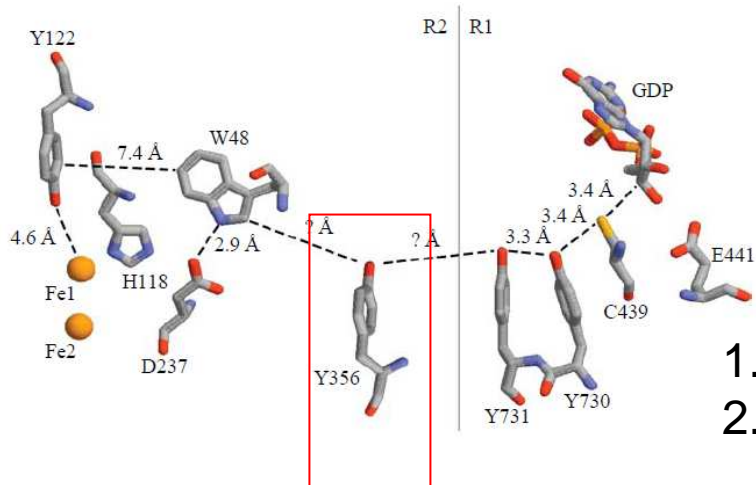
« orthogonal »

Avec cassure de liaisons

Transfert de radicaux



TAH= TE et TP concertés



Etude de la tyrosine 356 de R2:

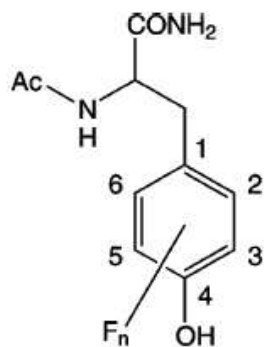
1. Tyrosines fluorées

1. Inhibition avec $E \uparrow$
2. L'état de protonation n'influe pas sur l'activité

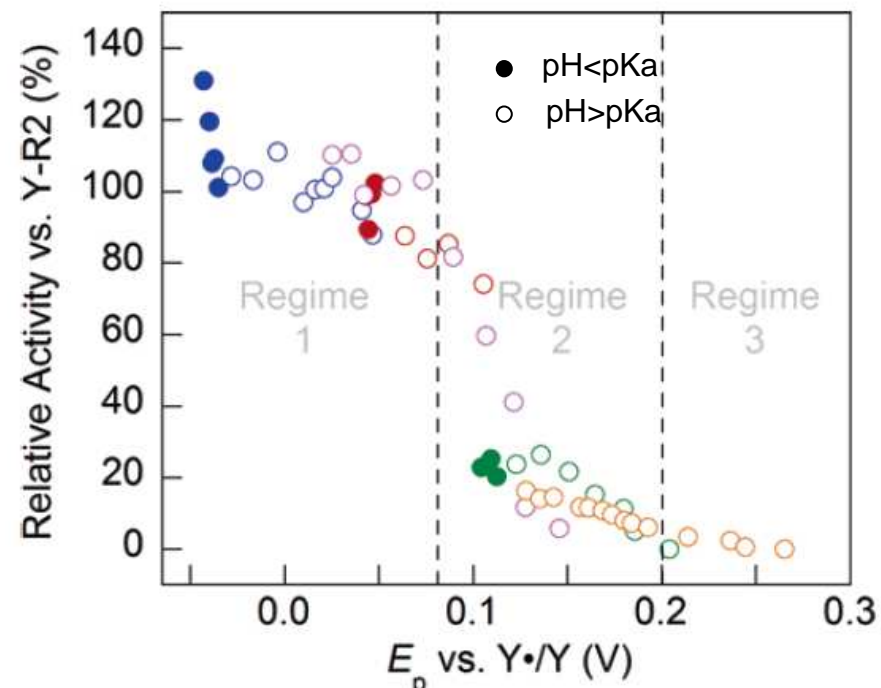
Nocera, Stubbe *Phil Trans R Soc B* 2006,
JACS 2006, *Biochemistry* 2011

Conclusions:

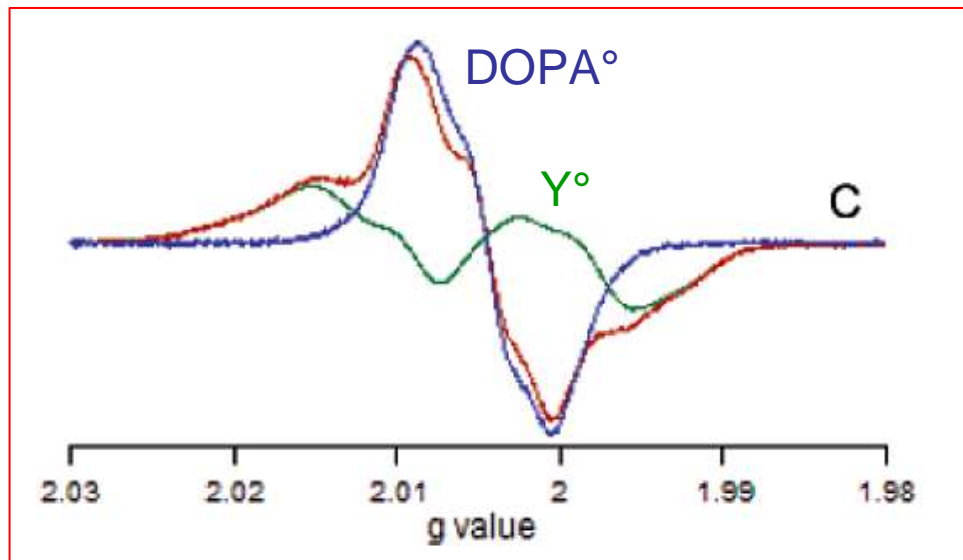
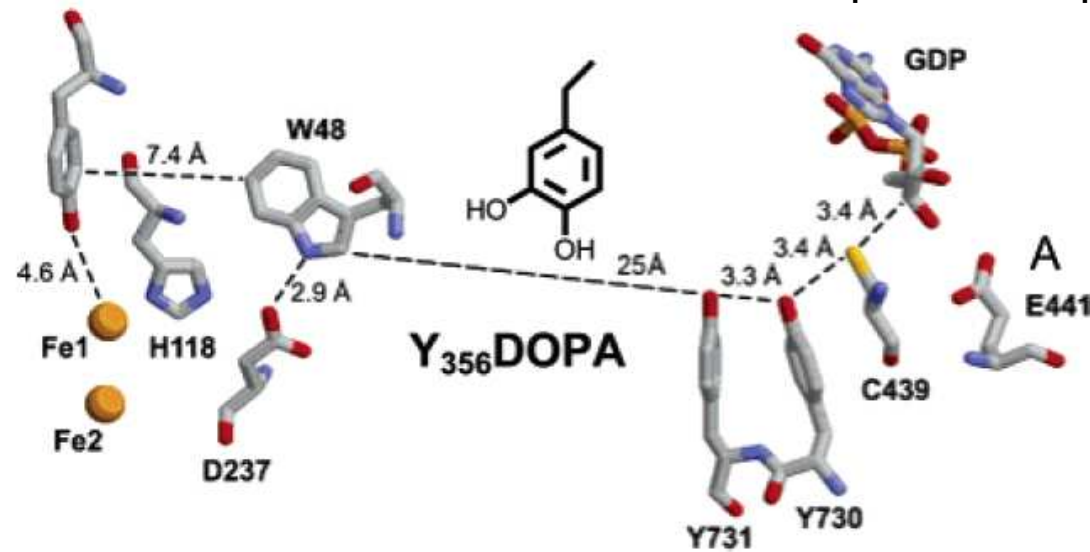
- Y356 est sur le chemin radicalaire
- Le transfert de protons est « orthogonal »



fluorotyrosine	pK_a	$E_p(Y \cdot / Y^-)$ mV
Ac-Y-NH ₂	9.9	642
Ac-3,5-F ₂ Y-NH ₂	7.2	755
Ac-2,3-F ₂ Y-NH ₂	7.8	810
Ac-2,3,5-F ₃ Y-NH ₂	6.4	853
Ac-2,3,6-F ₃ Y-NH ₂	7.0	911
Ac-F ₄ Y-NH ₂	5.6	968



Etude de la tyrosine 356 de R2:
2. Dopa: une trappe à radical

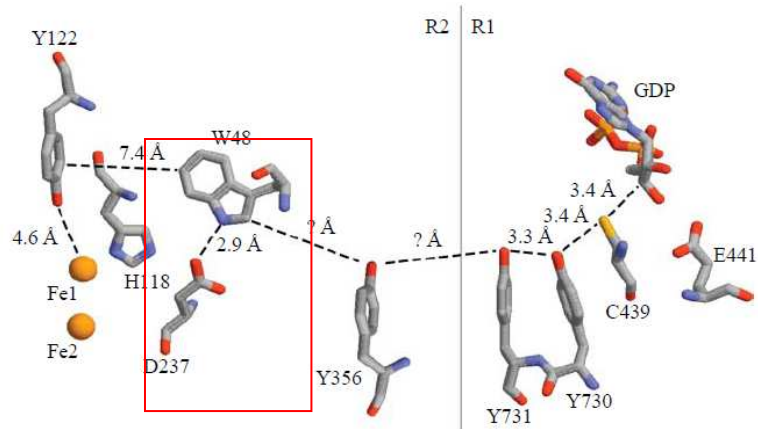


Un radical DOPA° est observé par RPE !

$E = 0.57 \text{ V}$

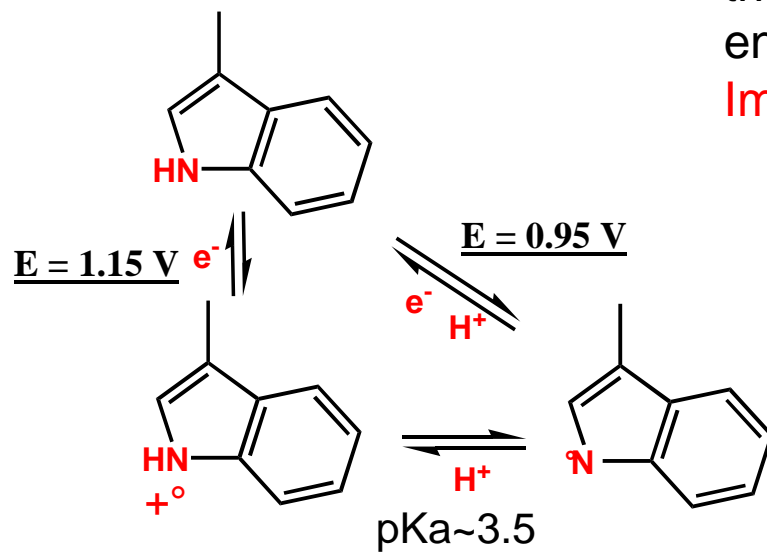


Pas de transfert à R1

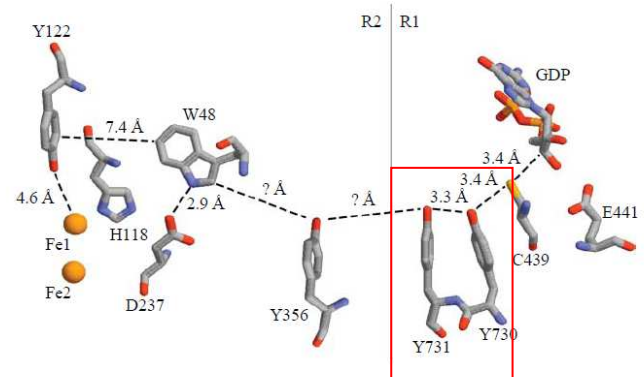


La déprotonation de W48 lève la barrière thermodynamique au transfert de radical entre Y122 et Y356

Importance de D237



Etude des tyrosines 730/731 de R1: 1. amino-tyrosine



Un radical $\text{NH}_2\text{-Y}^\bullet$ est observé par RPE !

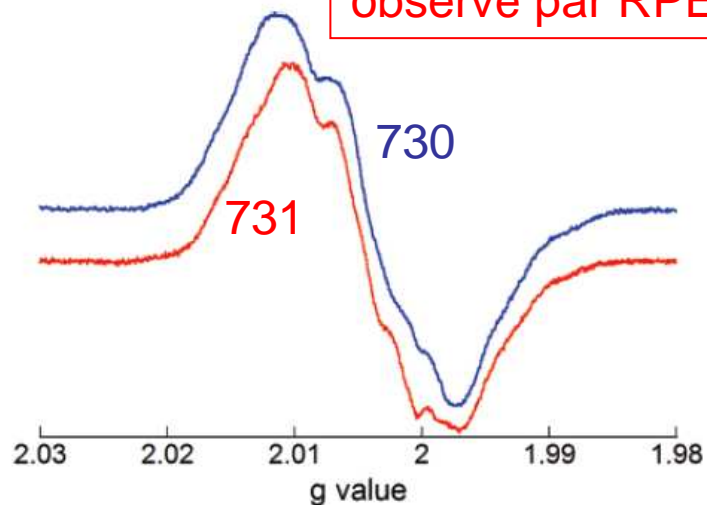


Figure 6. Comparison of the $\text{NH}_2\text{Y}_{730}^\bullet$ (blue, Figure 4) and $\text{NH}_2\text{Y}_{731}^\bullet$ (red, Figure S3).

Stubbe J, JACS 2007

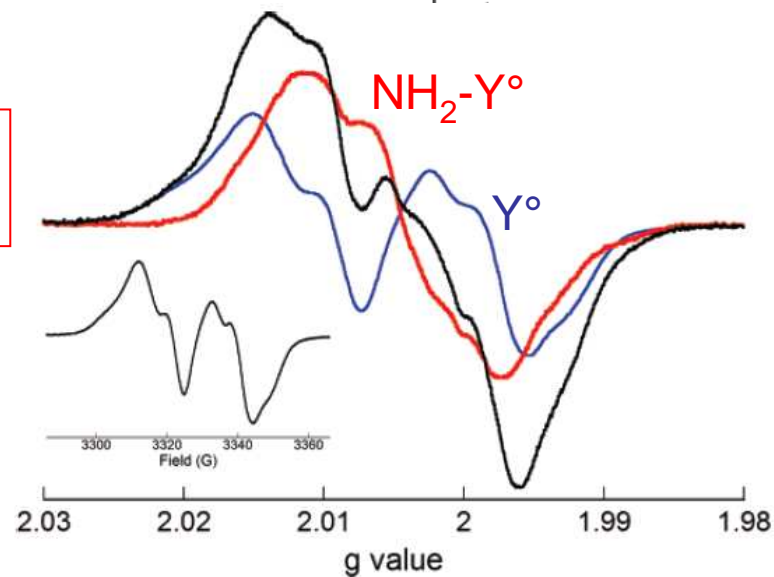
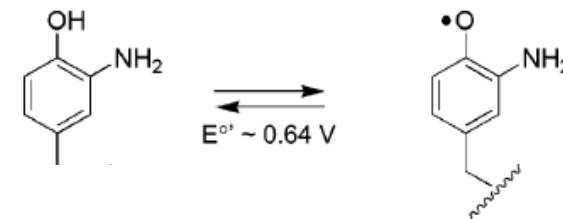
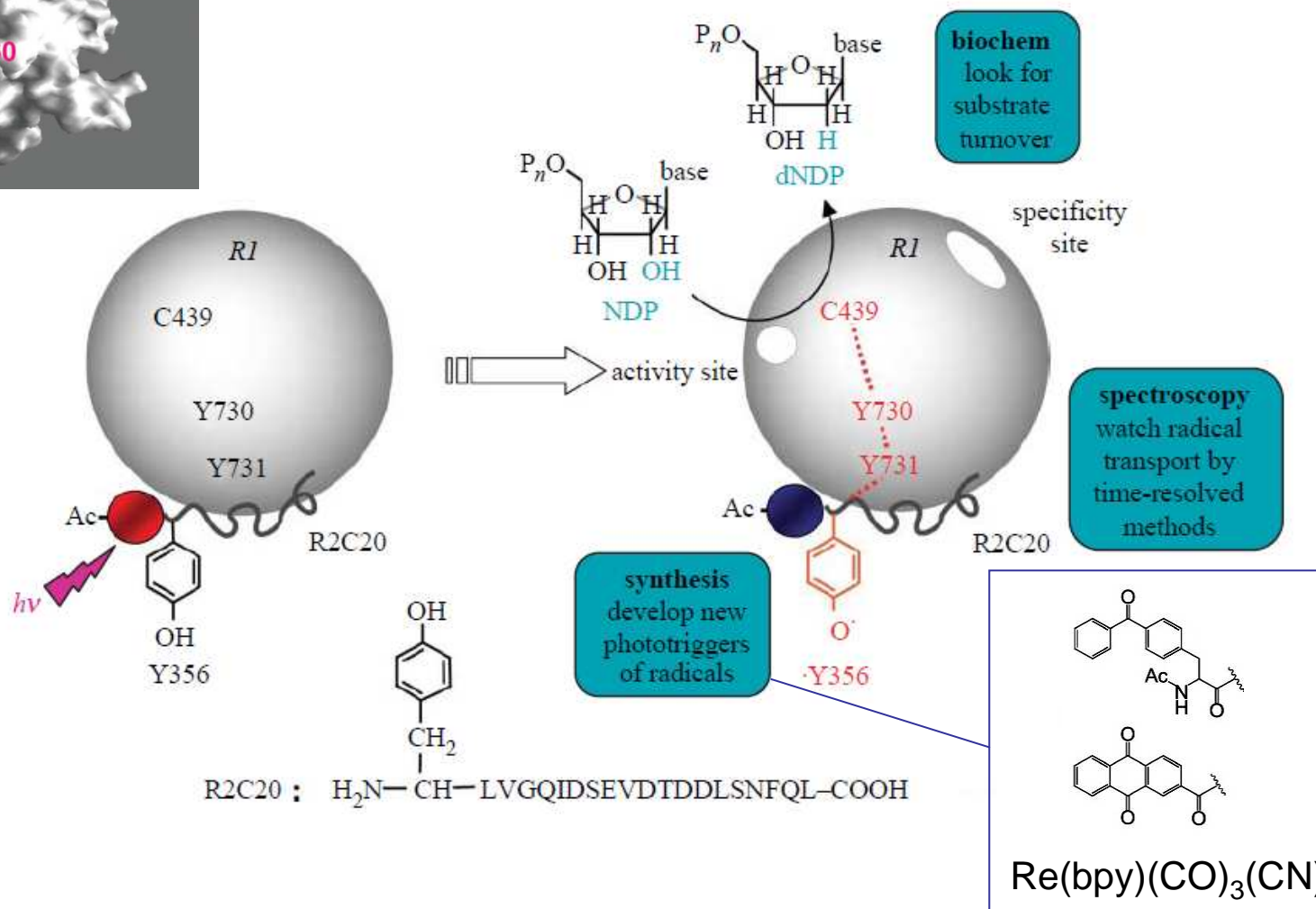
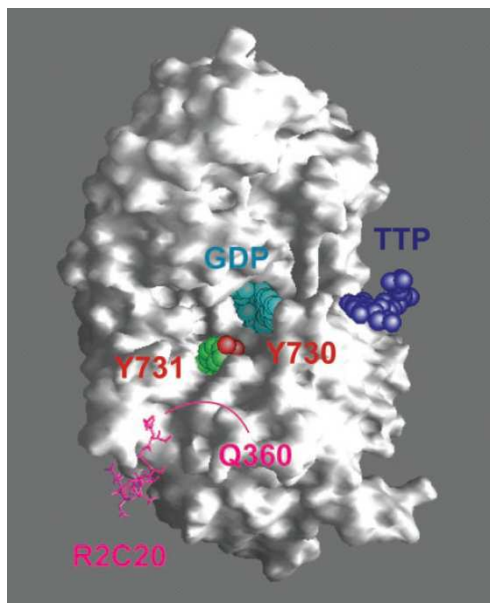


Table 3. Monitoring the Activity of $\text{NH}_2\text{Y-}\alpha 2\text{s}$ by Measuring Deoxynucleotide and N^\bullet Formation

$\alpha 2$ variant	spectrophotometric RNR assay (wt %) ^a	radioactive RNR assay (wt %) ^a	N_3ADP assay (% N^\bullet at 20 s, ^b % N^\bullet vs initial Y_{122}^\bullet) ^c
wt $\alpha 2$	100 ^a	100 ^a	52
$\text{Y}_{730}\text{NH}_2\text{Y-}\alpha 2$	4 ± 0.3	4 ± 0.5	19 ± 2, ^b 15 ± 2 ^c
$\text{Y}_{731}\text{NH}_2\text{Y-}\alpha 2$	7 ± 1	7 ± 0.5	20 ± 2, ^b 15 ± 2 ^c

Création de radicaux par voie photochimique

Nocera D, Stubbe J. PNAS 2004; JACS 2007 8500; JACS 2007 13828



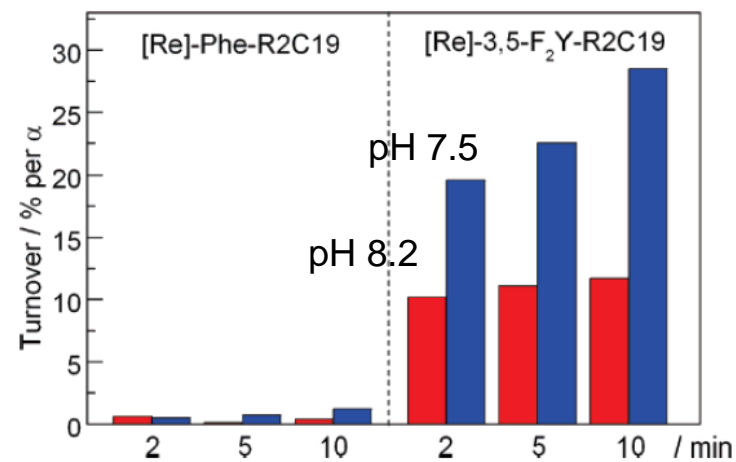
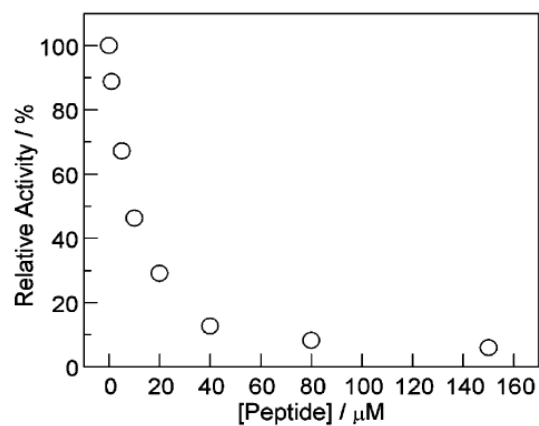
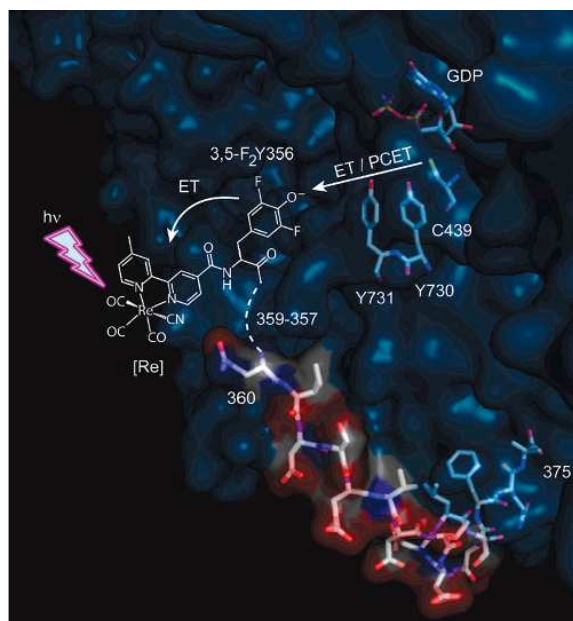
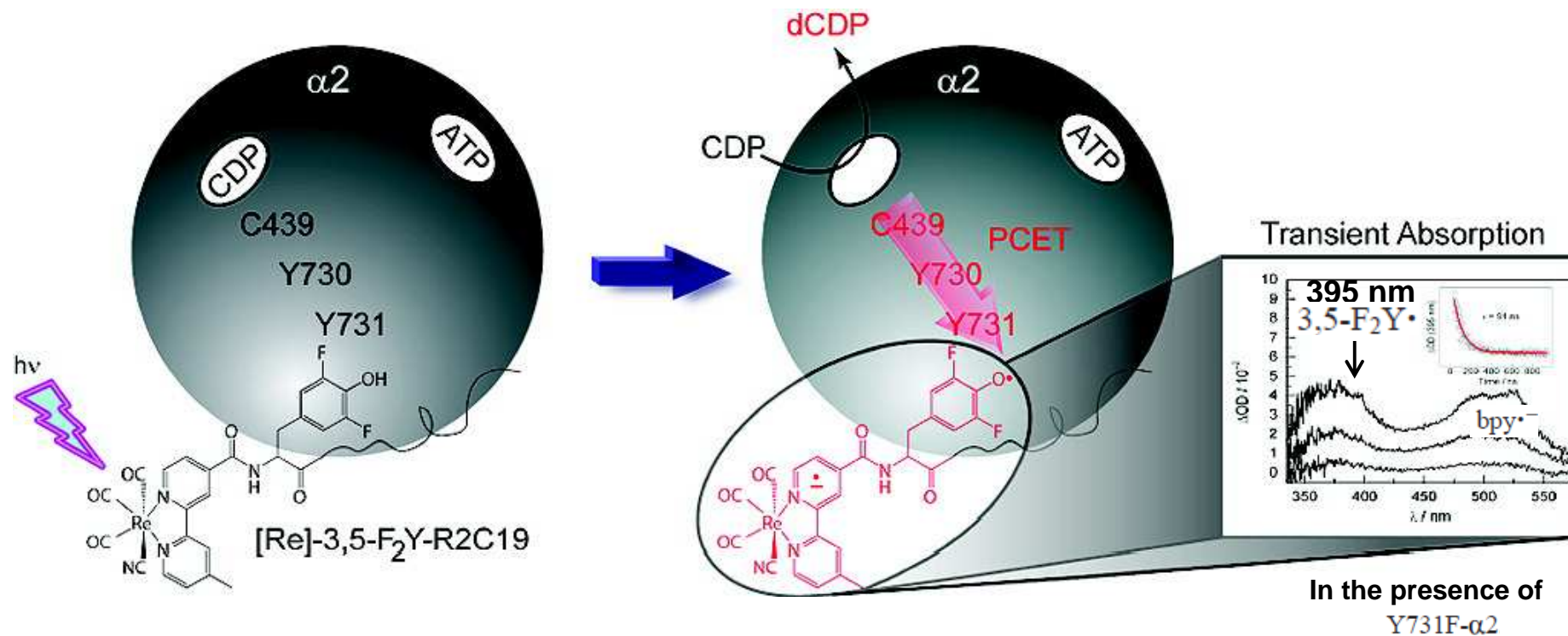
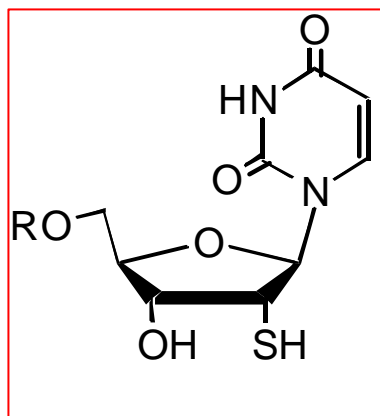


Figure 3. Competitive inhibition binding assay of [Re]-Y-R2C19 to $\alpha 2$.



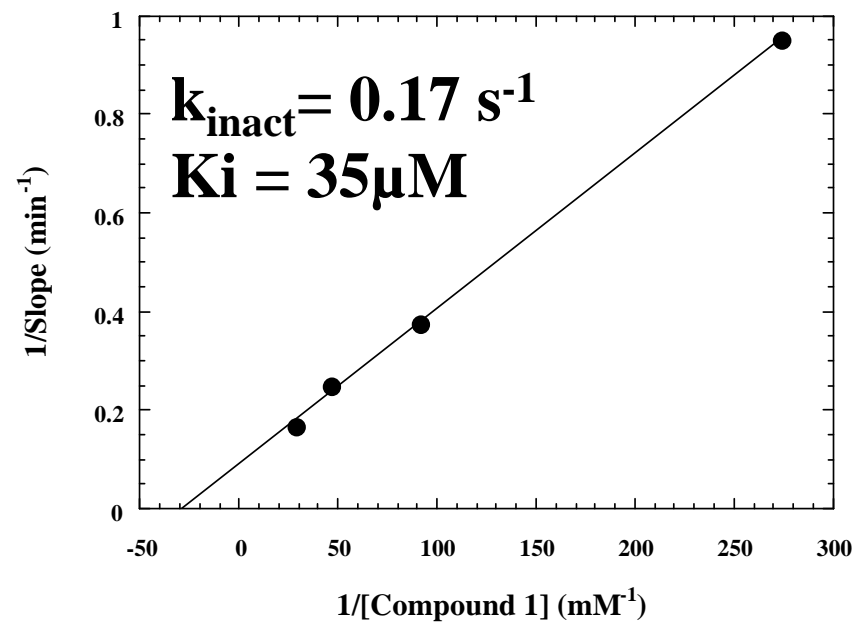
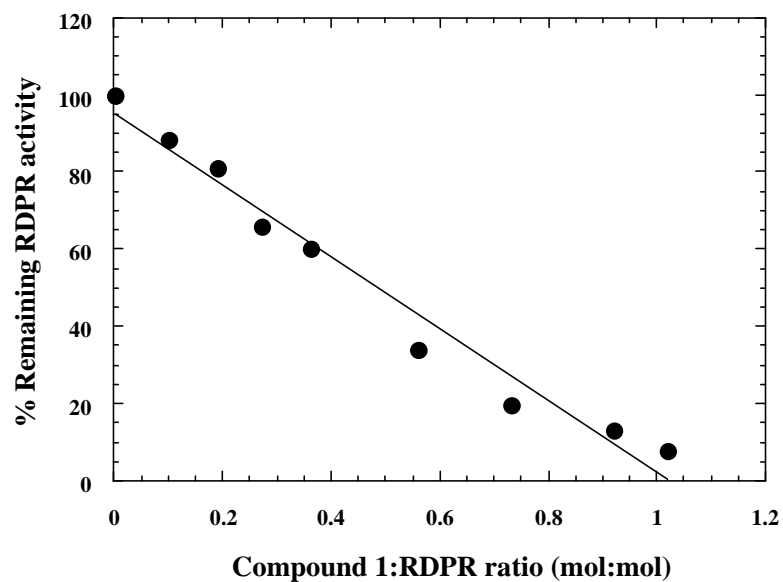
Transfert de radical de R2 à R1: Analogues de substrat

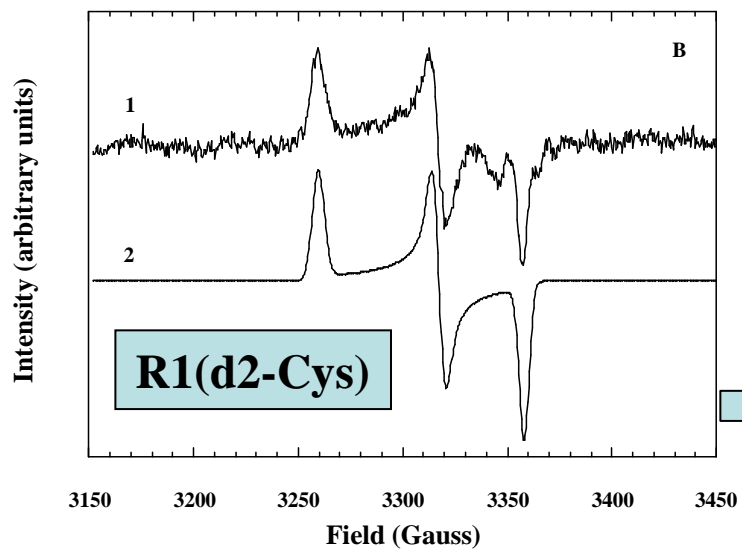
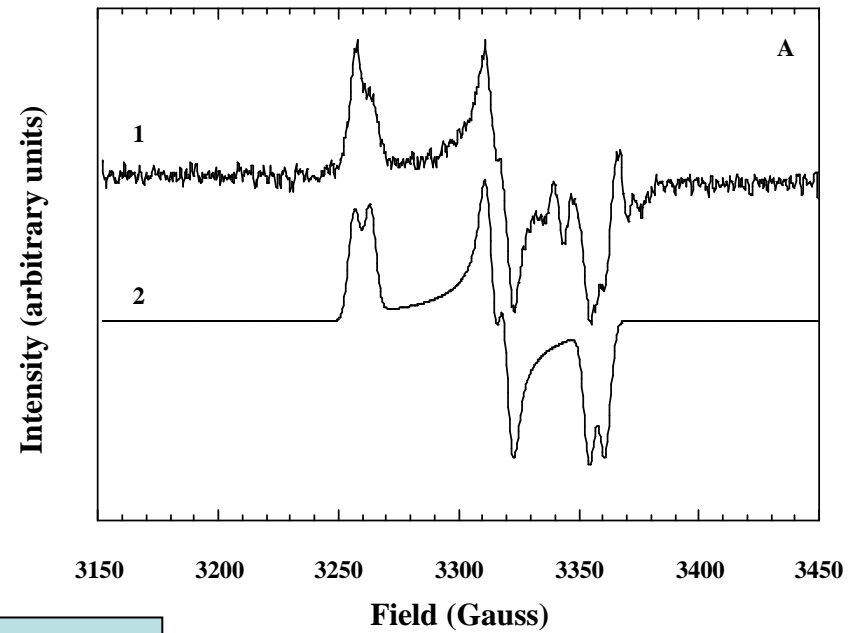
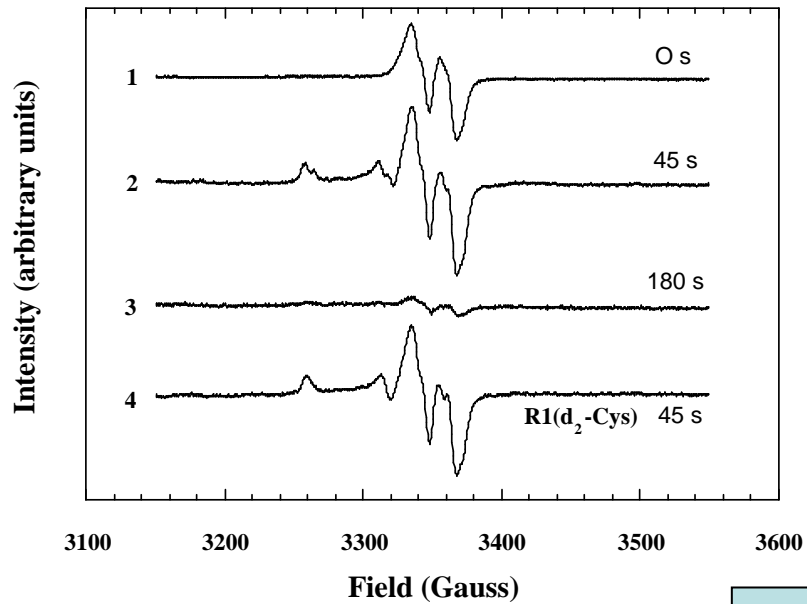
Inactivation of *Escherichia coli* ribonucleotide reductase by 2'-deoxy -2'-mercaptouridine 5'diphosphate - Electron paramagnetic resonance evidence for a transient protein perthiyl radical.

J. Covès, L. Le Hir de Fallois, J.L. Decout, L. Lepape, M. Fontecave.
Biochemistry, 1996 35, 8595-8602

Synthesis of 2'Deoxy-2'-mercaptouridine and cytidine derivatives as potential inhibitors of ribonucleotide diphosphate reductase : Thionitrites, disulfides and 2'-Deoxy-2'-mercaptouridine 5'-Diphosphate.

L. Le Hir de Fallois, J.L. Décout, M. Fontecave
J.Chem.Soc., Perkin Trans., 1997, 2587-2595





$$g_x = 2.06$$

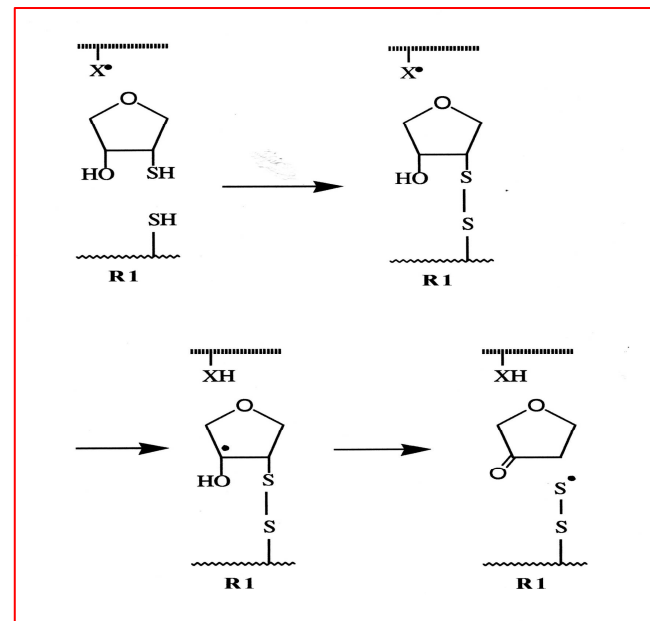
$$g_y = 2.026$$

$$g_z = 2.0019$$

$$g_{av} = 2.03$$

$$a \text{ (mT)} = 0.65$$

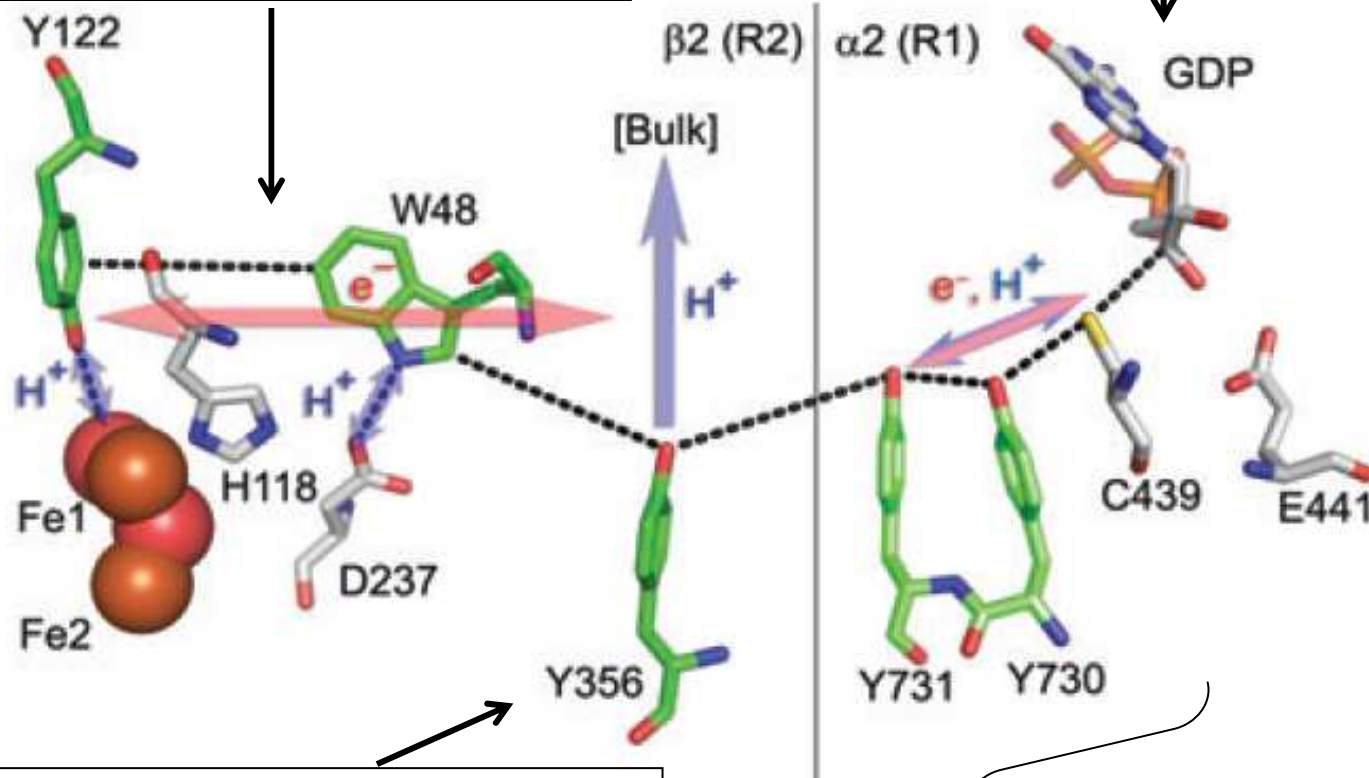
Radical
Persulfure
RSS^o
sur R1



CONCLUSION

Transfert d'électron
de W_{48} à Y_{122}°
 H_2O fournit un H^+ à Y^-
 D_{237} sert à baisser le E de W
« orthogonal »

Formation de Cys° endergonique
couplée
à une réaction exergonique (substrat)



Transfert d'électron
de Y_{356} à W_{48}
Assisté par un transfert de H^+
dans le milieu « orthogonal »

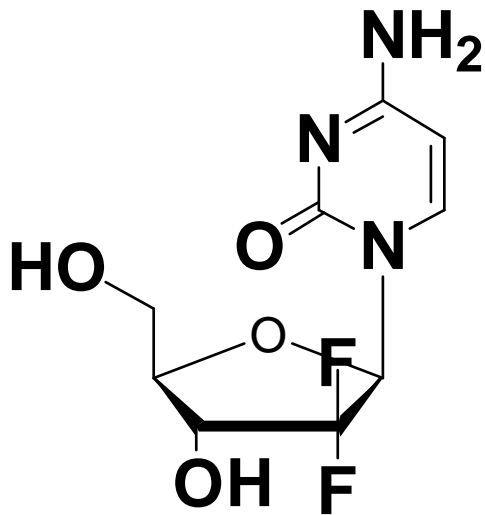
Abstraction d'atome H
ou transfert e^-/H^+ « colinéaire »

**Deux agents anticancéreux
Inhibiteurs de ribonucléotide réductase**

Gemcitabine (Eli-Lilly)

Clinical use against:

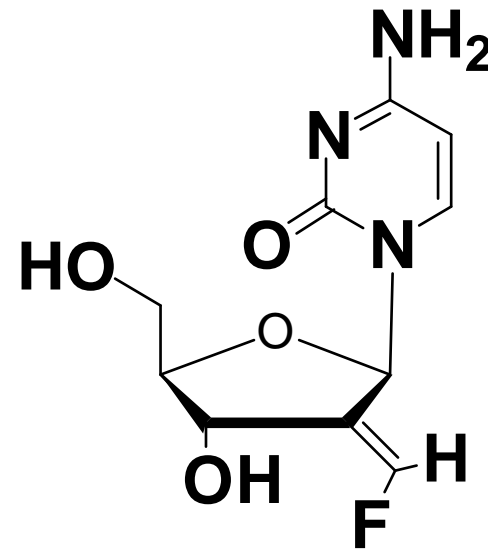
- solid tumors
- lung cancer (Pt)
- pancreas cancer



Tézacitabine (HMR)

Clinical trial against:

- solid tumors
- breast cancer





COLLÈGE
DE FRANCE
—1530—

Biosynthèse des précurseurs de l'ADN chez les organismes aérobies: du fer, de l'oxygène et des radicaux libres

Marc Fontecave

*Laboratoire de Chimie et Biologie des Métaux, Université Joseph Fourier, CNRS, CEA/DSV/iRTSV
CEA-Grenoble 17 rue des martyrs 38054 Grenoble cedex 9, France
mfontecave@cea.fr; Phone: (0033)438789103 ; Fax: (0033)438789124*

Collège de France, 11 Place Marcelin Berthelot, 75231 Paris Cedex 05

RiboNucléotide Réductase

