Transfert de radicaux dans les systèmes biologiques: Les ribonucléotide réductases

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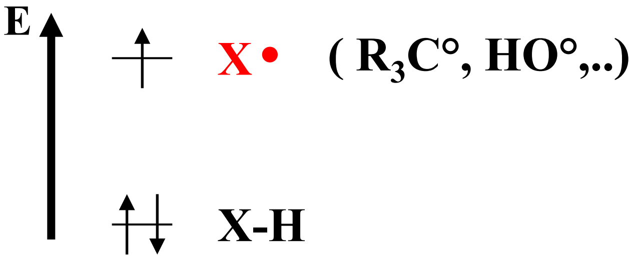
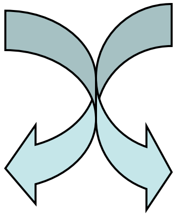
Radicaux libres

Le « dogme »: des espèces chimiques très réactives
incontrôlables et toxiques
incompatibles avec le vivant
stress oxydant: vieillissement, pathologies

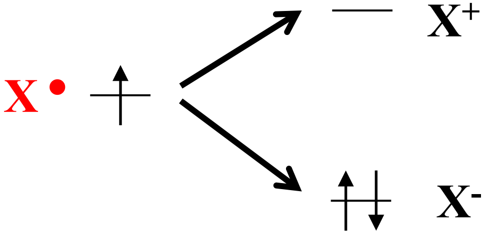


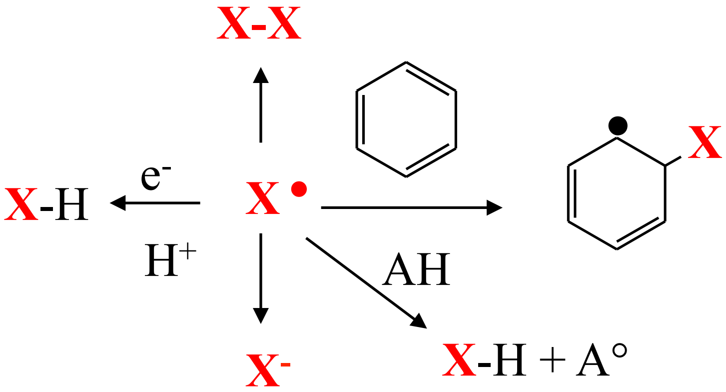


ADN, protéines, lipides, …

Stress oxydant

Réactions de X°: arrachement d'atomes H, coupure de liaisons, addition sur C insaturé, couplage radicalaire..





Radicaux libres

Le « dogme »: des espèces chimiques <u>très réactives</u>
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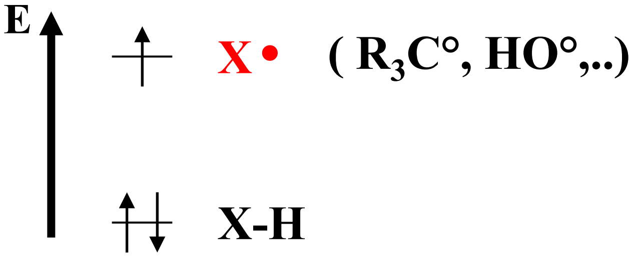
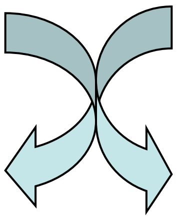




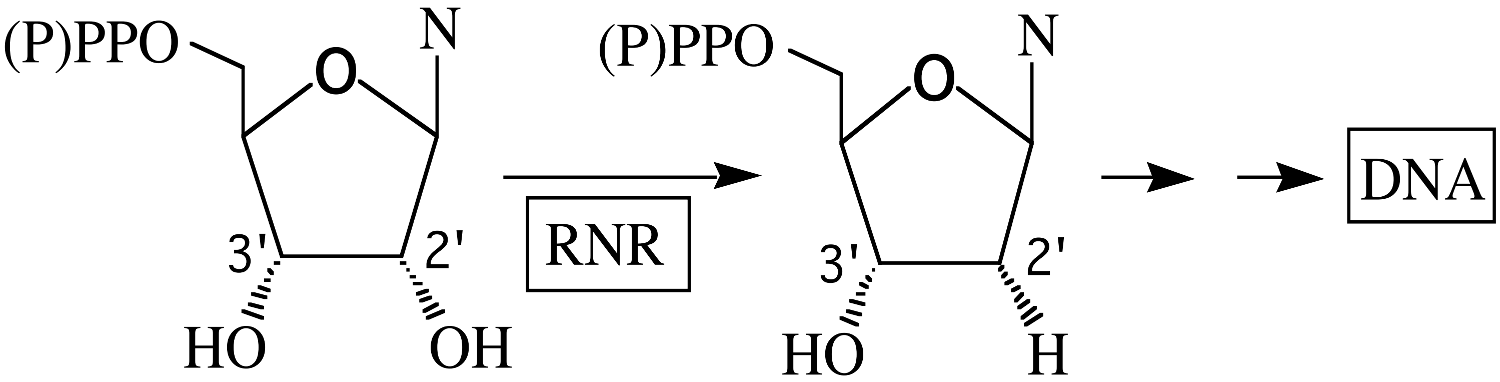
<u>Réactions de X°:</u> arrachement d'atomes H, coupure de liaisons, addition sur C insaturé, couplage radicalaire..

La réalité: des centaines de réactions biologiques « radicalaires » !!

$$\text{X-H} \xrightarrow[\text{enzyme}]{} [\text{X}\bullet] \longrightarrow \quad \longrightarrow \text{produit}$$

Le cas des ribonucléotide réductases et des enzymes de la famille <u>« Radical-SAM »</u>

RiboNucléotide Réductase

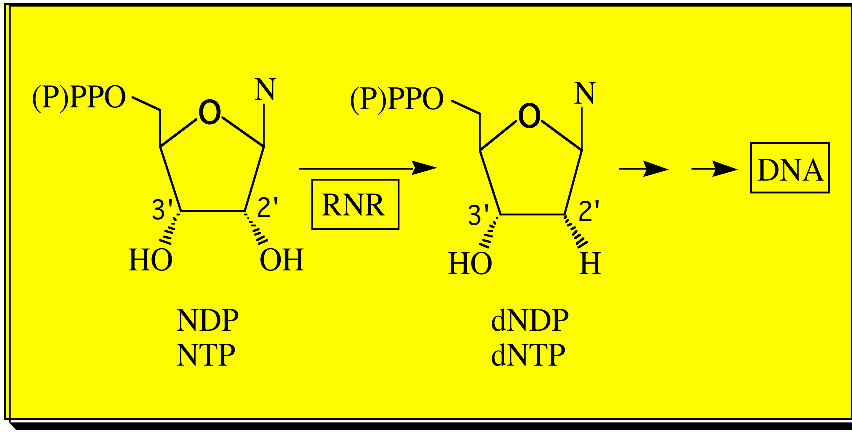


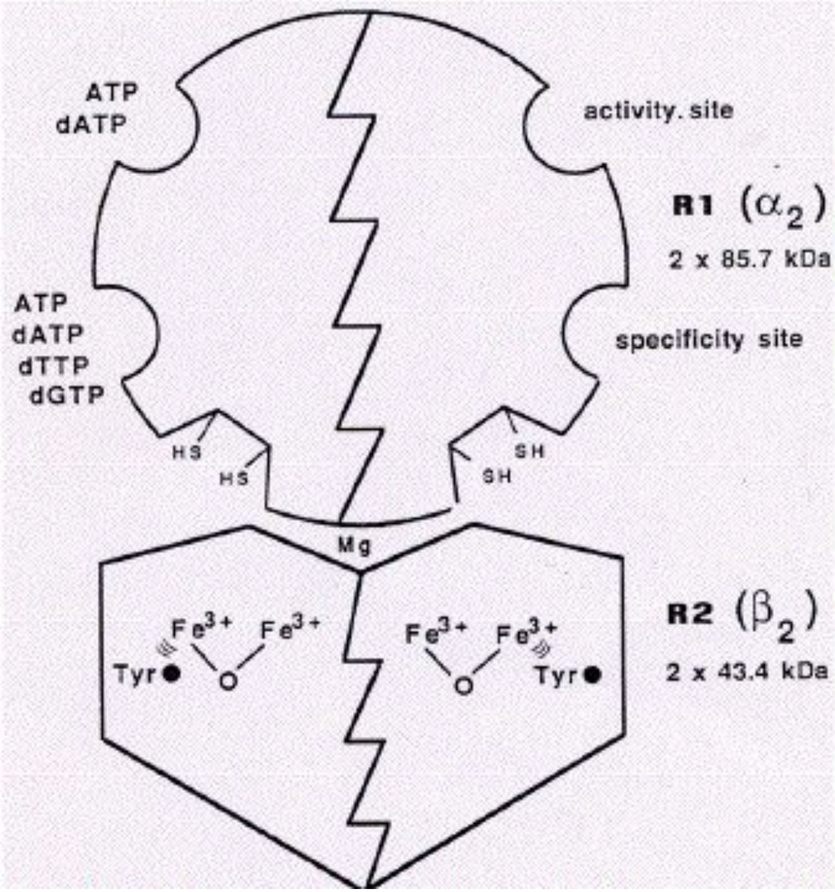
NDP
NTP

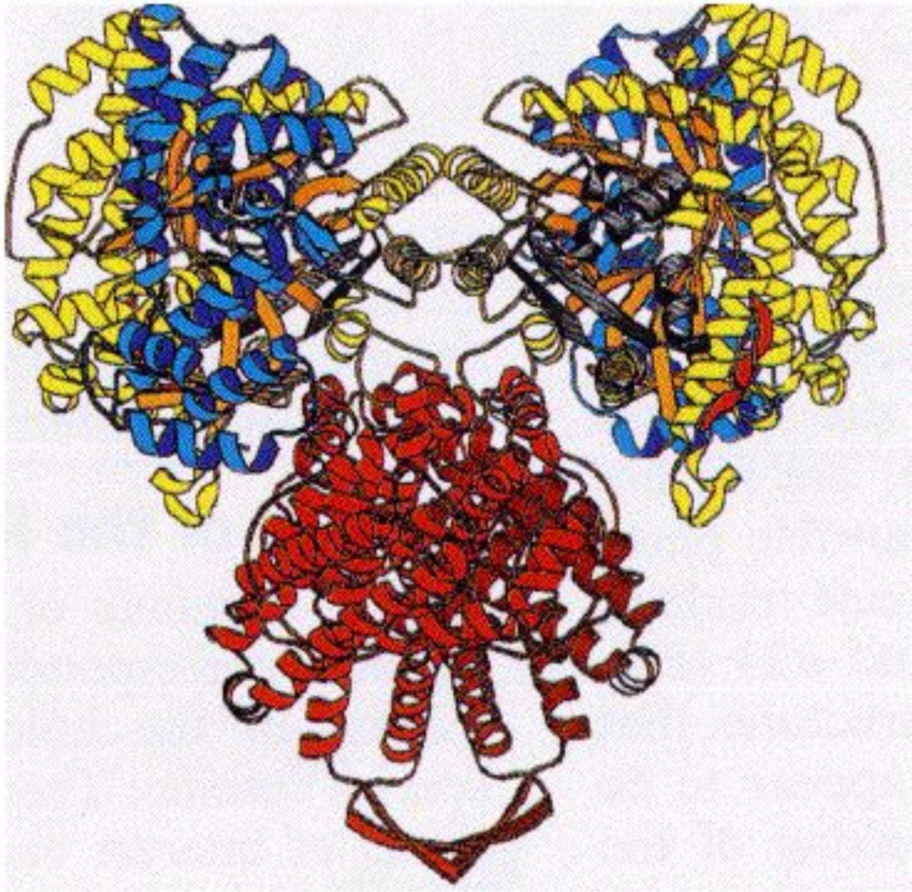
dNDP
dNTP

Ribonucléotide réductase
Structure et mécanismes

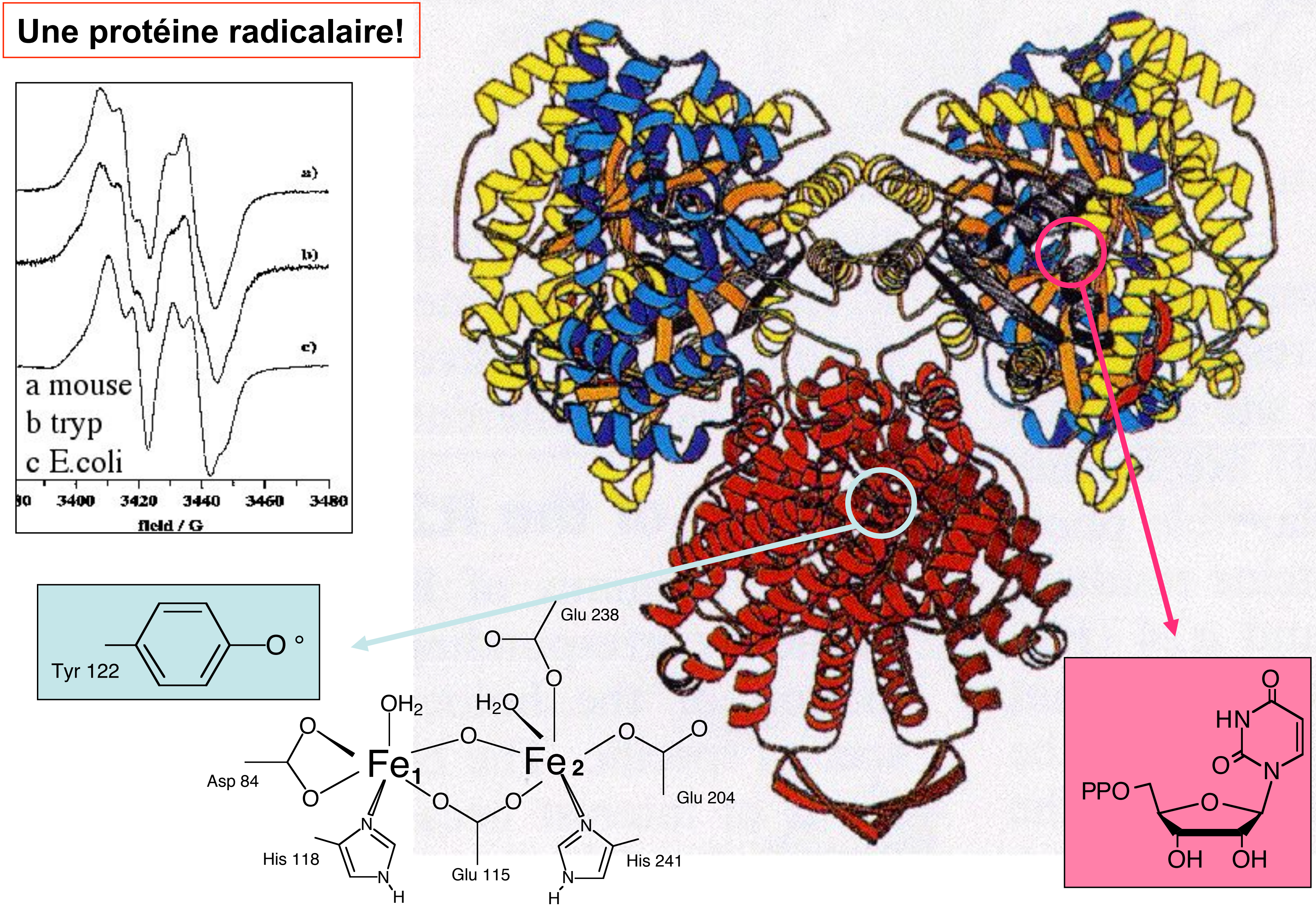
P. Reichard Annu. Rev. Biochem (2006)
H. Eklund Nature (1990); Nature (1994)
J. Stubbe Chem Rev (1998); Chem Rev (2003)







Une protéine radicalaire!



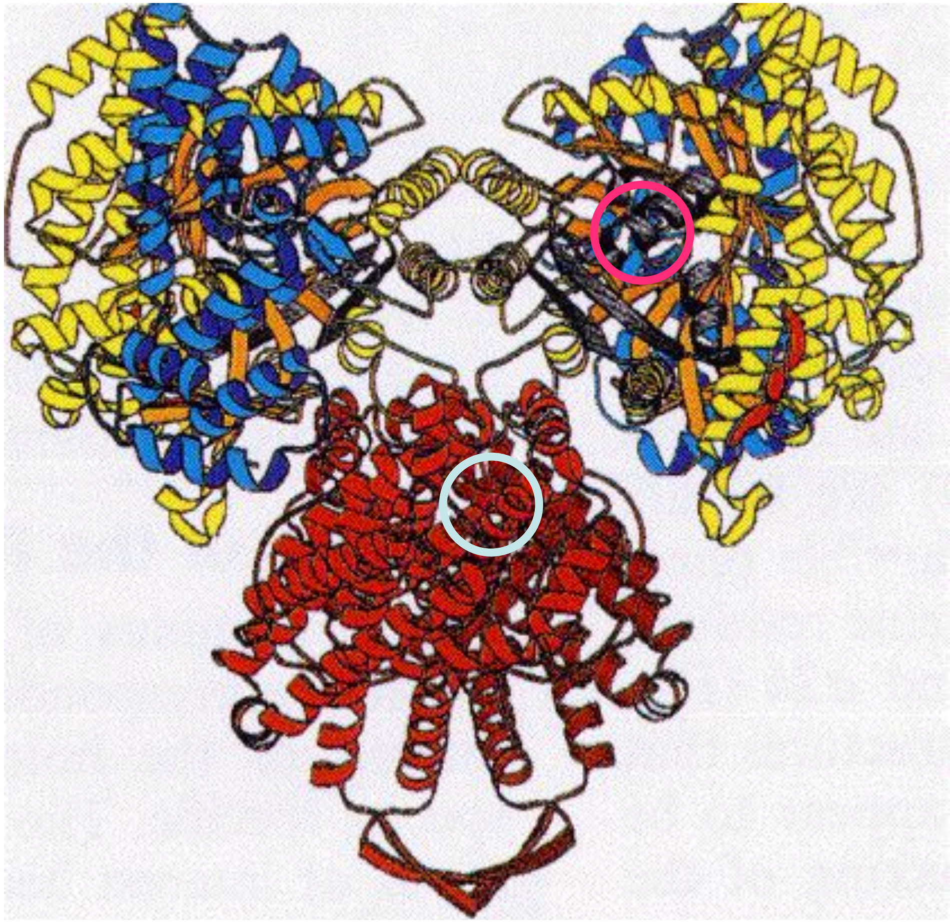
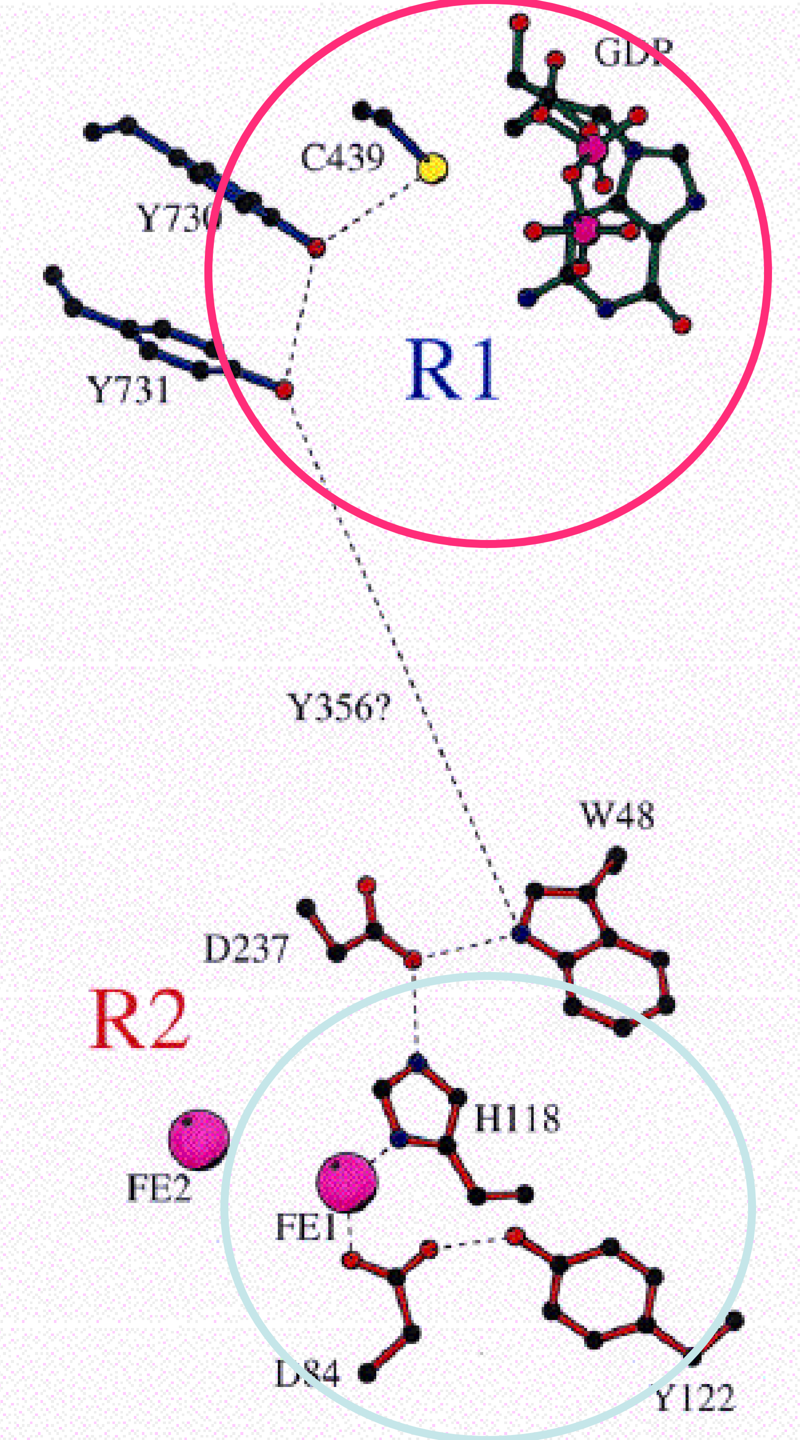
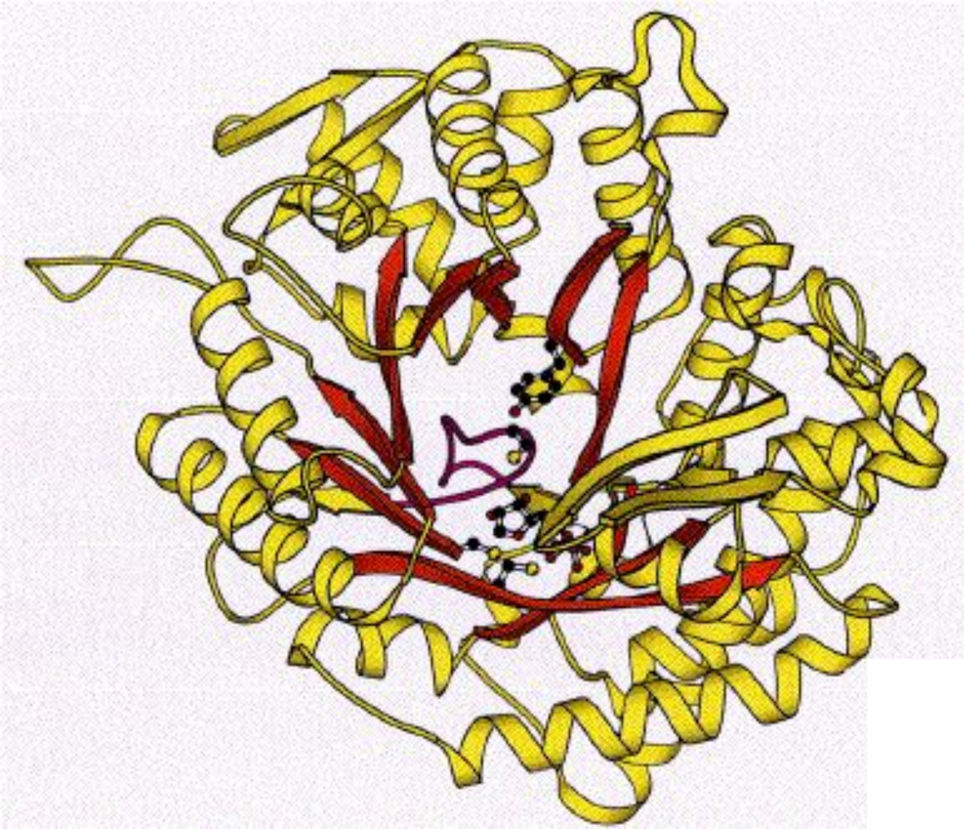
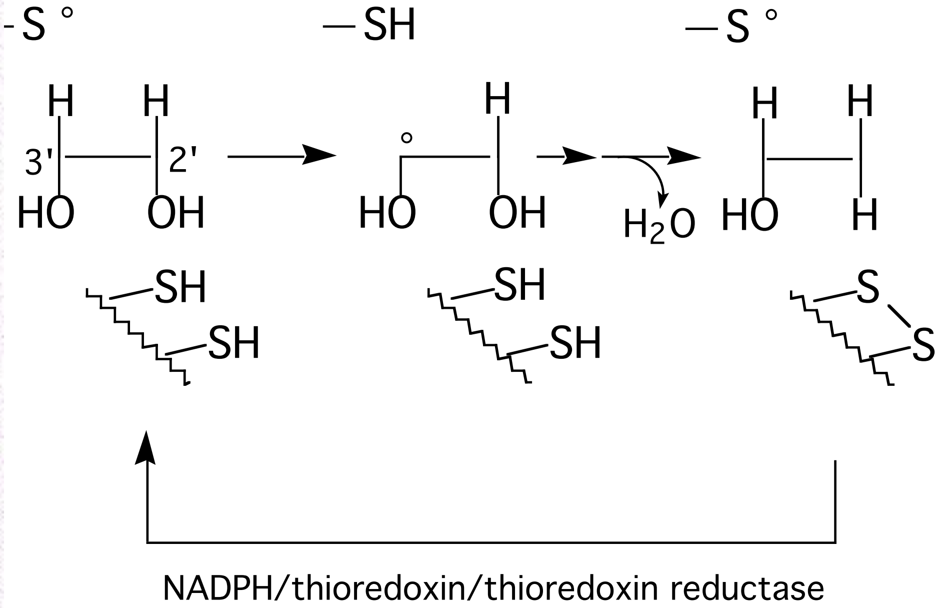


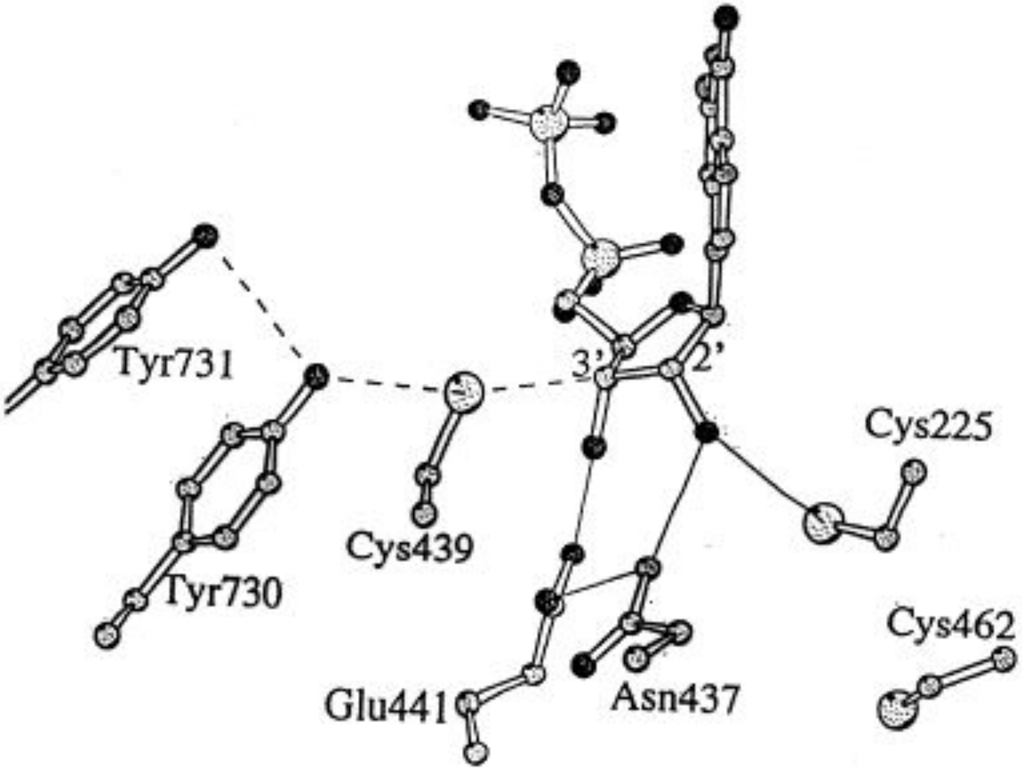
Table 3. Activity of Mutants on the Putative PCET Pathway in *E. coli* and Mouse R2

protein	iron/ R2	Y$^•$s/R2	SA (nmol/ min mg)	N$^•$ formation^c (s^{-1})	Y$^•$ loss^c (s^{-1})
E coli^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%)		
wt-R1			1650		
Y730F			26 (1.6%)		no loss
Y731F			26 (1.6%)		no loss

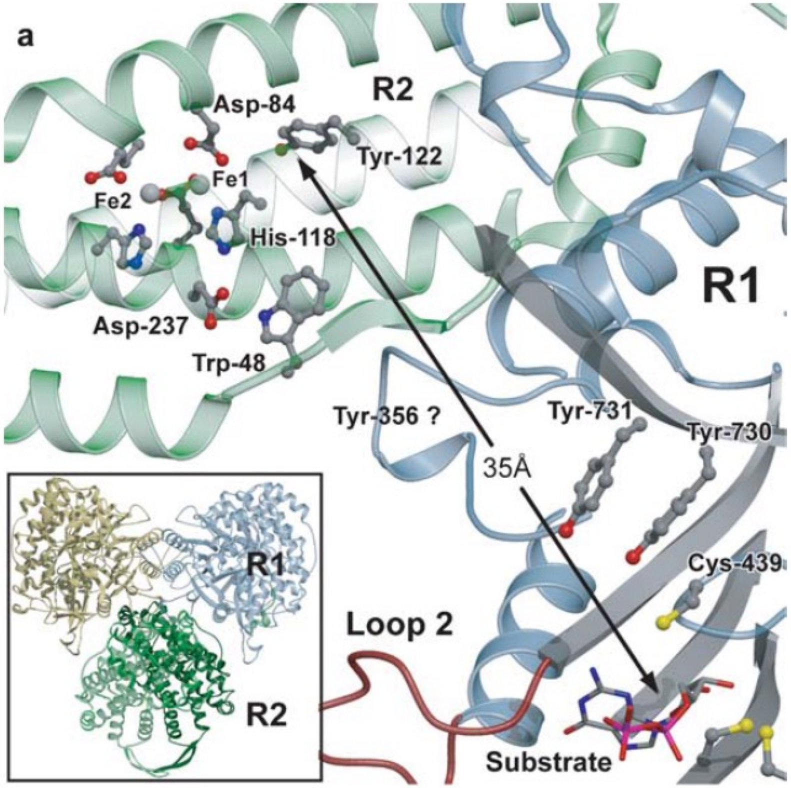





-S °
H
H
3'
2'
HO
OH
SH
SH
—SH
°
H
HO
OH
SH
SH
H₂O
—S °
H
H
HO
H
S
S
NADPH/thioredoxin/thioredoxin reductase


Tyr731
Tyr730
Cys439
3'
2'
Cys225
Glu441
Asn437
Cys462

Etude du transfert de radicaux dans la RNR



J Stubbe (MIT)

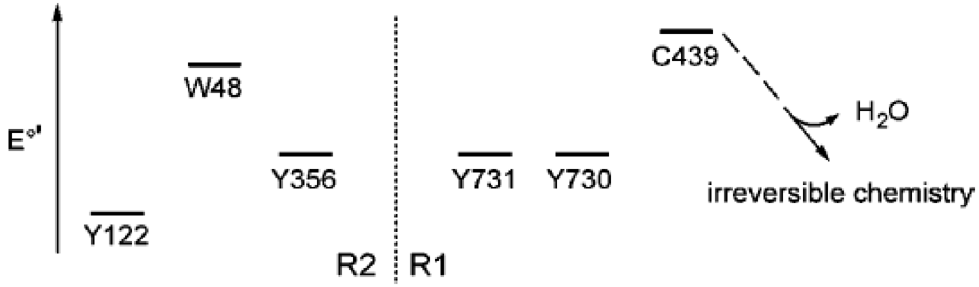
A

35 Å ↔ $k_{ET} = 10^{-6}$ s^{-1}
(théorie ET)

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

B

Contrainte thermodynamique
Transfert des électrons couplé
à des transferts de protons



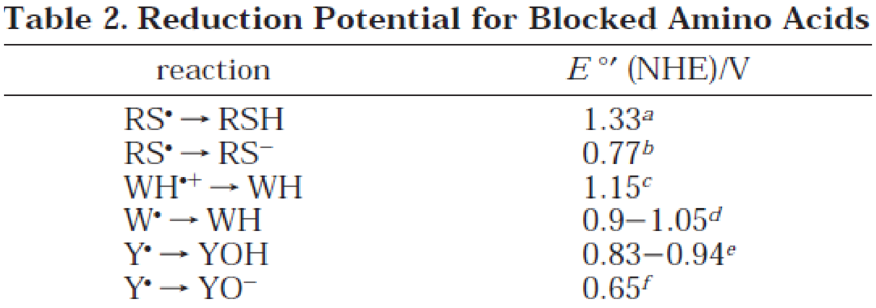


Table 2. Reduction Potential for Blocked Amino Acids

reaction	$E°'$ (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

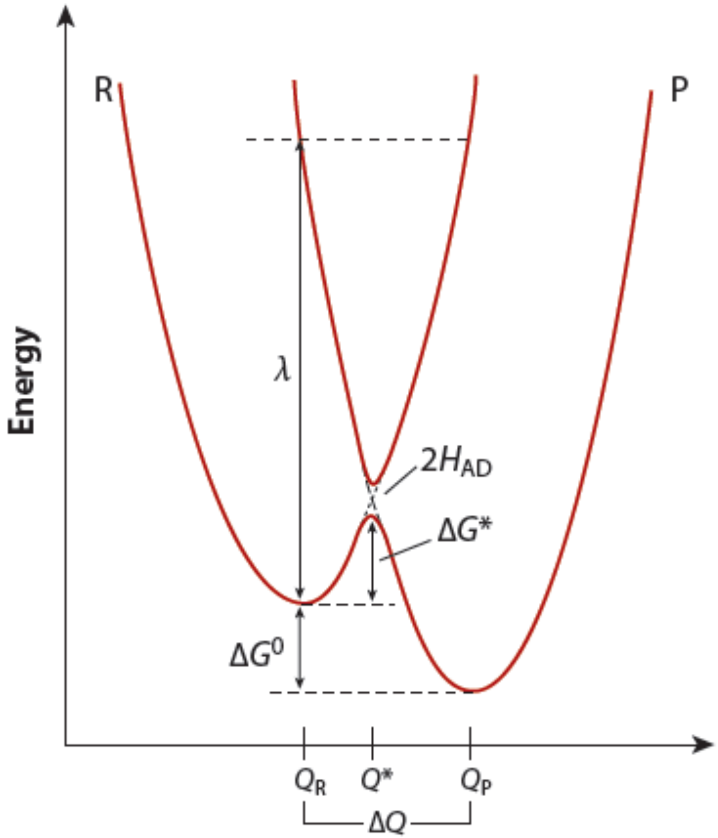
$$\underbrace{A_{ox} + B_{red}}_{R} \longrightarrow \underbrace{A_{red} + B_{ox}}_{P}$$

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

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

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

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.

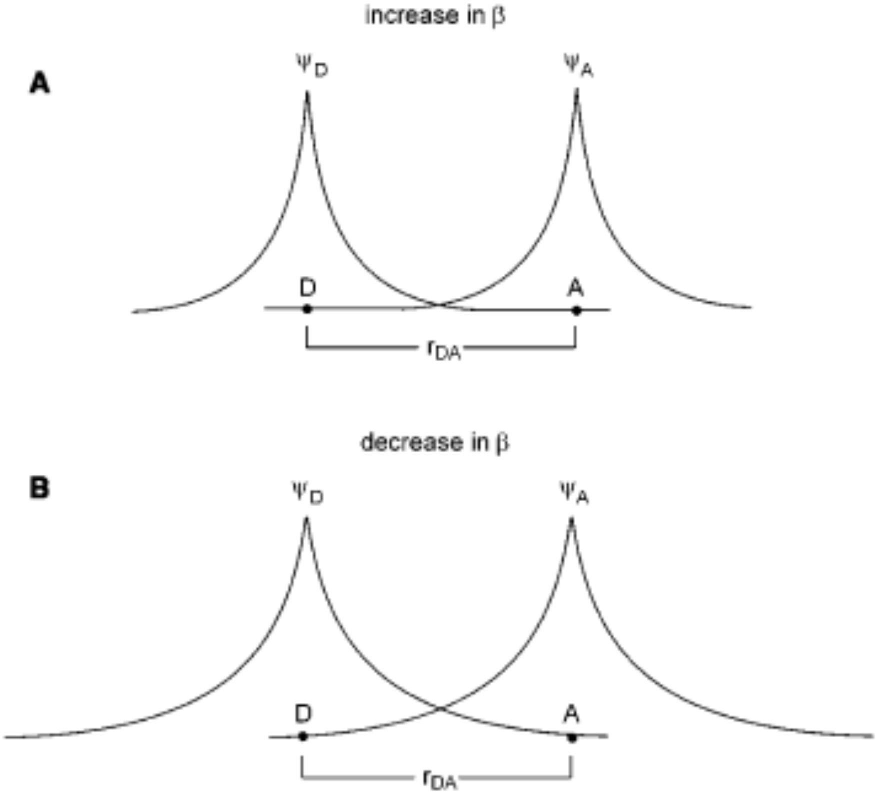
Transfert des électrons: effet de la distance

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

$$H_{\mathrm{AD}} = H_{\mathrm{AD}}{}^\circ \, \mathrm{e}^{-\beta(r_{\mathrm{DA}} - r_o)}$$

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

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



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

Transfert des électrons: méthodes

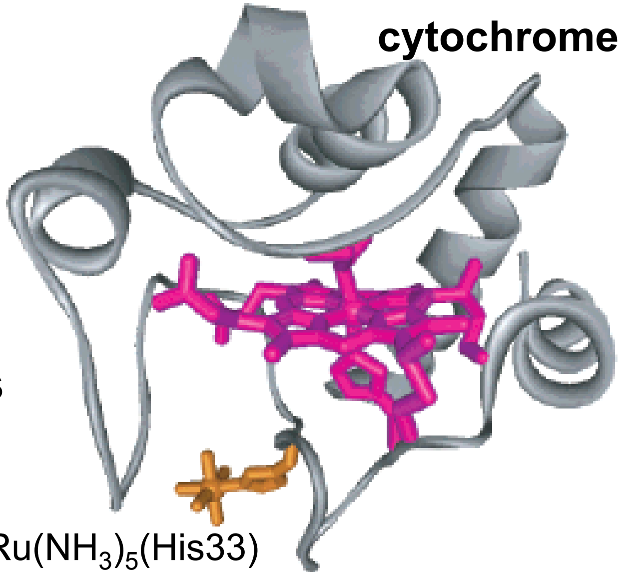
- **Complexes protéine-protéine**

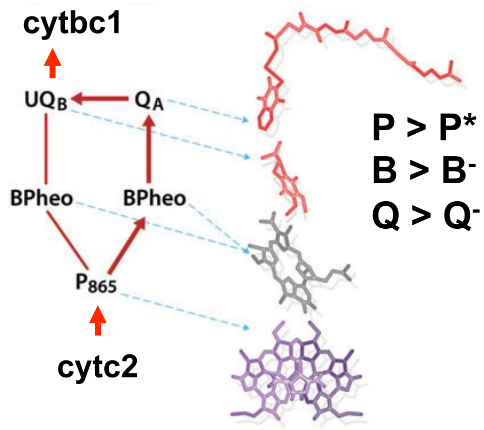
- **Marquage rédox de métalloprotéines**

Ex: cytochromes, protéines à Cu,.

- **Systèmes naturels (modifiés)**

Photosystèmes (non oxygéniques) bactériens





P > P* > P^+ (+0.45V)
B > B^- (-0.6V)
Q > Q^- (A:- 0.2V;
B:-0.08V)

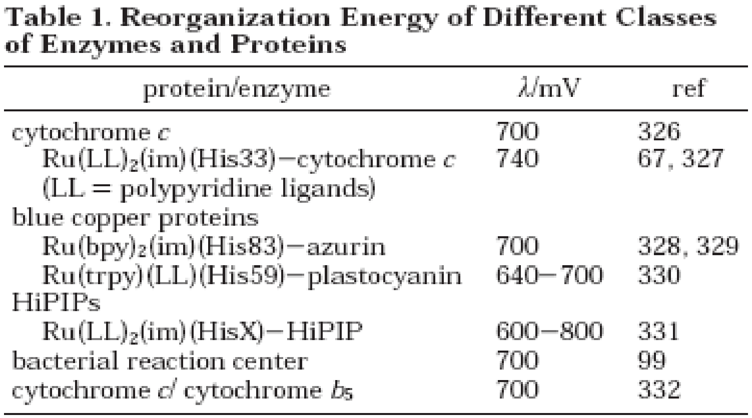
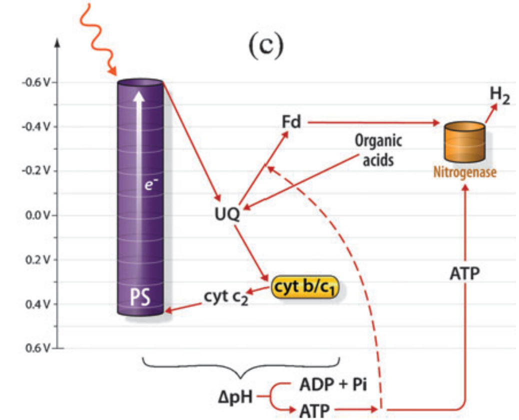
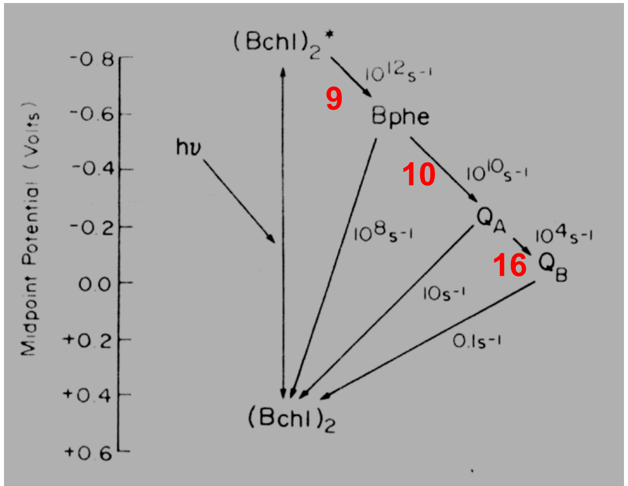


Table 1. Reorganization Energy of Different Classes of Enzymes and Proteins

protein/enzyme	λ/mV	ref
cytochrome *c*	700	326
Ru(LL)$_2$(im)(His33)−cytochrome *c*	740	67, 327
(LL = polypyridine ligands)		
blue copper proteins		
Ru(bpy)$_2$(im)(His83)−azurin	700	328, 329
Ru(trpy)(LL)(His59)−plastocyanin	640−700	330
HiPIPs		
Ru(LL)$_2$(im)(HisX)−HiPIP	600−800	331
bacterial reaction center	700	99
cytochrome *c*/ cytochrome *b*$_5$	700	332



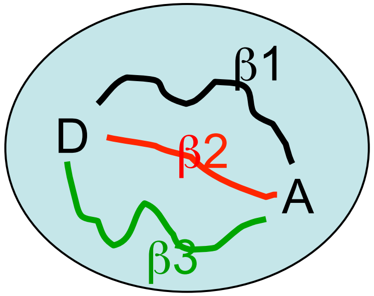


⇨ **λ = 0.7 eV (16 kcal.mol^{-1})**

⇨ **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 β= 1.4 Å^{-1})

β = 1.2 +/- 0.2 Å^{-1} → « Les deux théories convergent »
$\Delta k = 10^3\text{-}10^4$

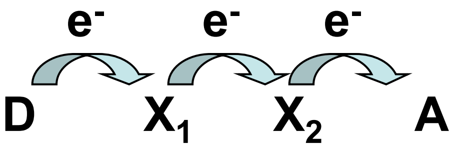
Vide: 3.4

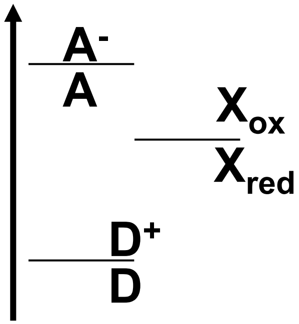
D-A covalent: 0.7

Verre organique (THF): 1.2

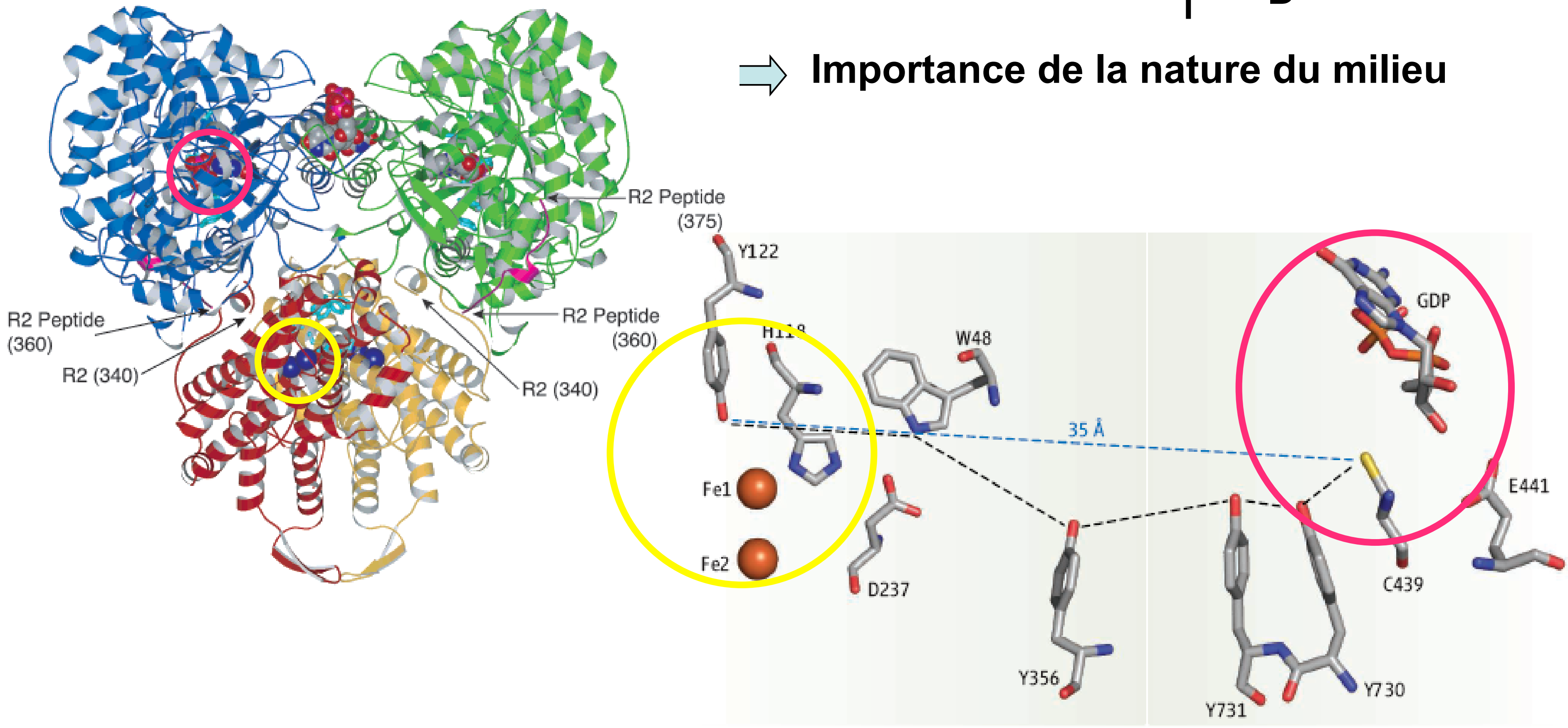
Au-delà de 25 Å ?: cofacteurs intermédiaires « multistep tunneling »

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



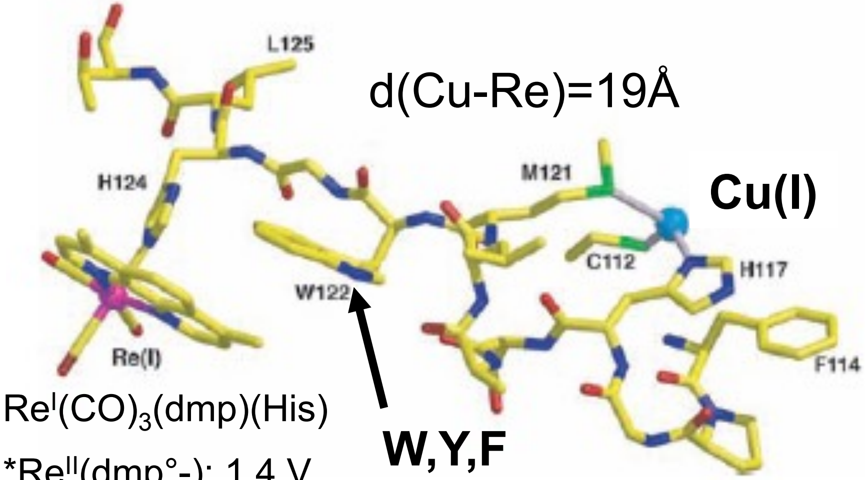


⇒ **Importance de la nature du milieu**



Tryptophan-Accelerated Electron Flow Through Proteins
Crystal Shih, *et al.*
Science **320**, 1760 (2008);
DOI: 10.1126/science.1158241

Modèle: Re-azurin(Cu)



d(Cu-Re)=19Å

Cu(I)

$Re^I(CO)_3(dmp)(His)$

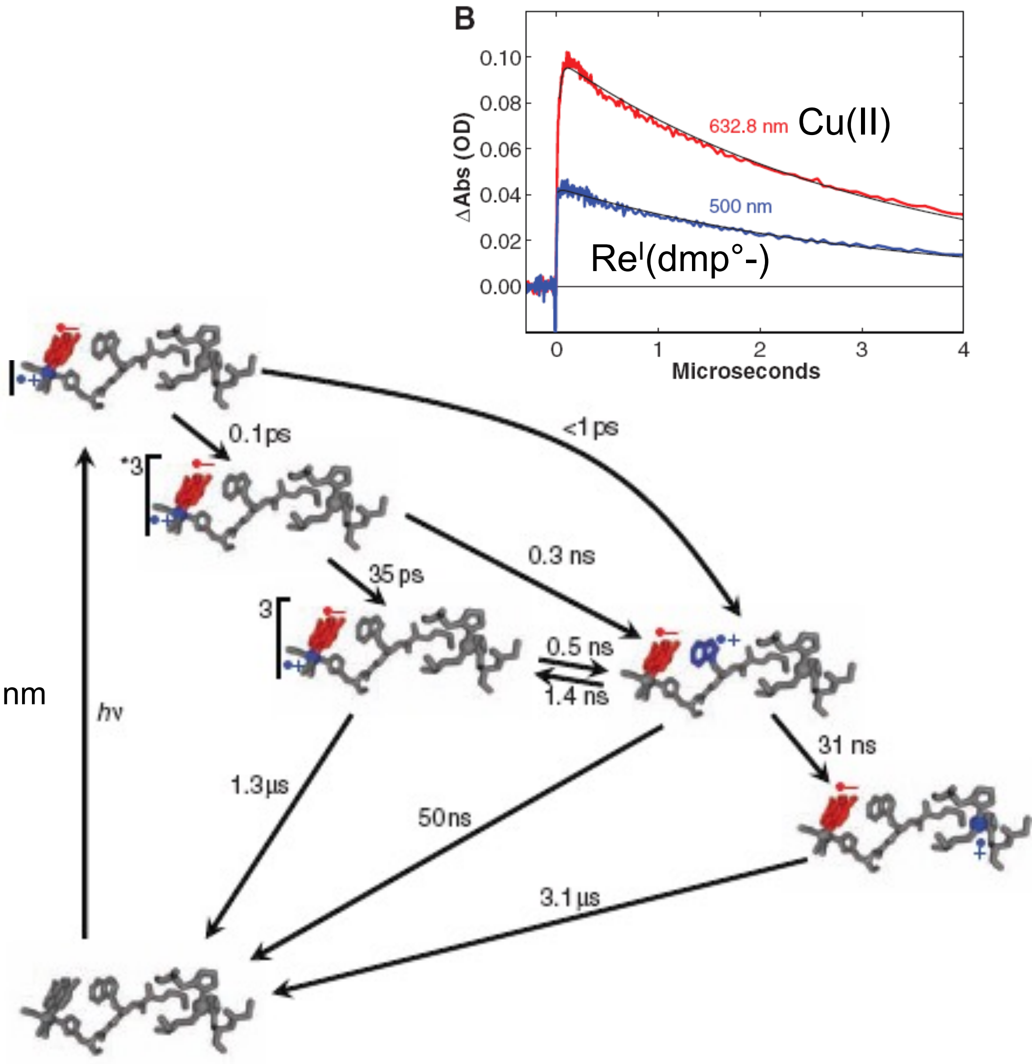
$^*Re^{II}(dmp°^-)$: 1.4 V

W,Y,F

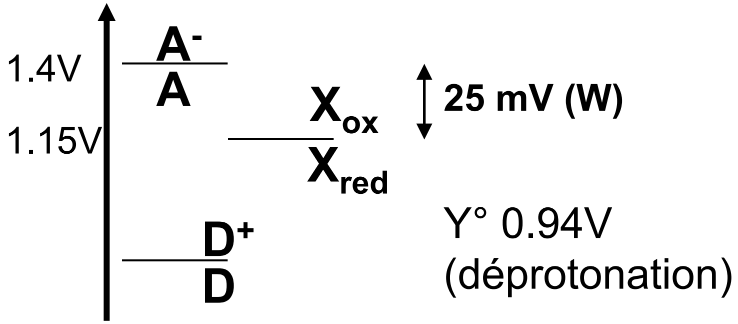
⇩

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 ».



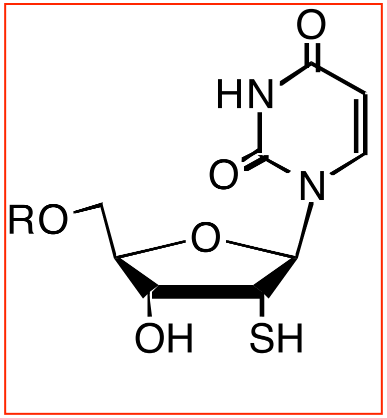
355 nm



25 mV (W)

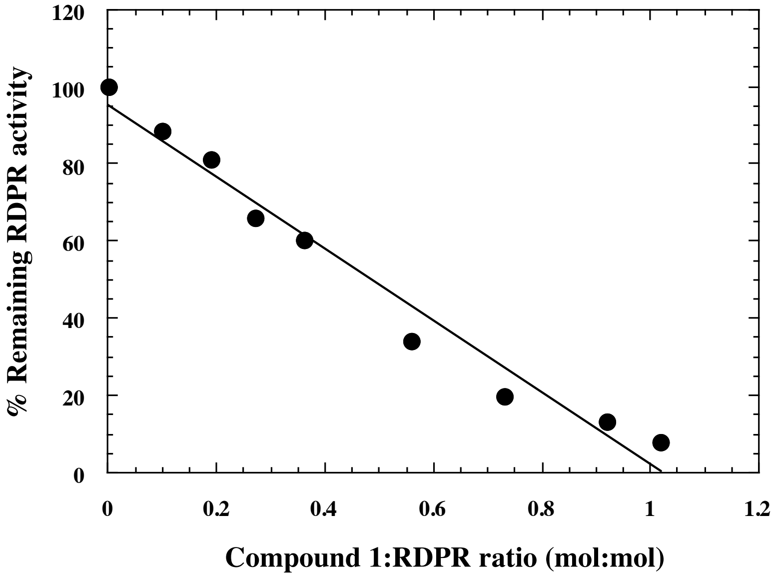
Y° 0.94V
(déprotonation)

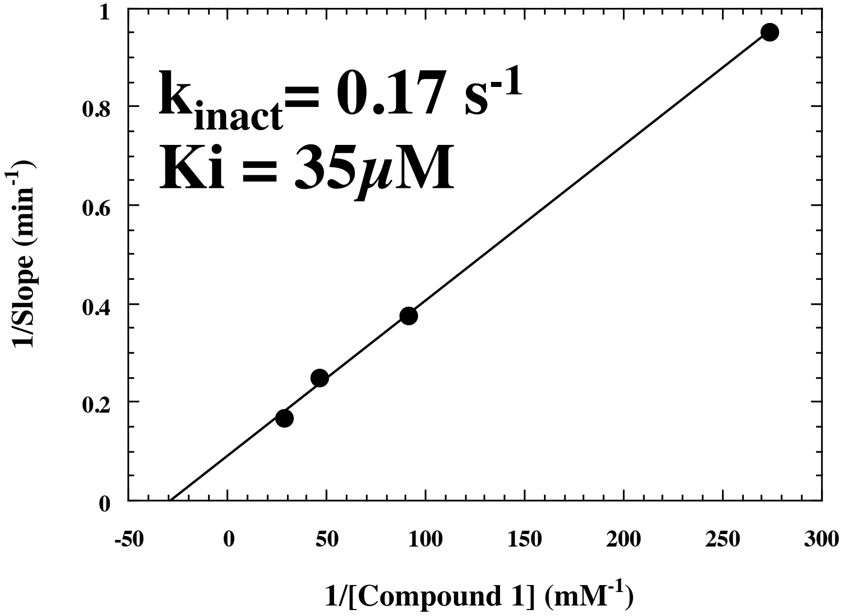
Transfert de radical de R2 à R1:
Analogues de substrat

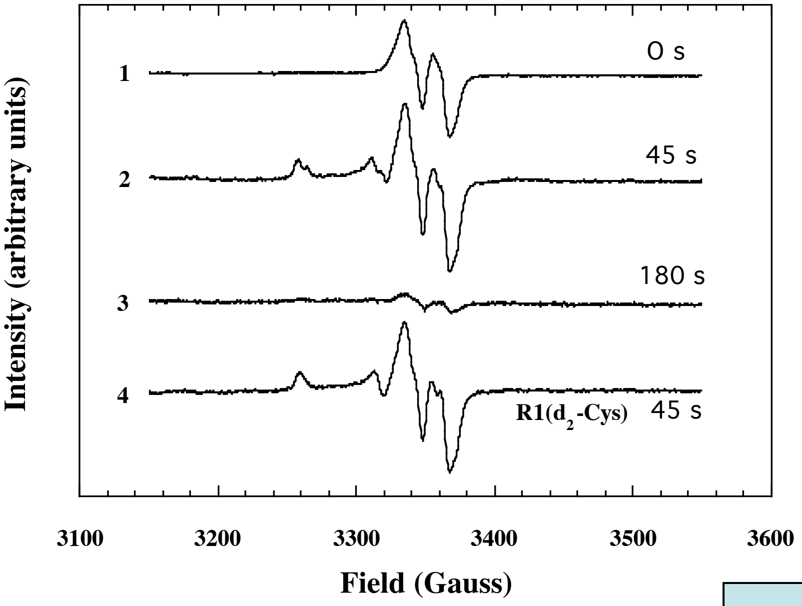


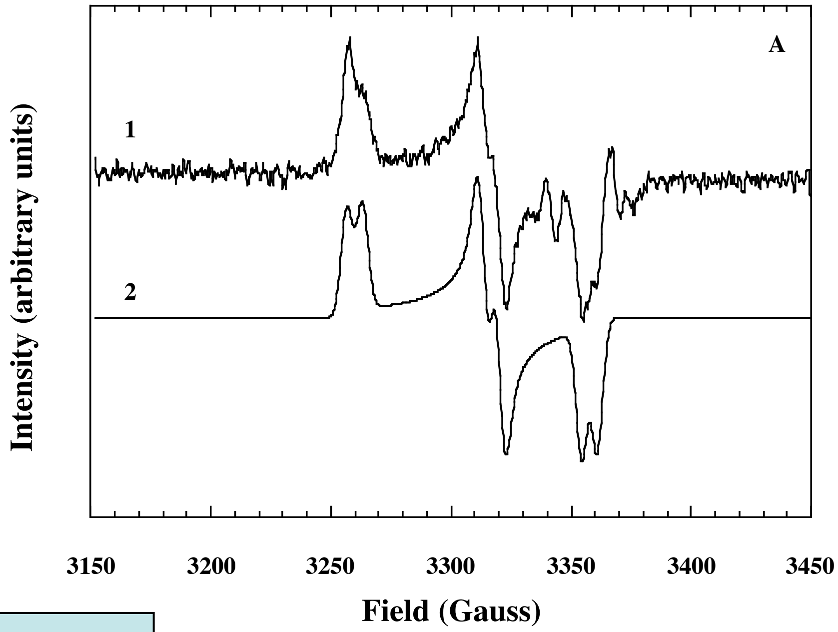
Inactivation of *Escherichia coli* ribonucleotide reductase by 2'-deoxy -2'-mercaptouridine 5'diphosphate - Electron paramagnetic resonance evidence for a transient protein perthiyl radical.
J. Covès, L. Le Hir de Fallois, J.L. Decout, L. Lepape, M. Fontecave.
Biochemistry, 1996 35, 8595-8602

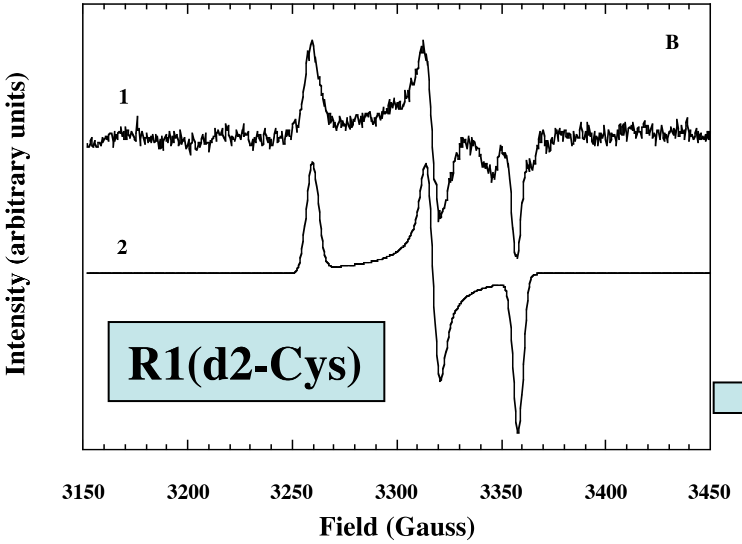
Synthesis of 2'Deoxy-2'-mercaptouridine and cytidine derivatives as potential inhibitors of ribonucleotide diphosphate reductase : Thionitrites, disulfides and 2'-Deoxy-2'-mercaptouridine 5'-Diphosphate.
L. Le Hir de Fallois, J.L. Décout, M. Fontecave
J.Chem.Soc., Perkin Trans., 1997, 2587-2595





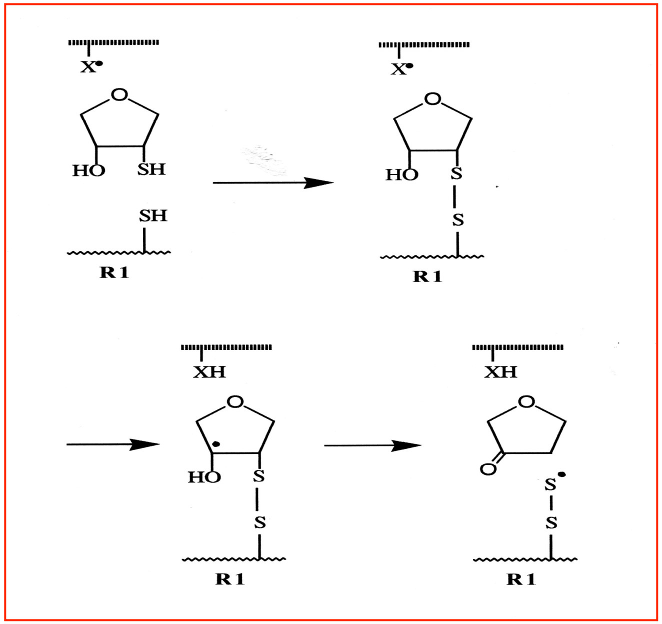






g_x= 2.06
g_y= 2.026
g_z= 2.0019
g_{av}= 2.03
a (mT)= 0.65

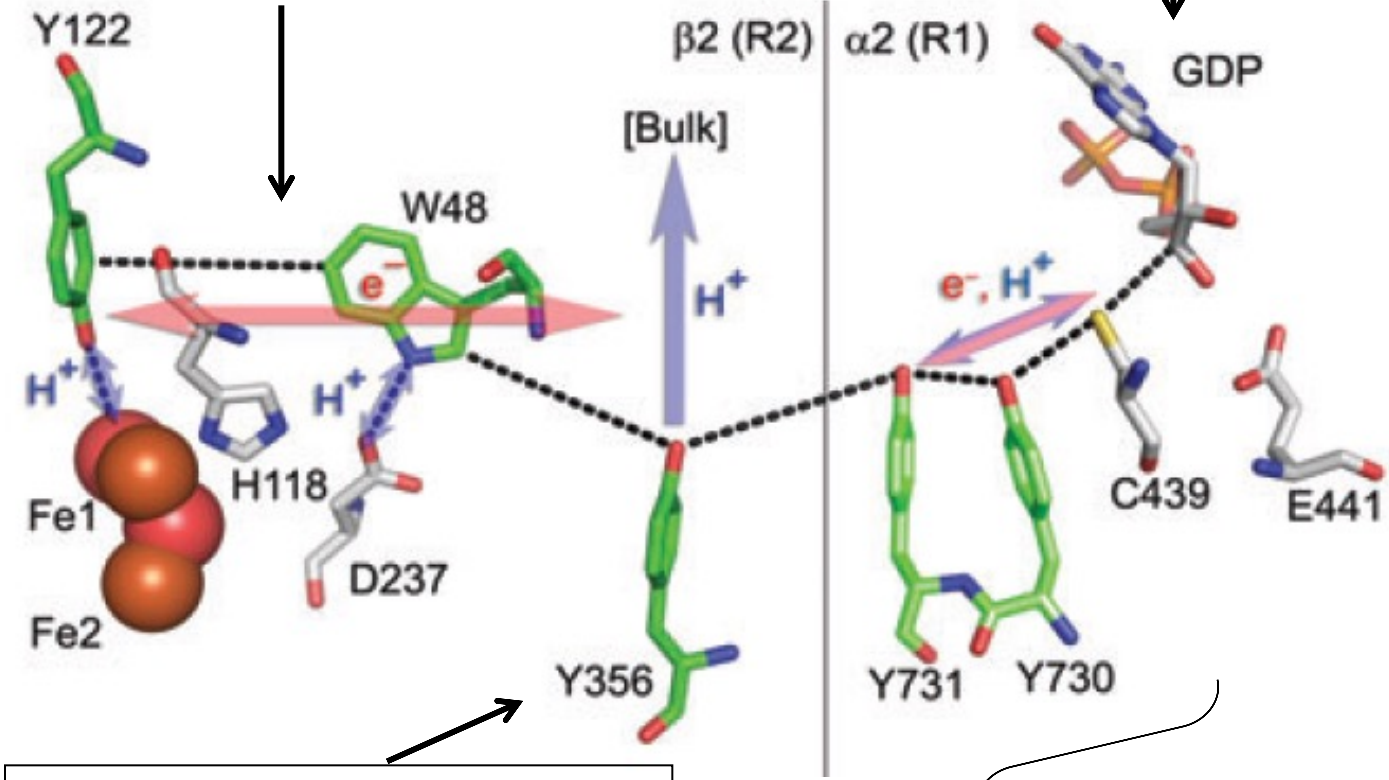
Radical
Persulfure
RSS°
sur R1



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 »

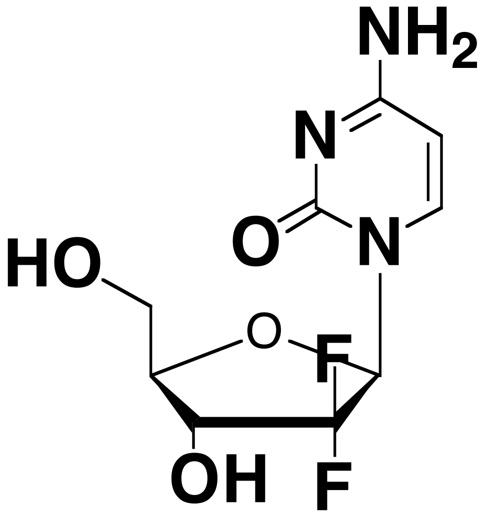
Deux agents anticancéreux
Inhibiteurs de ribonucléotide réductase

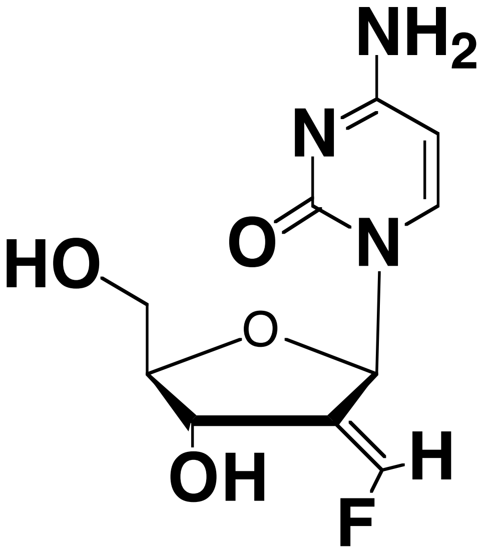
Gemcitabine (Eli-Lilly)

Clinical use against:
- solid tumors
- lung cancer (Pt)
- pancreas cancer

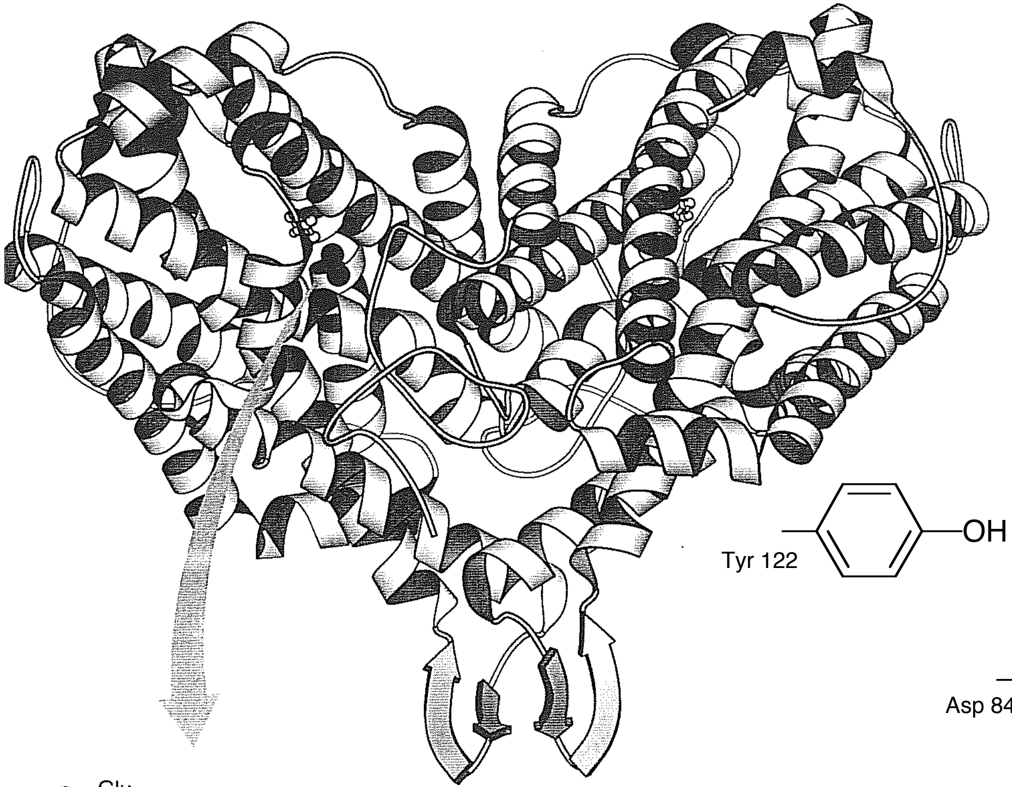
Tézacitabine (HMR)

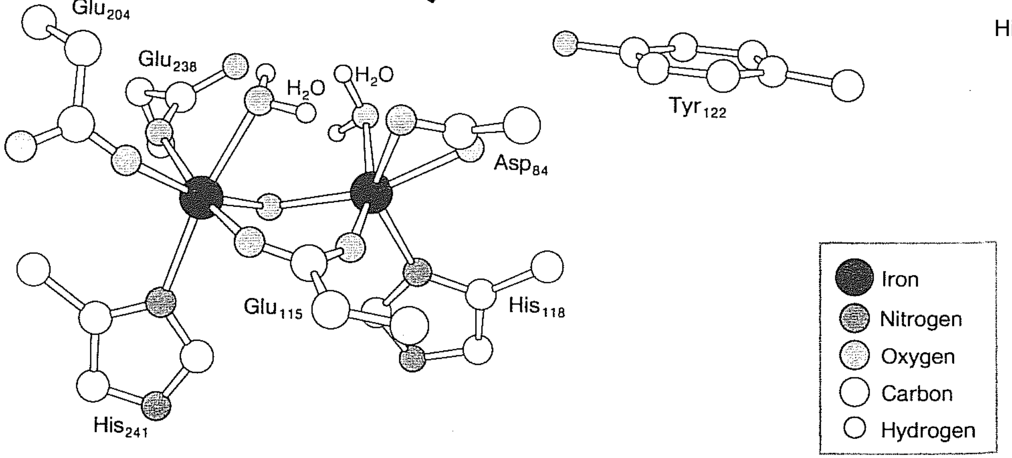
Clinical trial against:
- solid tumors
- breast cancer

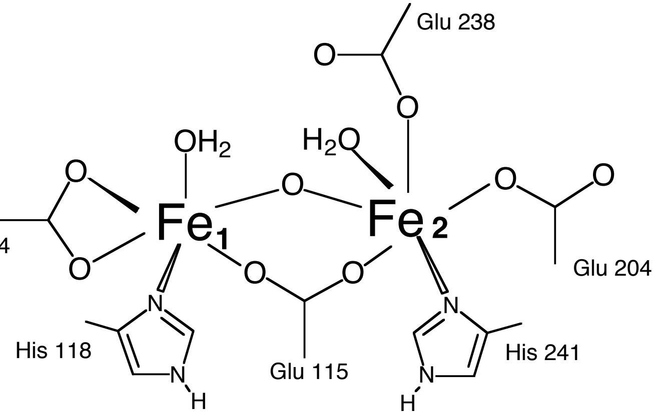




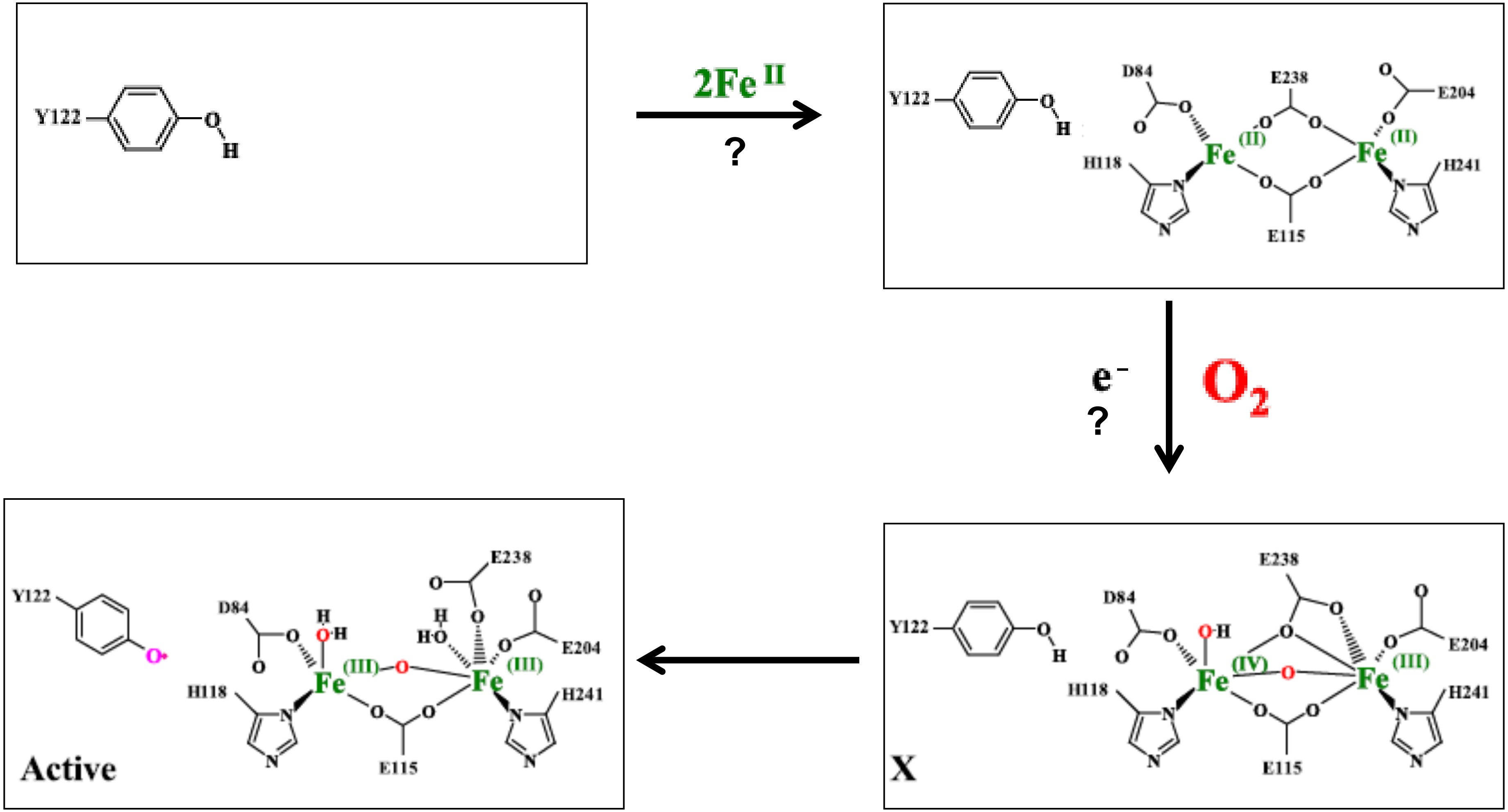
Formation du radical ?



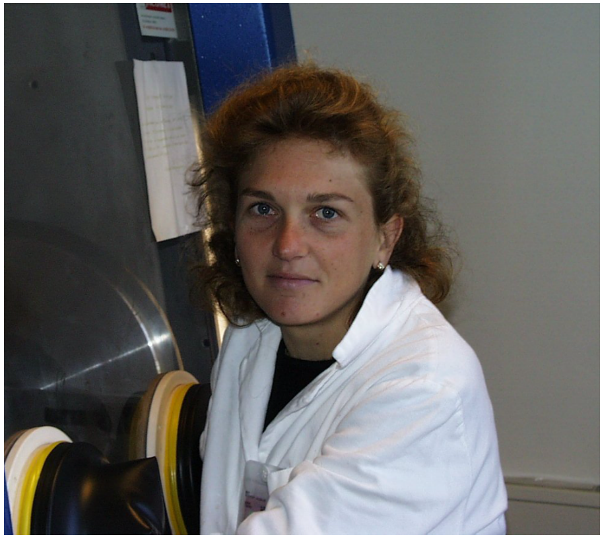




$$\text{R2-Y122-OH} + (\text{Fe(II) Fe(II)} + O_2 + H^+ + e^- \rightarrow$$
$$\text{R2-Y122-O}^\bullet + (\text{Fe(III)-O}^{2-}\text{-Fe(III)}) + H_2O$$



Peter Reichard (Stockholm)



Sandrine Ollagnier-de-Choudens

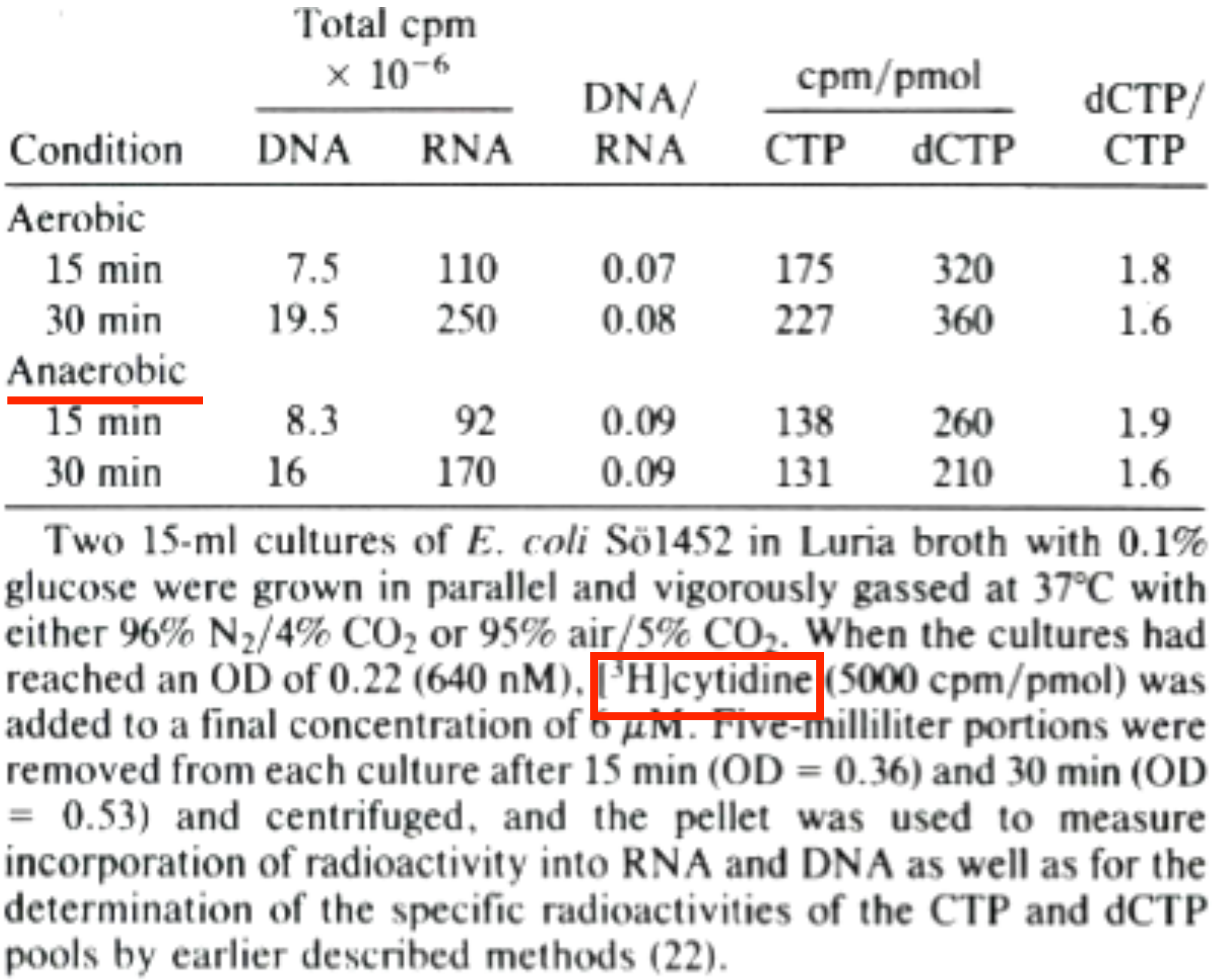
Etienne Mulliez

Comment les désoxyribonucléotides sont-ils biosynthétisés chez les organismes anaérobies ? (1986)

1987: comment E. coli en anaérobiose synthétise les désoxyribonucleotides ?

Oxygen-sensitive ribonucleoside triphosphate reductase in anaerobic *E. coli*
M. Fontecave, R. Eliasson, P. Reichard

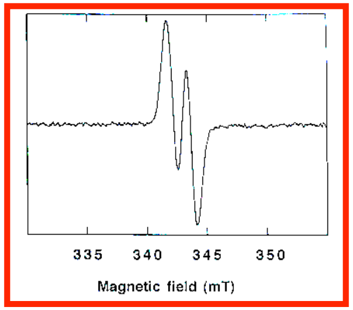
Proc. Natl. Acad. Sci. USA 1989, 86, 2147-2151



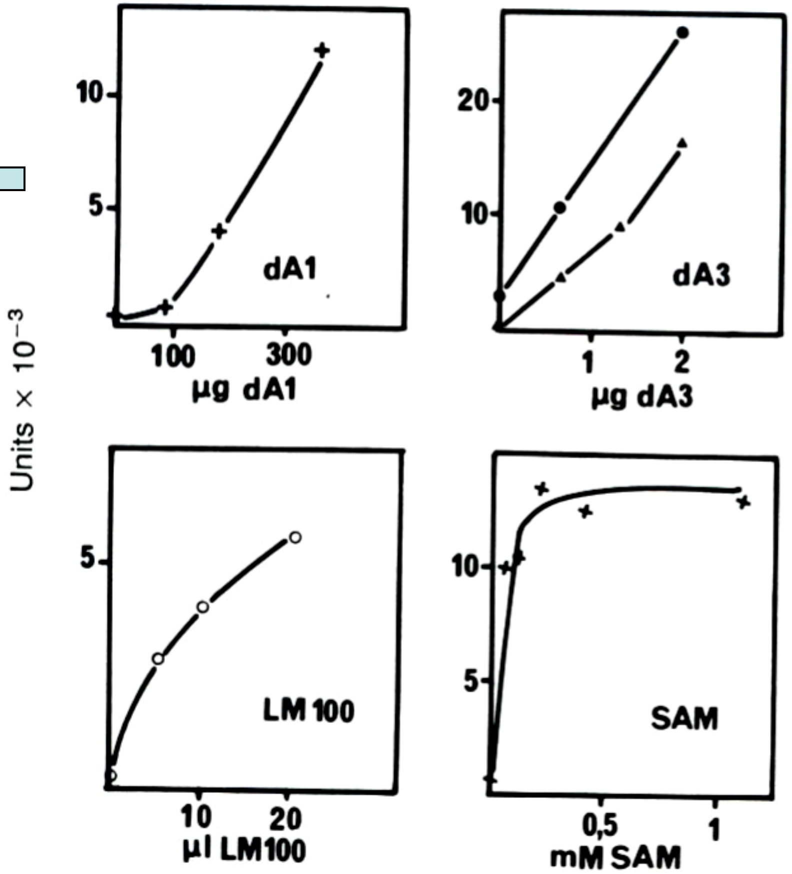
Condition	Total cpm × 10^{-6} DNA	Total cpm × 10^{-6} RNA	DNA/ RNA	cpm/pmol CTP	cpm/pmol dCTP	dCTP/ CTP
Aerobic						
15 min	7.5	110	0.07	175	320	1.8
30 min	19.5	250	0.08	227	360	1.6
Anaerobic						
15 min	8.3	92	0.09	138	260	1.9
30 min	16	170	0.09	131	210	1.6

Two 15-ml cultures of *E. coli* Sö1452 in Luria broth with 0.1% glucose were grown in parallel and vigorously gassed at 37°C with either 96% N_2/4% CO_2 or 95% air/5% CO_2. When the cultures had reached an OD of 0.22 (640 nM), [^{3}H]cytidine (5000 cpm/pmol) was added to a final concentration of 6 μM. Five-milliliter portions were removed from each culture after 15 min (OD = 0.36) and 30 min (OD = 0.53) and centrifuged, and the pellet was used to measure incorporation of radioactivity into RNA and DNA as well as for the determination of the specific radioactivities of the CTP and dCTP pools by earlier described methods (22).

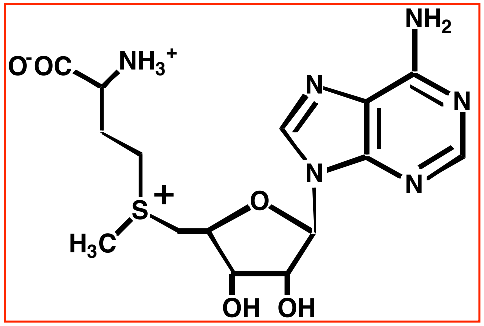
1989-1995: fractionnement et identification



Flavodoxine réductase



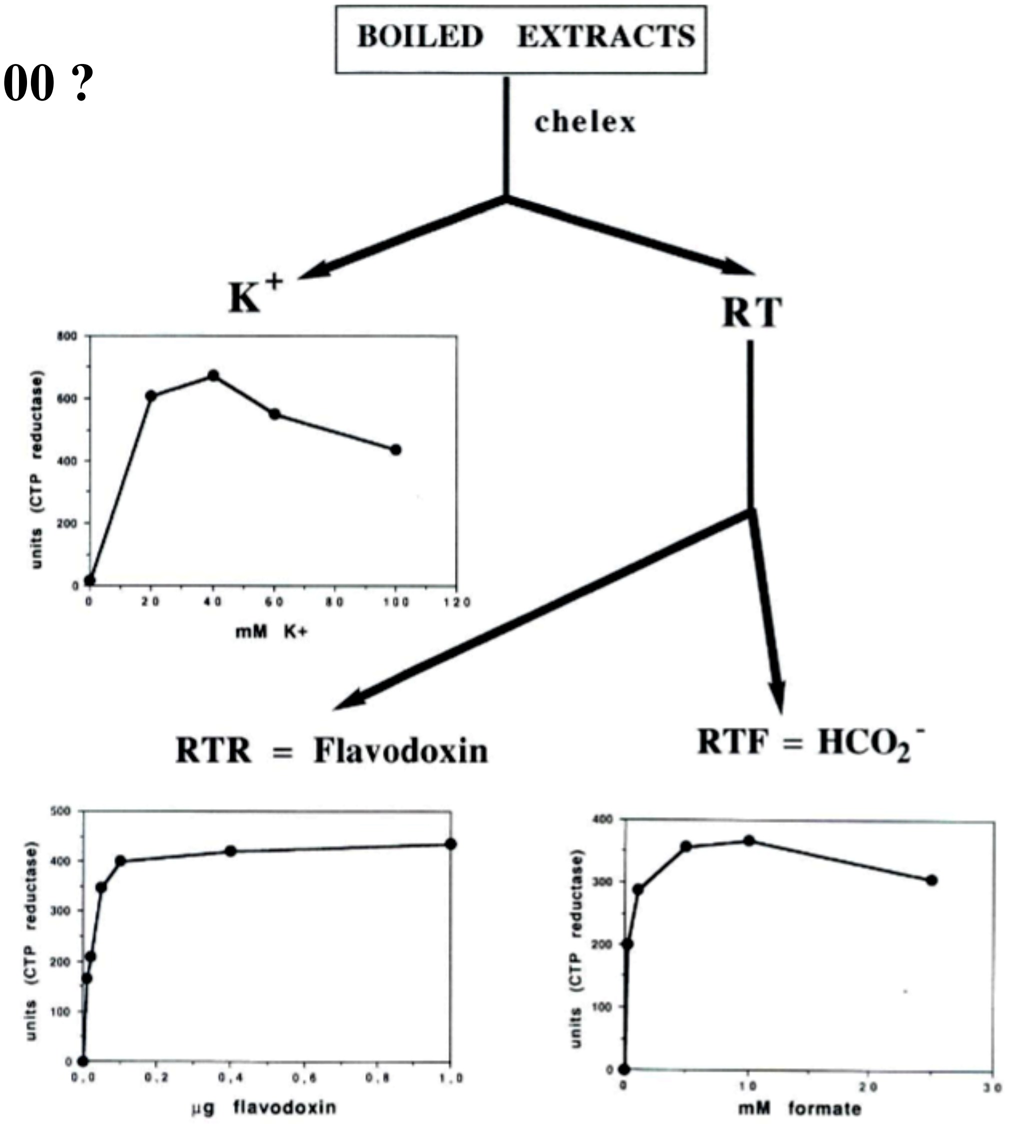
La RNR
-radical organique



The anaerobic ribonucleoside triphosphate reductase from *Escherichia Coli* requires **S-adenosylmethionine** as a cofactor
R. Eliasson, M. Fontecave, H. Jornvall, M. Krook, E. Pontis, P. Reichard
Proc. Natl. Acad. Sci. USA 1990, 87, 3314-3318

An iron-sulfur center and **a free radical** in the active anaerobic ribonucleotide reductase of *Escherichia coli*
E. Mulliez, M. Fontecave, J. Gaillard, P. Reichard
J. Biol. Chem. 1993, 268, 2296-2299

LM 100 ?



BOILED EXTRACTS

chelex

K^+

RT

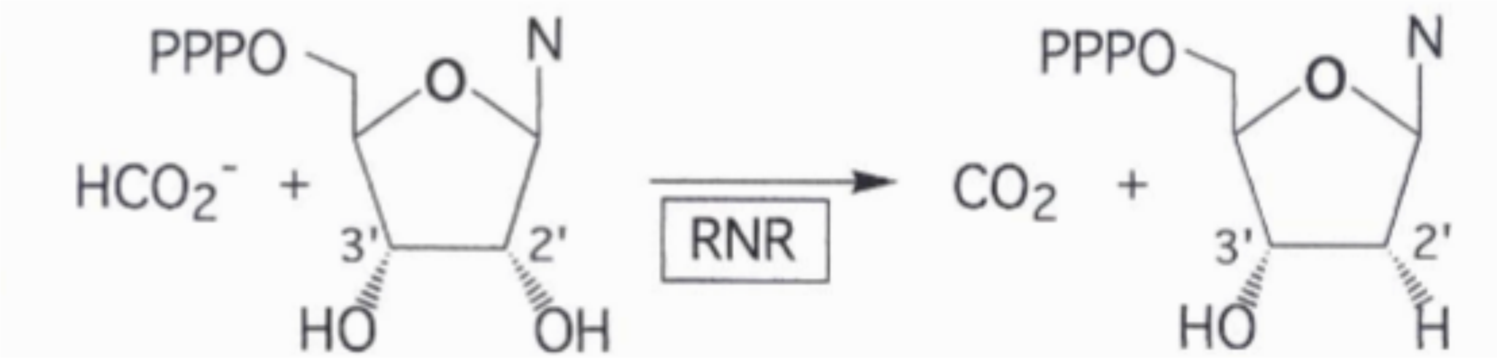
RTR = Flavodoxin

RTF = HCO_2^-

Flavodoxin is required for the activation of the anaerobic ribonucleotide reductase
V. Bianchi, R. Eliasson, M. Fontecave, E. Mulliez, D.M. Hoover, R.G. Matthews, P. Reichard
Biochem. Biophys. Res.Commun. 1993, 197, 792-797

Formate as hydrogen donor for the anaerobic ribonucleotide reductase from *Escherichia coli*
E. Mulliez, S. Ollagnier, M. Fontecave, R. Eliasson, P. Reichard
Proc. Natl. Acad. Sci. 1995, 92, 8759-8762

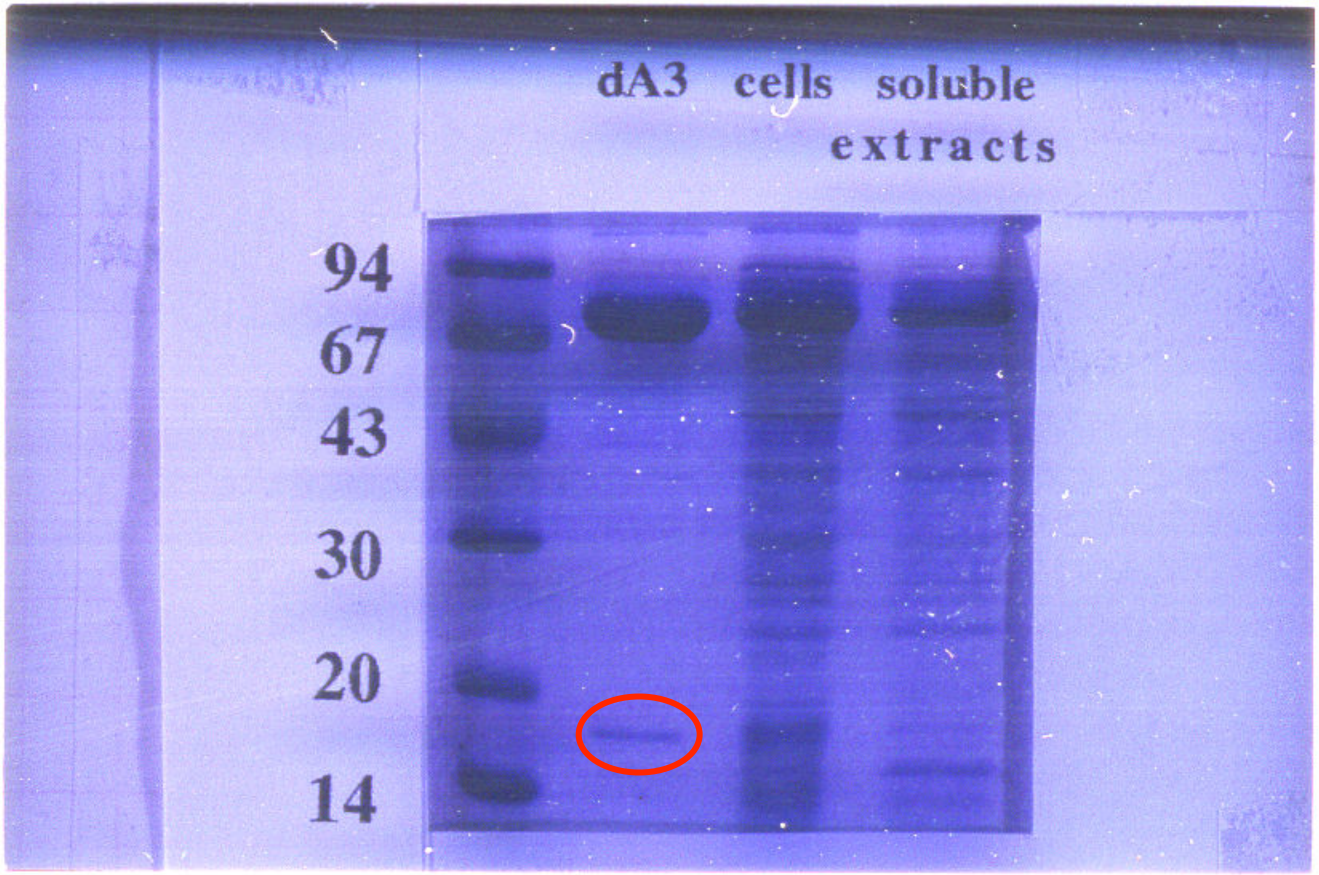
La ribonucléotide réductase anaérobie



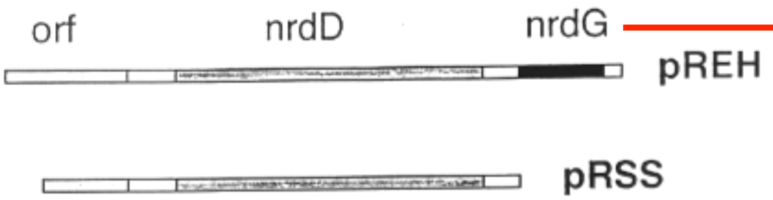
RNA

DNA

1995: La ribonucléotide réductase est un complexe à deux composants !!

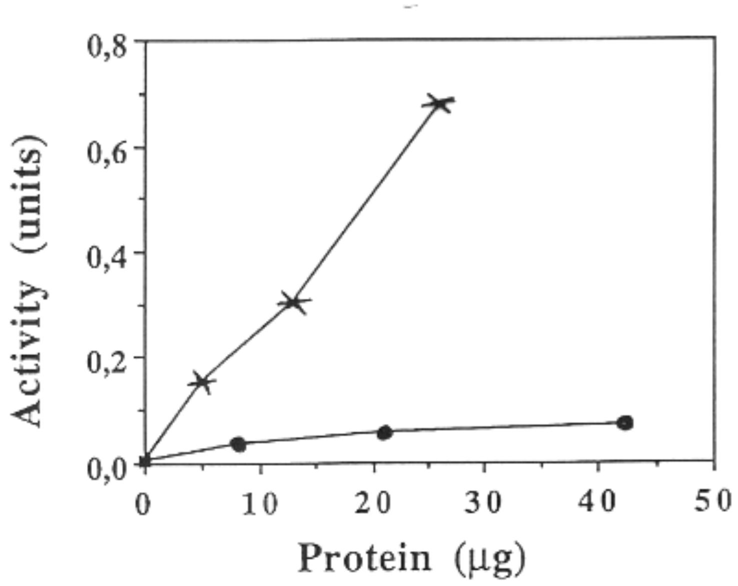


1995: La ribonucléotide réductase (nrdD) est active en présence d’ un «contaminant »: l’ activase (nrdG)



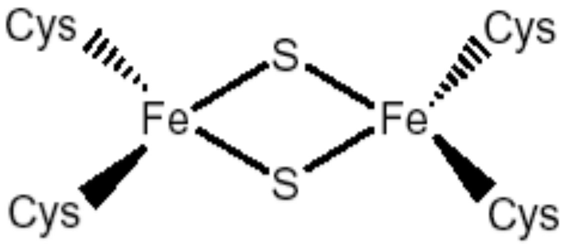
CX_3CX_2C

Activation of reductase prepared from E. coli carrying plasmid **pRSS** with extracts from bacteria carrying **pRSS** (●) or **pREH** (X).

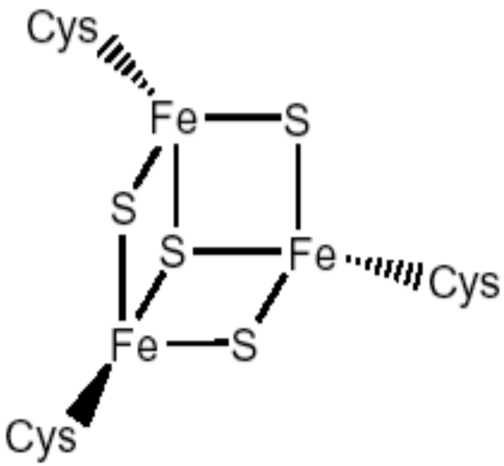


nrdG est une protéine fer-soufre
(1996-1999)

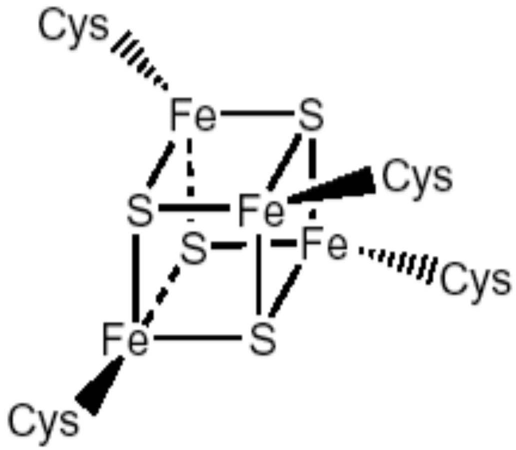
Centres fer-soufre



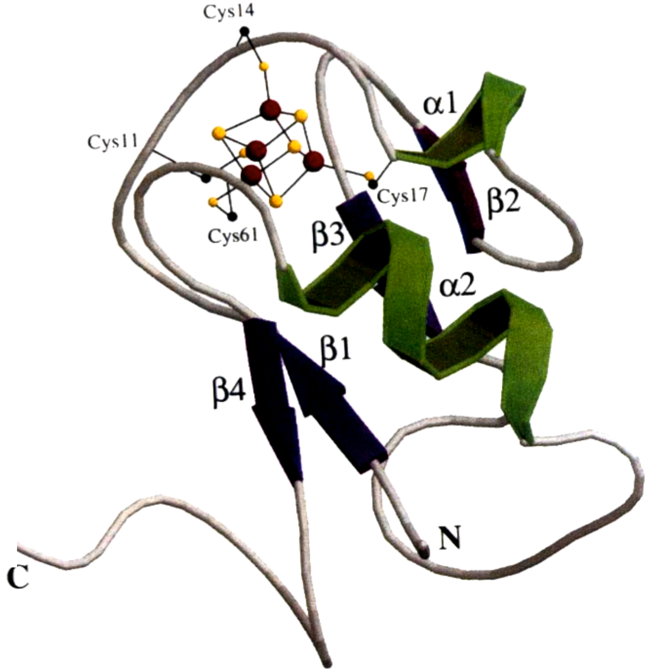
2Fe–2S



3Fe–4S

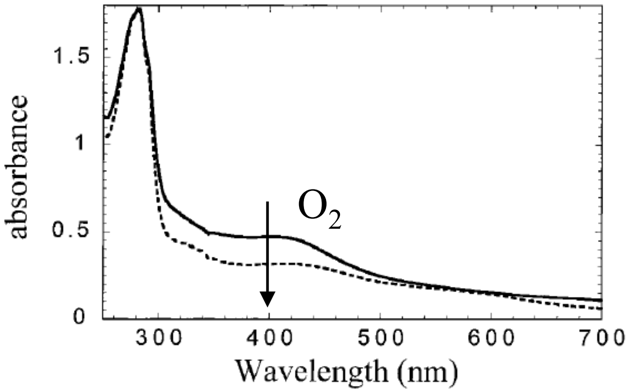


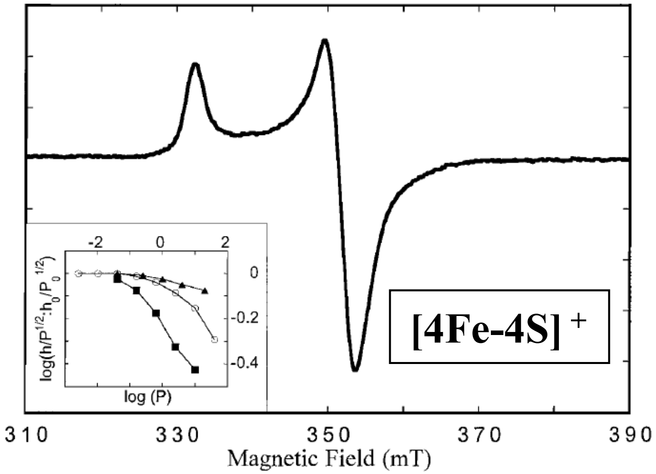
4Fe–4S



Activase
nrdG
2x17.5 kDa

J. Tamarit, M. Fontecave et al
J. Biol. Chem 1999 274 31291





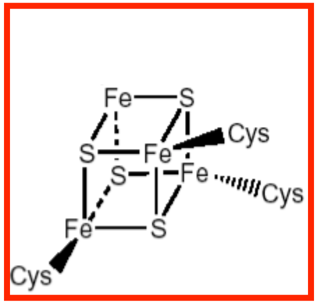
[4Fe-4S]$^{2+}$ **S=0**

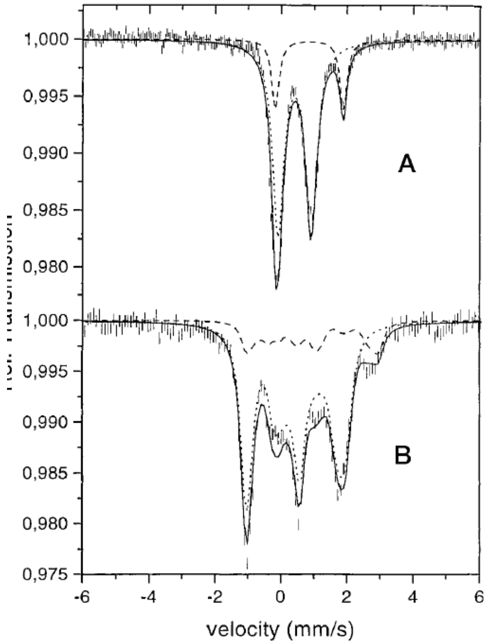
e^- ↓↑

[4Fe-4S]$^+$ **S=1/2**

O_2

[2Fe-2S]$^{2+}$



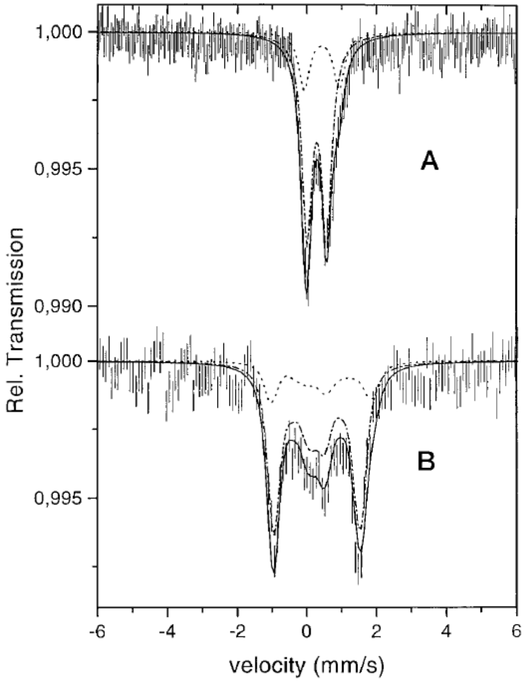


[4Fe-4S]$^{2+}$

A. 77K, 20 mT

B. 4.2K, 7T

O_2

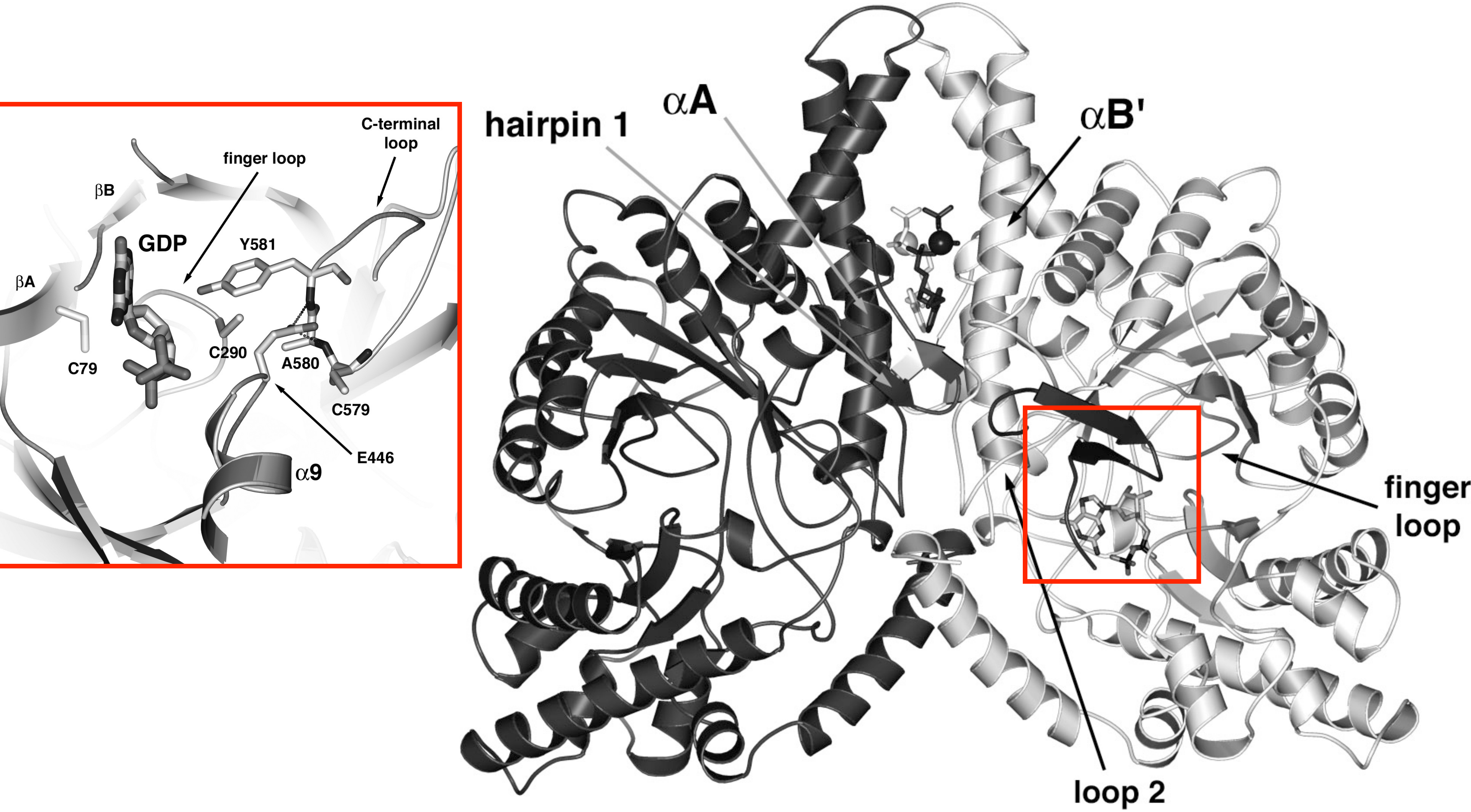


[2Fe-2S]$^{2+}$

A. 77K, 20 mT

B. 4.2K, 7T

1999: structure tridimensionnelle de la ribonucléotide réductase α2 (nrdD)



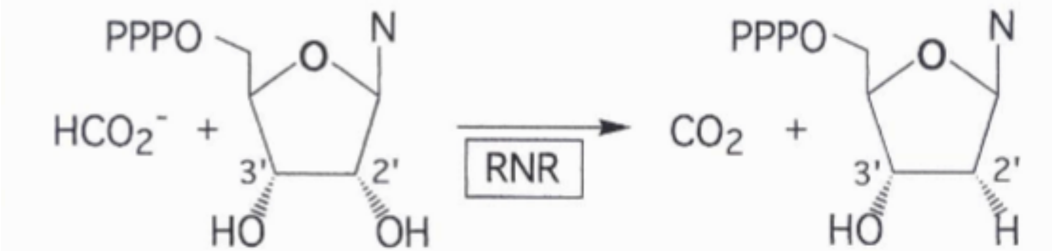
Logan, D.T., Andersson, J., Sjoberg, B.-M., and Nordlund, P. (1999). A glycyl radical site in the crystal structure of a class III ribonucleotide reductase. Science *283*, 1499–1504.

La ribonucléotide réductase: Une enzyme radicalaire

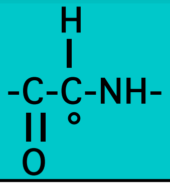
M. Fontecave, E. Mulliez, D. Logan

Progress in Nucleic Acid Research and Molecular Biology 2002, *72*, *95-127*

Proc. Natl Acad. Sci. 2003, 100, 3826-3831



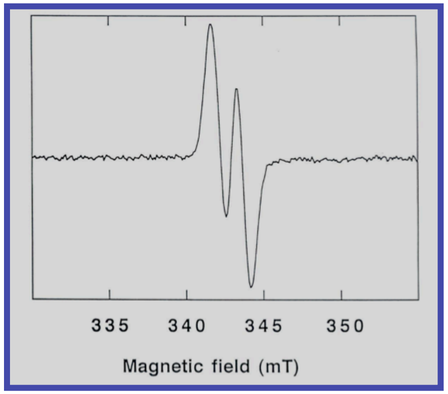
Réductase
nrdD
2x80kDa

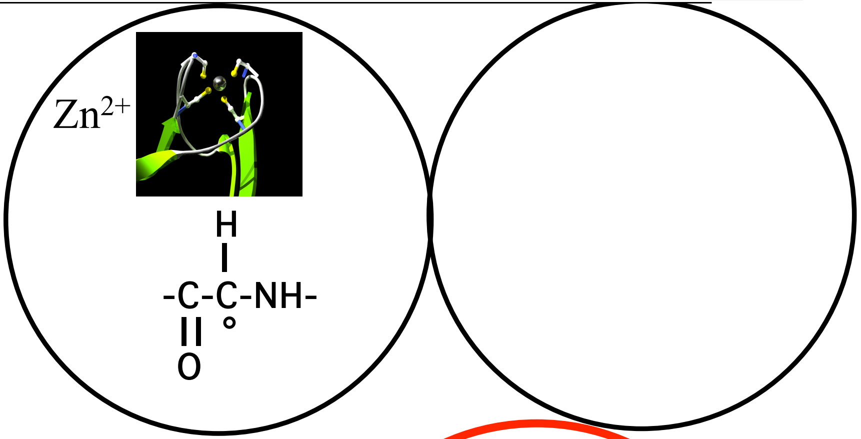


$-\underset{\underset{O}{\|}}{C}-\underset{\circ}{\overset{\overset{H}{|}}{C}}-NH-$

= glycyl radica

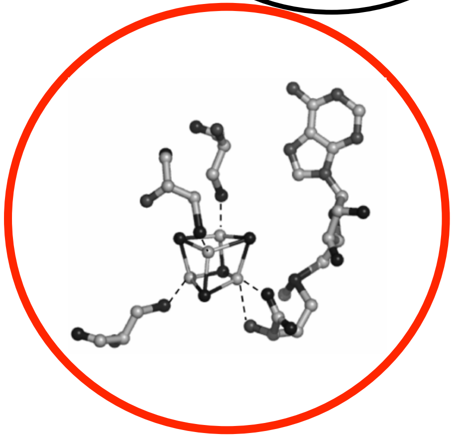
Protéine α





$-\underset{\underset{O}{\|}}{C}-\underset{\circ}{\overset{\overset{H}{|}}{C}}-NH-$

Activase
nrdG
2x17.5 kDa

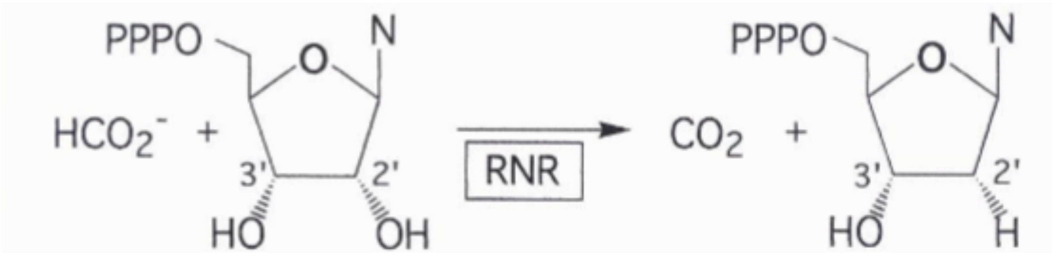


protéine β

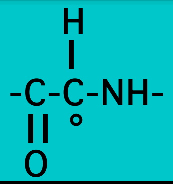
CX_3CX_2C

La ribonucléotide réductase: Une enzyme radicalaire

M. Fontecave, E. Mulliez, D. Logan
Progress in Nucleic Acid Research and Molecular Biology 2002, 72, 95-127
Proc. Natl Acad. Sci. 2003, 100, 3826-3831



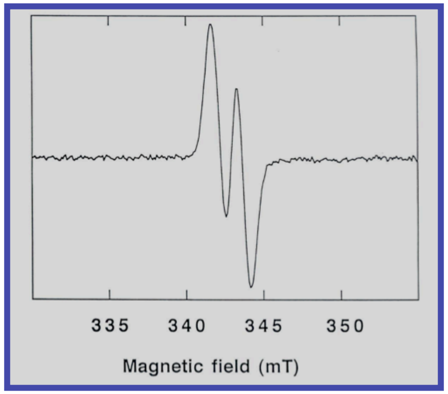
Réductase
nrdD
2x80kDa

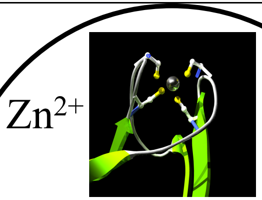


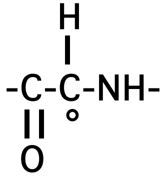
$$-\underset{\underset{O}{\|}}{C}-\overset{\overset{H}{|}}{\underset{\circ}{C}}-NH-$$

= glycyl radica

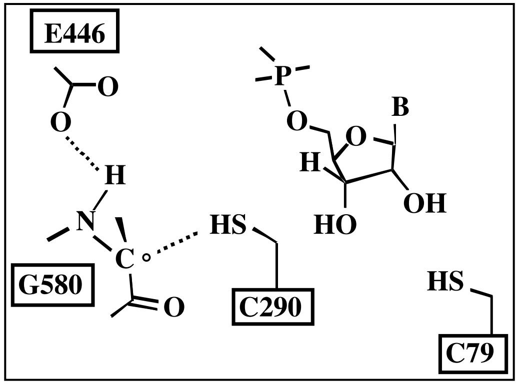
Protéine α



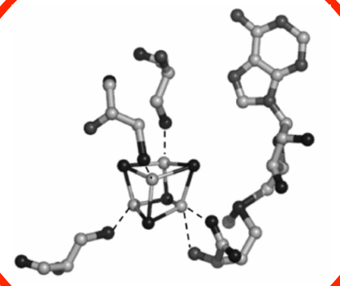




$$-\underset{\underset{O}{\|}}{C}-\overset{\overset{H}{|}}{\underset{\circ}{C}}-NH-$$

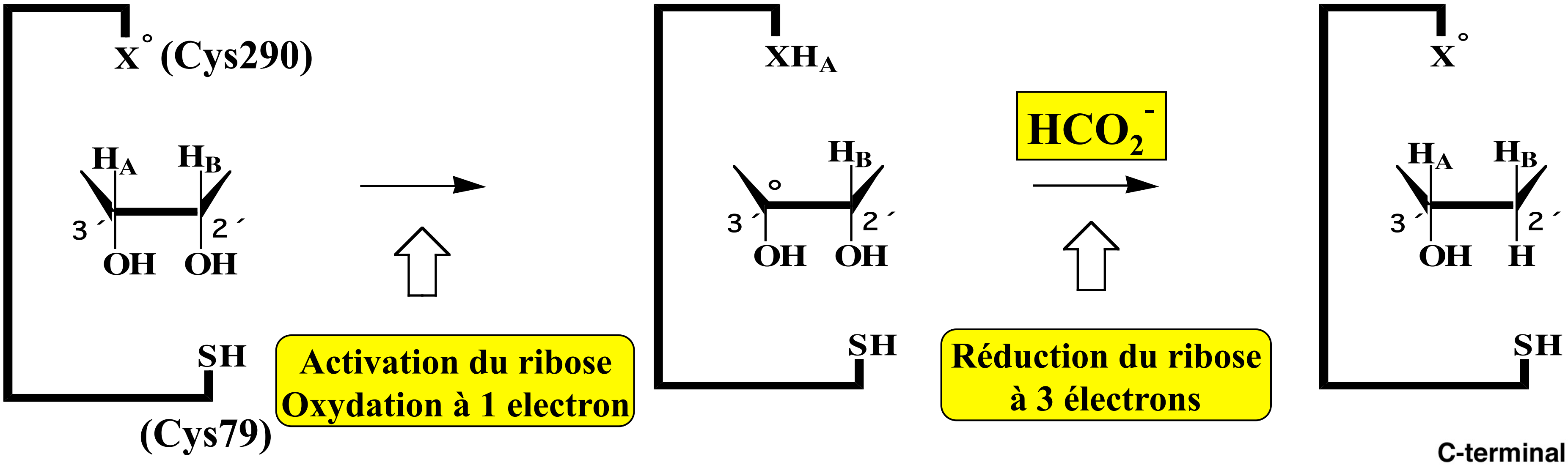


Activase
nrdG
2x17.5 kDa



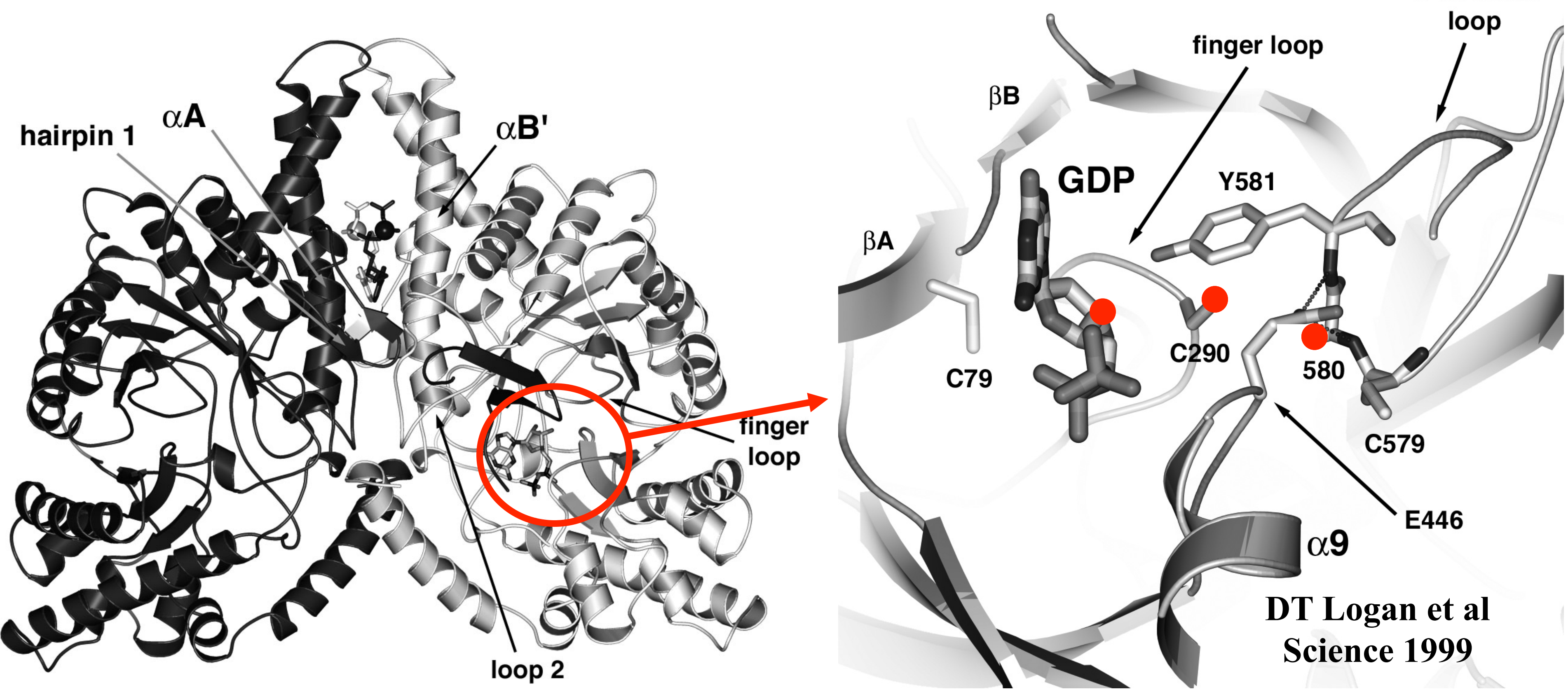
protéine β

CX_3CX_2C



Activation du ribose
Oxydation à 1 electron

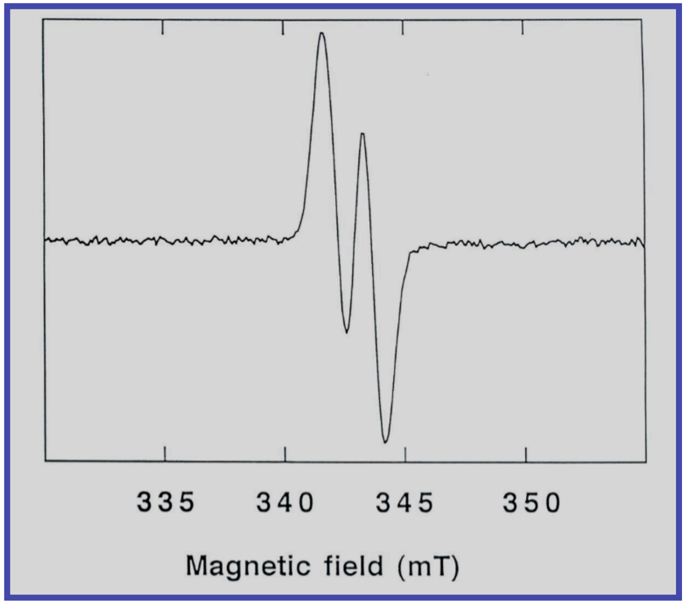
Réduction du ribose
à 3 électrons

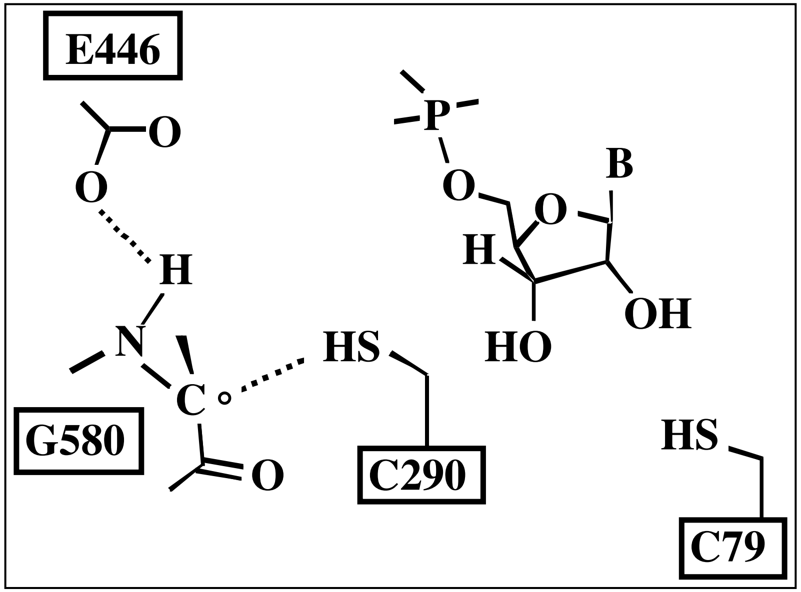


DT Logan et al
Science 1999

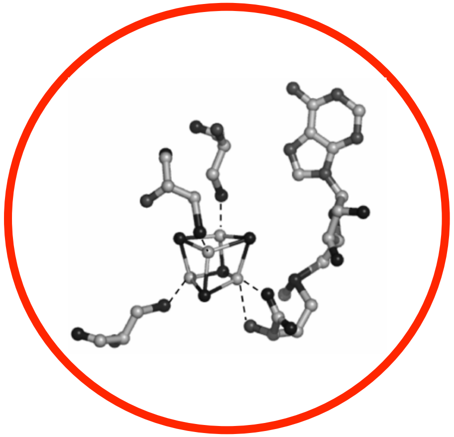
Formation du radical glycinyle ?

Fonction de l’activase (protéine β)





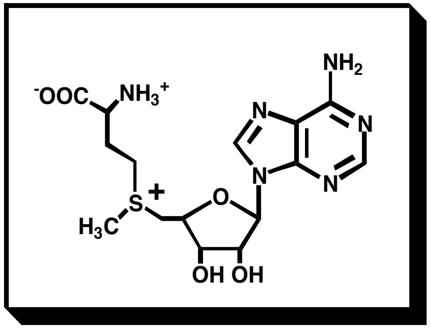
Activase
nrdG
2x17.5 kDa

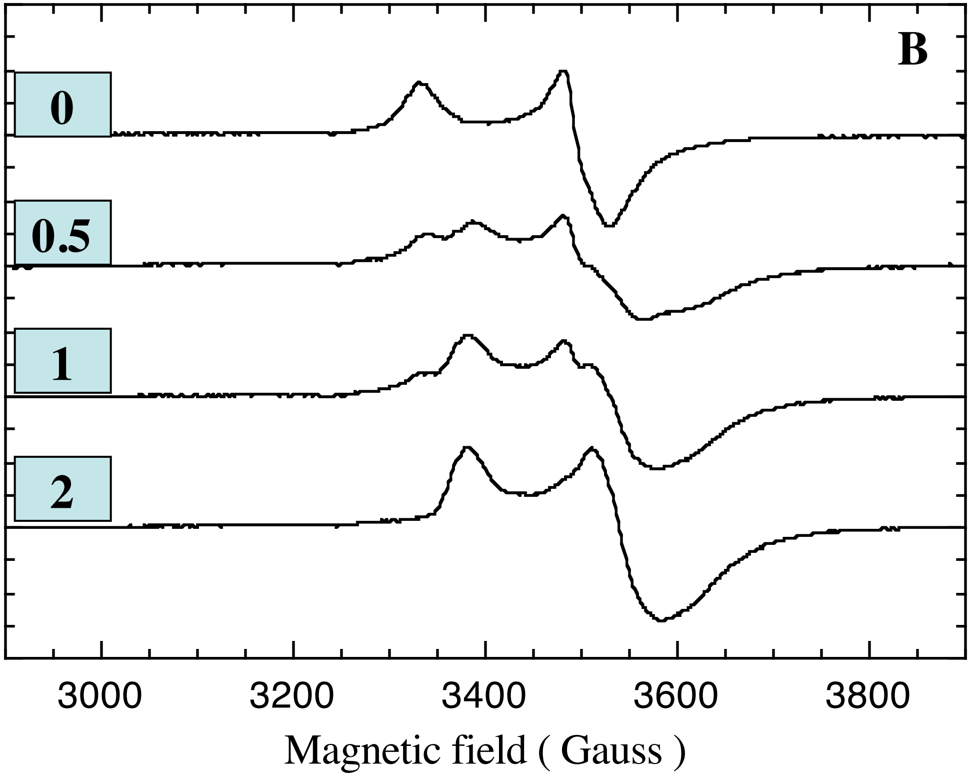


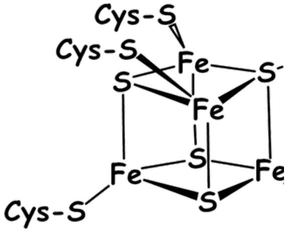
Un complexe [4Fe-4S]-SAM dans l'activase

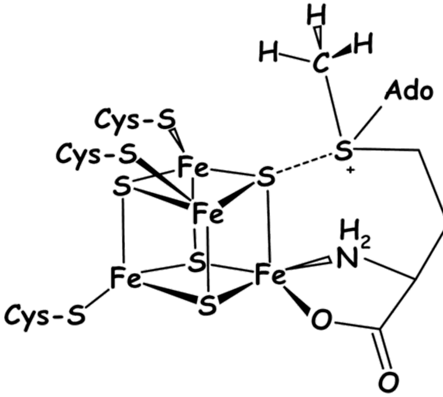
Protéine β

$$(4Fe\text{-}4S)^{+} \xrightarrow[\text{SAM}]{} (4Fe\text{-}4S)^{+}\text{---SAM}$$







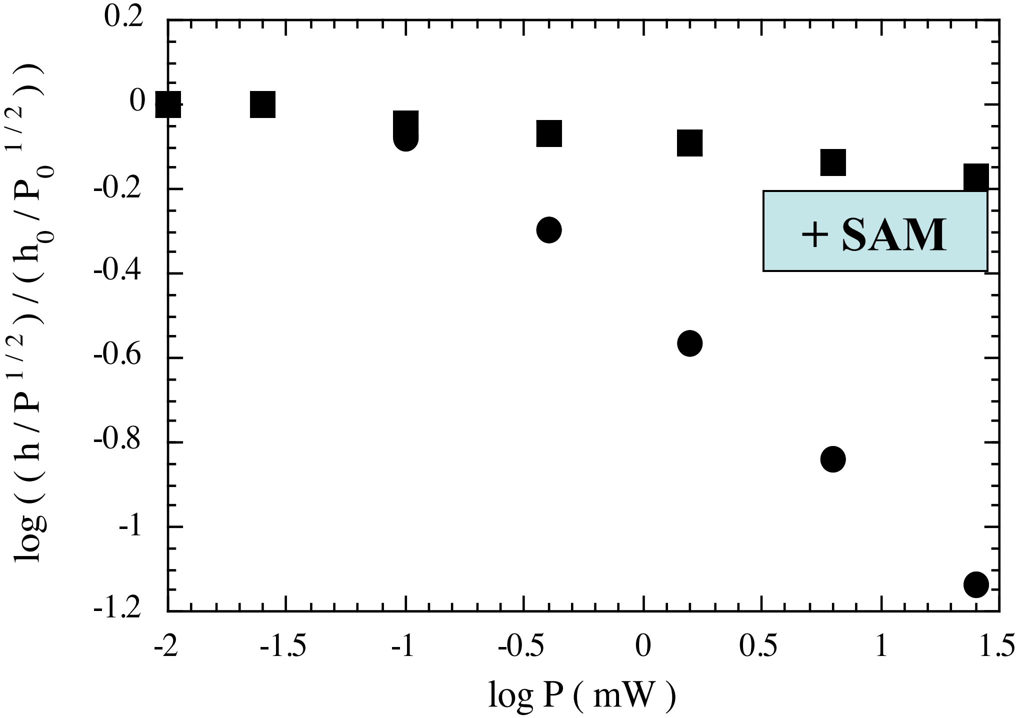


Resonance paramagnétique électronique

Un complexe [4Fe-4S]-SAM dans l’activase

$$(4Fe\text{-}4S)^{+} \xrightarrow[\text{SAM}]{} (4Fe\text{-}4S)^{+}\text{---SAM}$$

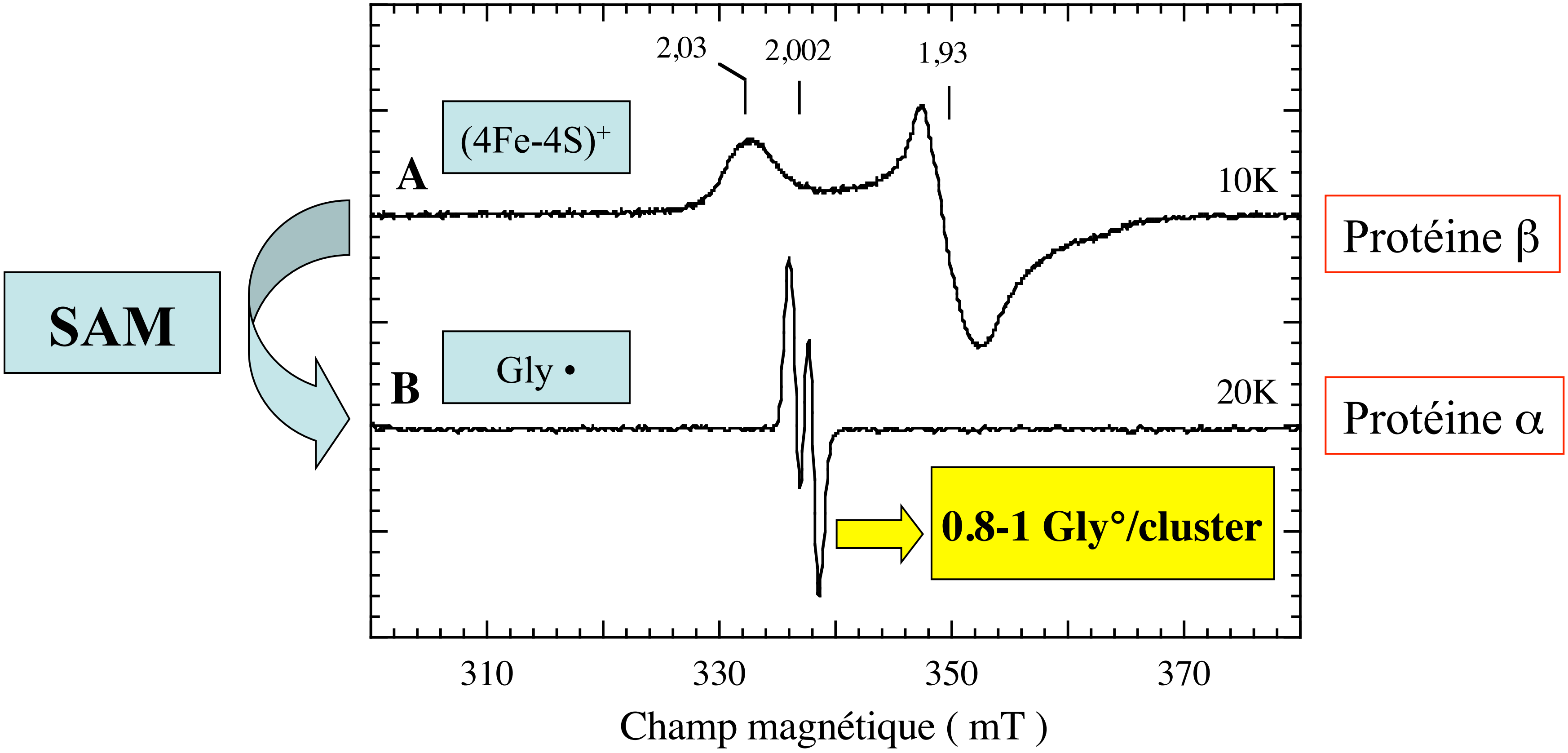
Figure 2



Activation de la ribonucléotide réductase: d'une espèce paramagnétique à une autre dans le complexe α2β2

A : 220 µM ; 25 µW ; $5*10^4$; 1 mT ; 9,45 GHz

B : 220 µM ; 0,25 µW ; $2*10^5$; 1 mT ; 9,45 GHz

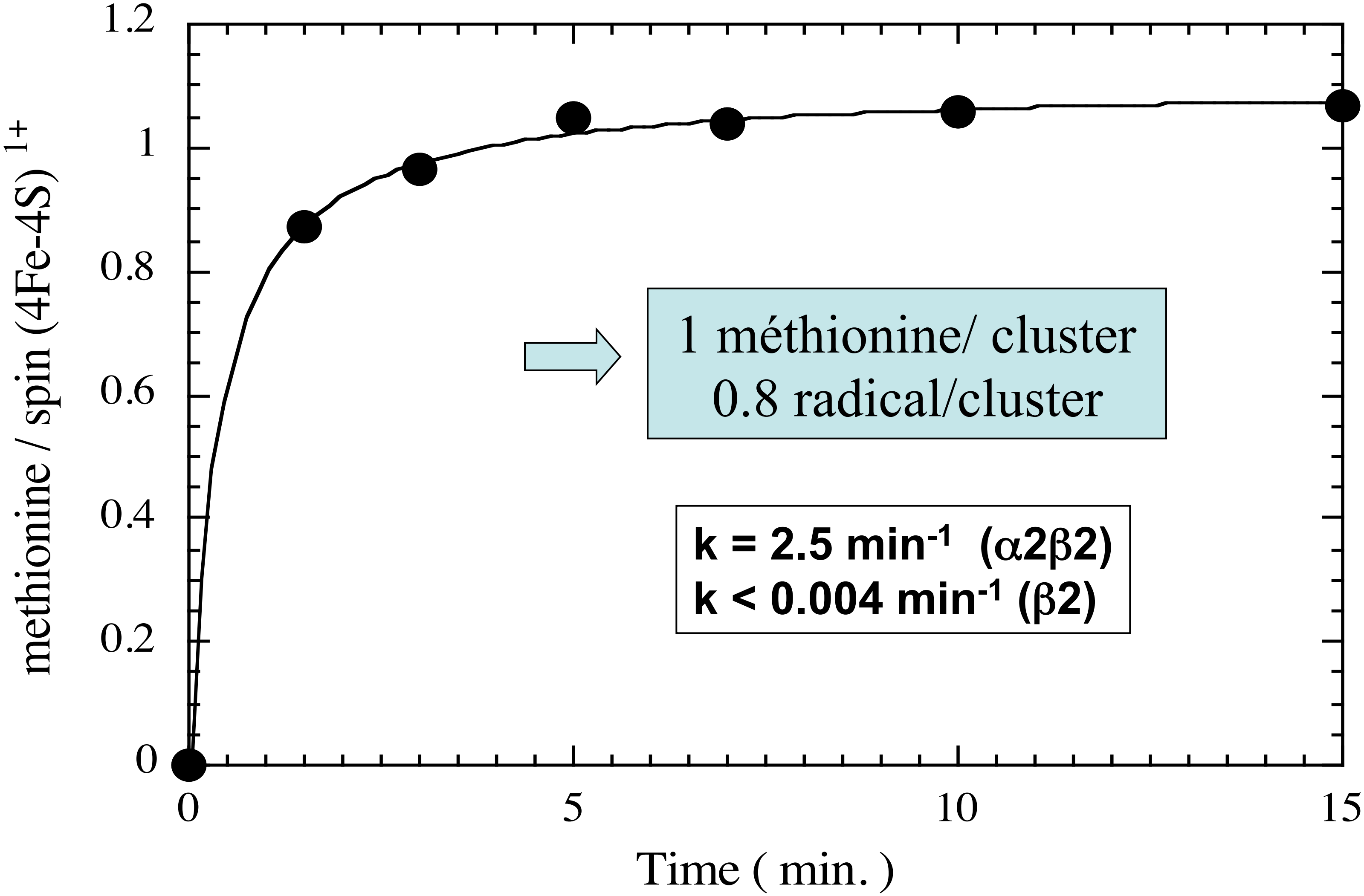


$$\text{- NH-CH}_2\text{-CO-} \xrightarrow[\beta\text{-[4Fe-4S]}^+]{\text{SAM}} \text{- NH-}\overset{\bullet}{\text{C}}\text{H-CO-}$$

α inactif

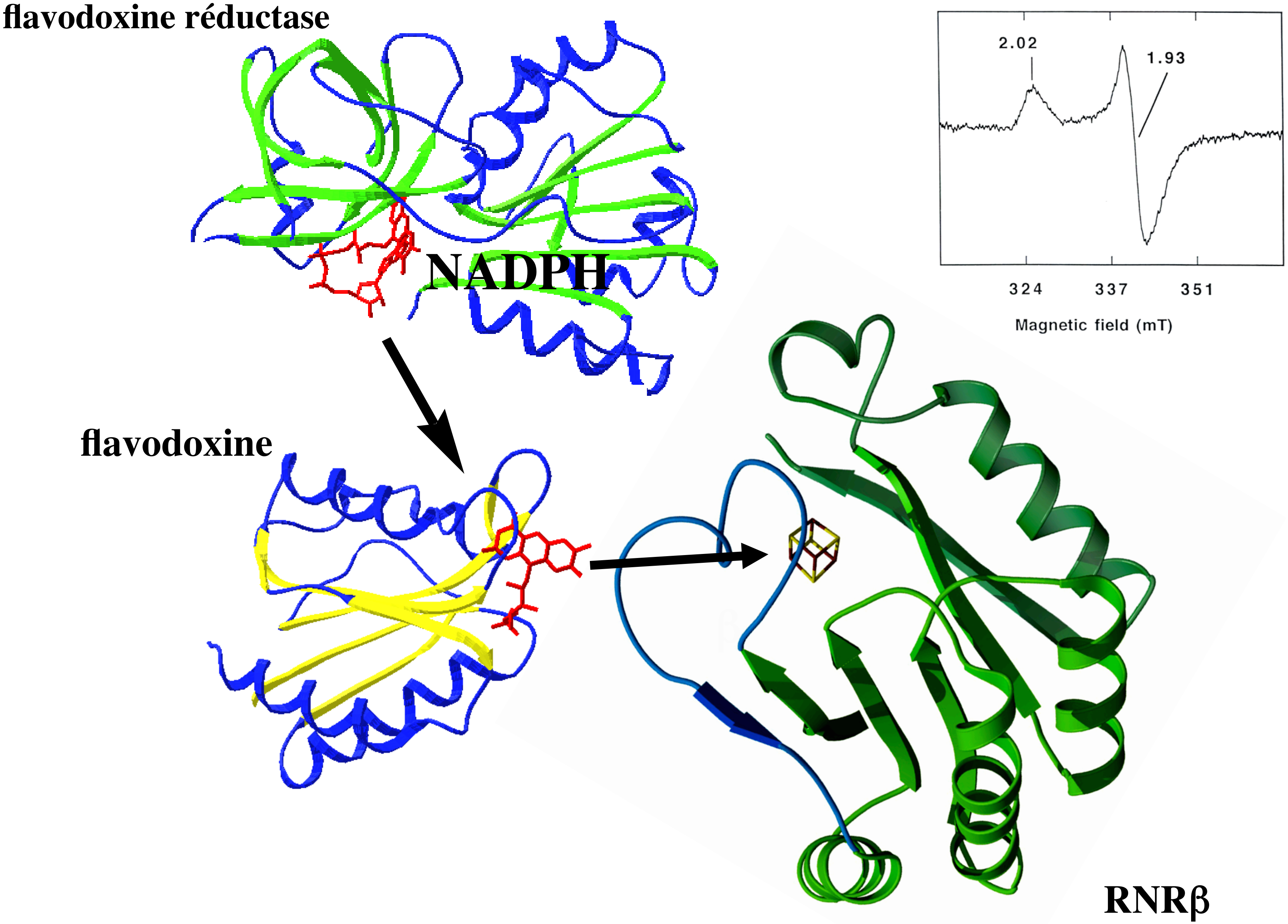
α actif

Clivage de SAM par le complexe α2β2



1 méthionine/ cluster
0.8 radical/cluster

k = 2.5 min^{-1} (α2β2)
k < 0.004 min^{-1} (β2)



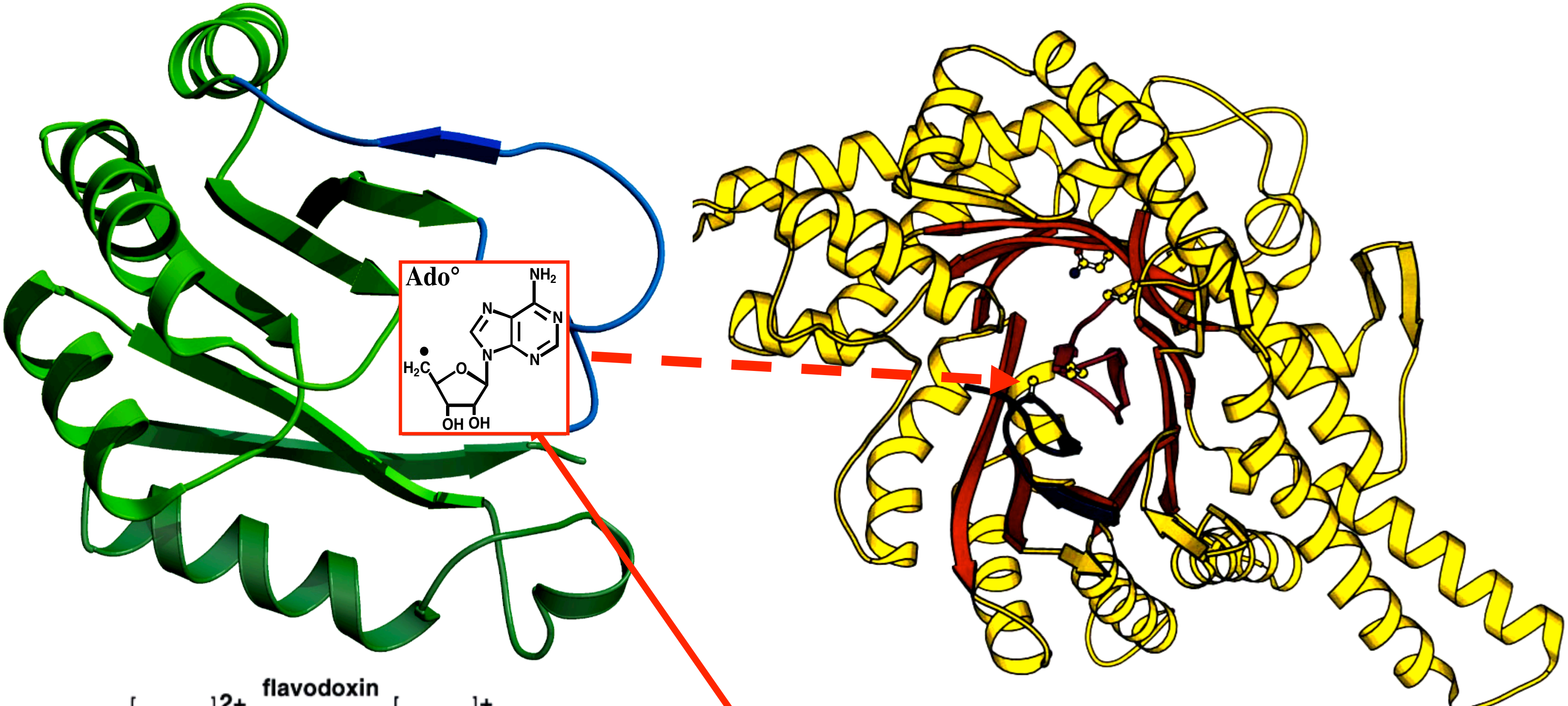
flavodoxine réductase

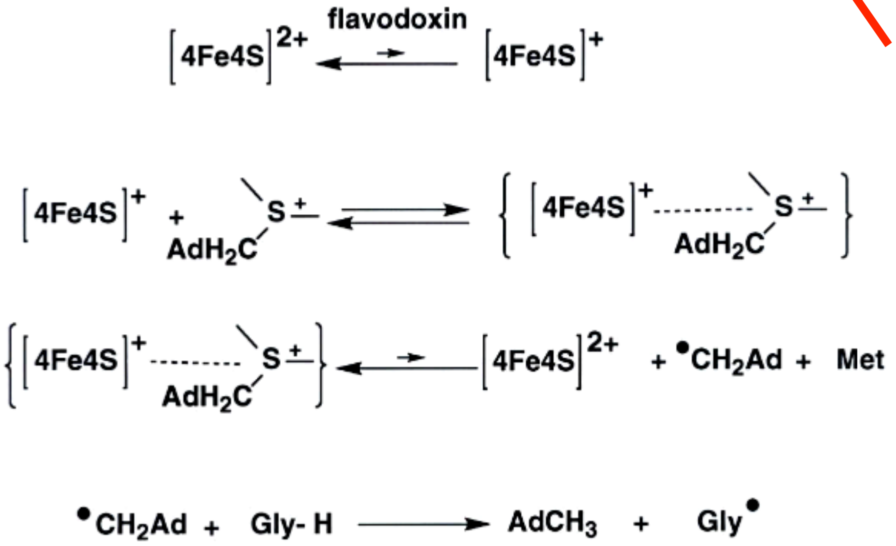
NADPH

flavodoxine

RNRβ

Un modèle structural de RNRβ : Y. Nicolet, IBS, Grenoble



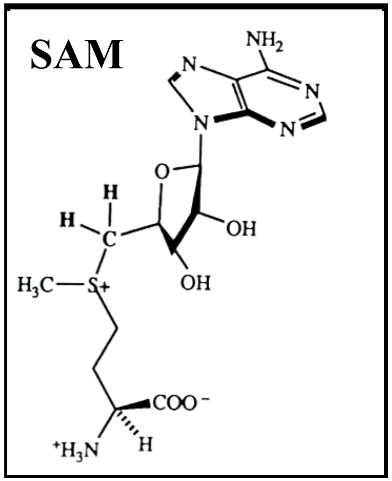


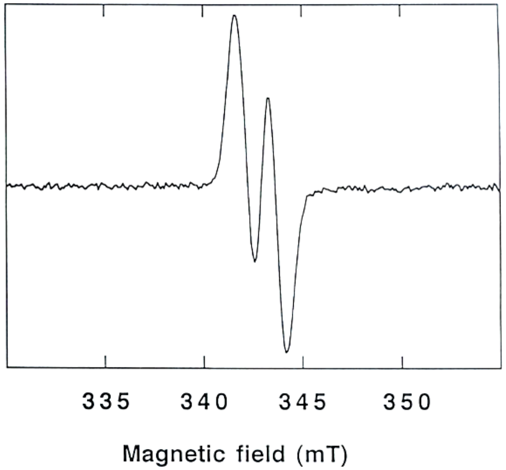
$$[\mathrm{4Fe4S}]^{2+} \overset{\text{flavodoxin}}{\rightleftarrows} [\mathrm{4Fe4S}]^{+}$$

$$[\mathrm{4Fe4S}]^{+} + \mathrm{AdH_2C}{-}\mathrm{S}^{+} \rightleftarrows \left\{ [\mathrm{4Fe4S}]^{+} \cdots\cdots \mathrm{S}^{+}{-}\mathrm{CH_2Ad} \right\}$$

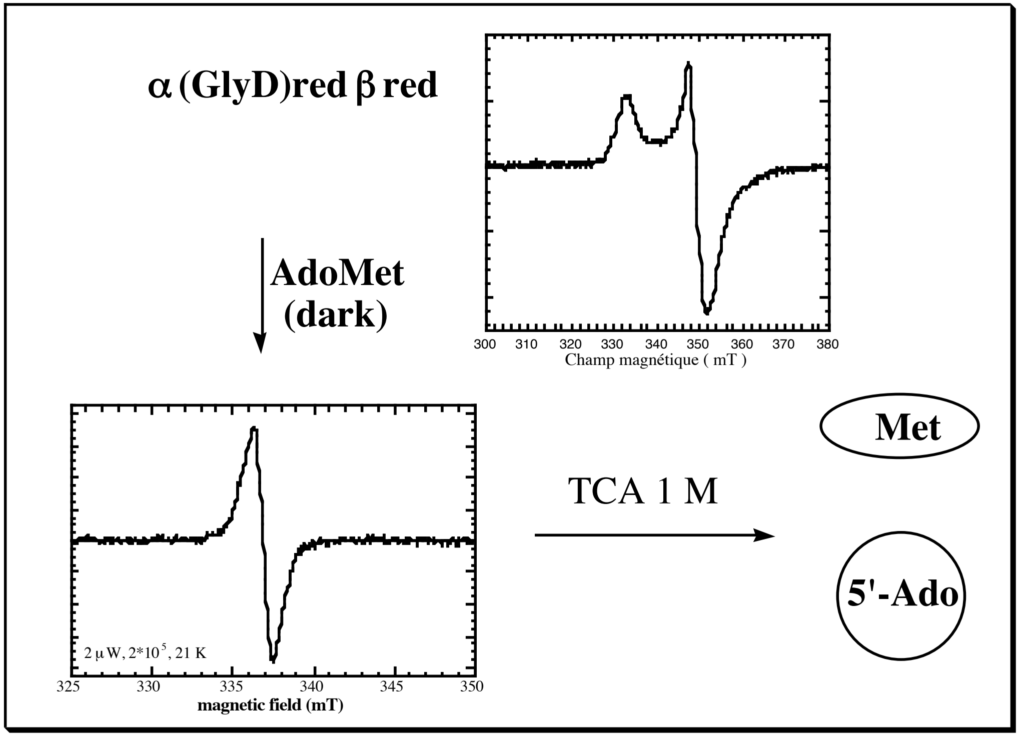
$$\left\{ [\mathrm{4Fe4S}]^{+} \cdots\cdots \mathrm{S}^{+}{-}\mathrm{CH_2Ad} \right\} \rightleftarrows [\mathrm{4Fe4S}]^{2+} + {}^{\bullet}\mathrm{CH_2Ad} + \mathrm{Met}$$

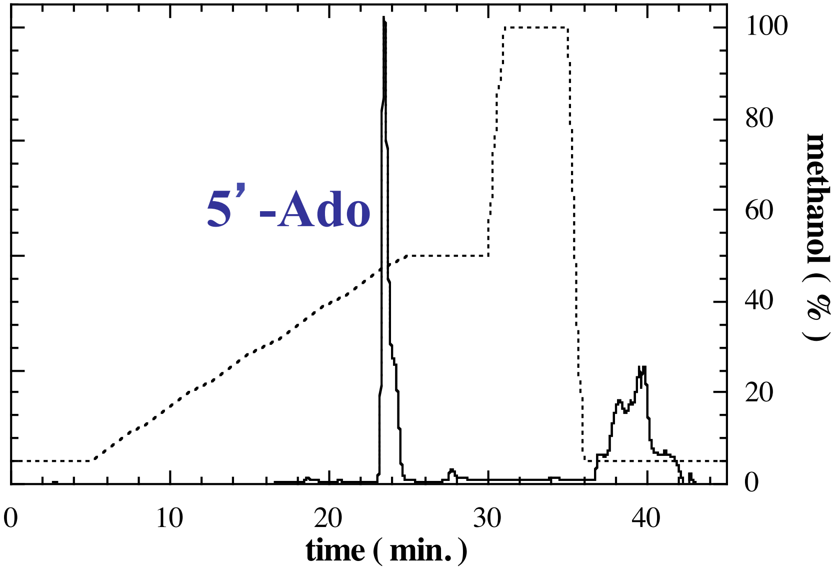
$${}^{\bullet}\mathrm{CH_2Ad} + \mathrm{Gly\text{-}H} \longrightarrow \mathrm{AdCH_3} + \mathrm{Gly}^{\bullet}$$

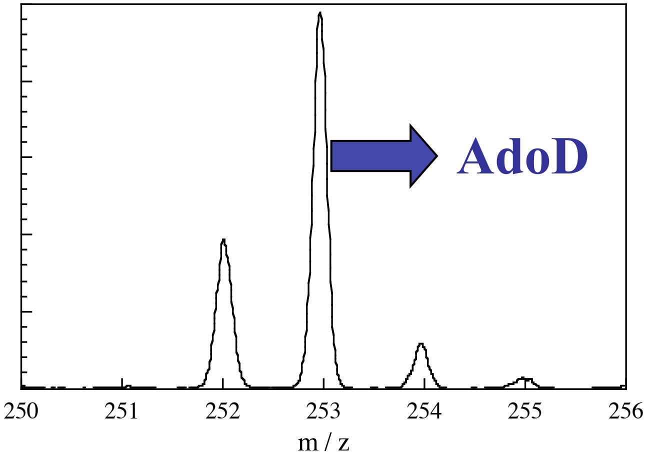


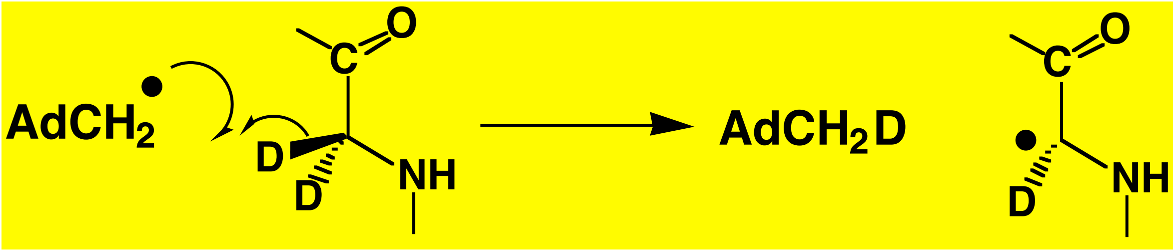


1 - REACTION





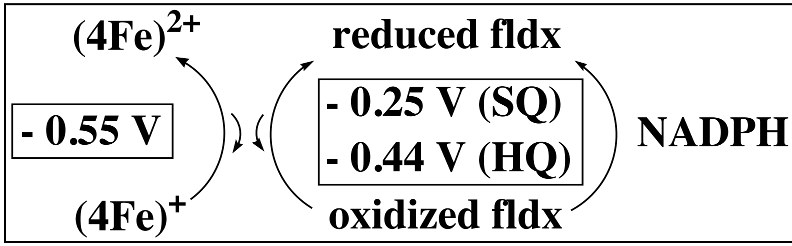


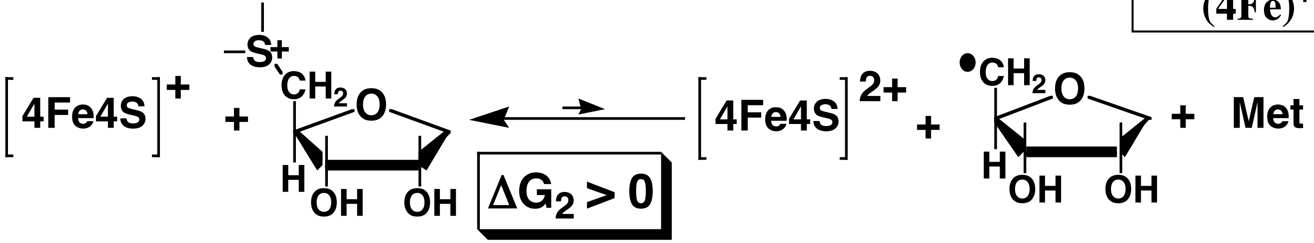


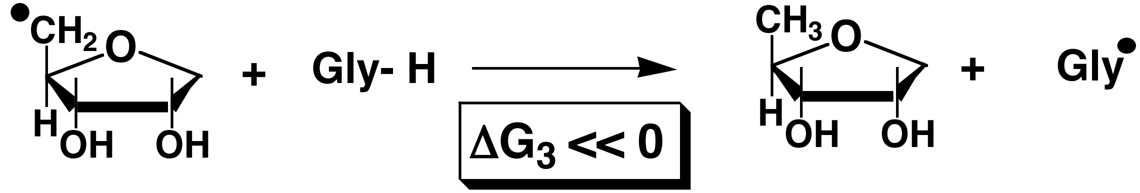
Un transfert de radical « direct »

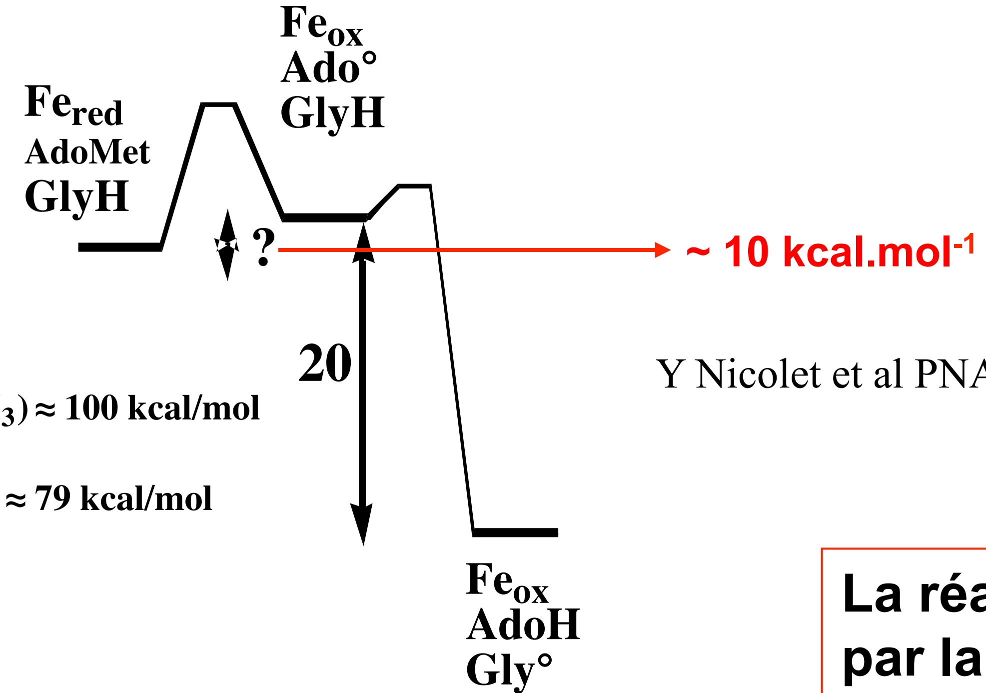
$$\left[4Fe4S\right]^{2+} \underset{\rightarrow}{\overset{flavodoxin}{\longleftarrow}} \left[4Fe4S\right]^{+}$$

$\boxed{\Delta G_1 > 0}$





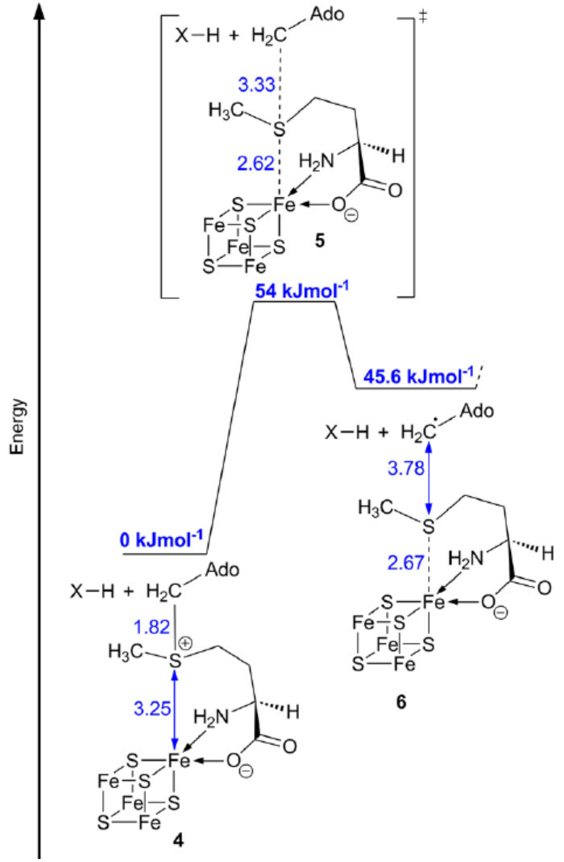




E_{C-H} (AdCH$_3$) ≈ 100 kcal/mol

E_{C-H} (GlyH) ≈ 79 kcal/mol

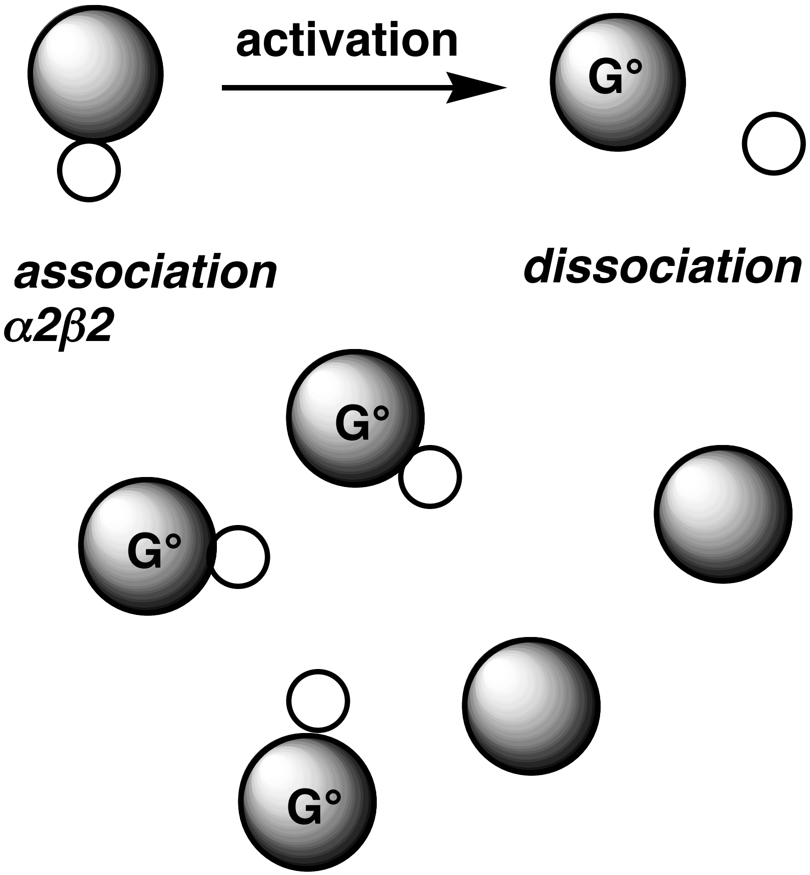
Y Nicolet et al PNAS 2009

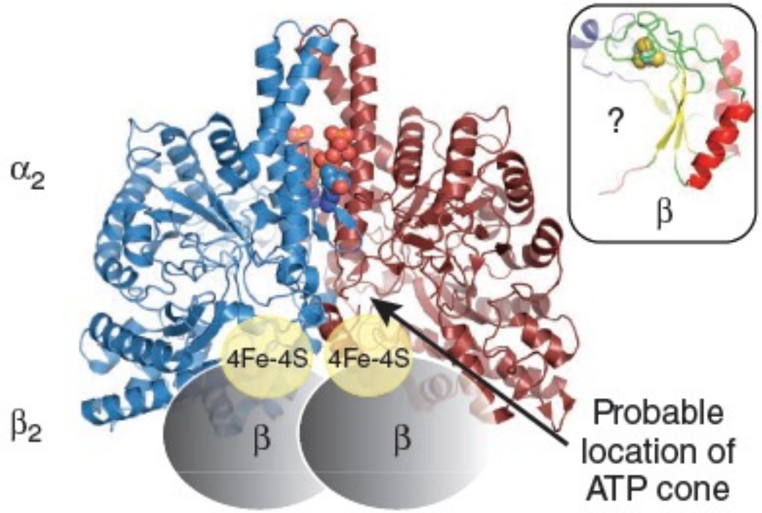


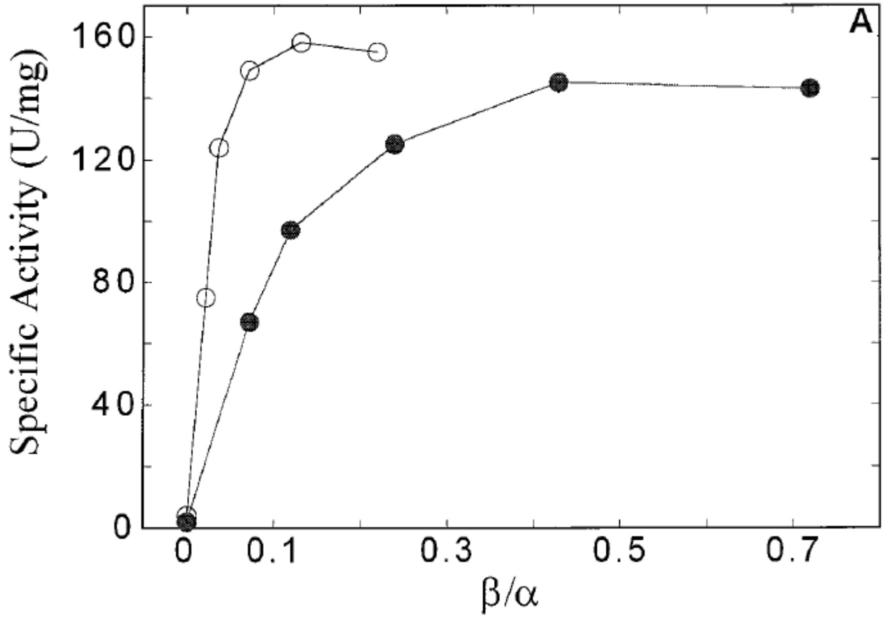
**La réaction est « tirée »
par la stabilité du radical Gly°**

Protéine β est une » activase

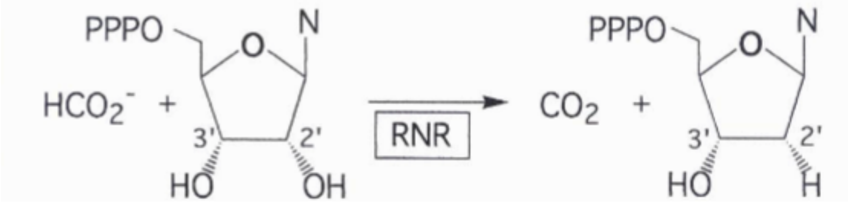
J. Tamarit, M. Fontecave et al
J. Biol. Chem 1999 274 31291



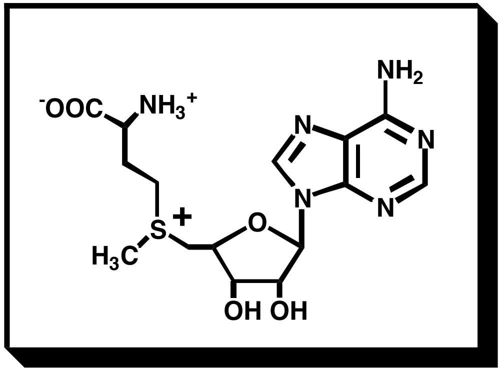


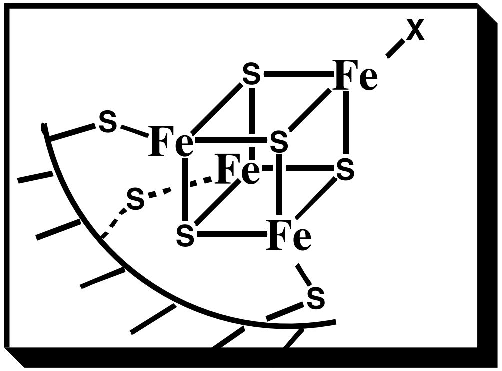


La ribonucléotide réductase anaérobie:
SAM (S-Adenosylméthionine) + cluster fer-soufre:
Un système de formation de radicaux



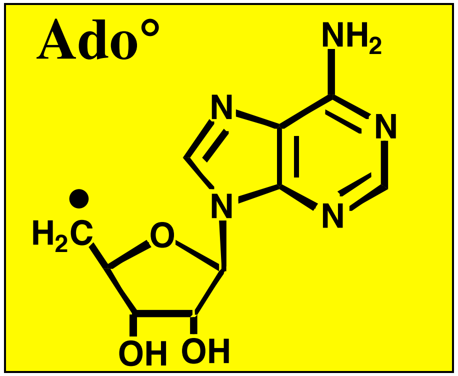
SAM



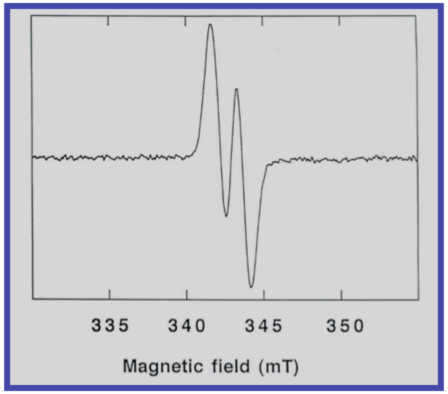


[4Fe-4S]

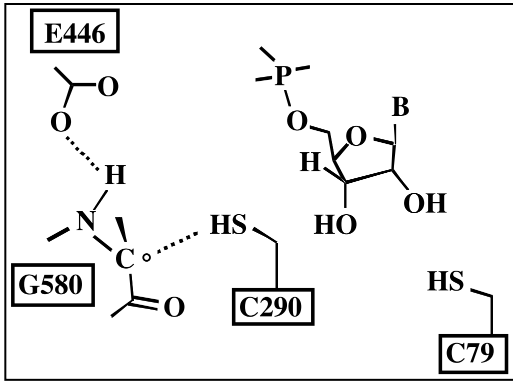
e- →



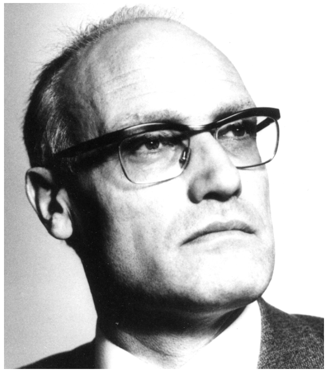
→



Radical glycinyle

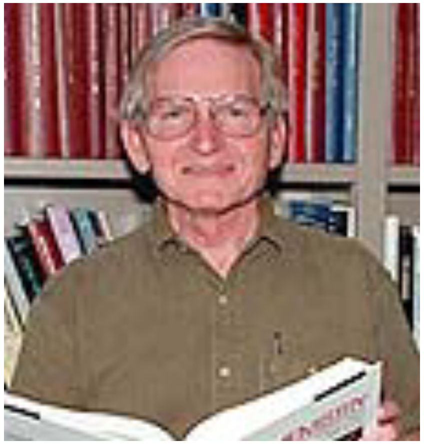


Enzymes Radical-SAM : les fondations



Pyruvate-formate lyase (J. Knappe):

- SAM est nécessaire (1965)
- SaM est clivée au cours de la réaction (1976)
- Un radical protéique (1984), un radical glycinyle (1992)



Lysine aminomutase (P. Frey):

- SAM is nécessaire (Barker, 1970)
- SAM une source de Ado° (1987)
- une enzyme fer-soufre (1991)

Ribonucléotide réductase (P. Reichard, M. Fontecave):

- une nouvelle ribonucléotide réductase (1987)
- SAM est nécessaire (1989)
- un cluster [4Fe-4S] (1993) impliqué dans le clivage de SAM (1996)
- Un radical glycinyle (1993)

Enzymes Radical-SAM

HJ Sofia Nucleic Acids Res (2001) 29 1097

Cys-X-X-X-**Cys**-X-X-**Cys**

											enzyme	*fonction*
RNR	G	C	V	H	E	C	P	G	C	Y	**-ribonucleotide reductase**	**Synthèse ADN sis**
PFL	G	C	L	M	R	C	L	Y	C	H	-pyruvate-formate lyase	**Métabolisme pyruvate**
BioB	Y	C	P	E	D	C	G	Y	C	S	**-biotin synthase**	**Synthèse biotineis**
BSS	G	C	P	L	R	C	P	W	C	S	-benzylsuccinate synthase	**Métabolisme toluene**
LS	I	C	T	R	R	C	P	F	C	D	-lipoate synthase	**Synthèse lipoate**
SPL	G	C	M	G	H	C	H	Y	C	Y	**-spore photoproduct lyase**	**Réparation ADNuir**
LAM	M	C	S	M	Y	C	R	H	C	T	-lysine aminomutase	**Métabolisme lysine**
miaB	G	C	N	K	Y	C	T	Y	C	V	**- miaB gene product**	**Modification ARNt**

La même chimie radicalaire pour:

Biosynthèse de:

-Cofacteurs (lipoate, PQQ, molybdoptérine…)

-Antibiotiques (désosamine, mitomycine, fosfomycine,…)

-Vitamines (biotine, thiamine,…)

-Alkaloïdes

-Chlorophylle

Métabolisme de:

-Sucres

-Amino-acides

-Hydrocarbures

Modification de:

-ARNs de transfert

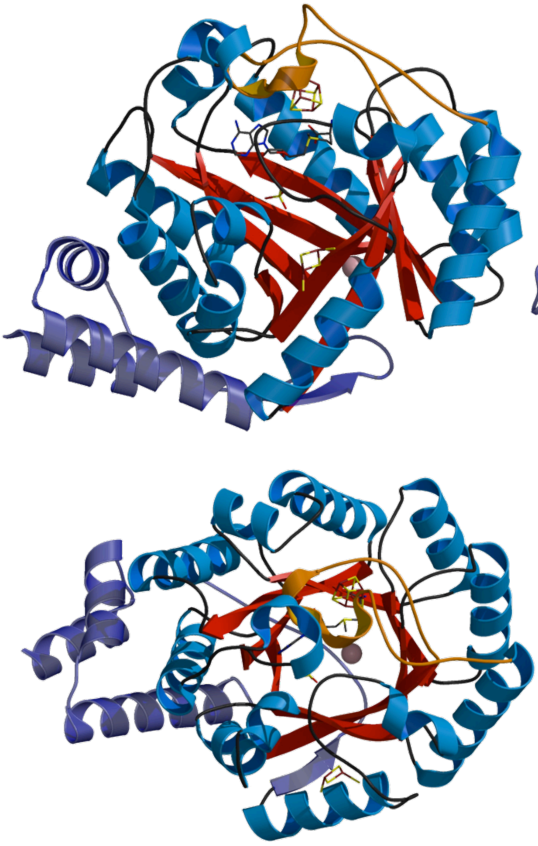
-Enzymes

Réparation de:

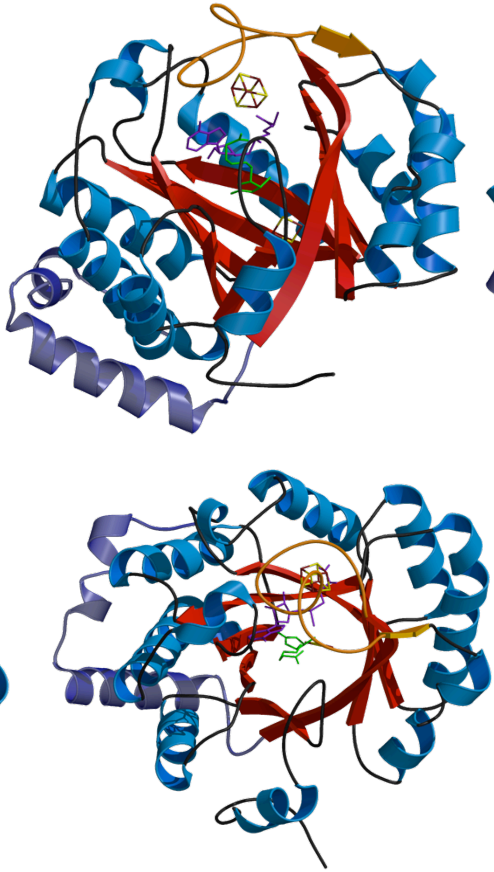
- ADN

La superfamille « Radical-SAM »:
structures

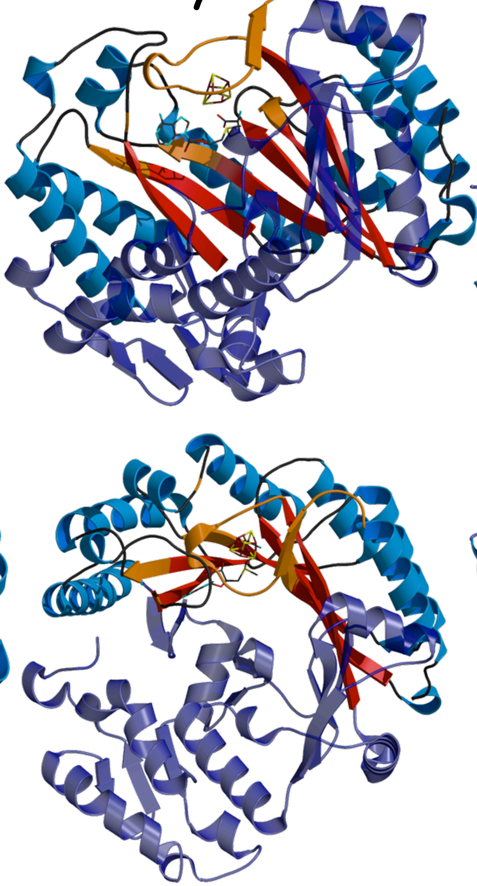
HydE 1.5 Å
maturase



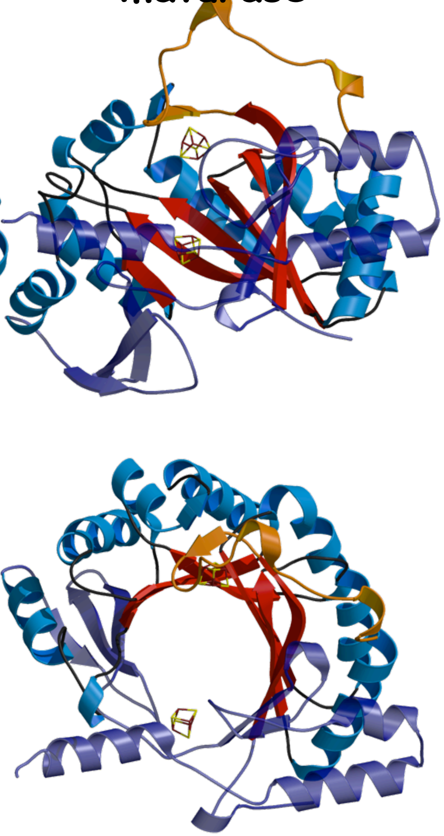
BioB 3.4 Å
Biotine synthase



HemN 2.07 Å
Coproporphyrinogen
synthase



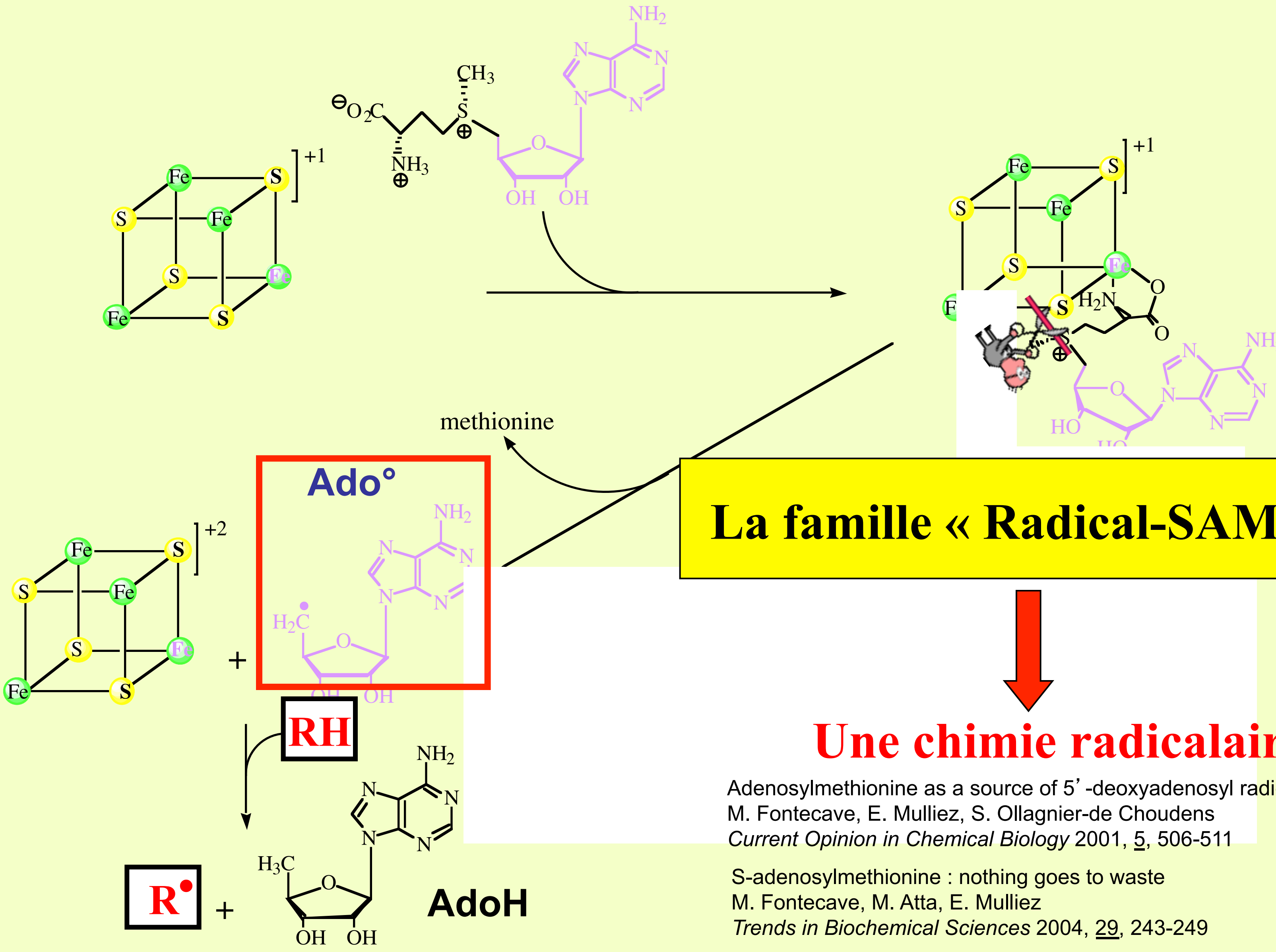
MoaA 2.2 Å
maturase



rmsd: - 2.3 Å (292 c_{α}) 3.7 Å (192 c_{α}) 4.3 Å (232 c_{α})

Y. Nicolet et al Nucleic Acids Research, 2004, (32) 4015–4025
C. Drennan et al, Chem Rev 2011

Figure 6



La famille « Radical-SAM » :

Une chimie radicalaire

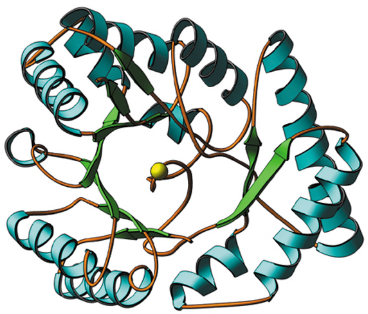
Adenosylmethionine as a source of 5’-deoxyadenosyl radicals
M. Fontecave, E. Mulliez, S. Ollagnier-de Choudens
Current Opinion in Chemical Biology 2001, 5, 506-511

S-adenosylmethionine : nothing goes to waste
M. Fontecave, M. Atta, E. Mulliez
Trends in Biochemical Sciences 2004, 29, 243-249

S-Adenosylmethionine-dependent radical-based modification of biological macromolecules
M. Atta, E. Mulliez, S. Arragain, F. Forouhar, J. F. Hunt, M. Fontecave
Curr. Op. Struct. Biol. 2010, 20, 684-692

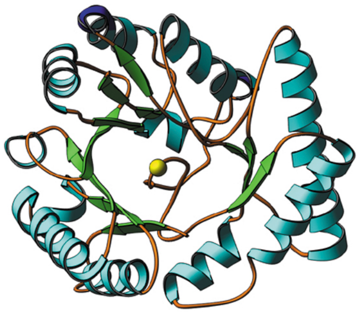
RH: protéine (glycine) ou substrat

Les 3 classes de ribonucléotide réductases: Des enzymes radicalaires



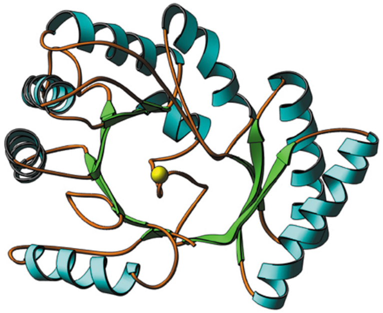
Class I

Eucaryotes
(HS, plantes,..)



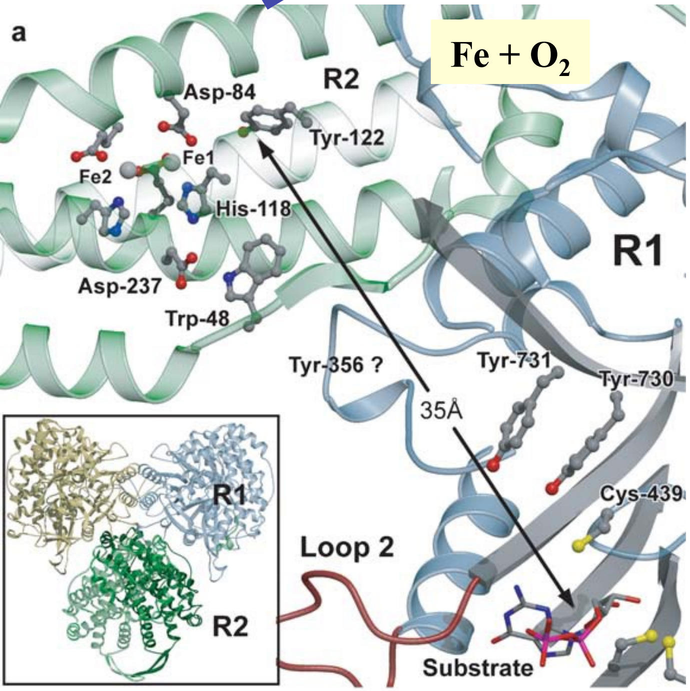
Class II

Bactéries
archaebactéries

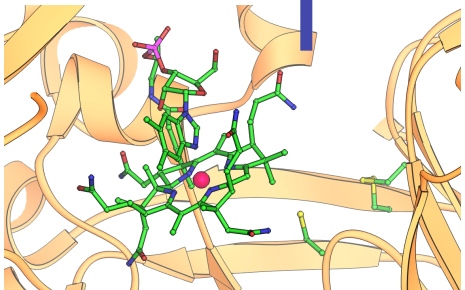


Class III

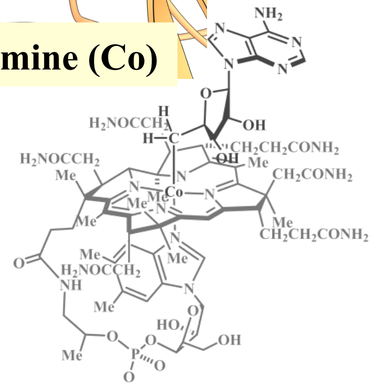
microorganismes
anaérobies



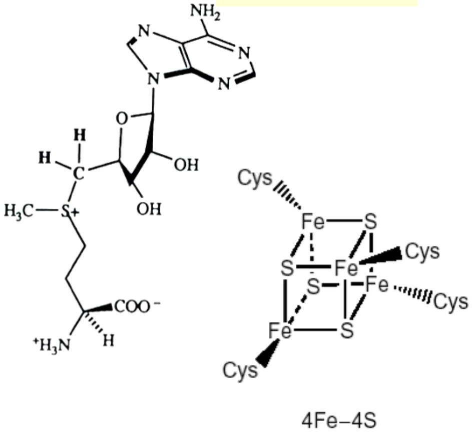
Fe + O_2



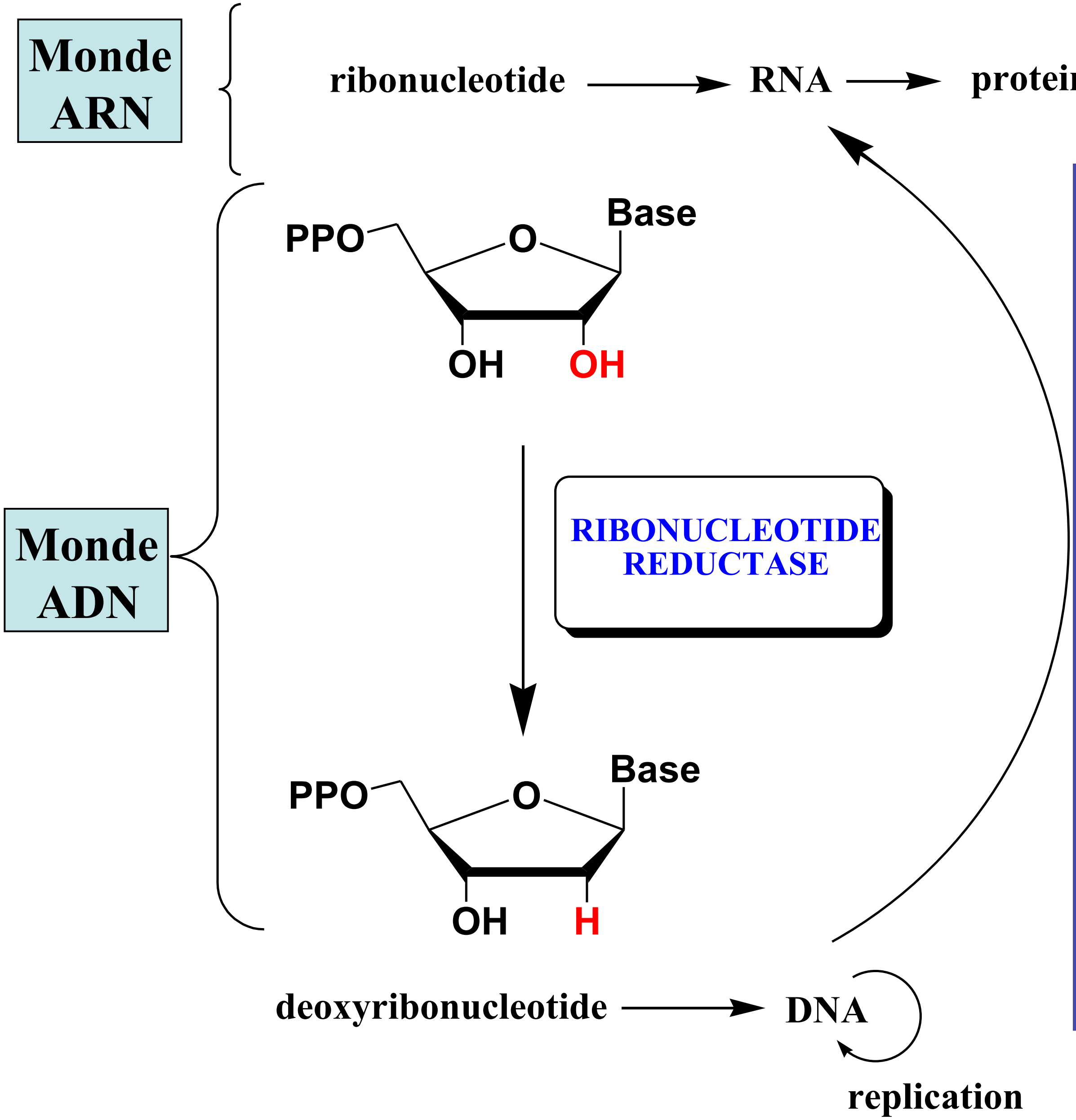
Adenosylcobalamine (Co)



Fe + SAM



Du monde à ARN au monde à ADN: la RNR primitive ?



Monde ARN

ribonucleotide → RNA → protein

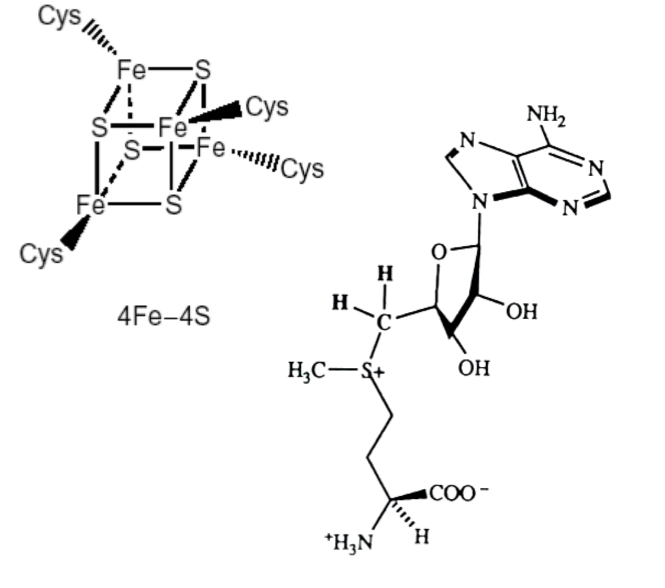
Monde ADN

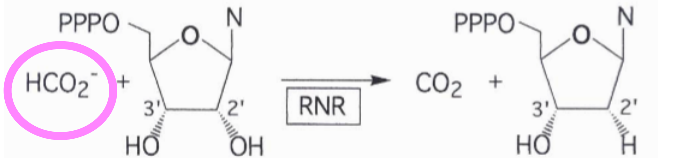
RIBONUCLEOTIDE REDUCTASE

deoxyribonucleotide → DNA

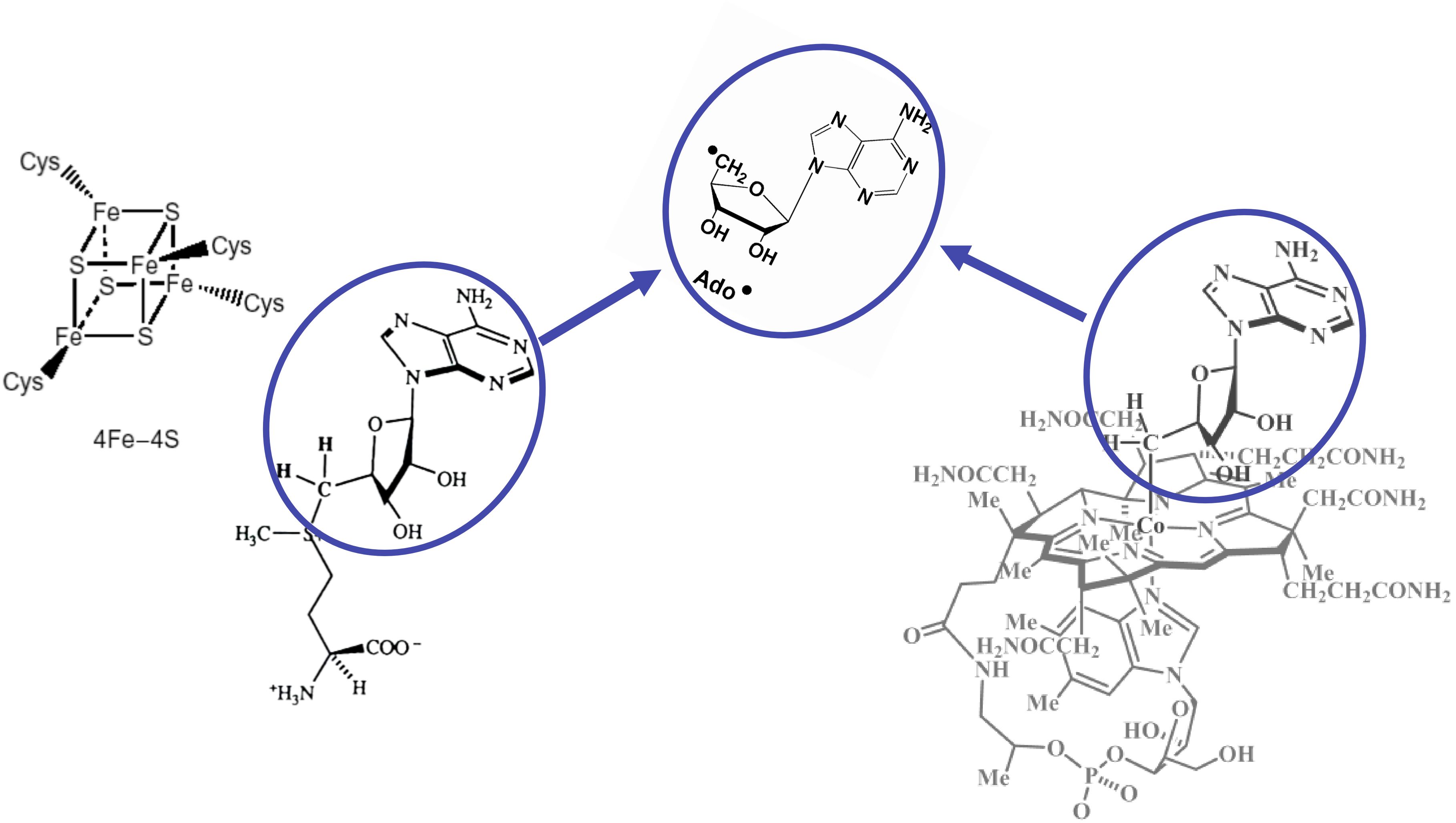
replication

anaérobiose





S-Adenosylmethionine, un donneur de radicaux 5’-deoxyadénosyles:
Une adénosylcobalamine (cofacteur B12) primitive ?



[Fe-S] + S-Adénosylméthionine

Adénosylcobalamine

→

évolution

Transfert de radicaux dans les systèmes biologiques: Les ribonucléotide réductases

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mfontecave@cea.fr; Phone: (0033)438789103 ; Fax: (0033)438789124

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