# Biosynthèse des précurseurs de l'ADN chez les organismes *anaérobies* : du fer, de la S-adénosylméthionine et des radicaux libres

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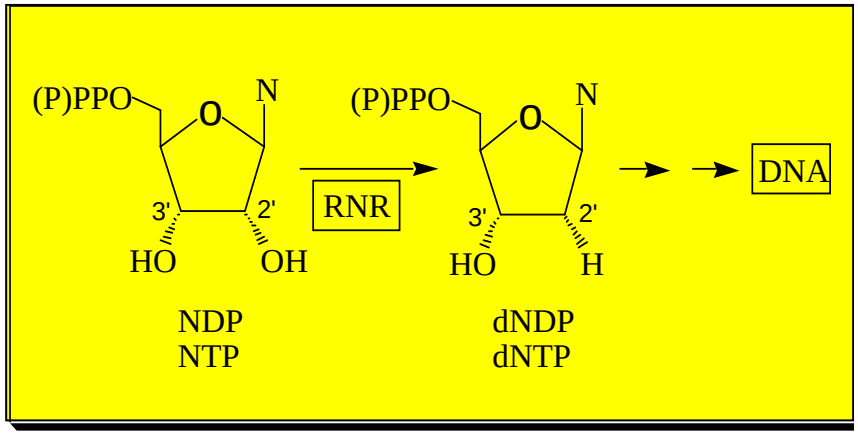
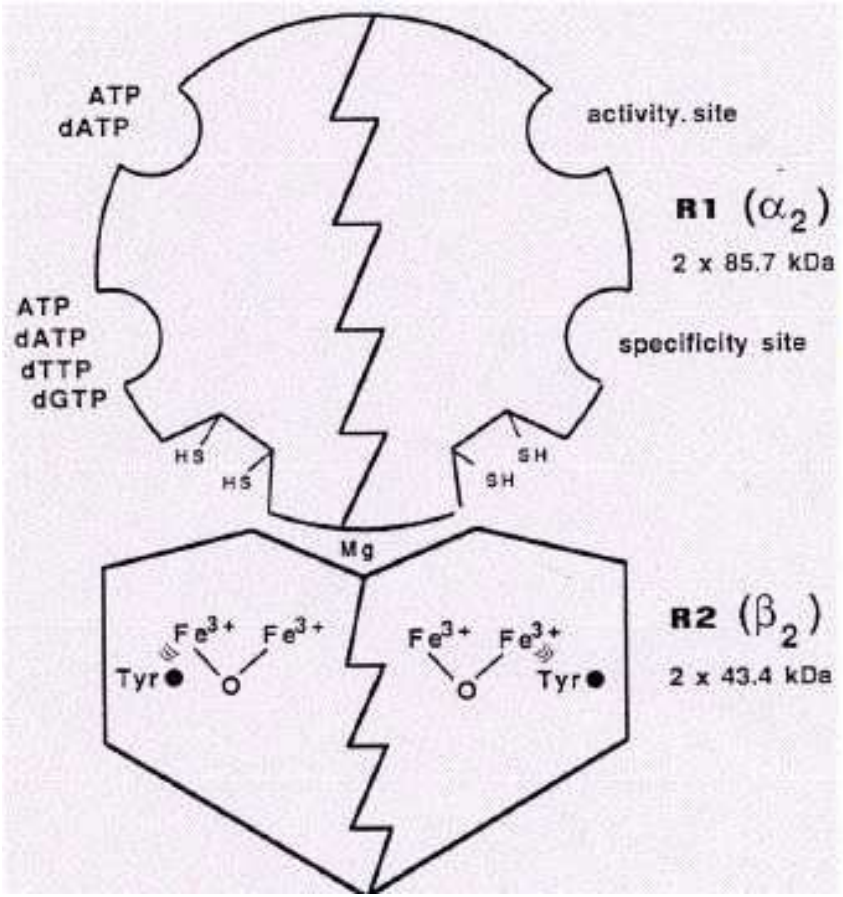
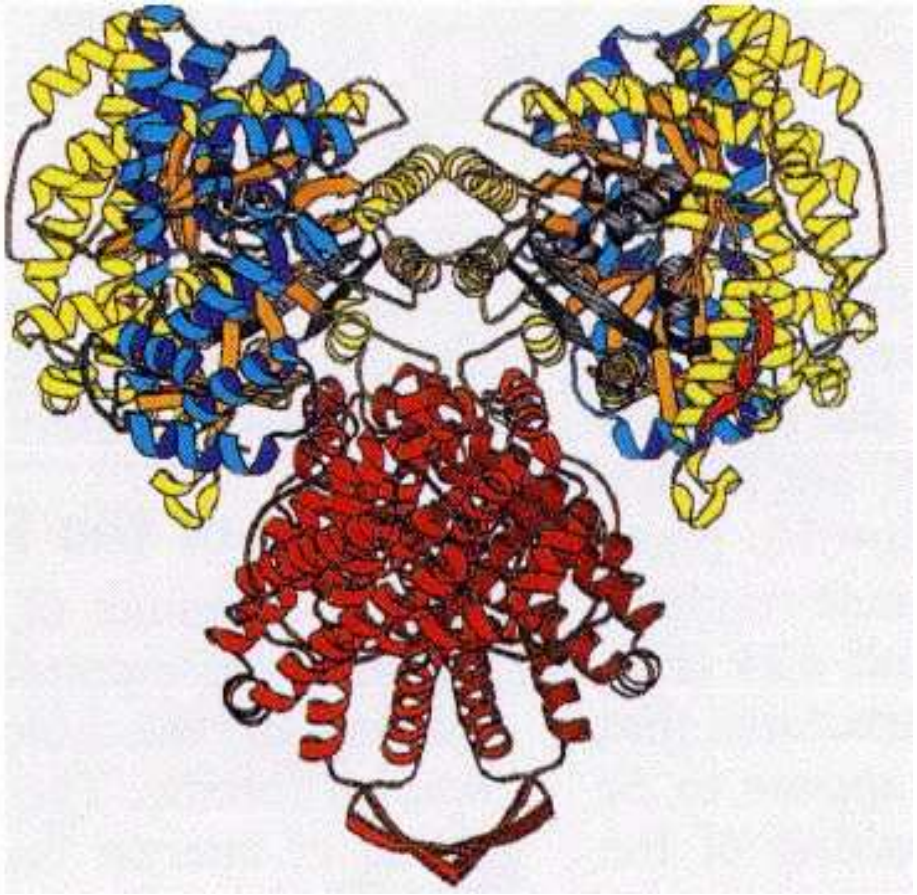
# Ribonucléotide réductase chez les aérobes

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

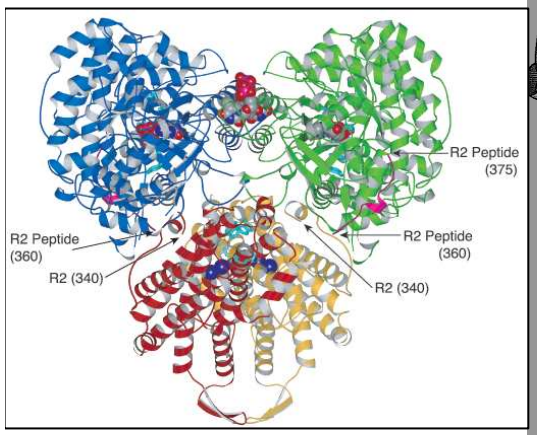


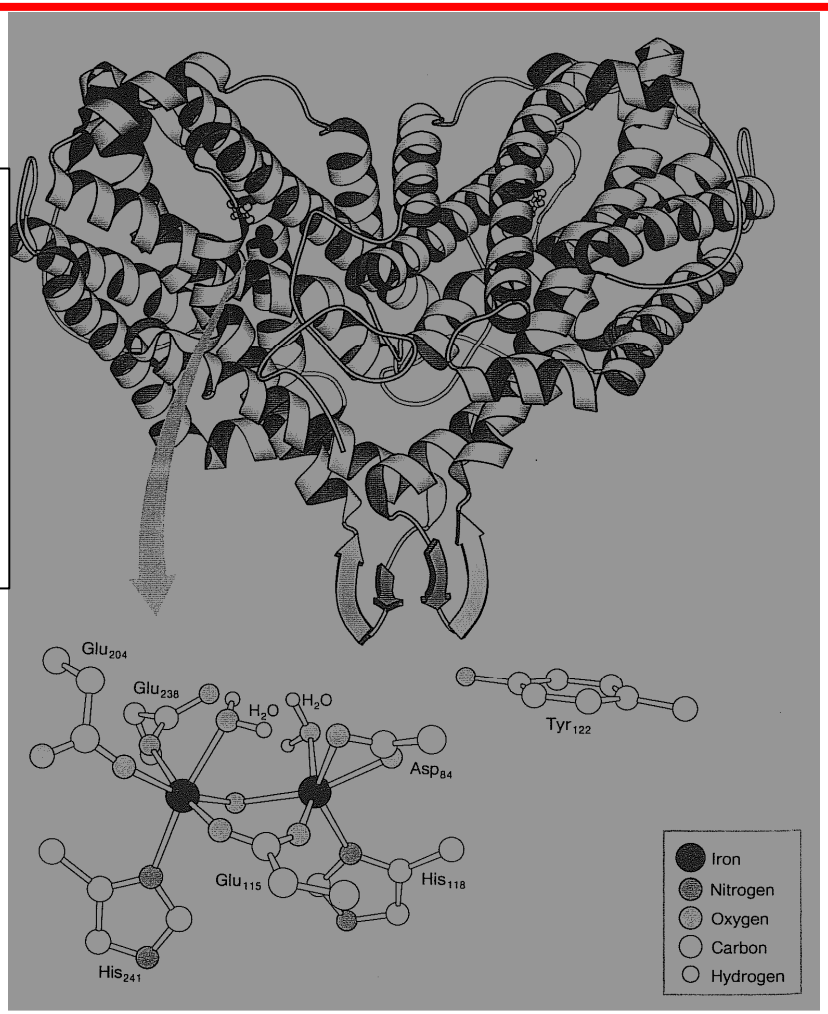



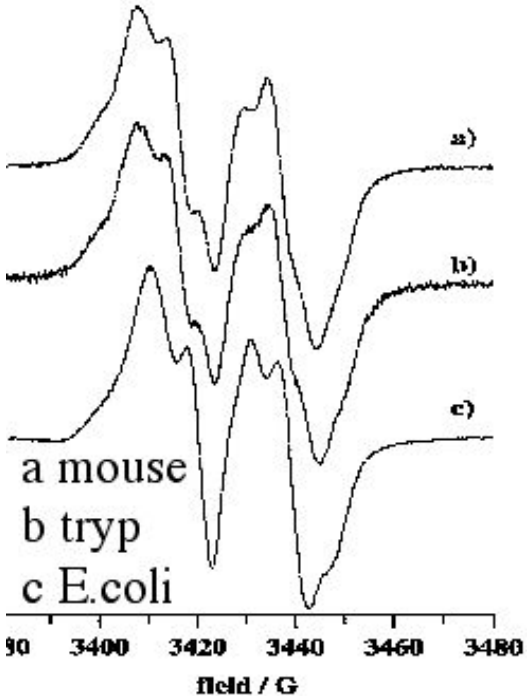



# Du fer, de l'oxygène et des radicaux

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



**Sandrine Ollagnier-de-Choudens**

**Peter Reichard (Stockholm)**

**Etienne Mulliez**

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

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

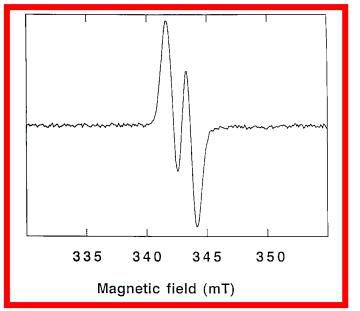
Oxygen-sensitive ribonucleoside triphosphate reductase in anaerobic *E. coli*
M. Fontecave, R. Eliasson, P. Reichard

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

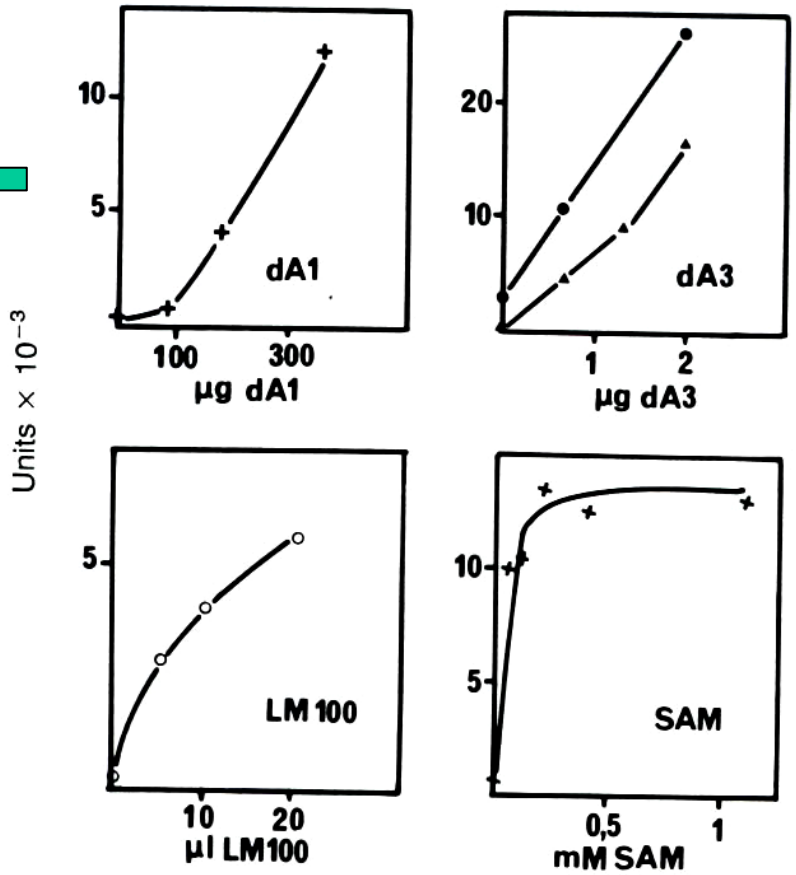
| Condition | Total cpm × $10^{-6}$ DNA | Total cpm × $10^{-6}$ RNA | DNA/RNA | cpm/pmol CTP | cpm/pmol dCTP | dCTP/CTP |
|---|---|---|---|---|---|---|
| Aerobic | | | | | | |
| 15 min | 7.5 | 110 | 0.07 | 175 | 320 | 1.8 |
| 30 min | 19.5 | 250 | 0.08 | 227 | 360 | 1.6 |
| Anaerobic | | | | | | |
| 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).

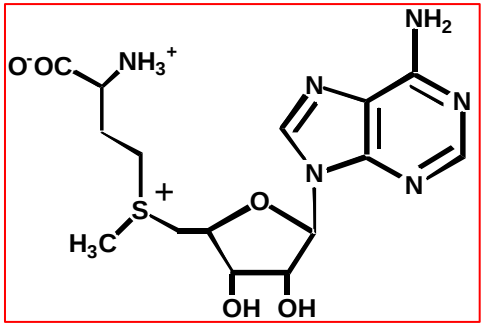
# 1989-1995: fractionnement et identification




**Flavodoxine réductase**



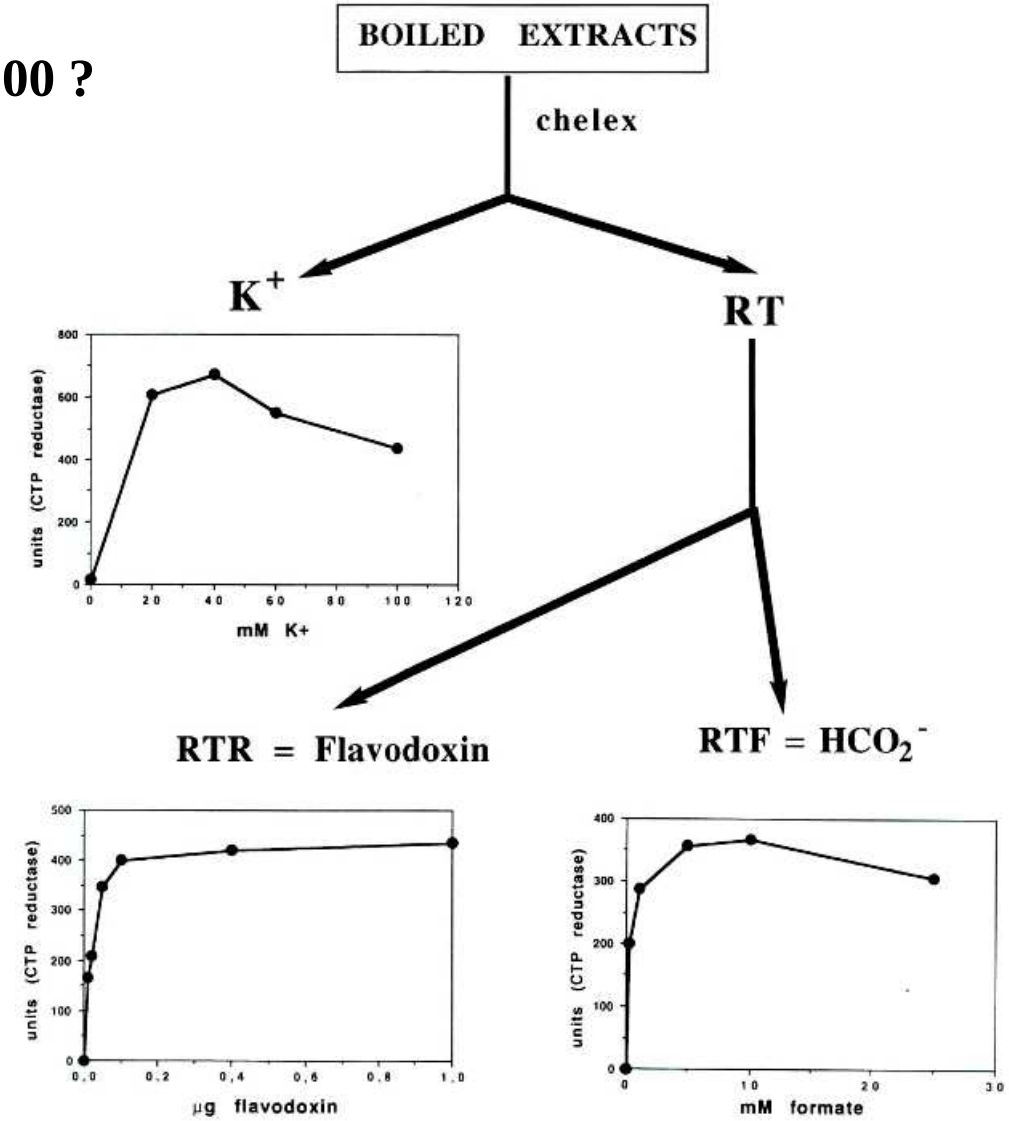

**La RNR -radical organique**




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

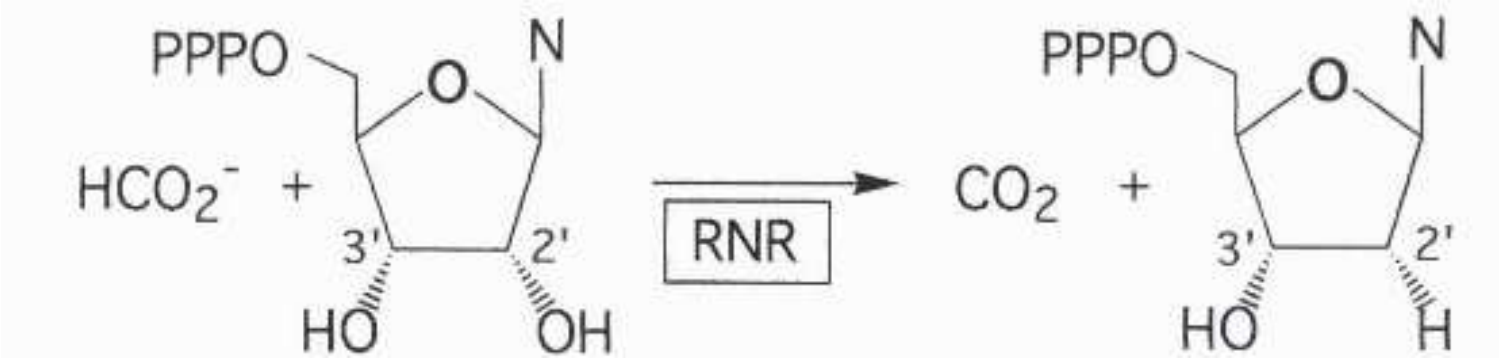
# LM 100 ?




**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

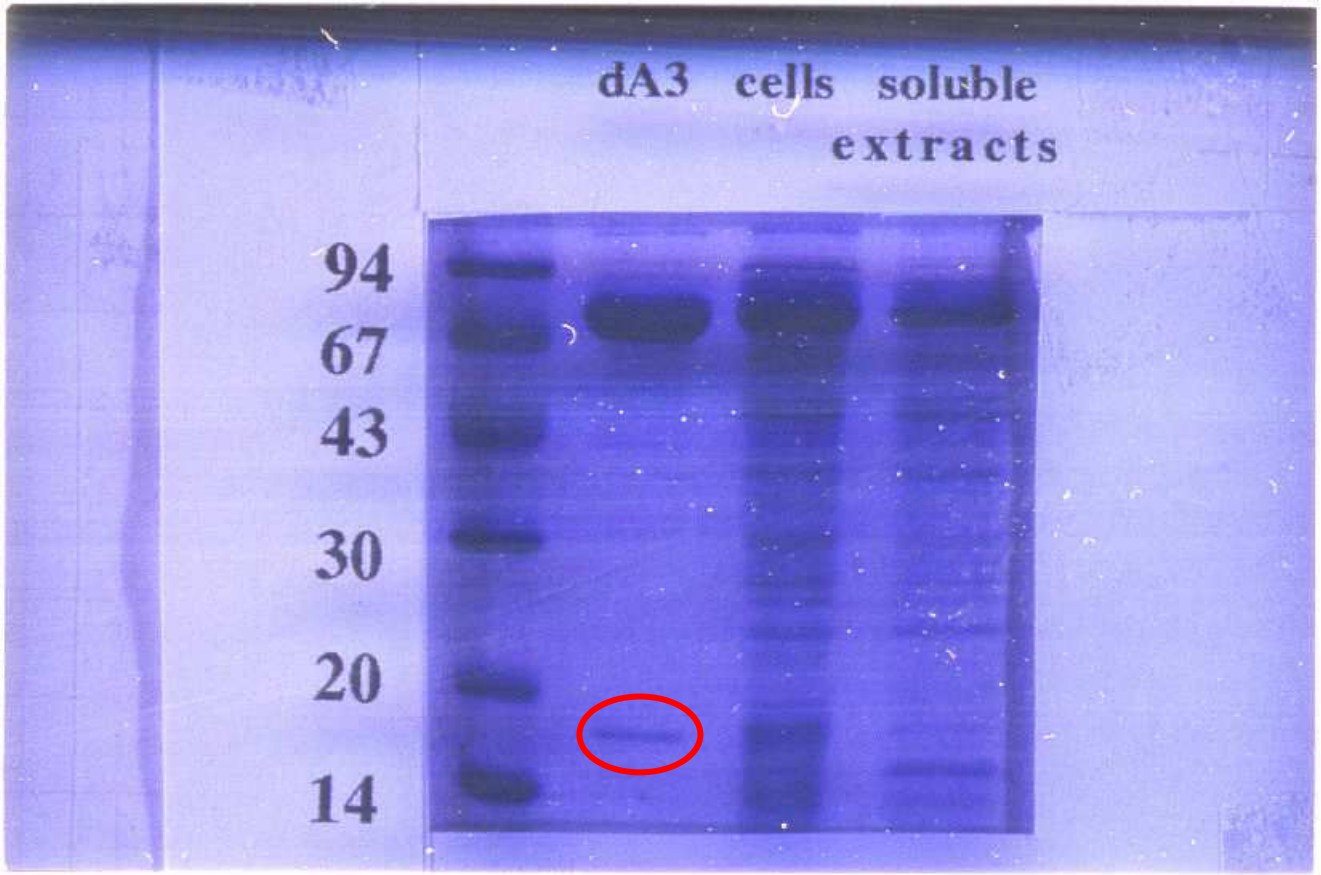
**Formate** as hydrogen donor for the anaerobic ribonucleotide reductase from *Escherichia coli*
E. Mulliez, S. Ollagnier, M. Fontecave, R. Eliasson, P. Reichard
*Proc. Natl. Acad. Sci.* 1995, 92, 8759-8762

# La ribonucléotide réductase anaérobie

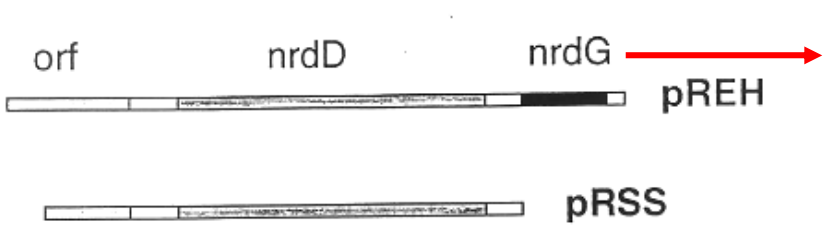



**RNA** **DNA**

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

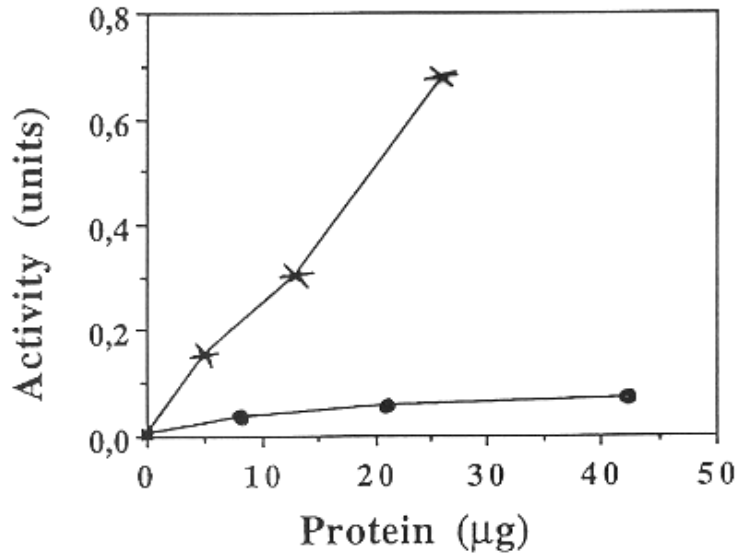


# 1995: La ribonucléotide réductase (nrdD) est active en présence d'un «contaminant »: l'activase (nrdG)



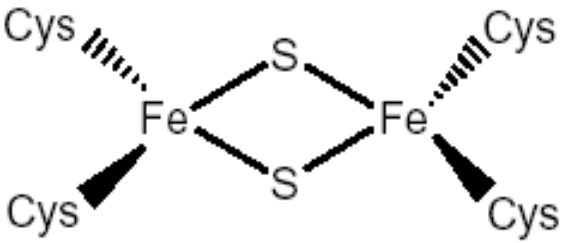

$CX_3CX_2C$

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

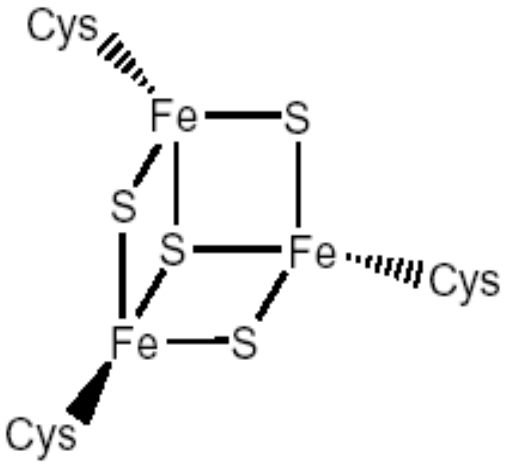



**nrdG est une protéine fer-soufre**
**(1996-1999)**

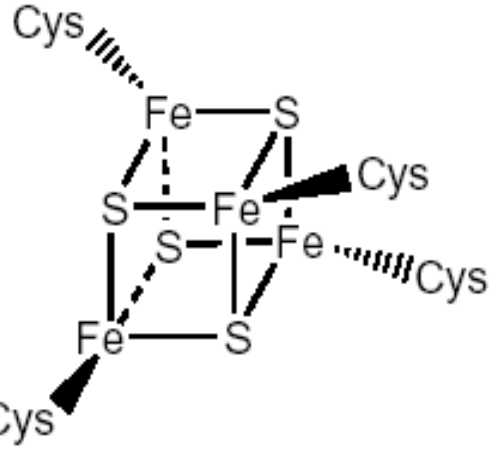
# Centres fer-soufre



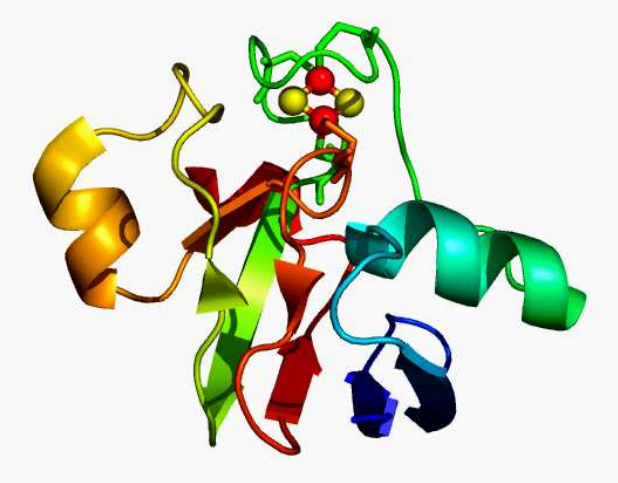

2Fe–2S

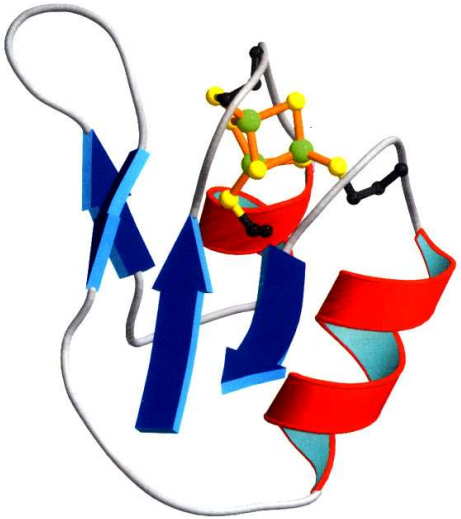


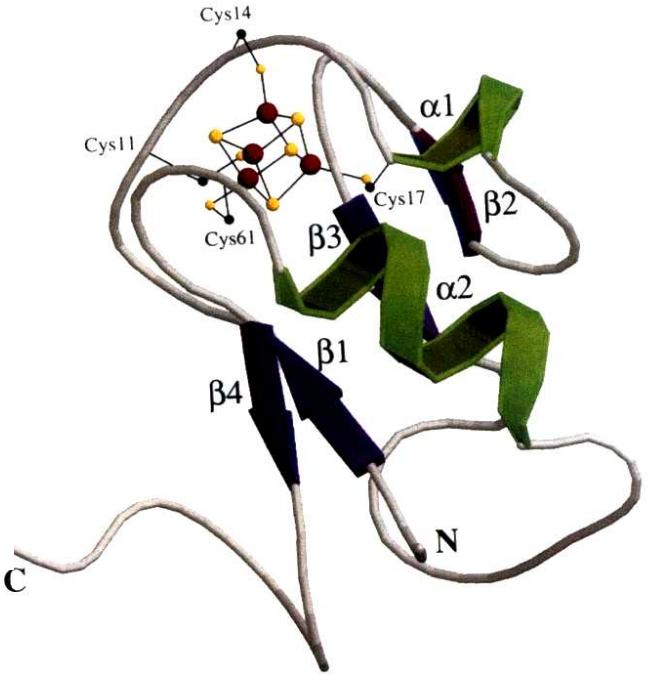

3Fe–4S




4Fe–4S







**Activase**
**nrdG**
**2x17.5 kDa**

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

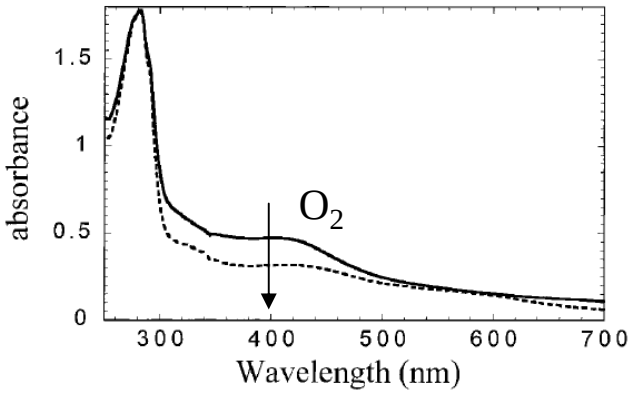
$e^-$

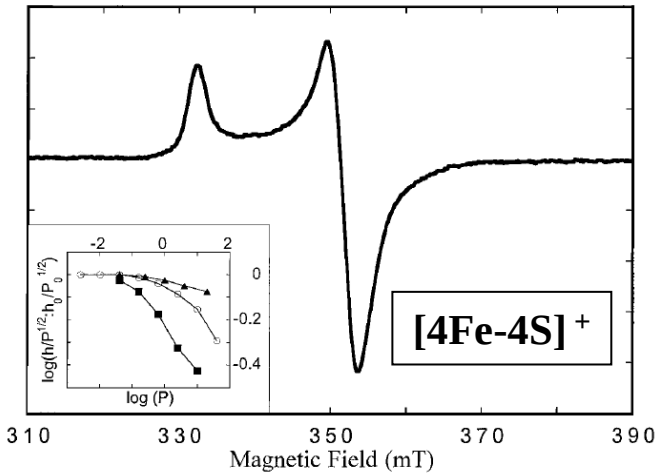
**[4Fe-4S]$^{+}$** **S=1/2**

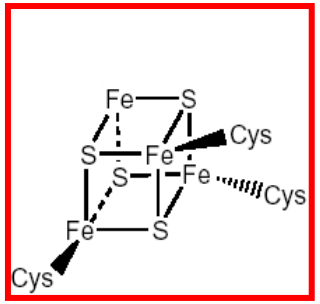
$O_2$

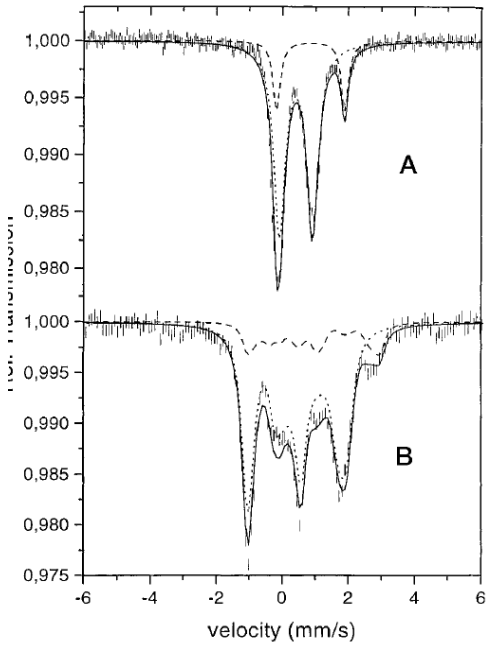
**[2Fe-2S]$^{2+}$**

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



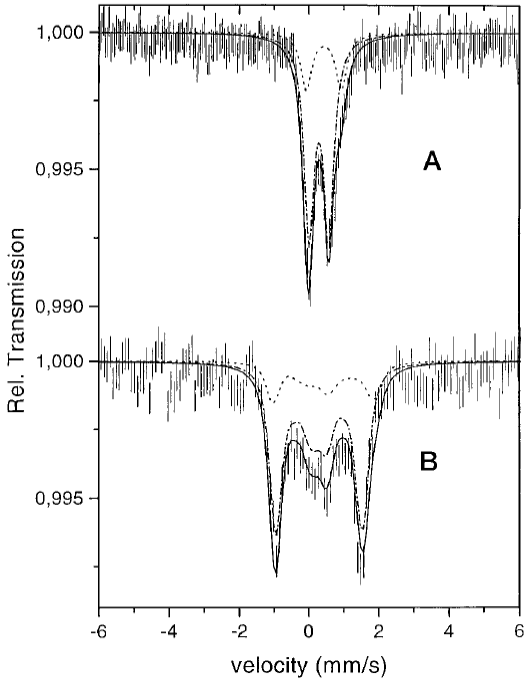




**[4Fe-4S]$^{2+}$**

A. 77K, 20 mT

B. 4.2K, 7T

**$O_2$**




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

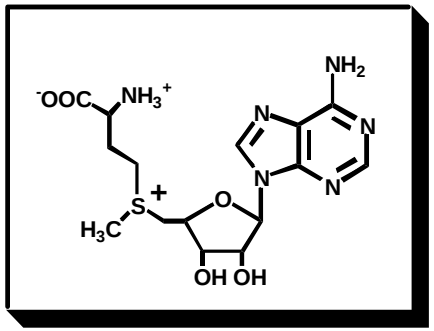
A. 77K, 20 mT

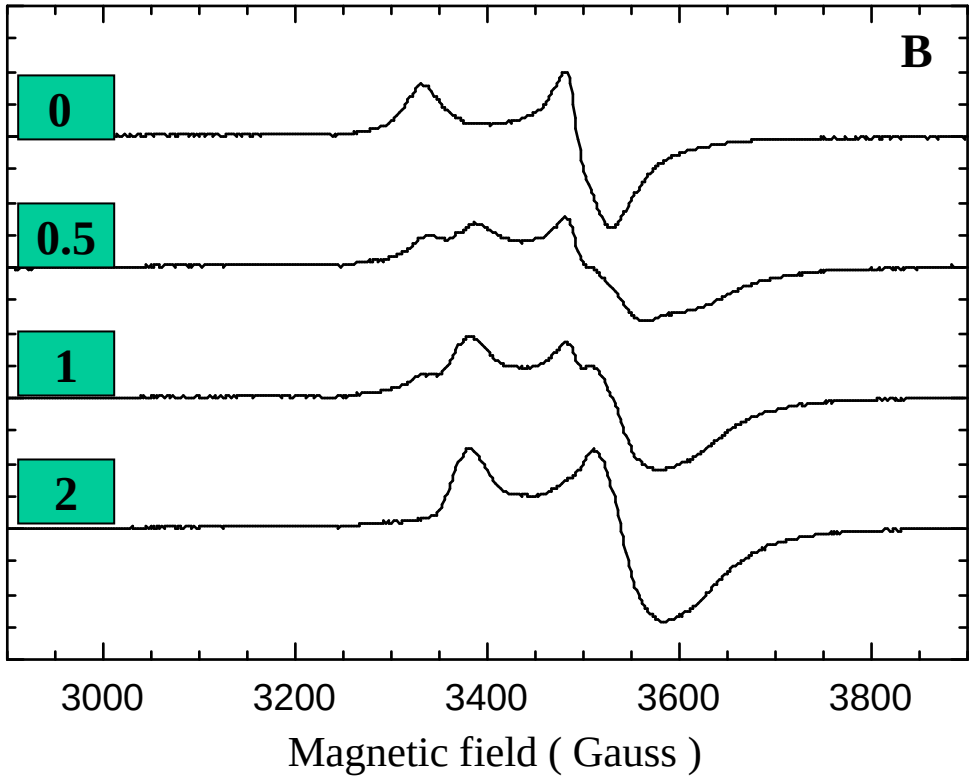
B. 4.2K, 7T

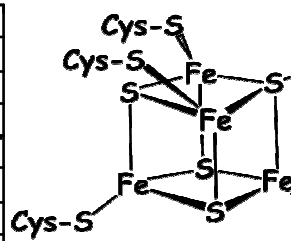
# Un complexe [4Fe-4S]-SAM dans l'activase

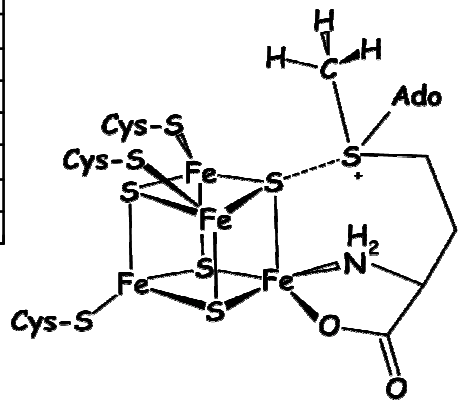
Protéine β

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



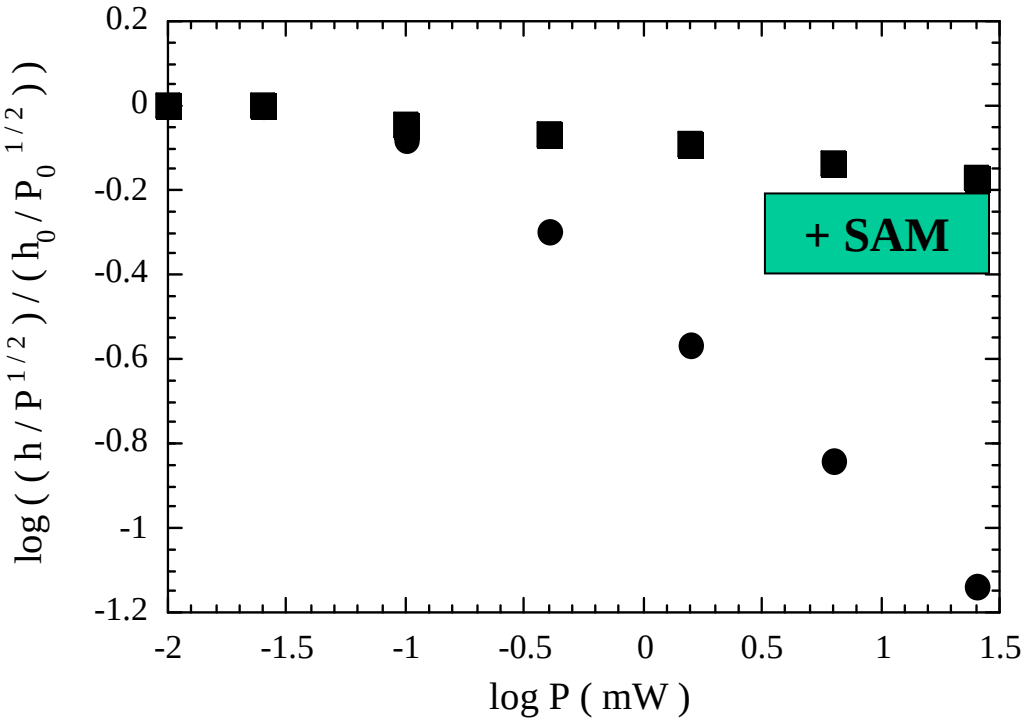




Resonance paramagnétique électronique

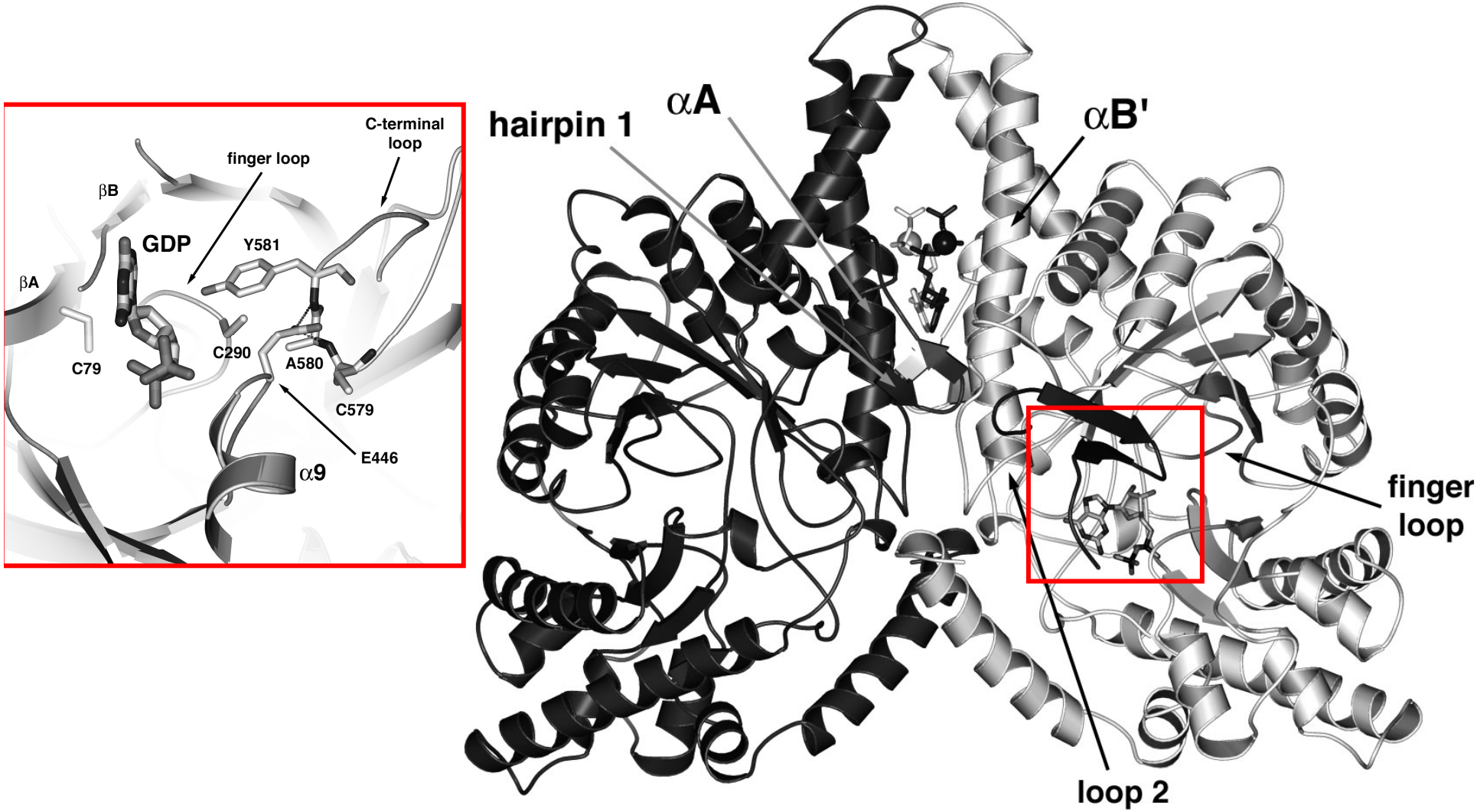
# Un complexe [4Fe-4S]-SAM dans l'activase

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

Figure 2

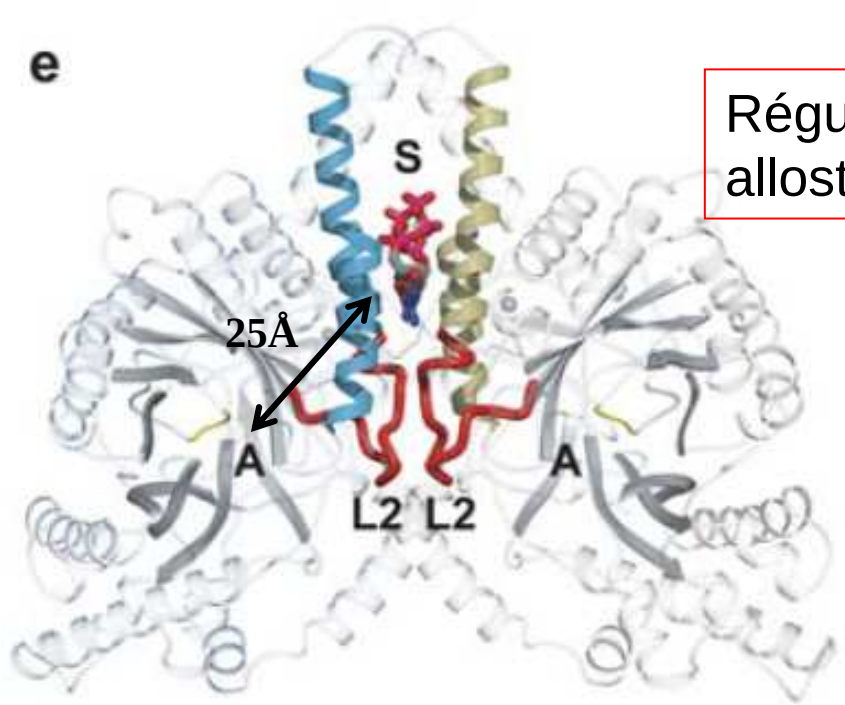


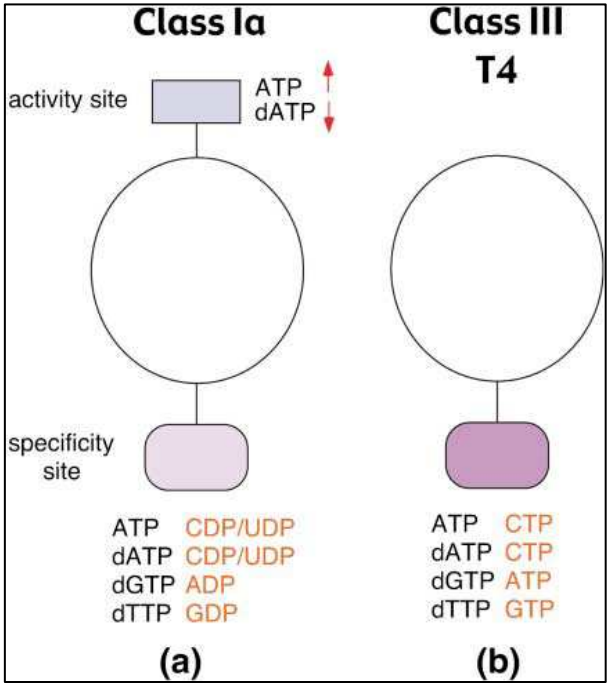
# 1999: structure tridimensionnelle de la ribonucléotide réductase α2 (nrdD)



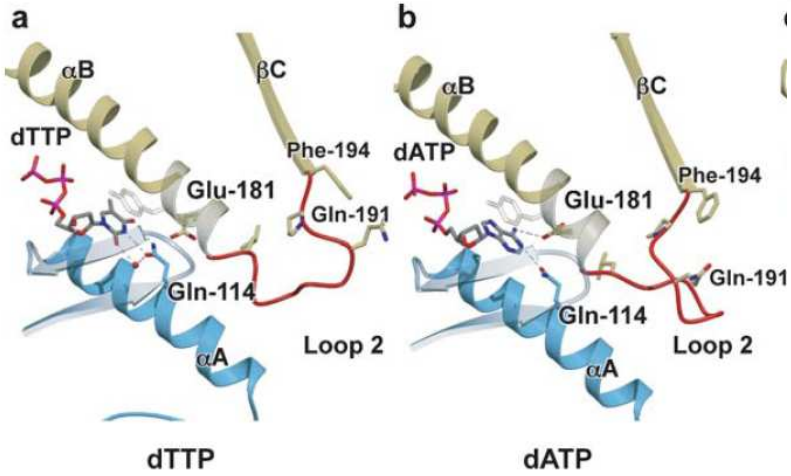

Logan, D.T., Andersson, J., Sjoberg, B.-M., and Nordlund, P. (1999). A glycyl radical site in the crystal structure of a class III ribonucleotide reductase. Science *283*, 1499–1504.

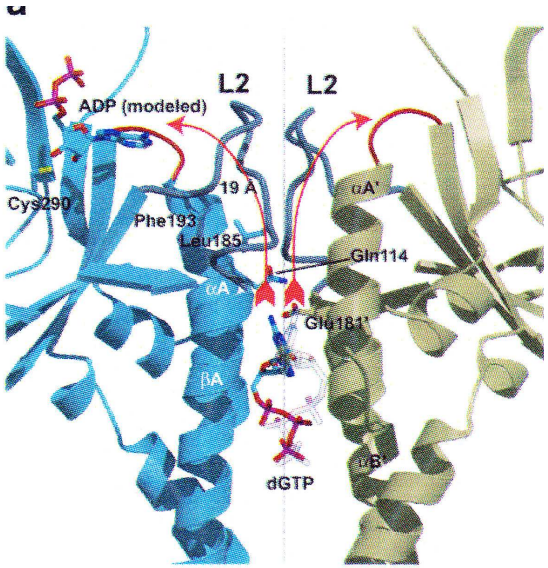
Régulation allostérique

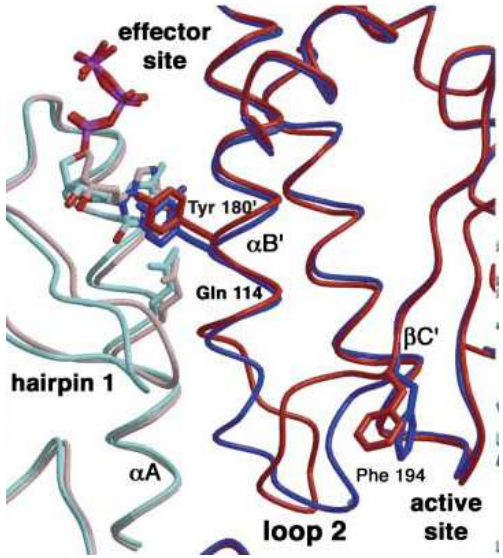


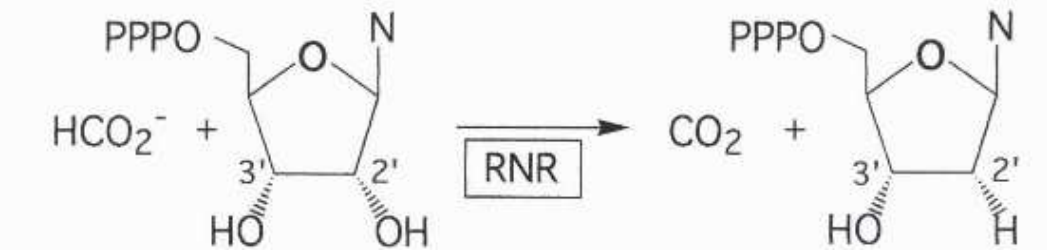

dATP
dTTP



# La ribonucléotide réductase: Une enzyme radicalaire

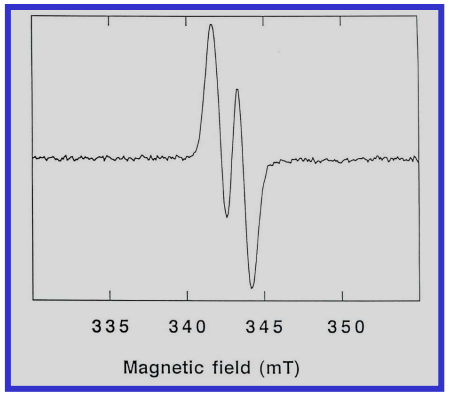


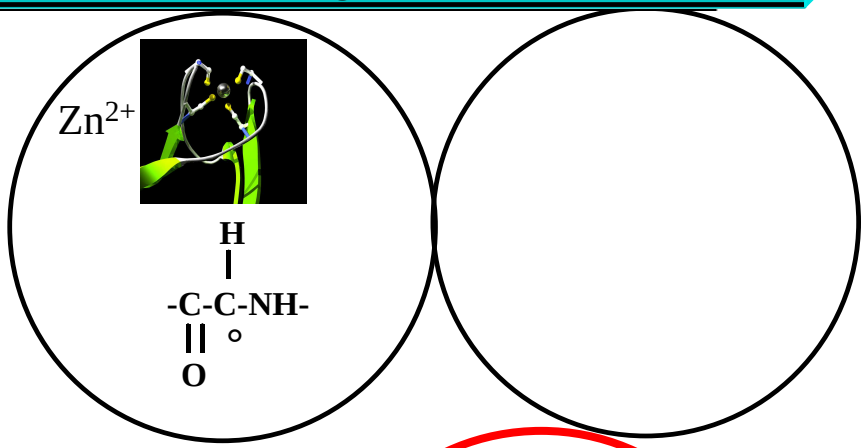

**M. Fontecave, E. Mulliez, D. Logan**

*Progress in Nucleic Acid Research and Molecular Biology 2002, 72, 95-127*
*Proc. Natl Acad. Sci.* 2003, 100, 3826-3831

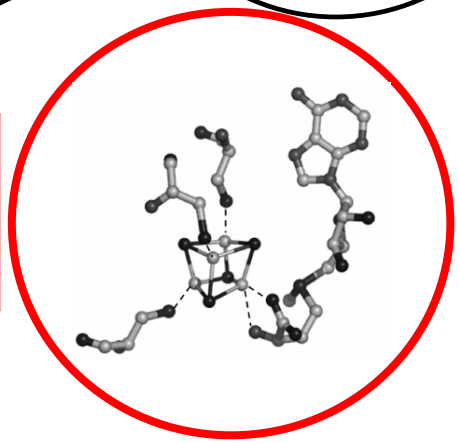
**Réductase nrdD 2x80kDa**

-C(H)-C(=O)-NH- = glycyl radical

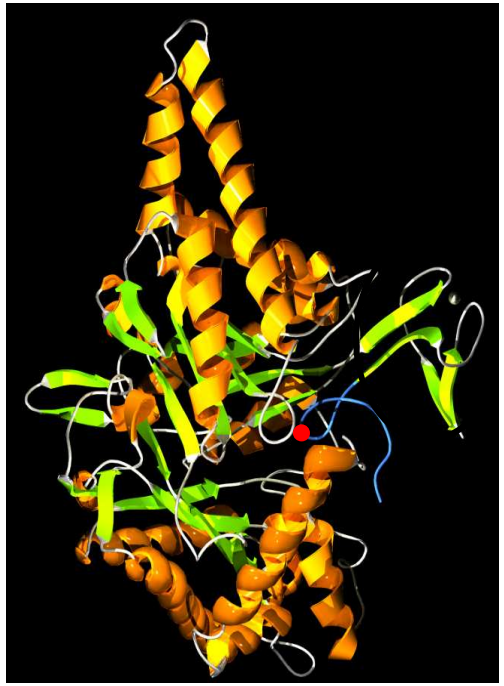



**Activase nrdG 2x17.5 kDa**



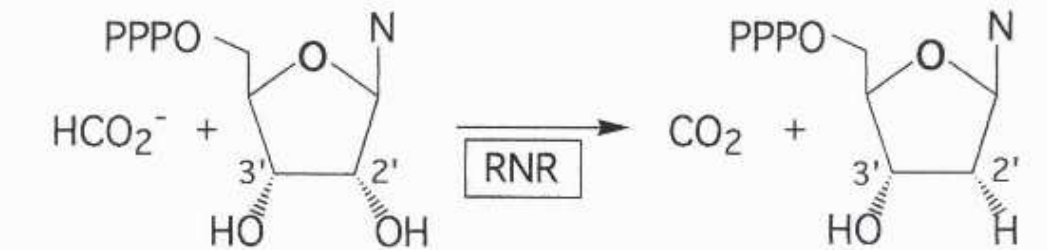
**Protéine α**



**protéine β**

$CX_3CX_2C$

# La ribonucléotide réductase: Une enzyme radicalaire

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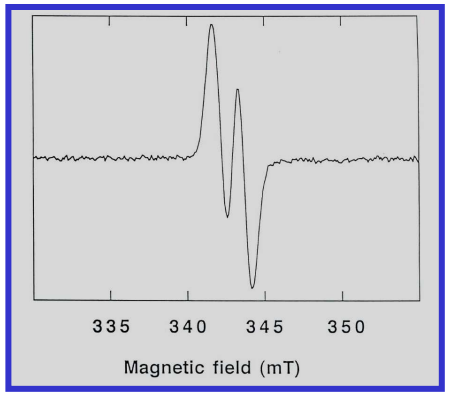
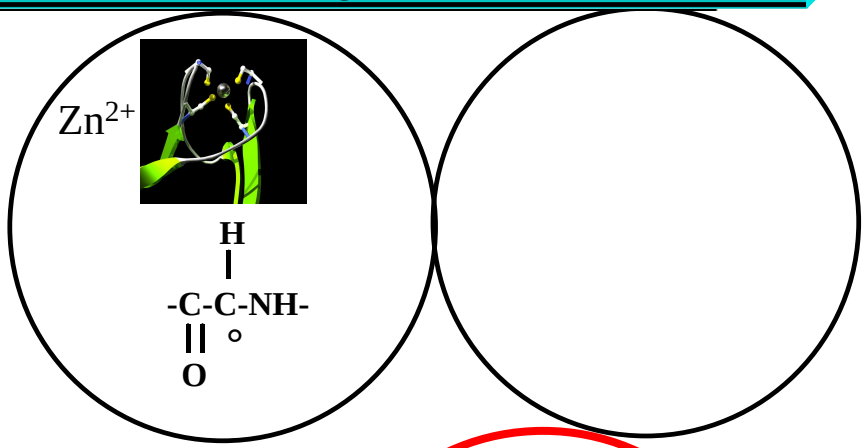
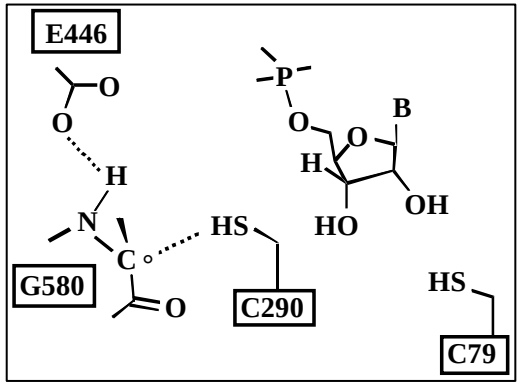
**Réductase nrdD 2x80kDa**

-C(H)-C(=O)-NH- (°) = glycyl radical

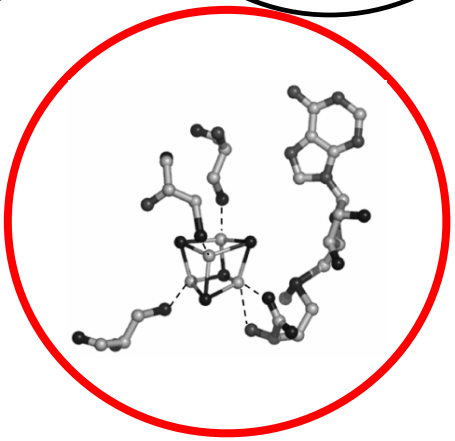
**Protéine α**




**Activase nrdG 2x17.5 kDa**

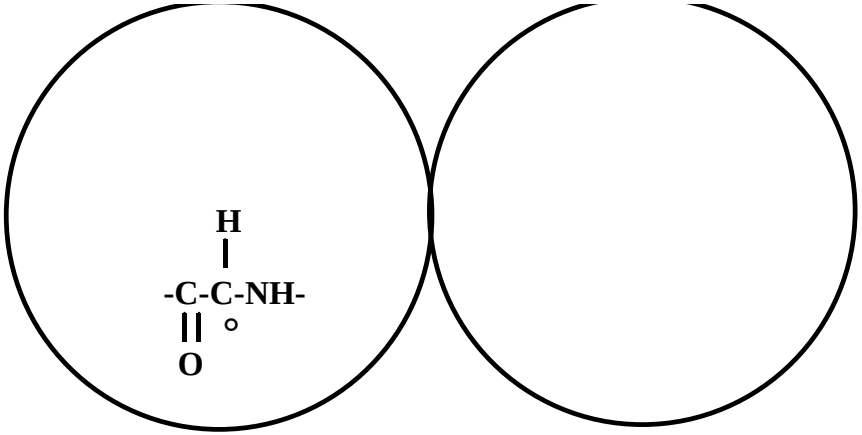


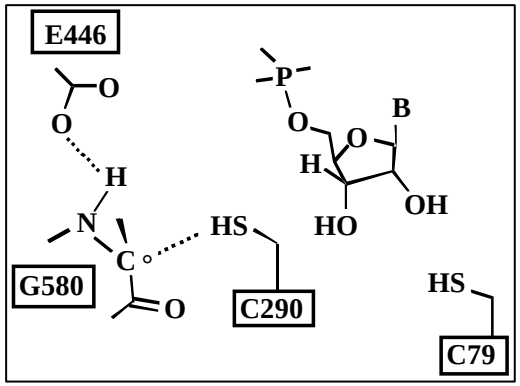
**protéine β**

**$CX_3CX_2C$**

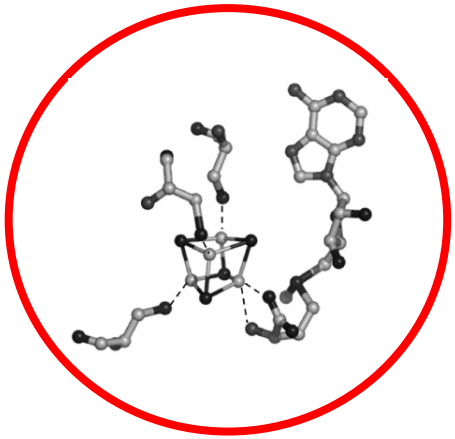
Très grande sensibilité à l'air

**Protéine α**

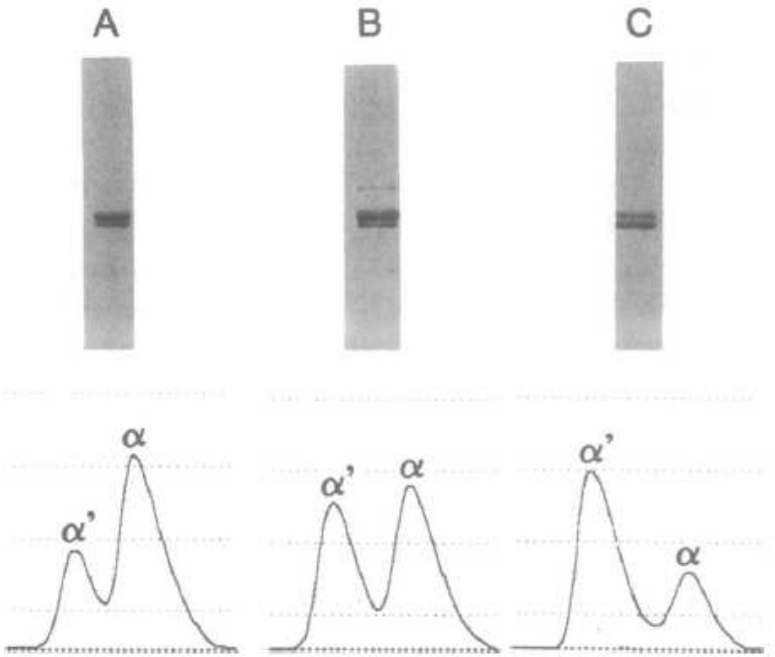


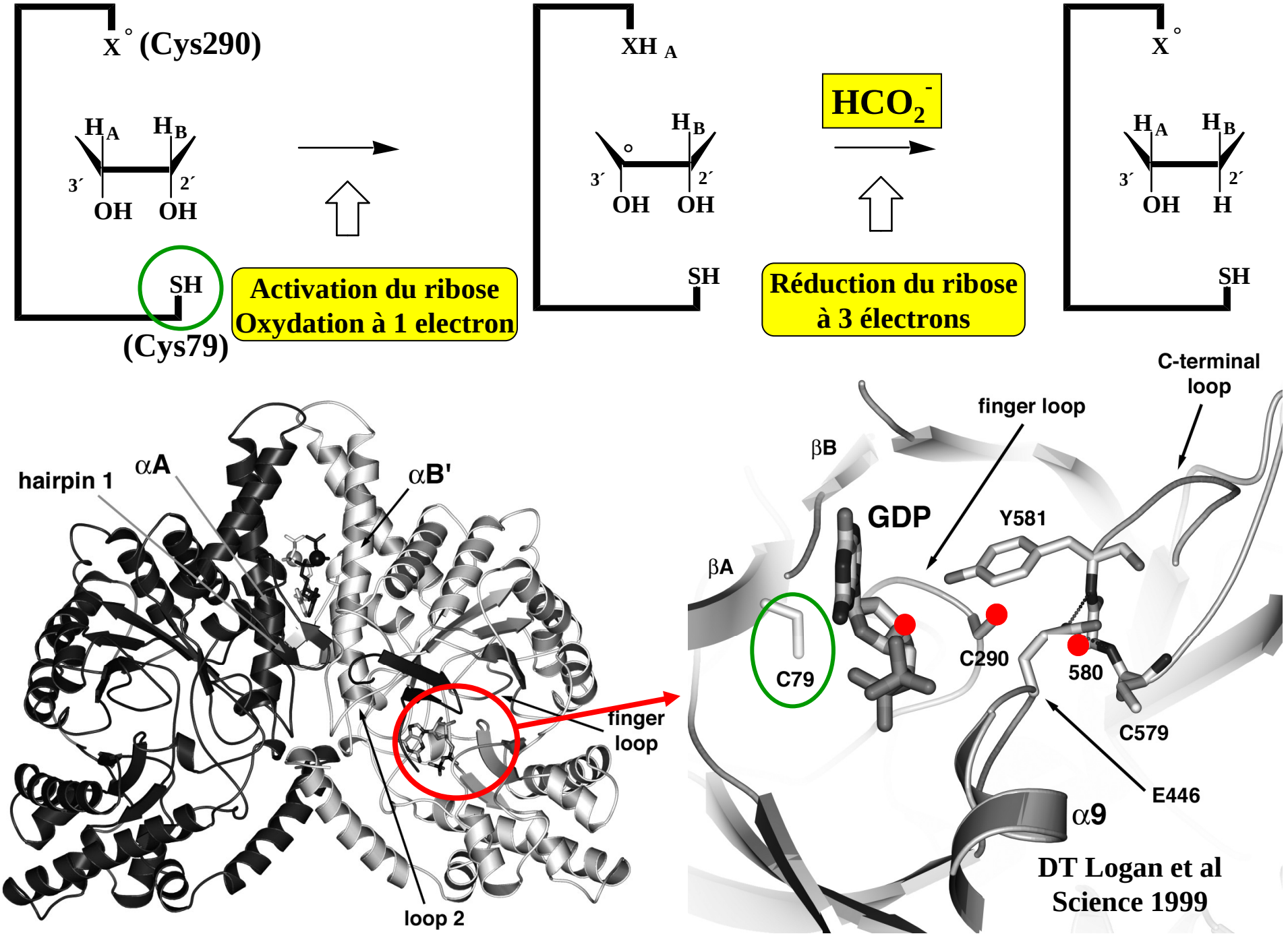



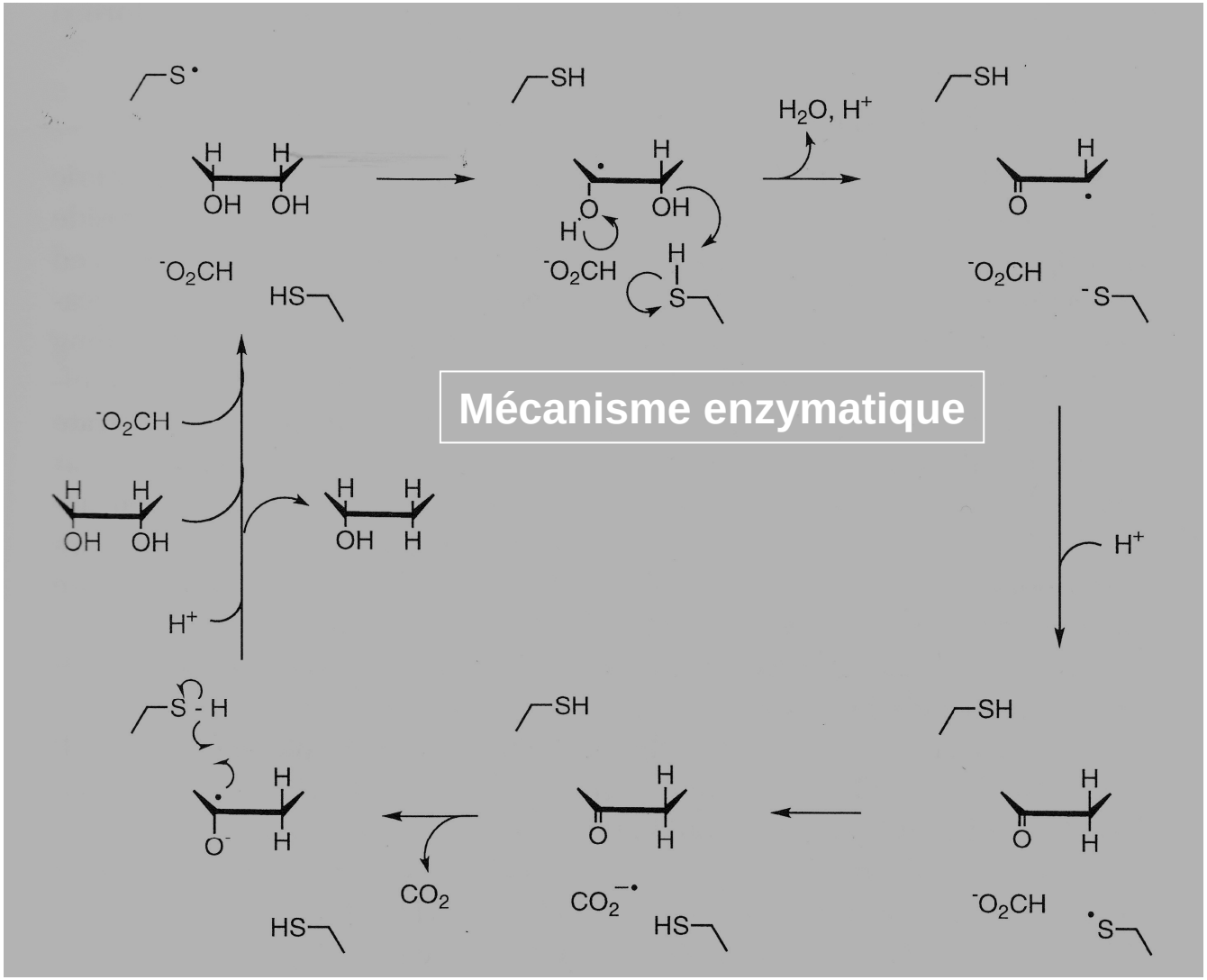

**protéine β**



[4Fe-4S]→[3Fe-4S]→[2Fe-2S]→[ ]




X° (Cys290)
H_A H_B
3´ 2´
OH OH
SH
(Cys79)
Activation du ribose
Oxydation à 1 electron
XH A
H_B
3´ 2´
OH OH
SH
HCO2-
Réduction du ribose
à 3 électrons
X°
H_A H_B
3´ 2´
OH H
SH
hairpin 1
αA
αB'
finger
loop
loop 2
C-terminal
loop
finger loop
βB
GDP
Y581
βA
C79
C290
580
C579
E446
α9
DT Logan et al
Science 1999




Marquage: [$^3$H]formate→ $^3$H dans solvant et non dans le produit

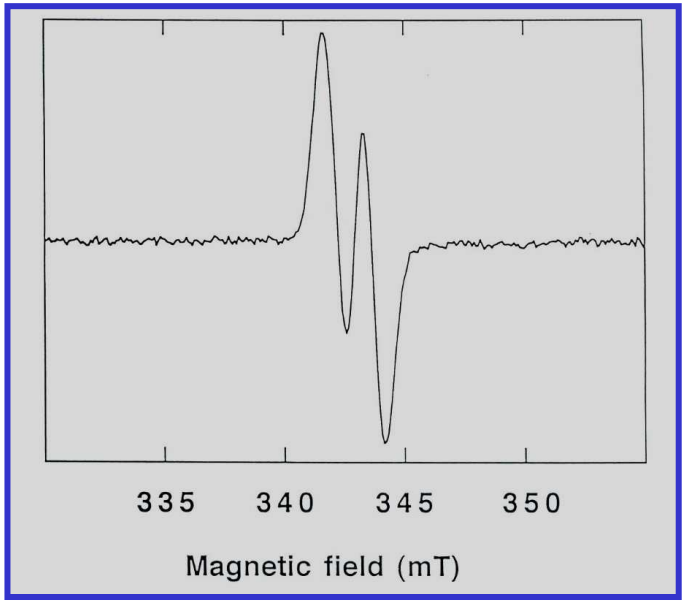
$D_2O$→D en position 2' (retention configuration)

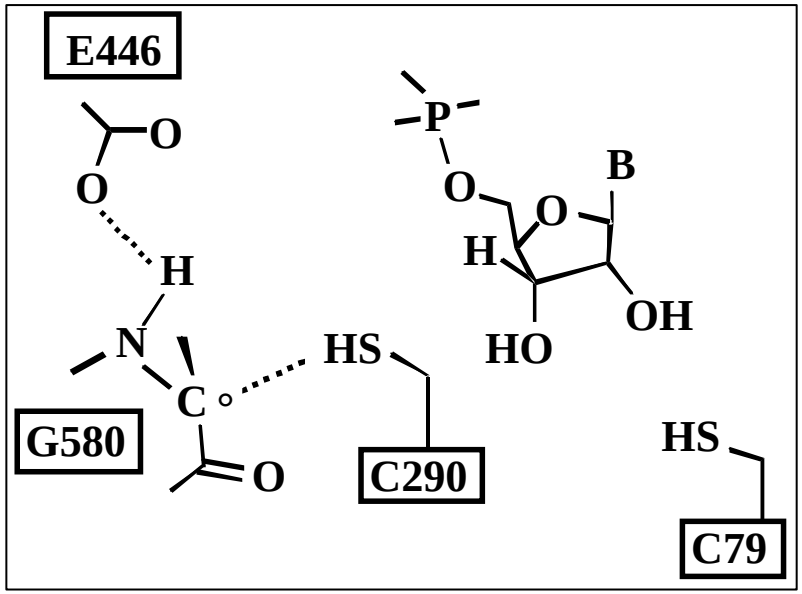
2% D en position 3'

P. Reichard, M. Fontecave et al.. BBRC 1995 214 28-35

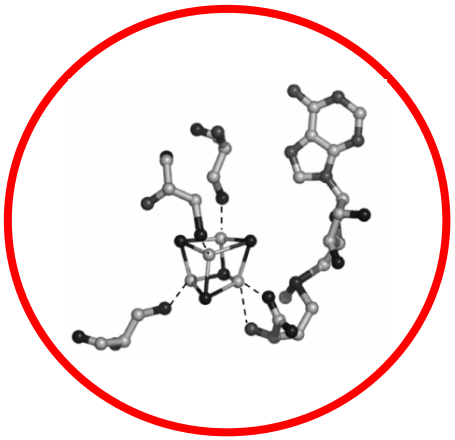
# Formation du radical glycinyle ?

# Fonction de l'activase (protéine β)



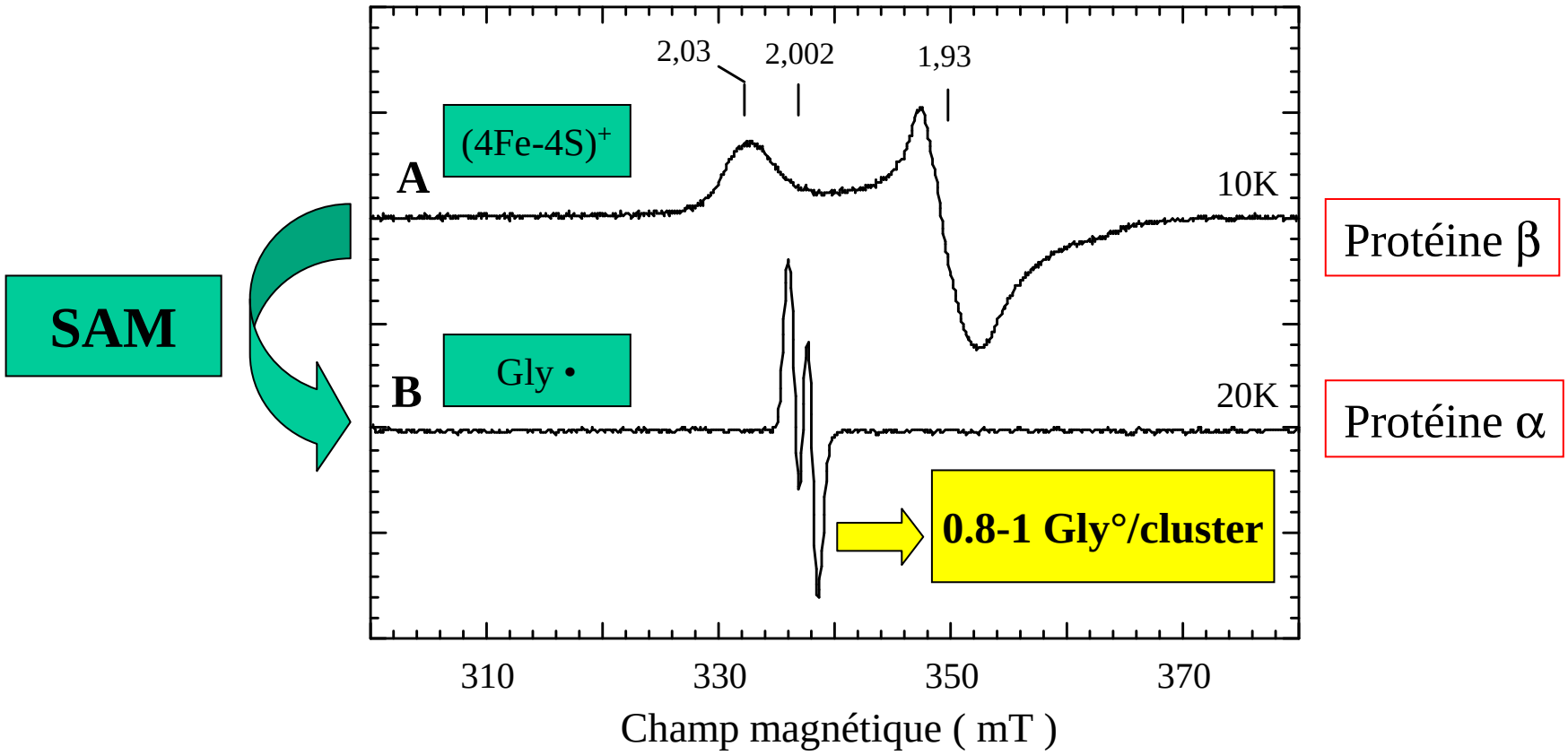

**Activase**
**nrdG**
**2x17.5 kDa**



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

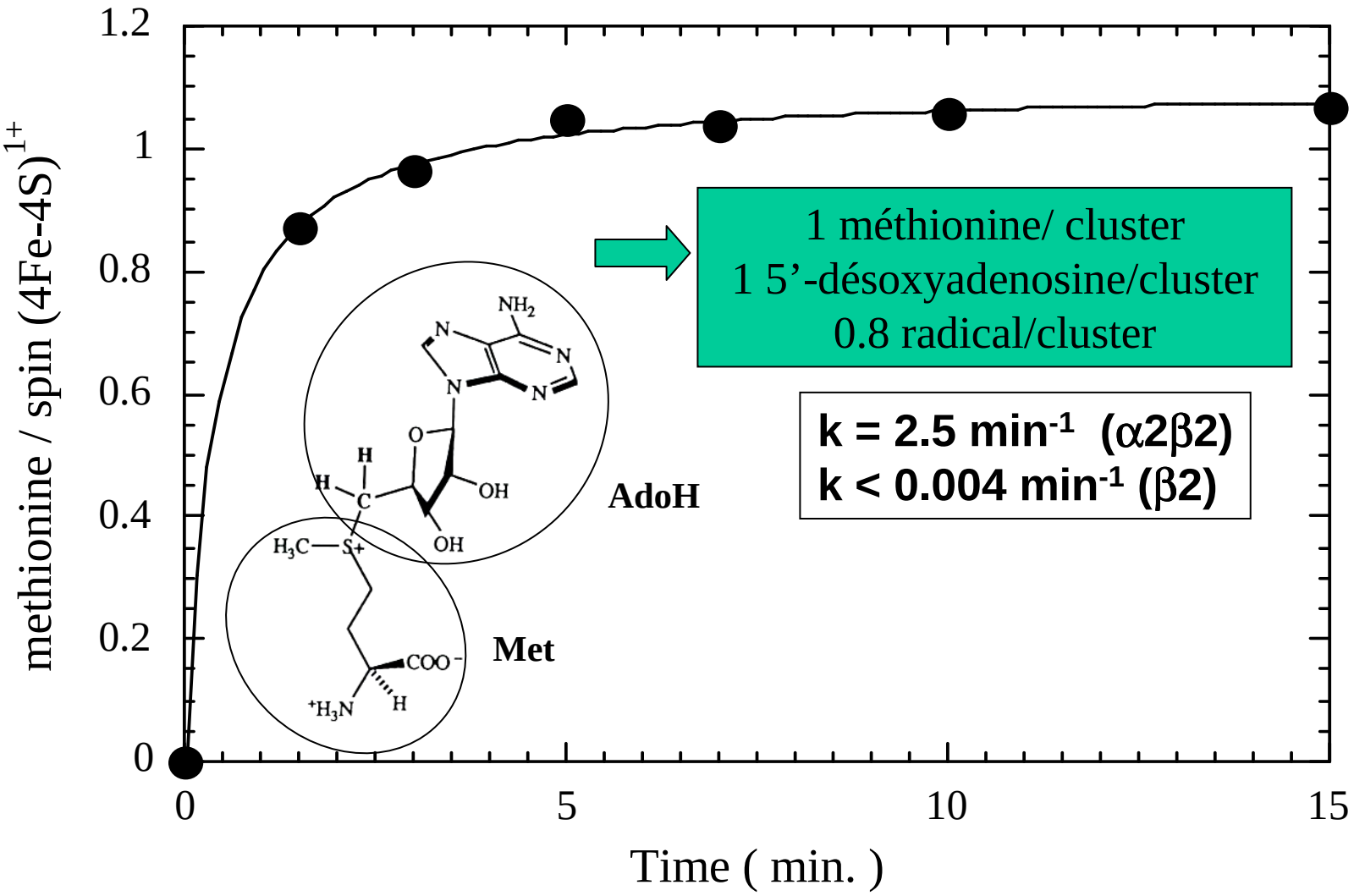
A : 220 µM ; 25 µW ; $5*10^4$ ; 1 mT ; 9,45 GHz

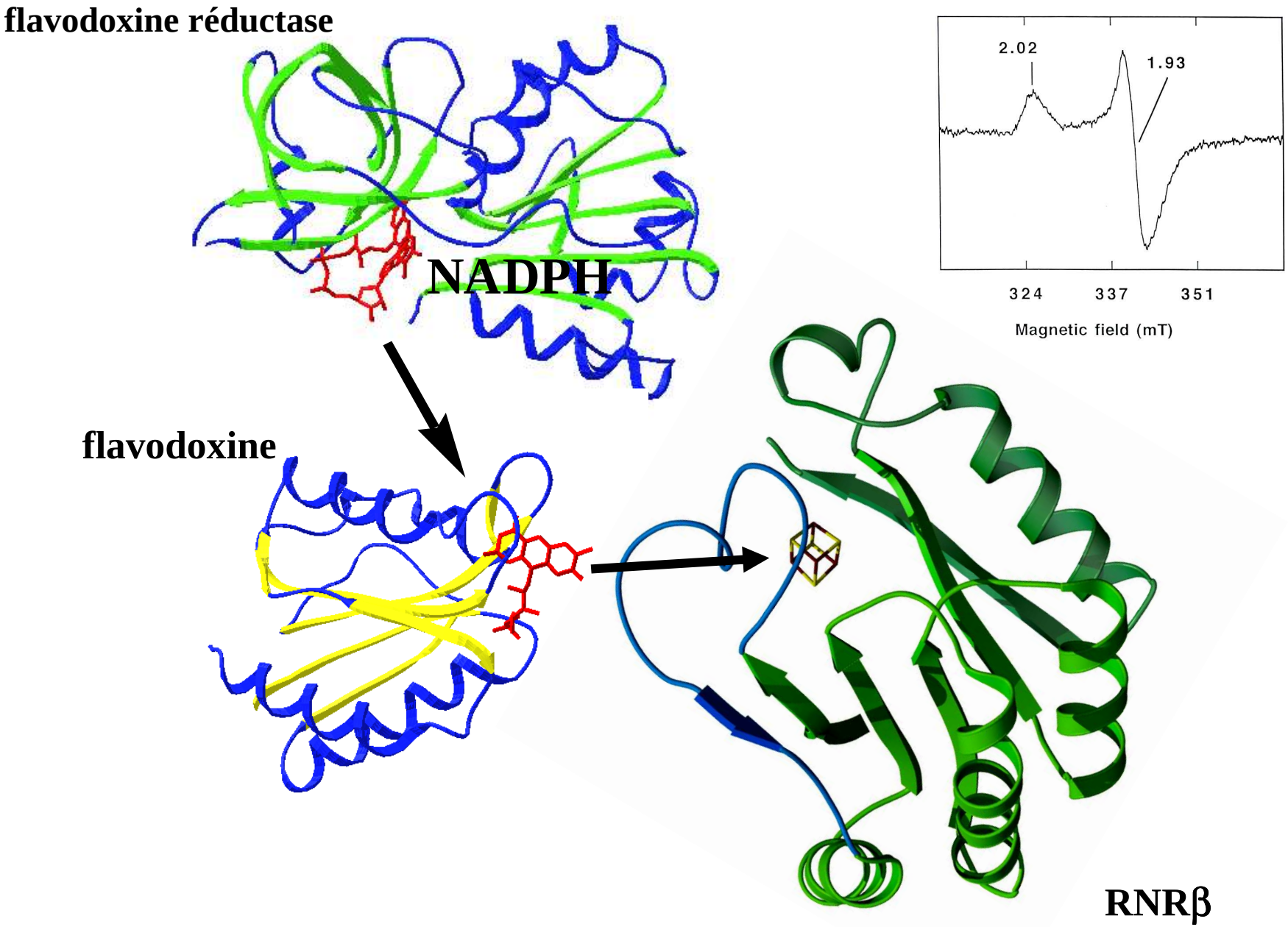
B : 220 µM ; 0,25µW ; $2*10^5$ ; 1 mT ; 9,45 GHz



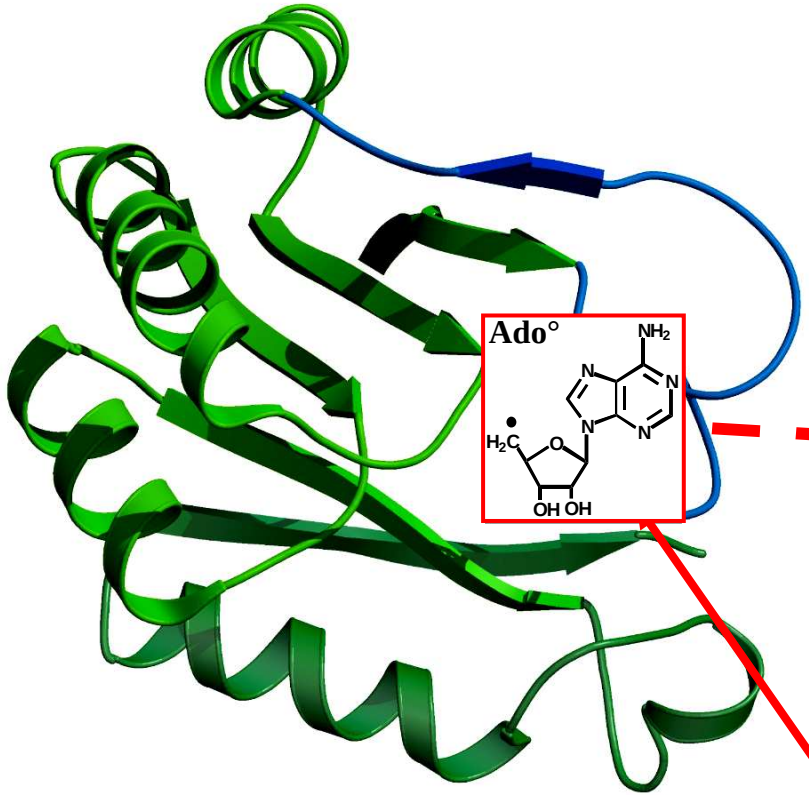

- NH-$CH_2$-CO- (α inactif) —SAM, β-[4Fe-4S]+→ - NH-$\dot{C}$H-CO- (α actif)

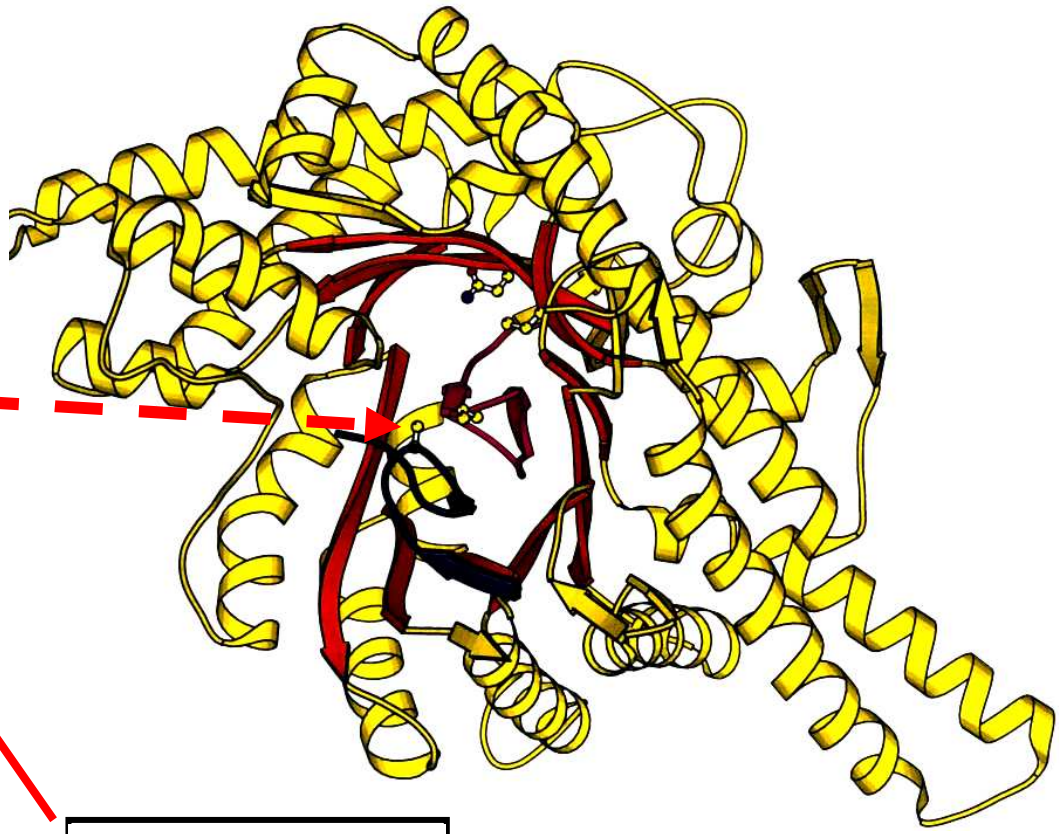
# Clivage de SAM par le complexe α2β2






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



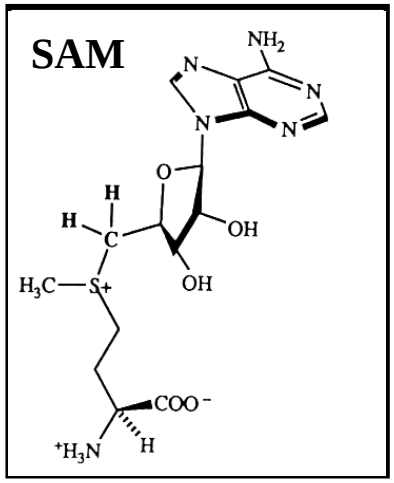



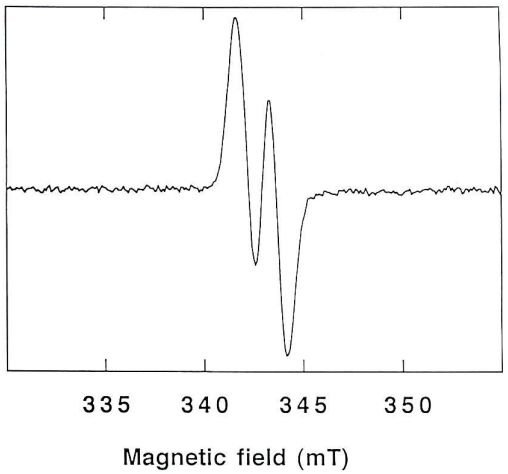
$$[4Fe4S]^{2+} \xrightarrow{\text{flavodoxin}} [4Fe4S]^{+}$$

$$[4Fe4S]^{+} + AdH_2C\text{–}S^{+} \rightleftharpoons \{[4Fe4S]^{+} \cdots AdH_2C\text{–}S^{+}\}$$

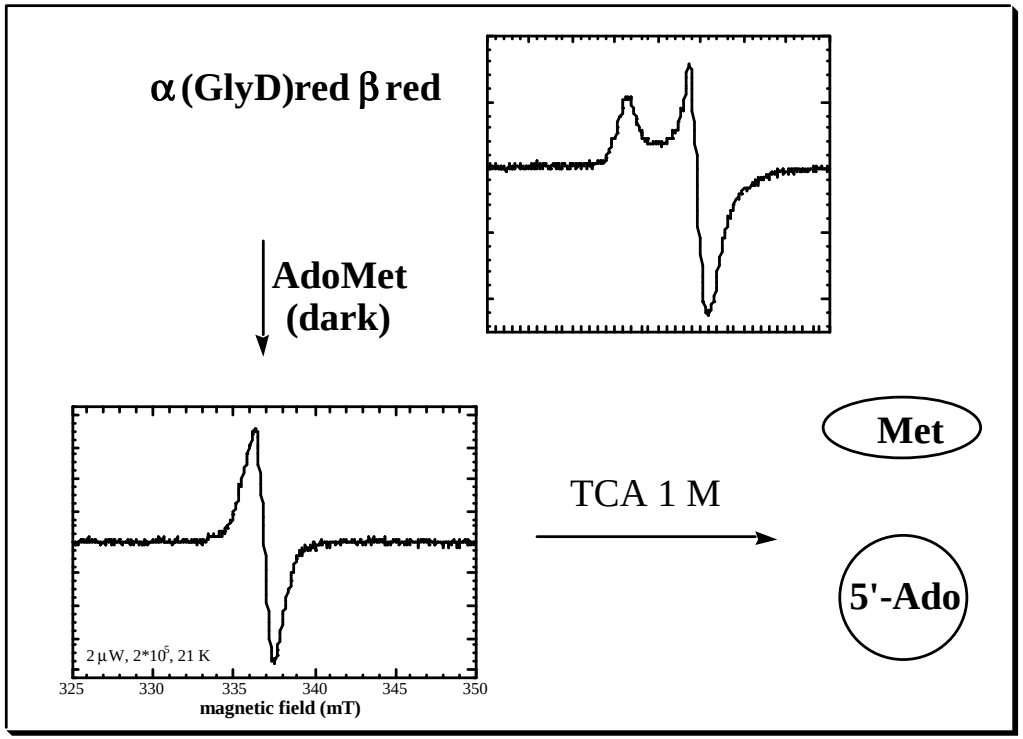
$$\{[4Fe4S]^{+} \cdots AdH_2C\text{–}S^{+}\} \rightarrow [4Fe4S]^{2+} + {}^{\bullet}CH_2Ad + Met$$

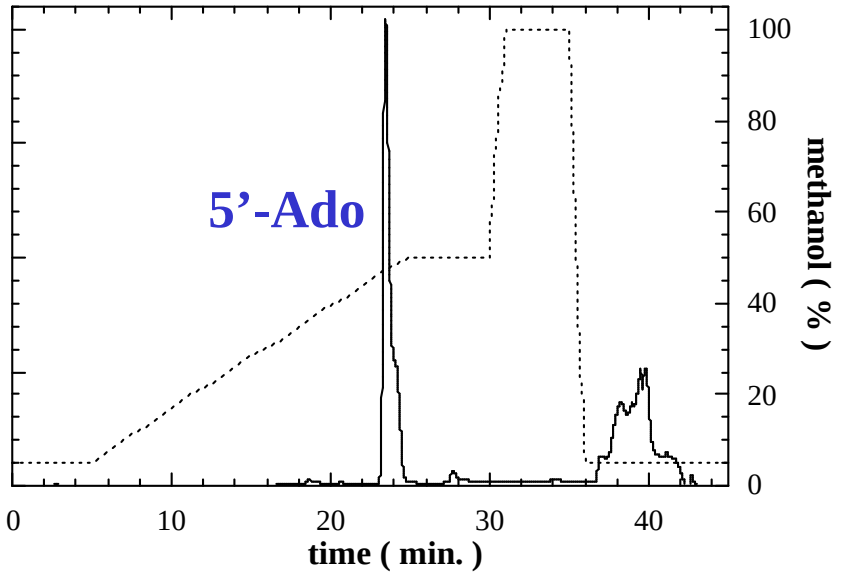
$${}^{\bullet}CH_2Ad + Gly\text{-}H \longrightarrow AdCH_3 + Gly^{\bullet}$$

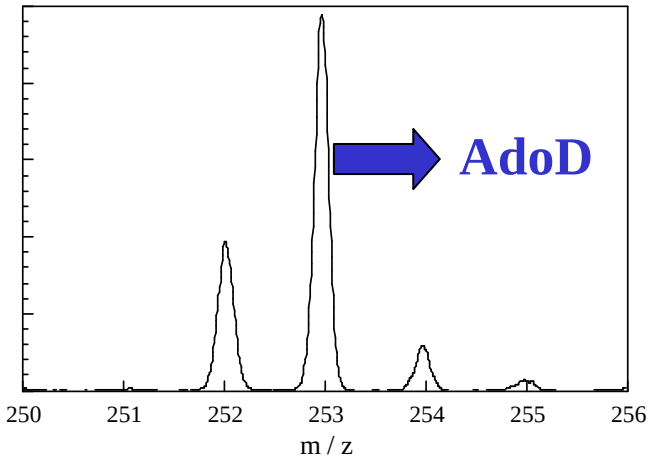


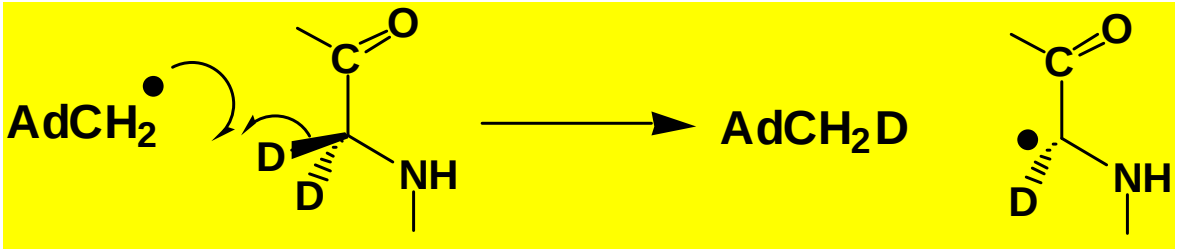



## 1 - REACTION

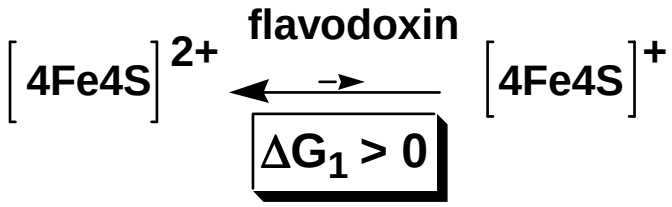


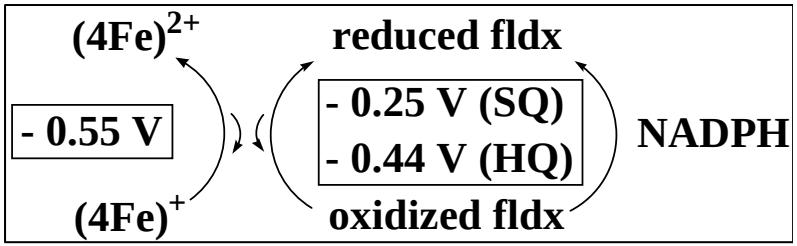



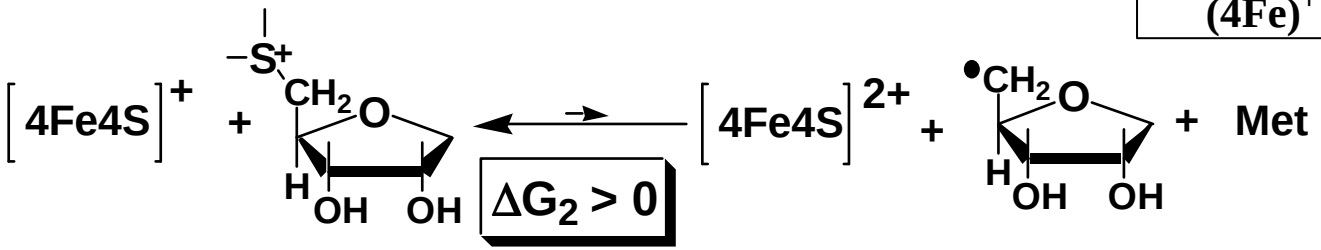



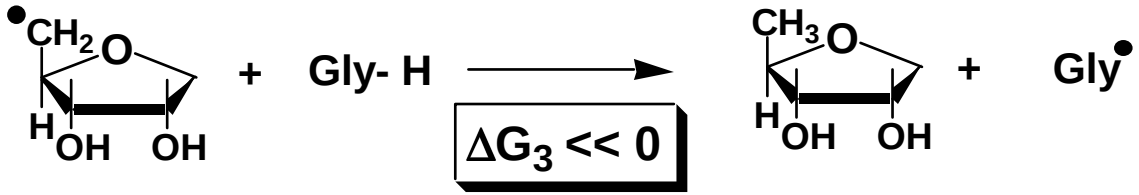



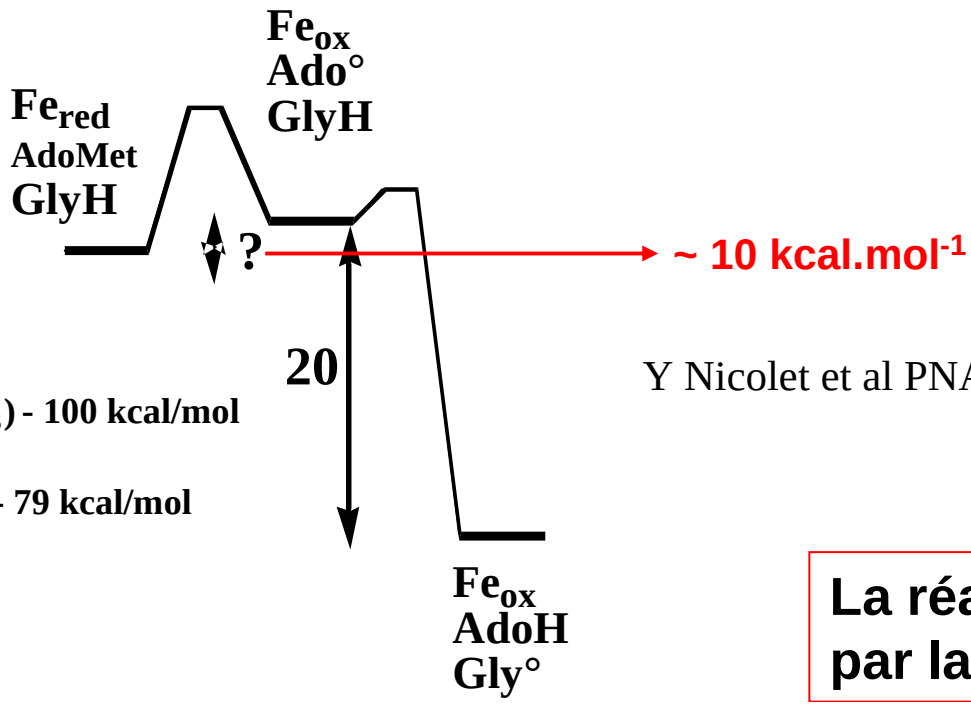

**Un transfert de radical « direct »**



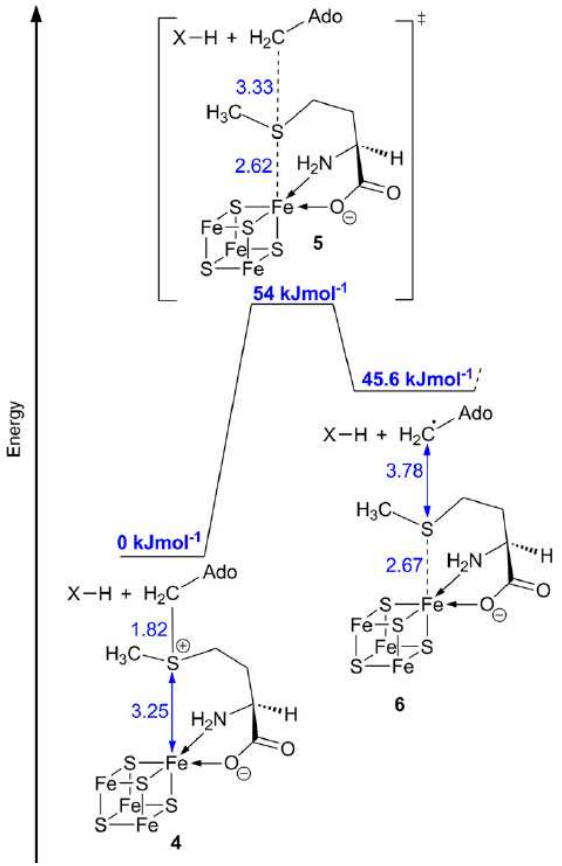




$E_{C-H}$ ($AdCH_3$) - 100 kcal/mol

$E_{C-H}$ (GlyH) - 79 kcal/mol

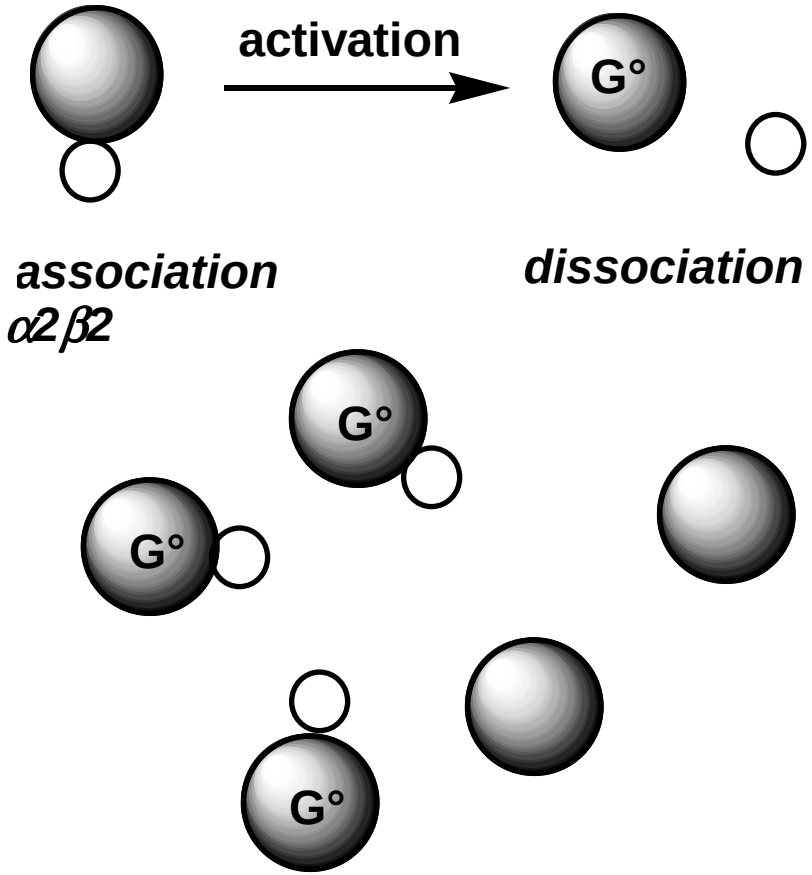
Y Nicolet et al PNAS 2009

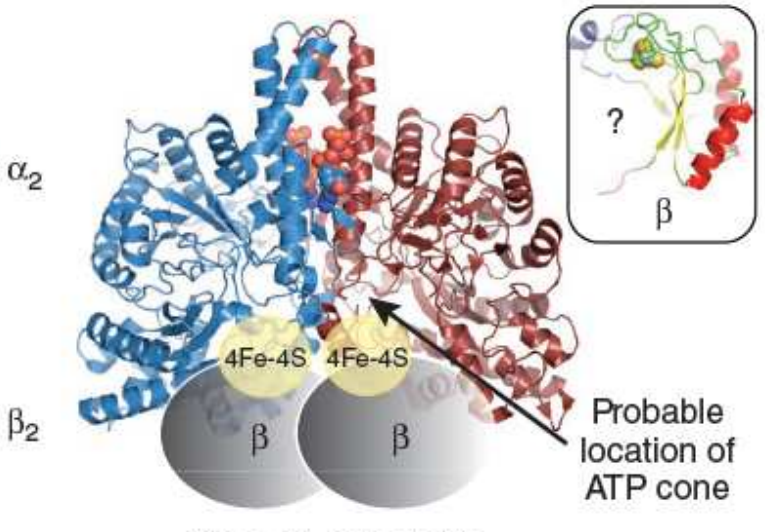


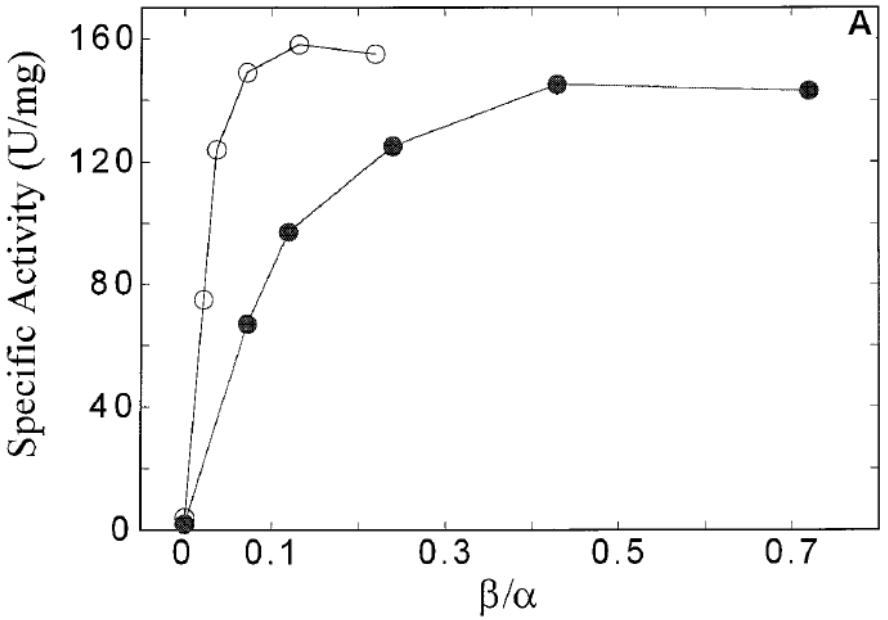

**La réaction est « tirée »**
**par la stabilité du radical Gly°**

# Protéine β est une » activase

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



# Biosynthèse des précurseurs de l'ADN chez les organismes *anaérobies* : du fer, de la S-adénosylméthionine et des radicaux libres

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