



COLLÈGE  
DE FRANCE  
—1530—

**Une découverte récente en enzymologie :  
la famille des métalloenzymes  
« Radical-SAM (S-AdénosylMéthionine) »**

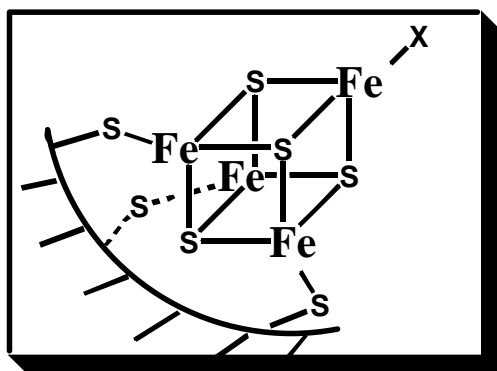
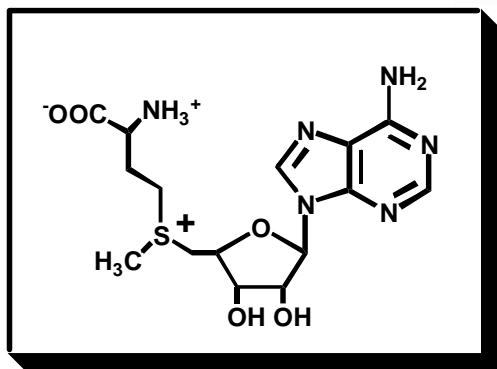
**Marc Fontecave**

*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*

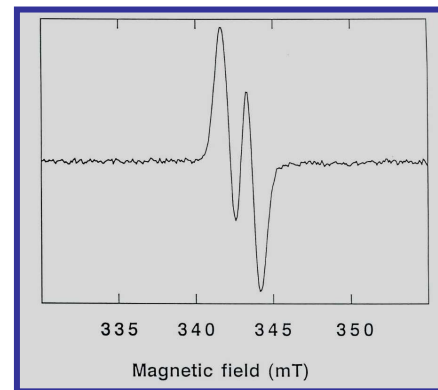
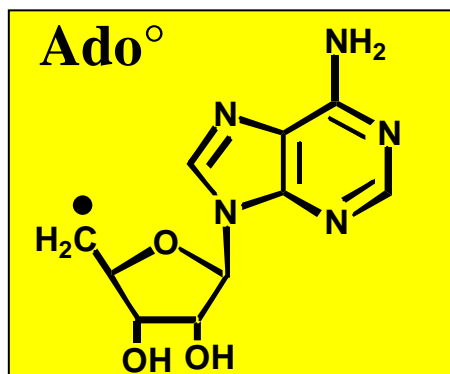
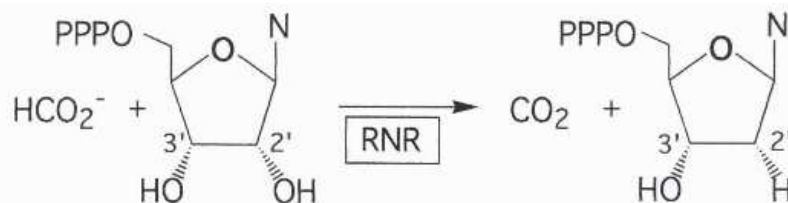
*Collège de France, 11 Place Marcelin Berthelot, 75231 Paris Cedex 05*

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

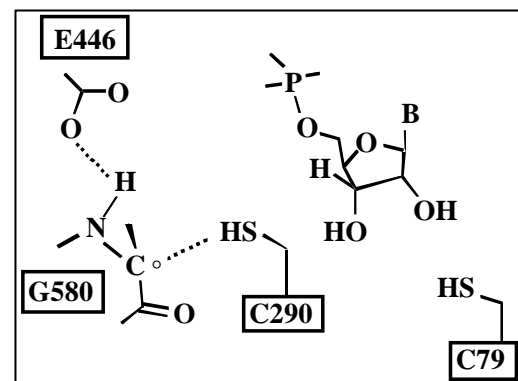
**SAM**



**[4Fe-4S]**



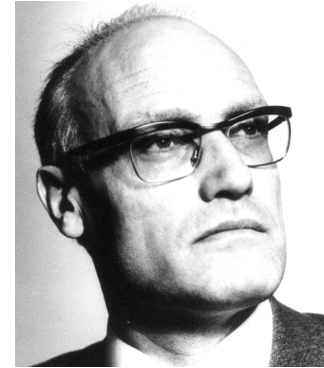
**Radical  
glycinyle**



# Enzymes Radical-SAM : les fondations

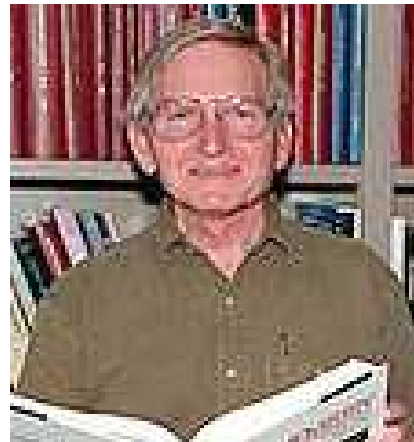
## Pyruvate-formate lyase (J. Knappe):

- 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<sup>o</sup> (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**

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 biotine**

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 ADN**

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,...)
- Alcaloïdes
- Chlorophylle

### **Métabolisme** de:

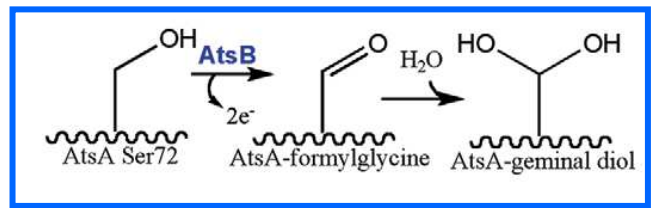
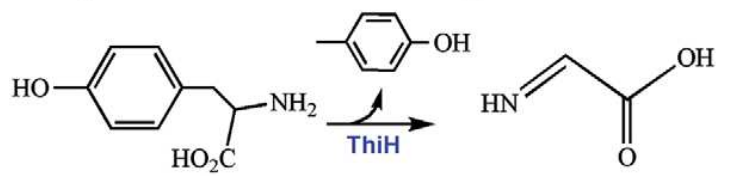
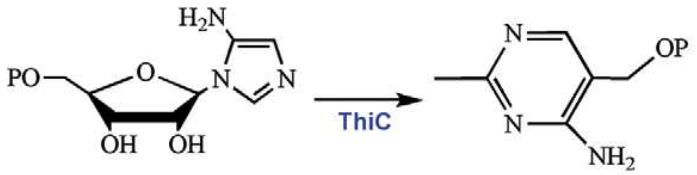
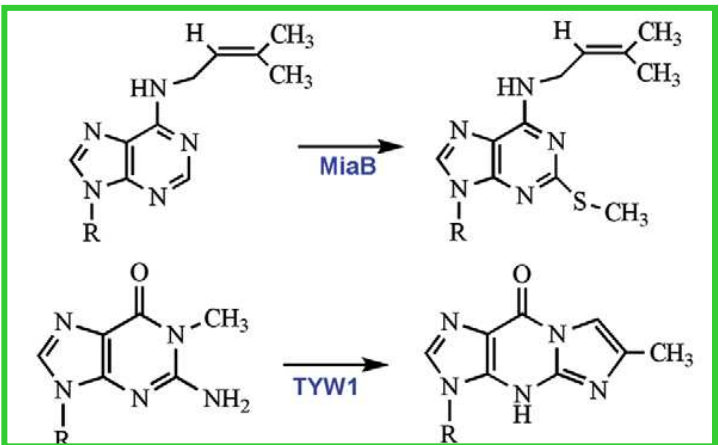
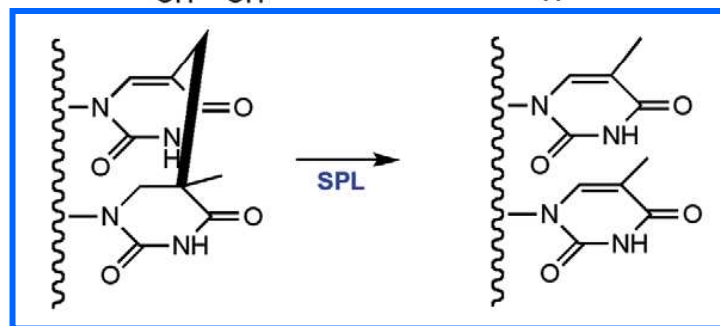
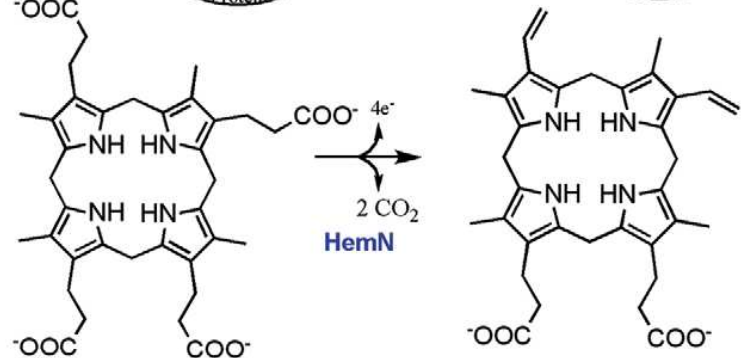
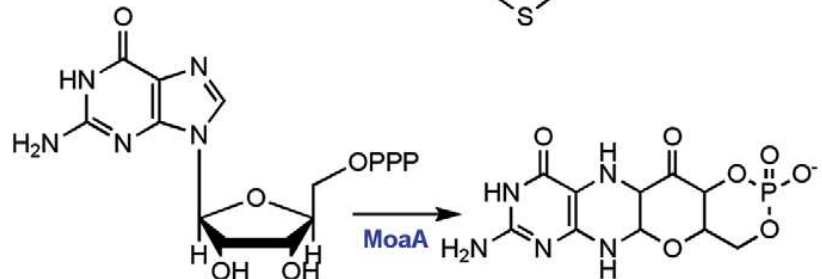
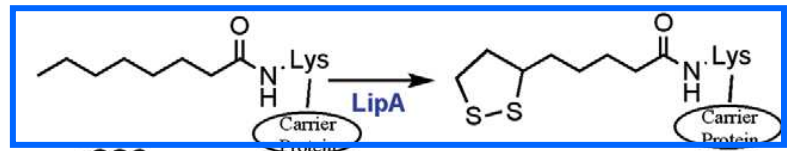
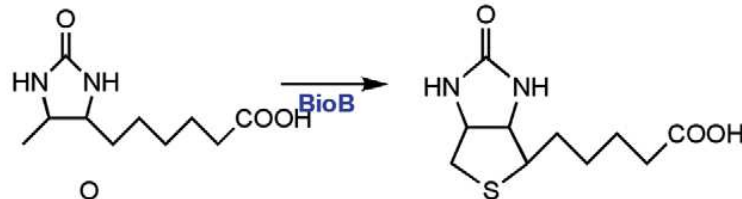
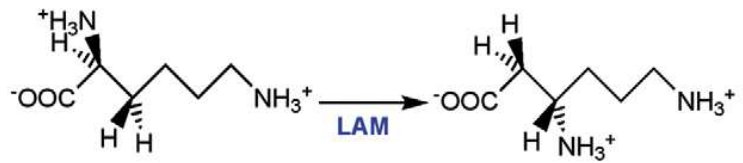
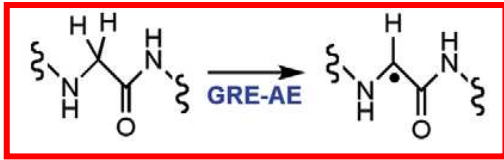
- Sucres
- Amino-acides
- Hydrocarbures

### **Modification** de:

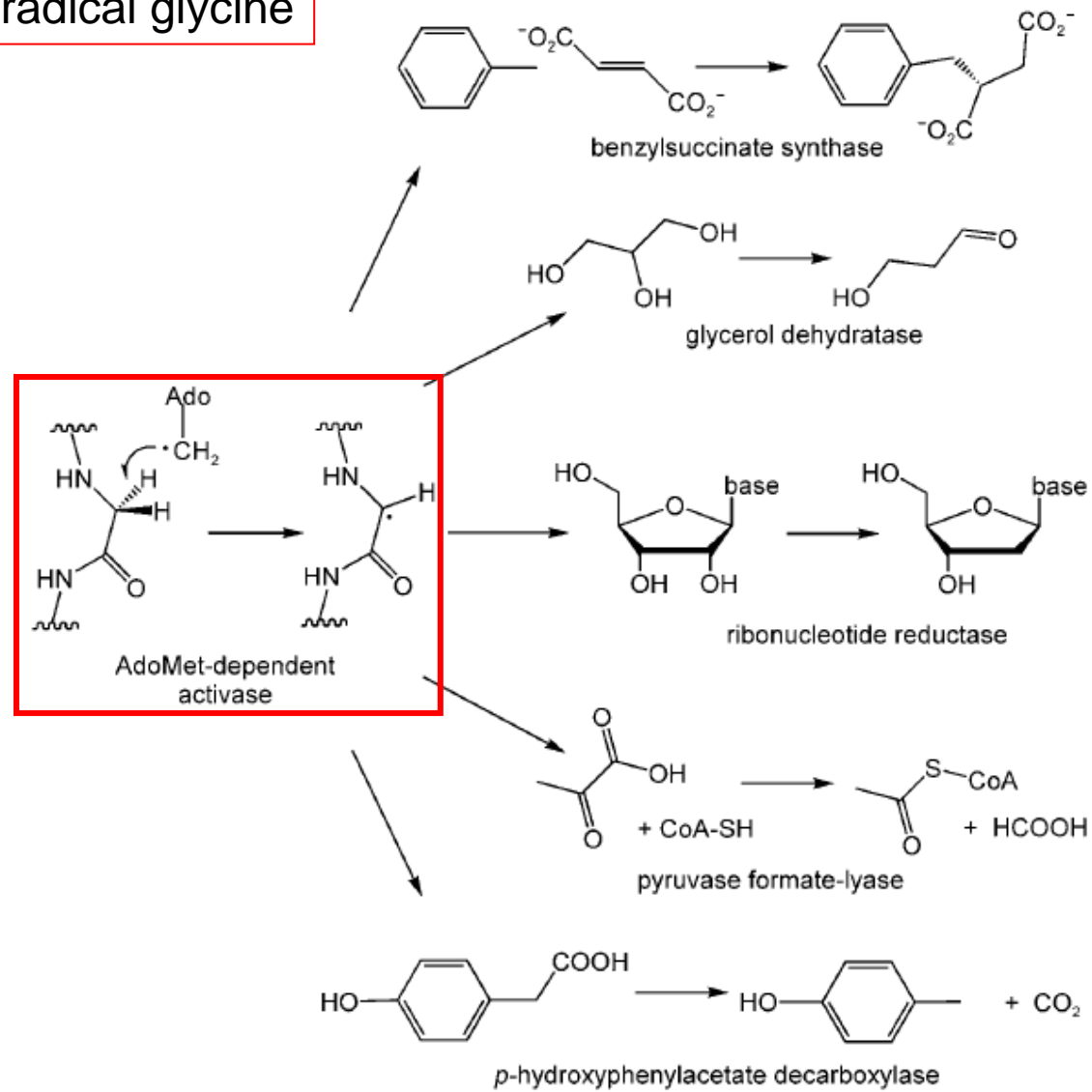
- ARNs de transfert
- Enzymes

### **Réparation** de:

- ADN



# Enzymes à radical glycine

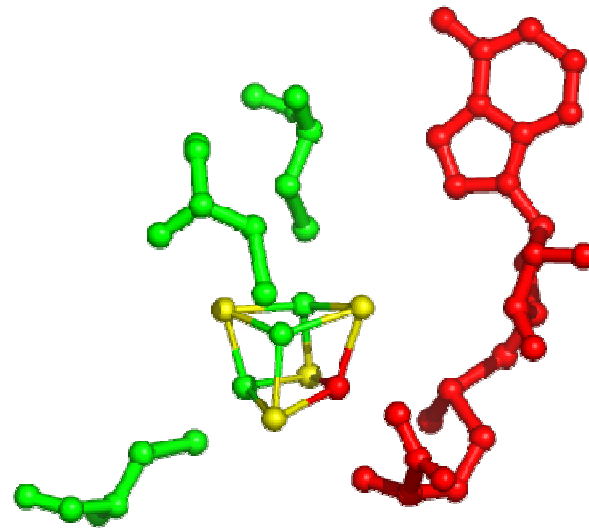
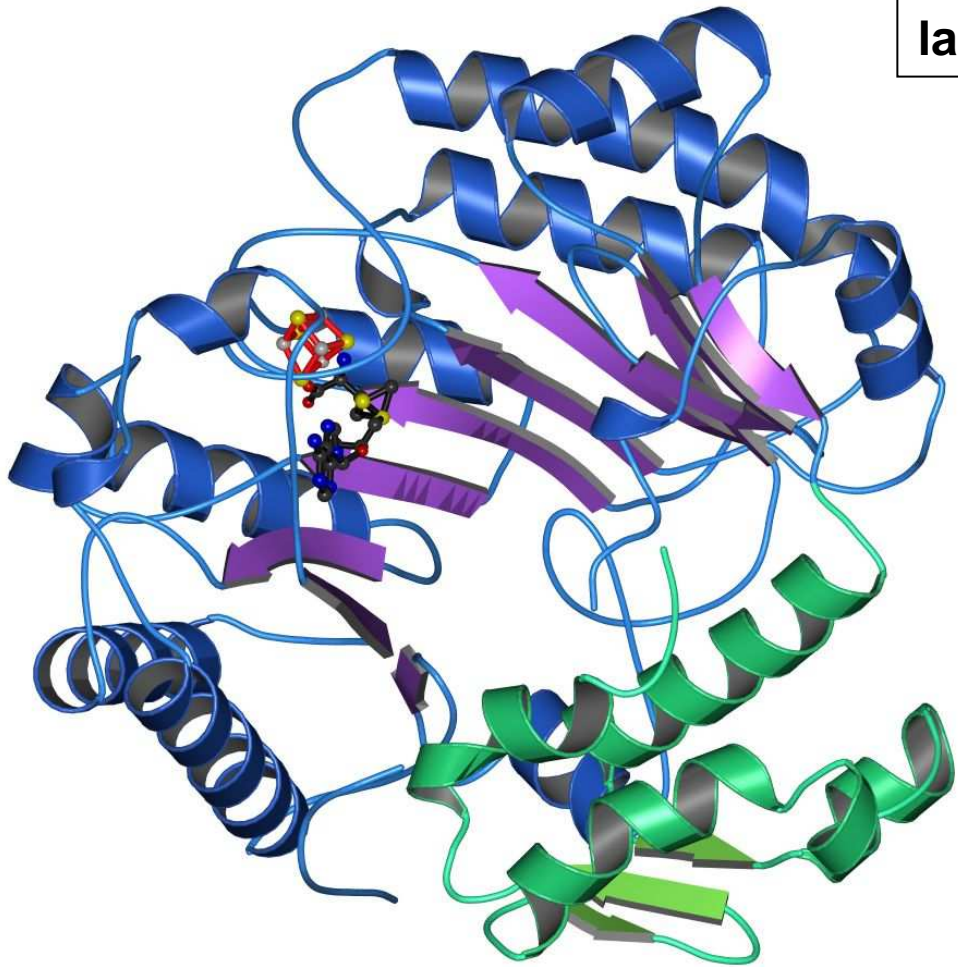


Un Cluster [4Fe-4S]  
Un complexe Cluster-SAM (2000.....)

Spectroscopies magnétiques (RPE, ENDOR, HYSCORE,...)  
Cristallographie rayons X

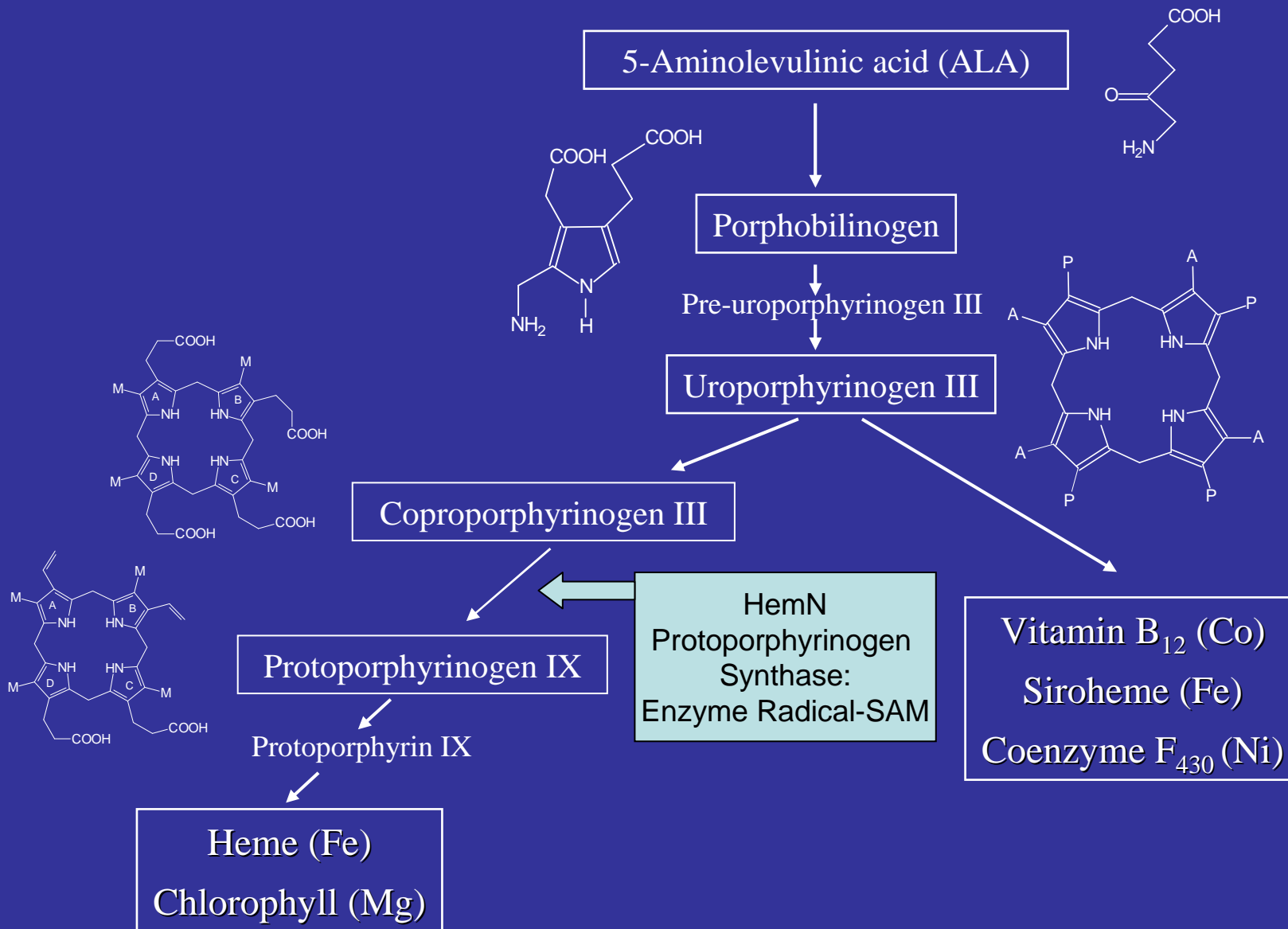
### la protoporphyrinogène synthase

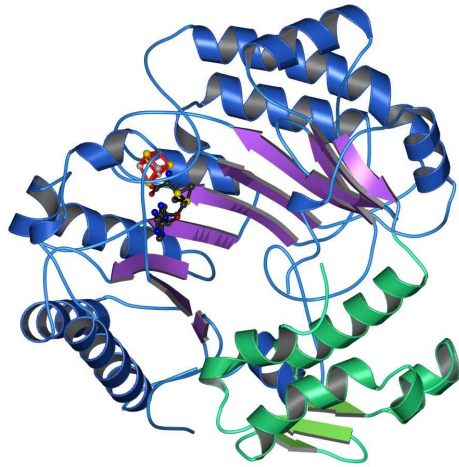
D. Jahn et al, EMBO J (2003) 6214



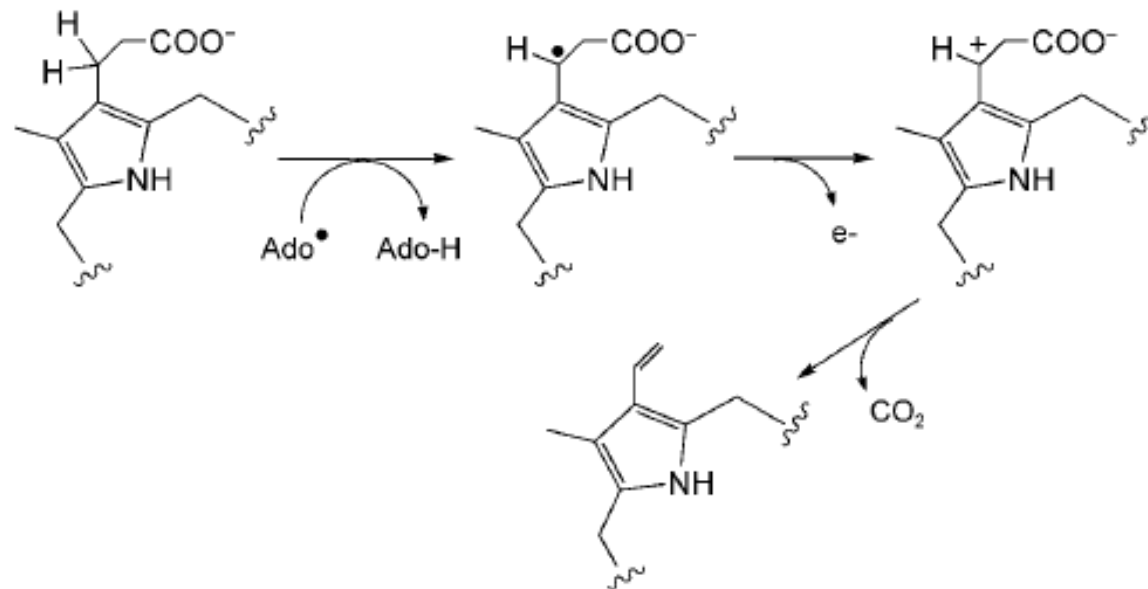
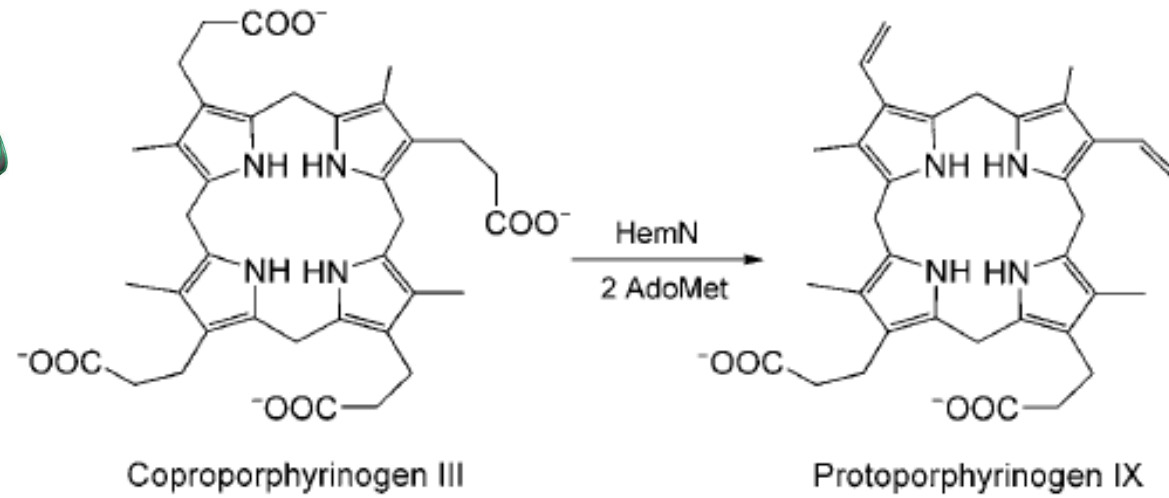


# Biosynthèse des tétrapyrroles (hèmes, etc...)



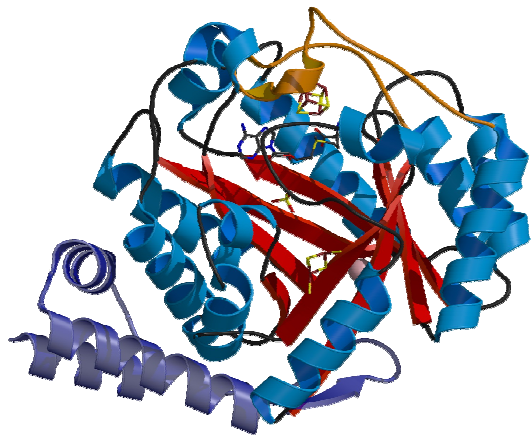


# la protoporphyrinogène synthase

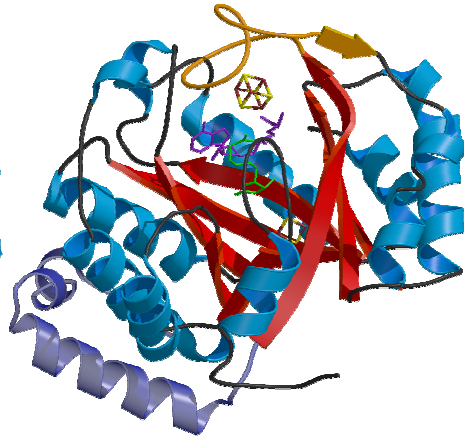


**La superfamille « Radical-SAM »:**  
**structures**

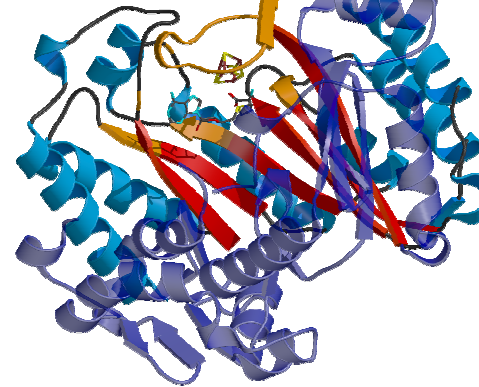
HydE 1.5 Å  
 maturase



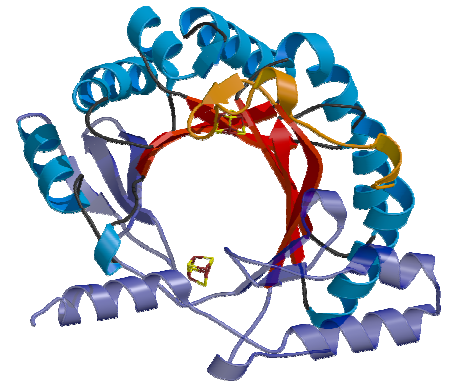
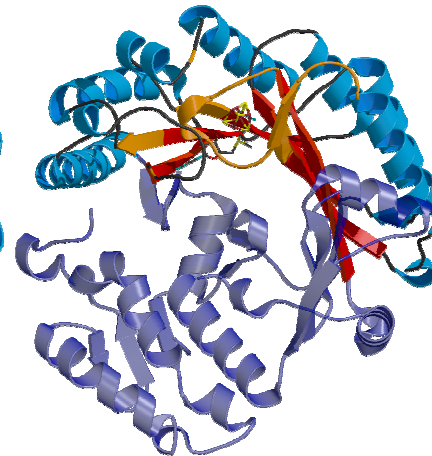
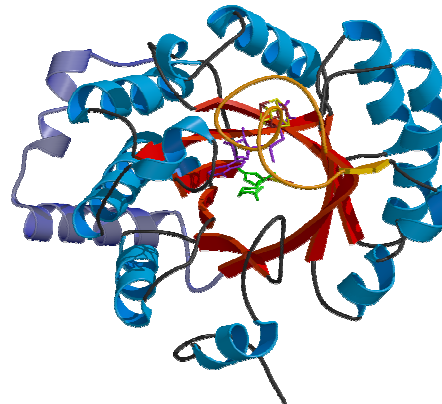
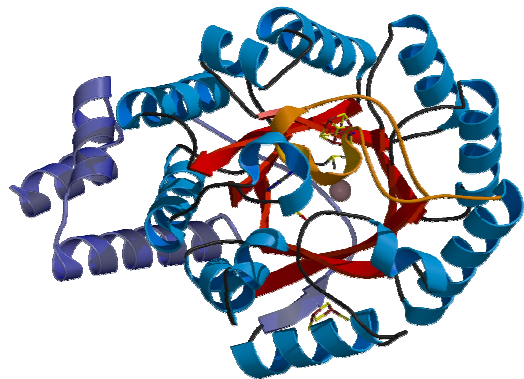
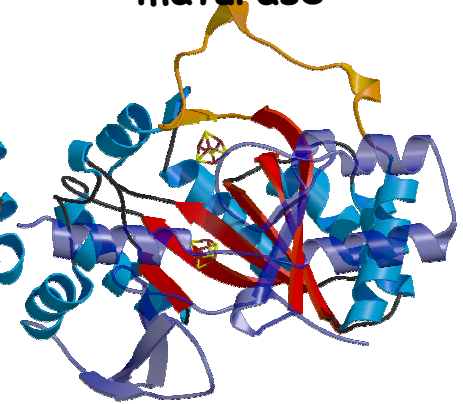
BioB 3.4 Å  
 Biotine synthase



HemN 2.07 Å  
 Coproporphyrinogen  
 synthase



MoaA 2.2 Å  
 maturase



rmsd: -

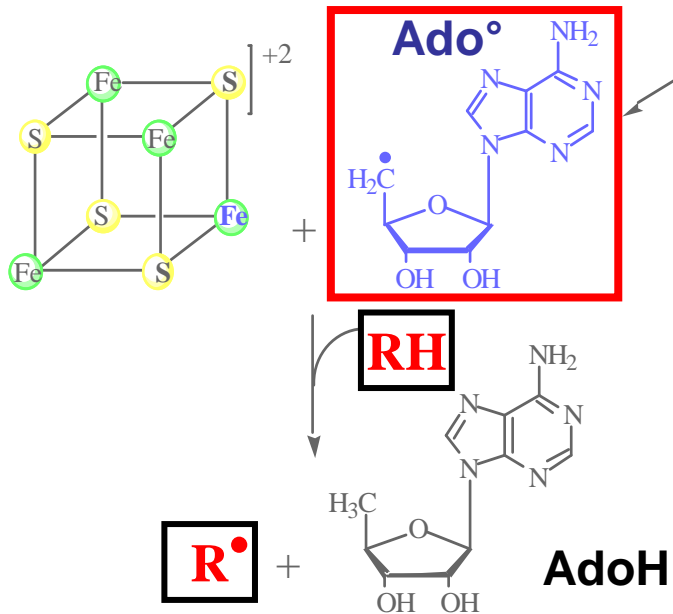
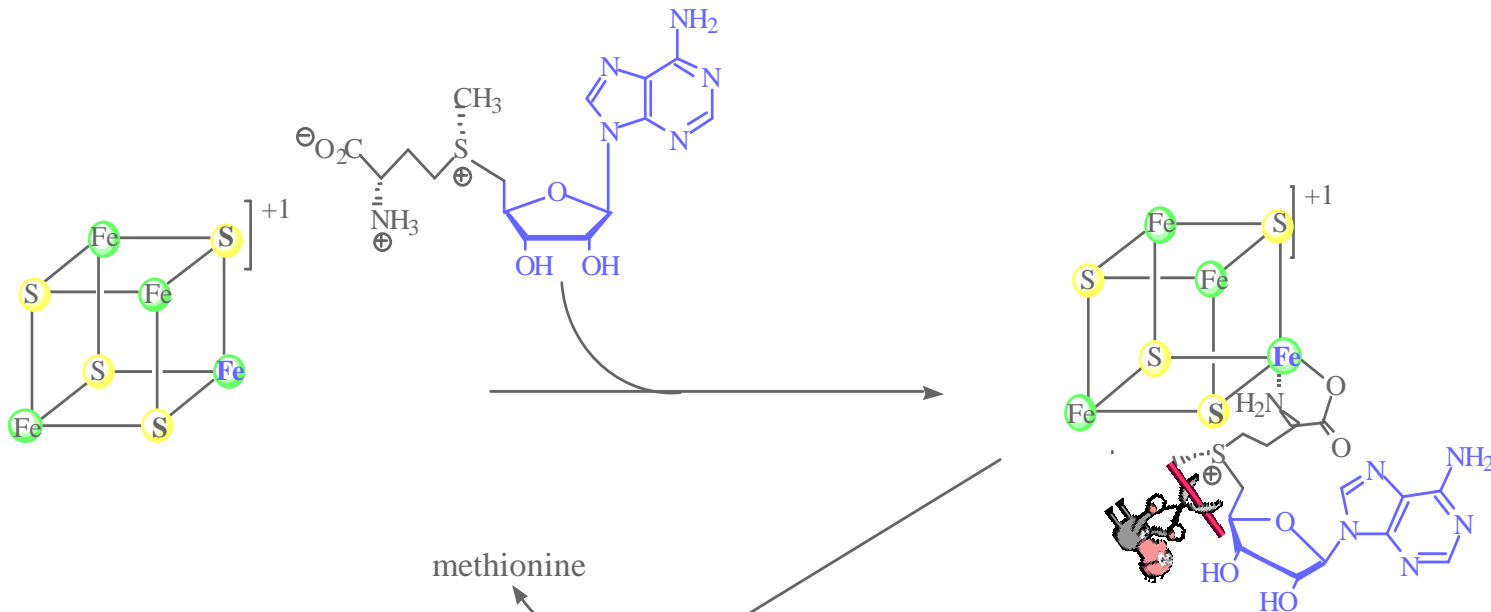
2.3 Å (292  $c_{\alpha}$ )

3.7 Å (192  $c_{\alpha}$ )

4.3 Å (232  $c_{\alpha}$ )

Y. Nicolet et al Nucleic Acids Research, 2004, (32) 4015–4025

C. Drennan et al, Chem Rev 2011



**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

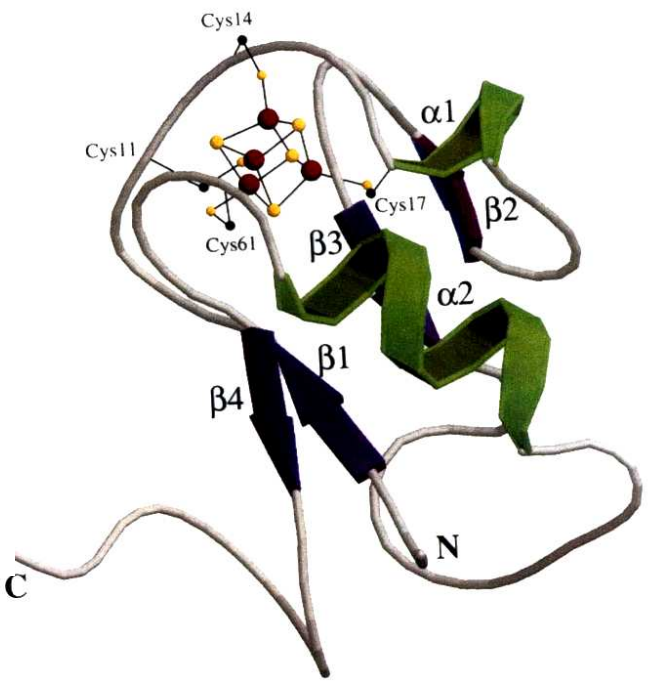
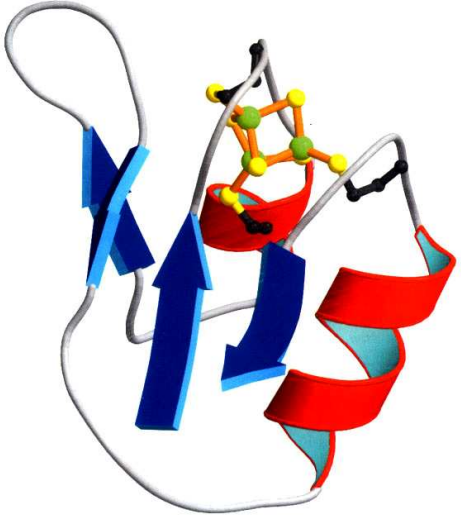
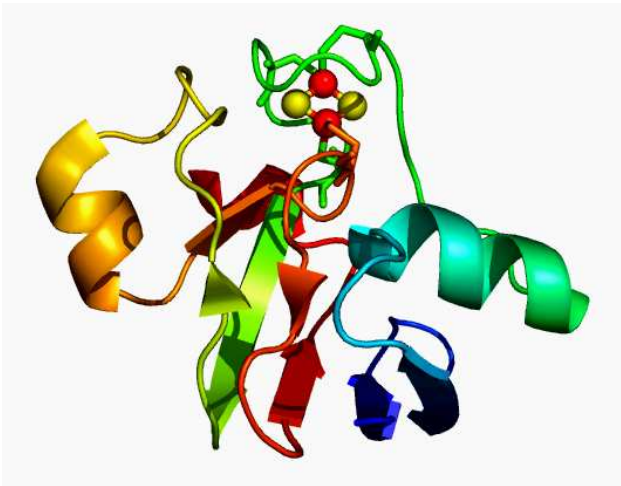
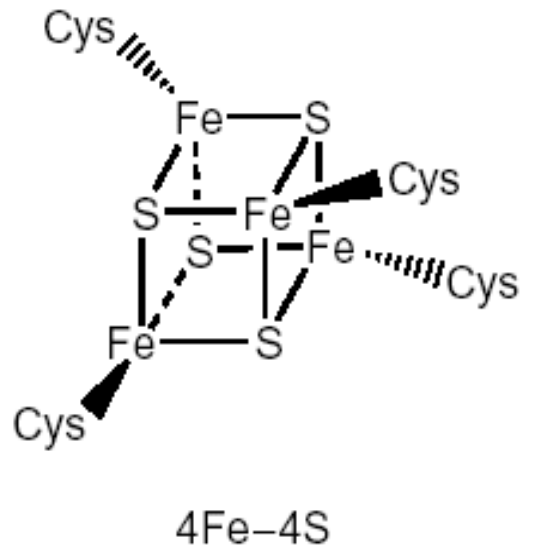
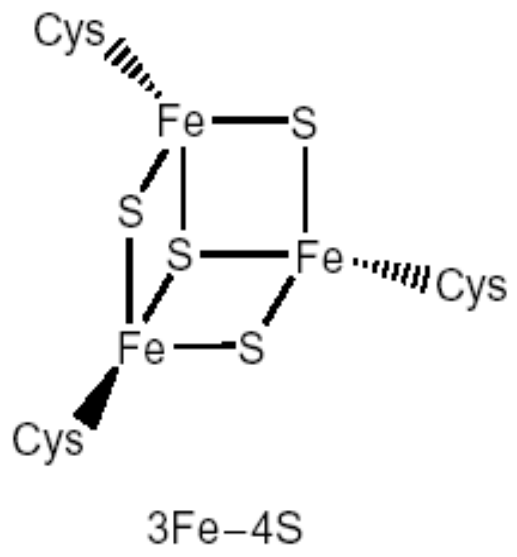
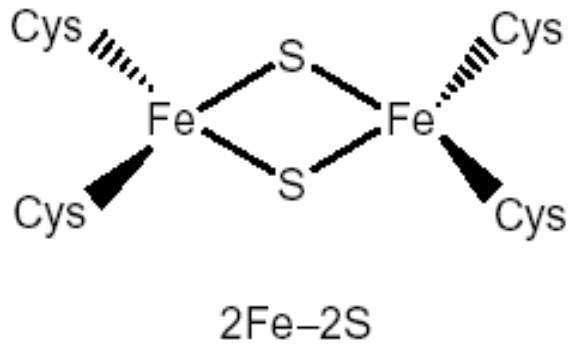
S-adenosylmethionine : nothing goes to waste  
 M. Fontecave, M. Atta, E. Mulliez  
*Trends in Biochemical Sciences* 2004, 29, 243-249

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**

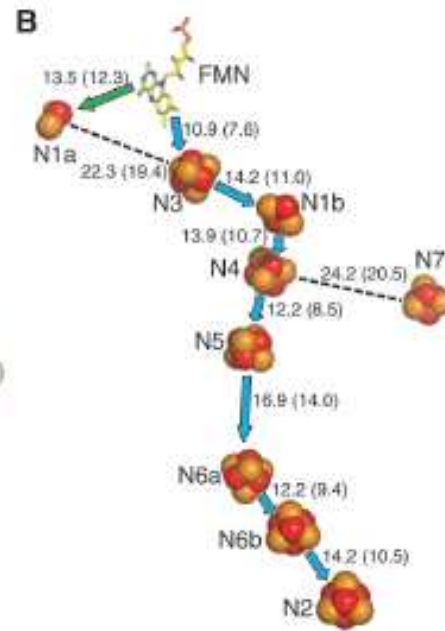
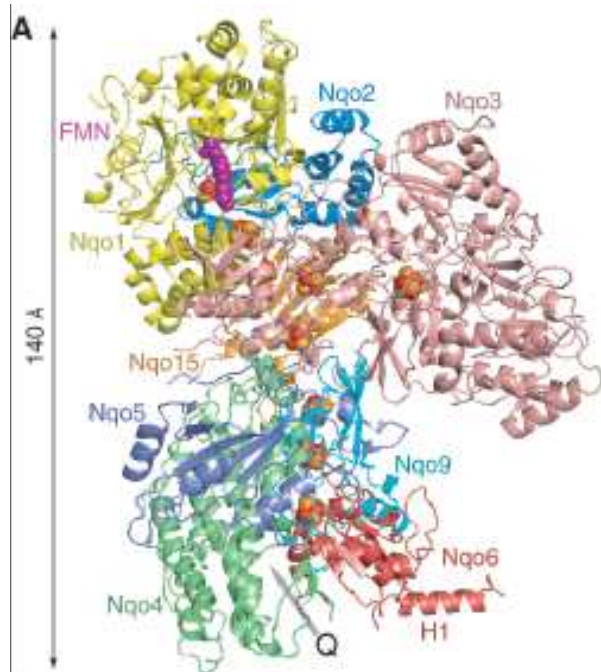
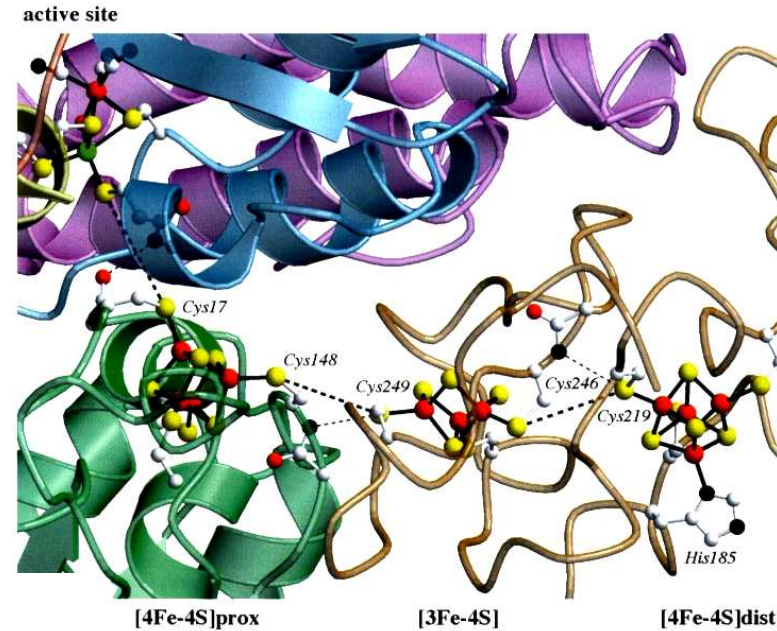
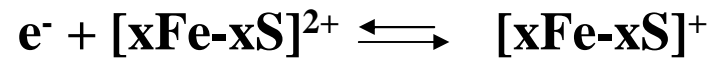
# Clusters fer-Soufre





# Clusters Fer-Soufre en Biologie

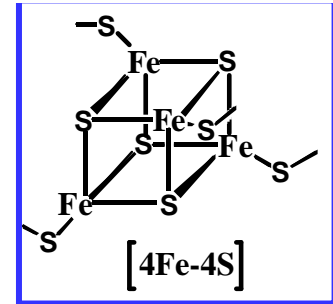
➤ Transport et transfert d'électrons  
(photosynthèse, respiration,...) (1960....)



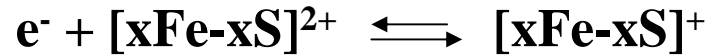
**Complexe I de *Thermus thermophilus***  
7 sous unités, 9 clusters FeS  
(Science 2006)

# Clusters fer-soufre en biologie

Iron-sulfur clusters : ever expanding roles  
M. Fontecave  
*Nature Chemical Biology* 2006, 2, 171-174



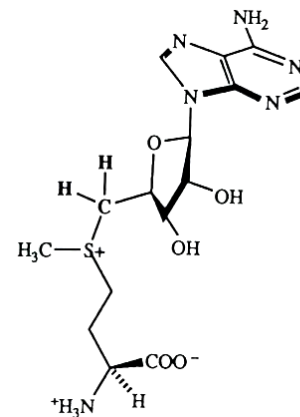
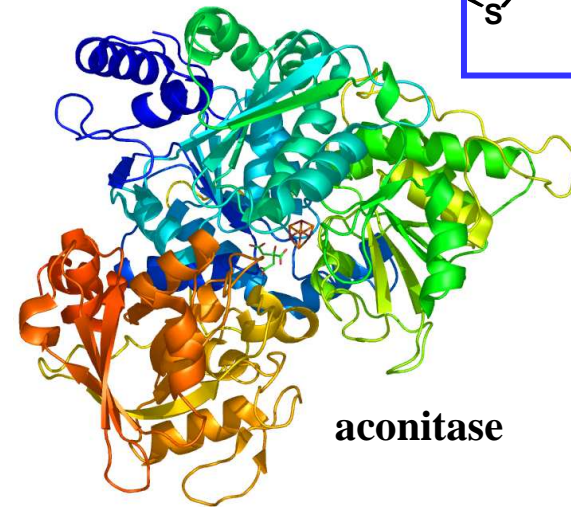
➤ **Transport et transfert d'électrons**  
(photosynthèse, respiration,...) (1960....)



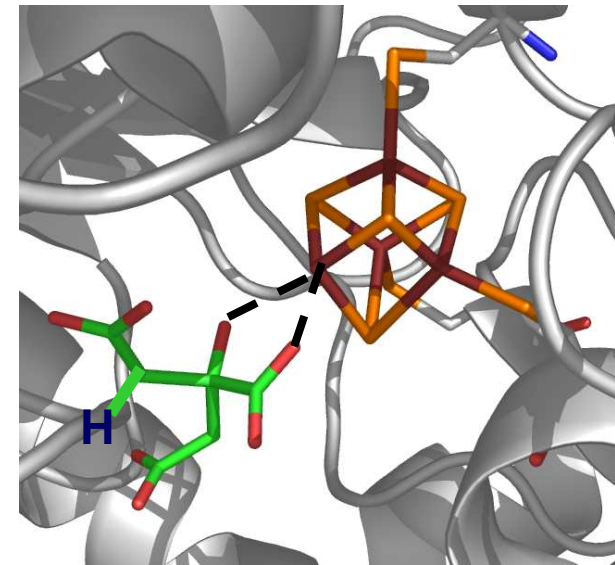
➤ **Catalyse non rédox**  
(déhydratases, **ACONITASE**,...) (1970....)

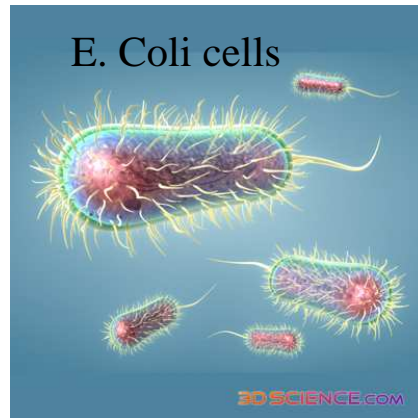
➤ **Modulation de l'expression des gènes**  
(FNR, SoxR, IRP,...) (1990....)

➤ **Catalyse rédox**  
(enzymes **Radical-SAM**) (2000....)



**SAM:**  
**S-adenosylmethionine**





# Iron-Sulfuromics

- Combien de protéines Fe-S chez **E. coli**?
- Abondance relative des divers clusters ?
- Combien de motifs « cystéine » différents ?

➡ ~ **90** protéines [Fe-S]  
(potentiellement **200**)

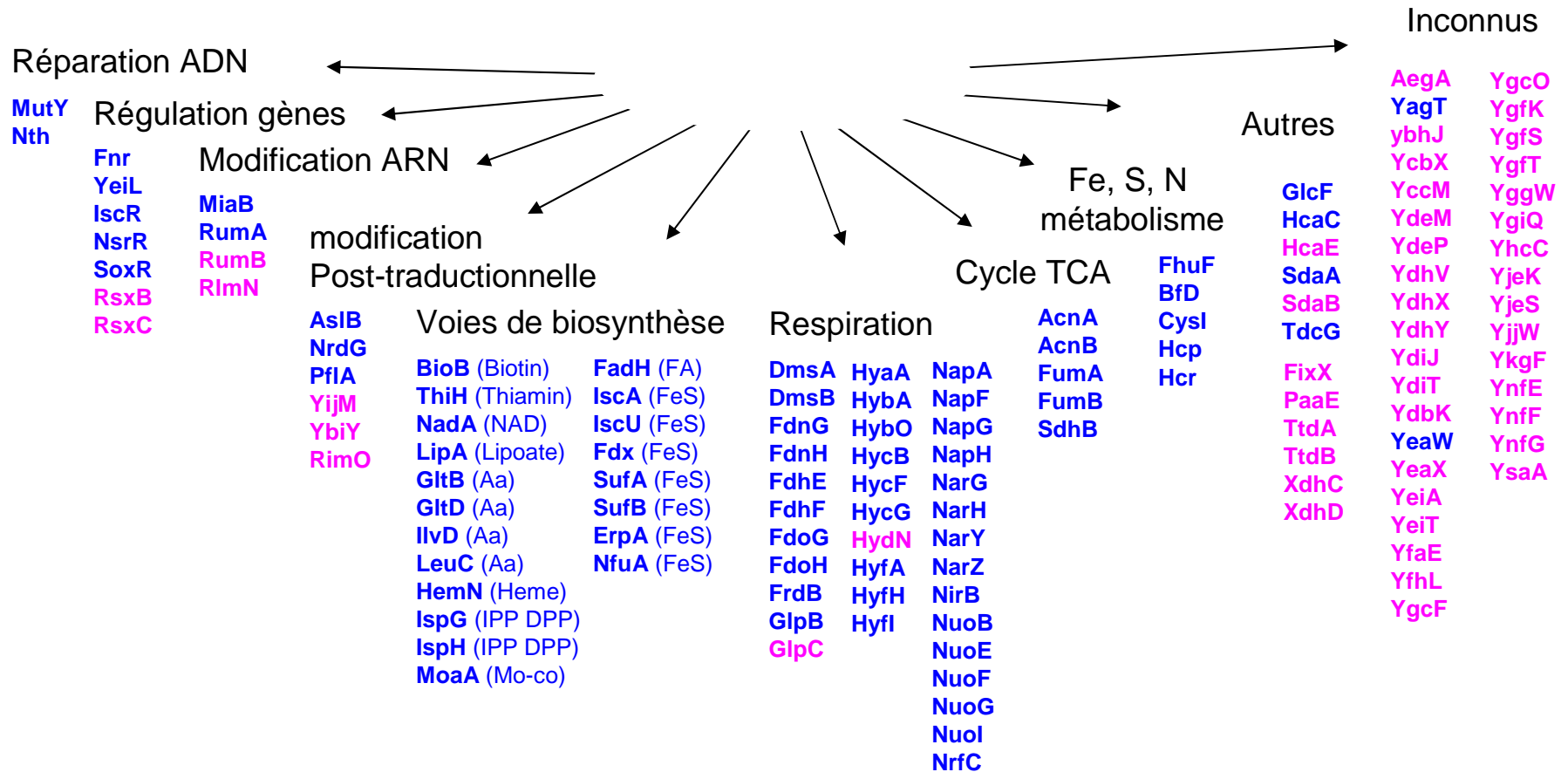
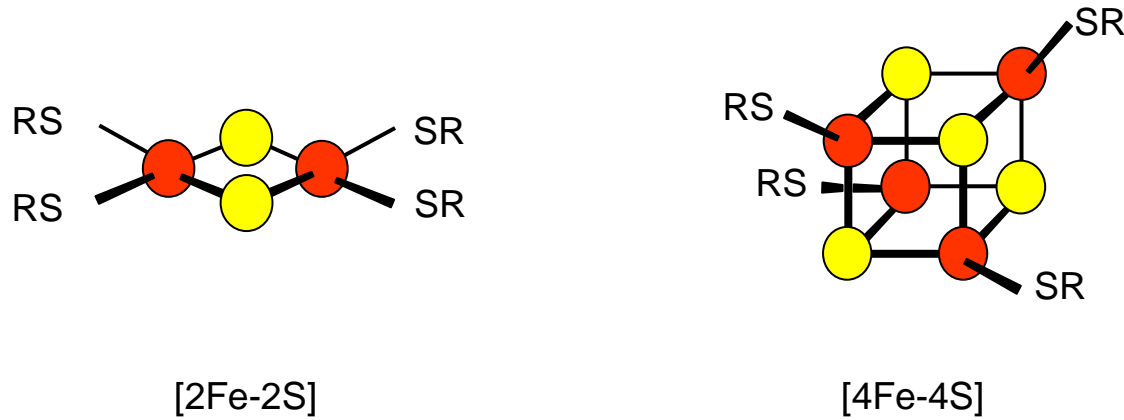
- |                            |      |   |                 |
|----------------------------|------|---|-----------------|
| -Transfert d'électrons:    | 55%  | ➡ | <b>Total 27</b> |
| -Non Rédox:                | 17%  |   |                 |
| -Rédox (Radical-SAM):      | 10%  |   |                 |
| -Senseurr/Régulateur:      | 6%   |   |                 |
| -Structural:               | < 4% |   |                 |
| -Biosynthèse des clusters: | 8%   |   |                 |

➡ [2Fe-2S]: < 10%                      [4Fe-4S]: > 90%

➡ **Plus de 30 signatures !**

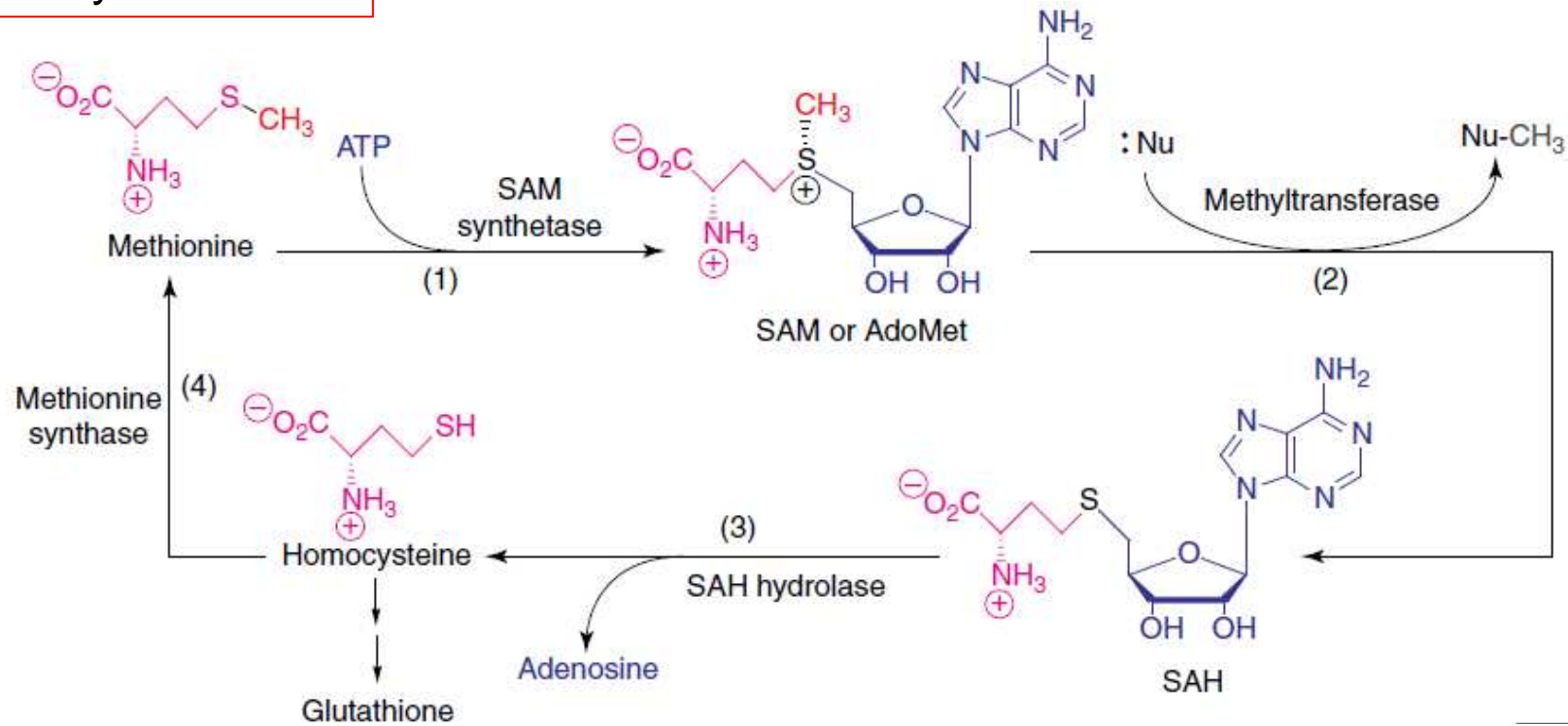
CX<sub>2</sub>CX<sub>2</sub>CX<sub>3</sub>C (72), CX<sub>2</sub>CX<sub>4</sub>CX<sub>3</sub>C (20), CX<sub>2</sub>CX<sub>2</sub>CX<sub>7</sub>C (16),.....





# S-adenosyl-méthionine en Biologie

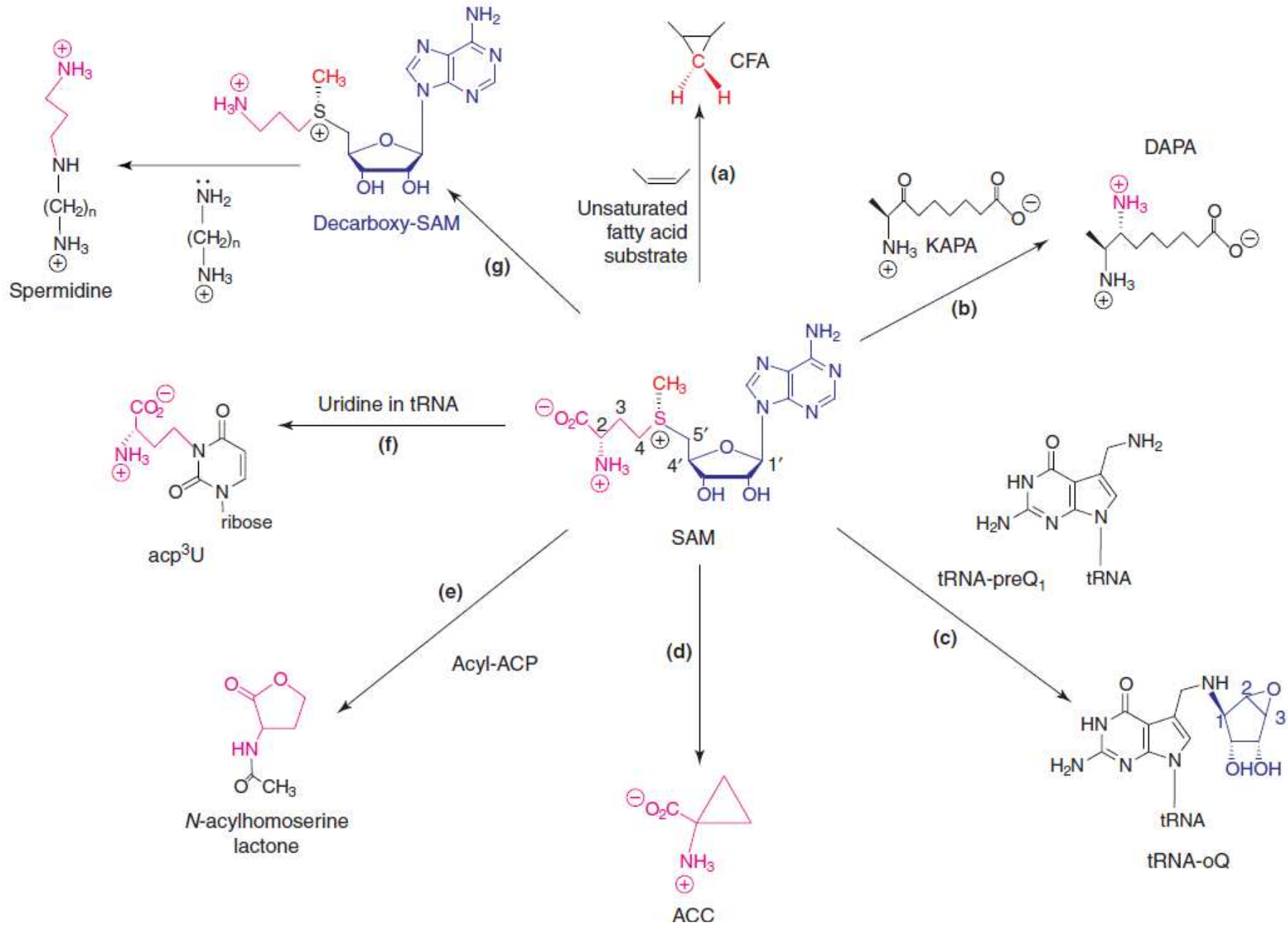
## Le cycle de SAM



# S-adenosylmethionine : nothing goes to waste

M. Fontecave, M. Atta, E. Mulliez

*Trends in Biochemical Sciences* 2004, 29, 243-249

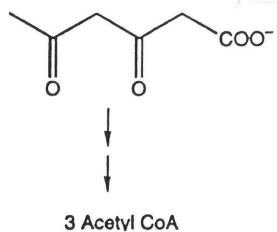
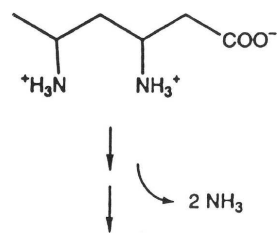
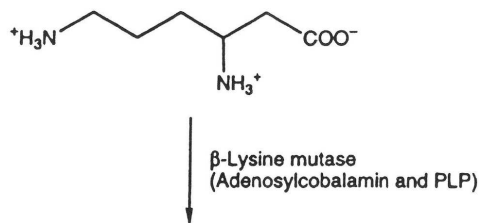
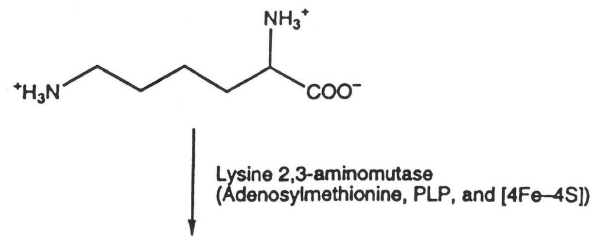
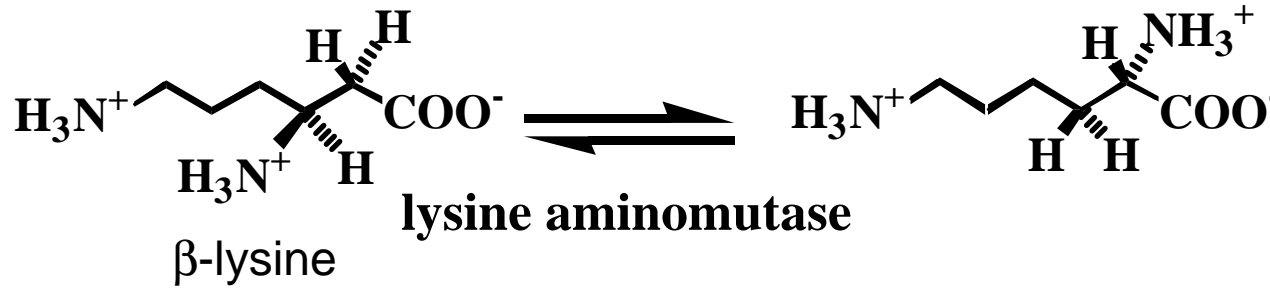


Un exemple d'enzyme « Radical-SAM »  
la Lysine AminoMutase (LAM):  
Mécanismes, structures

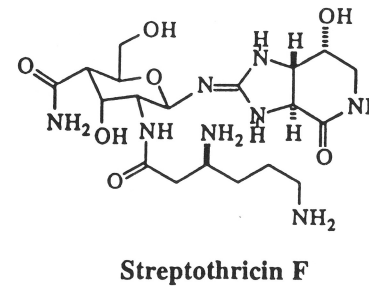


Perry Frey  
Université du Wisconsin

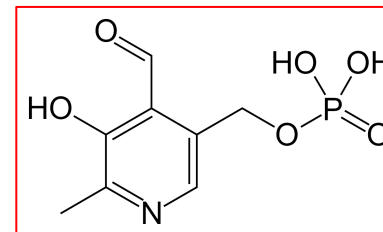
P Frey et al, Adv in Free radical Chemistry 1999; ABB 2000; Chem Rev 2003 103 2129  
Marsh ChemBioChem 2009  
Broderick Curr Op Chem Biol 2010

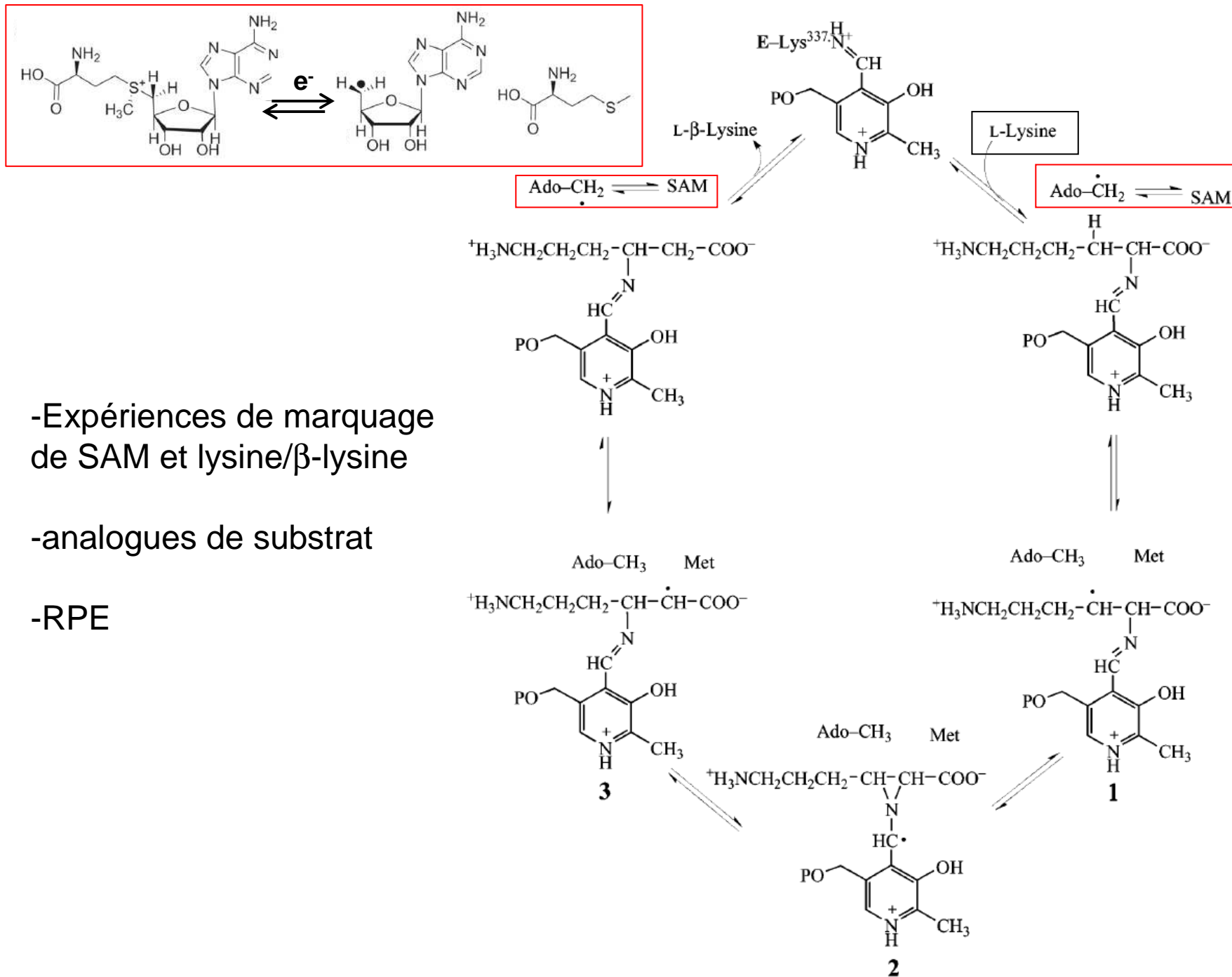


- 1<sup>ère</sup> étape de décomposition de la lysine (source de C et N)
- $\beta$ -lysine utilisée dans la synthèse d'antibiotiques



- 3-*pro*-R H migre en 2-*pro*-R
- Migration de NH<sub>3</sub> avec inversion de configuration
- Enzyme à cofacteur **pyridoxal-phosphate**

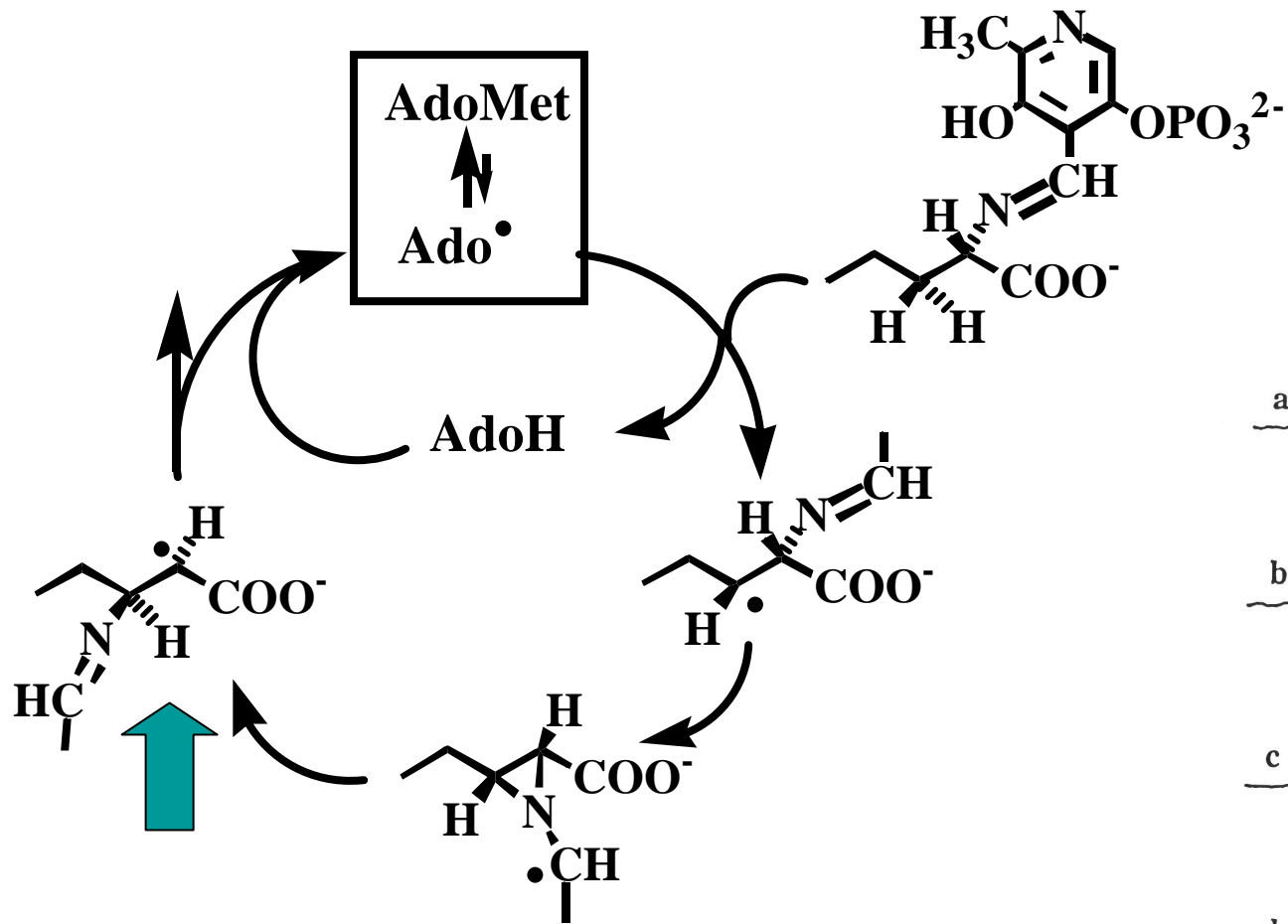




-Expériences de marquage de SAM et lysine/ $\beta$ -lysine

-analogues de substrat

-RPE



Un intermédiaire radicalaire  
(P. Frey Biochemistry 1992)

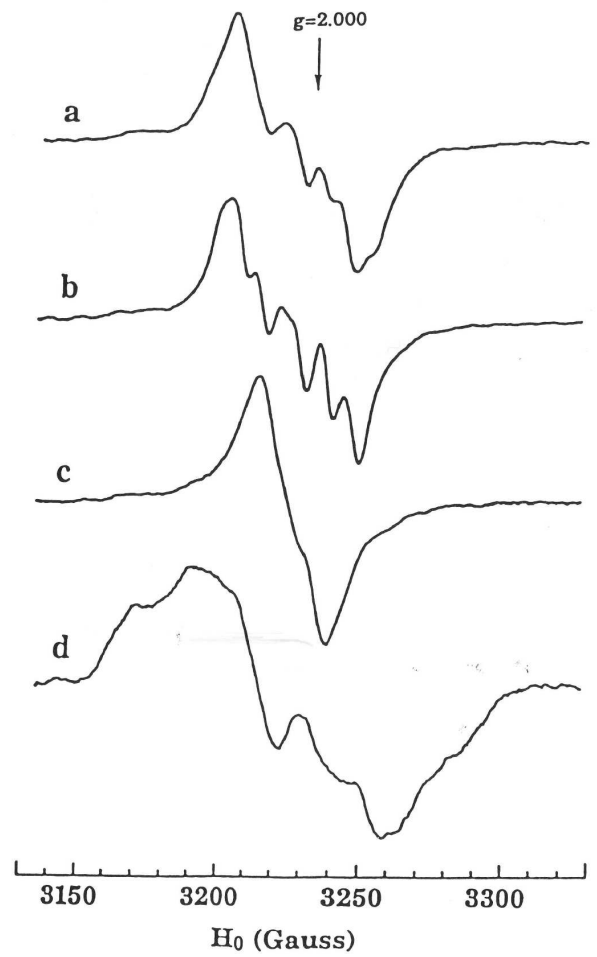
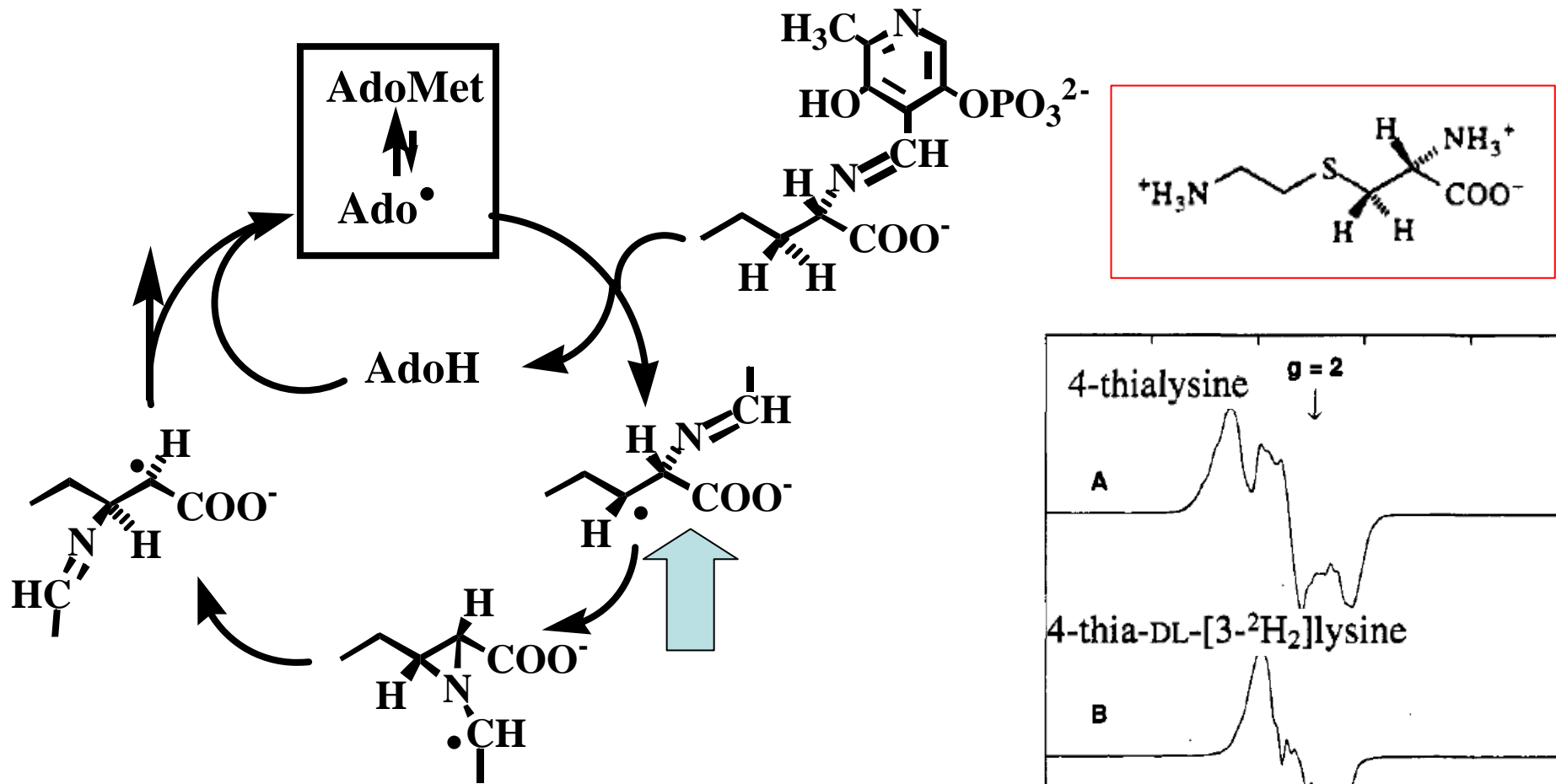
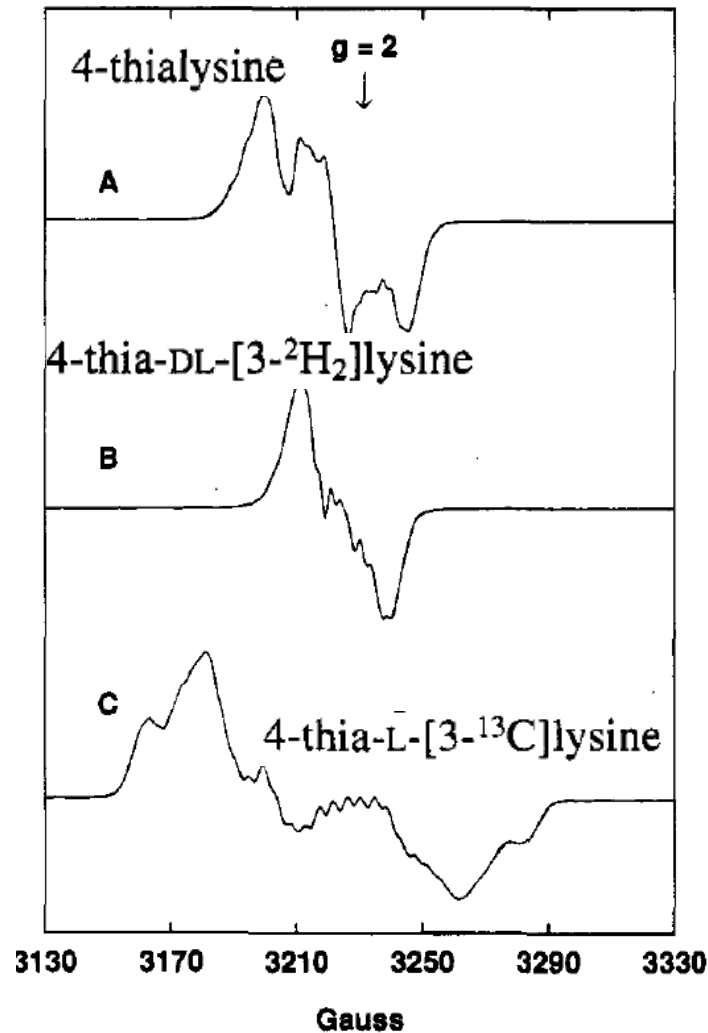


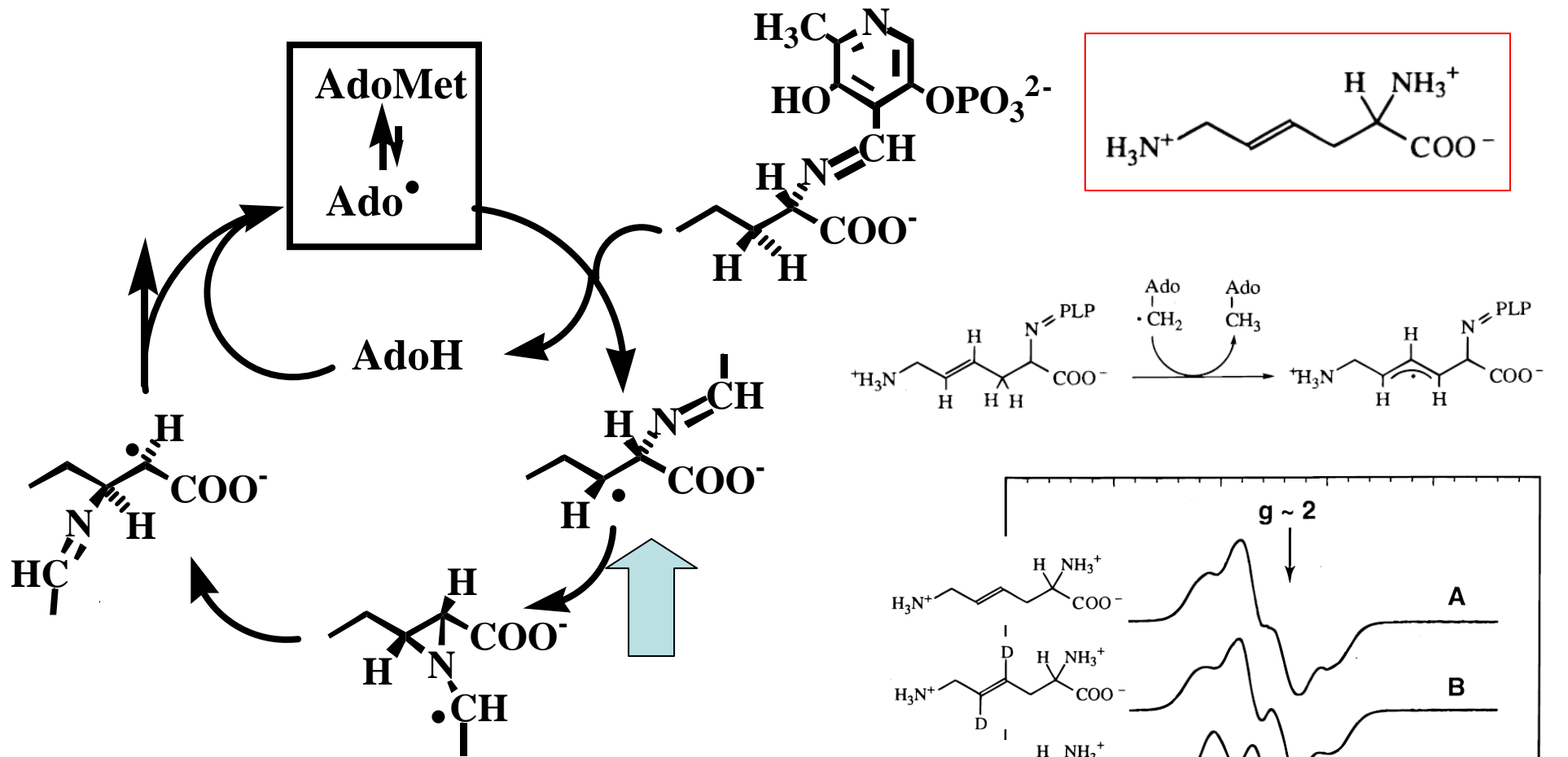
Figure 4. EPR spectra (77 K) of steady-state reactions LAM with SAM and isotopically labeled lysine. (a) L-lysine; (b) L-[3,3,4,4,5,5,6,6- $^2\text{H}_8$ ]lysine; (c) DL-[2- $^2\text{H}$ ]lysine; (d) L-[2- $^{13}\text{C}$ ]lysine. Adapted from reference 19 with permission from the American Chemical Society.



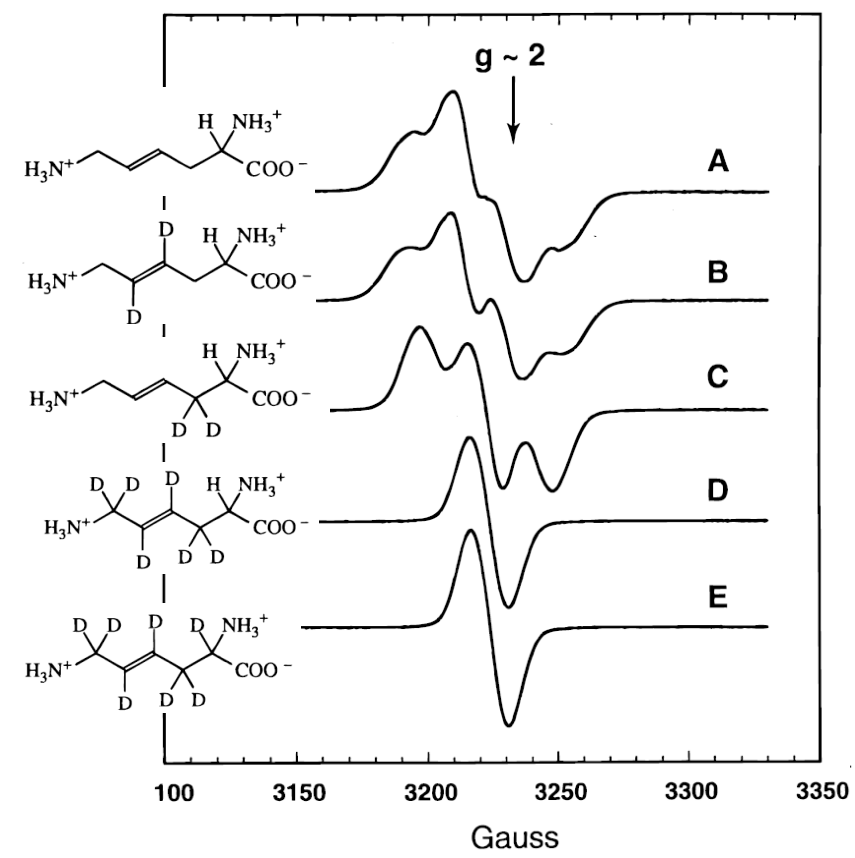
Analogues de substrat 1: **4-thialysine**, un substrat  
 Un autre intermédiaire radicalaire  
 P. Frey Biochemistry 1995

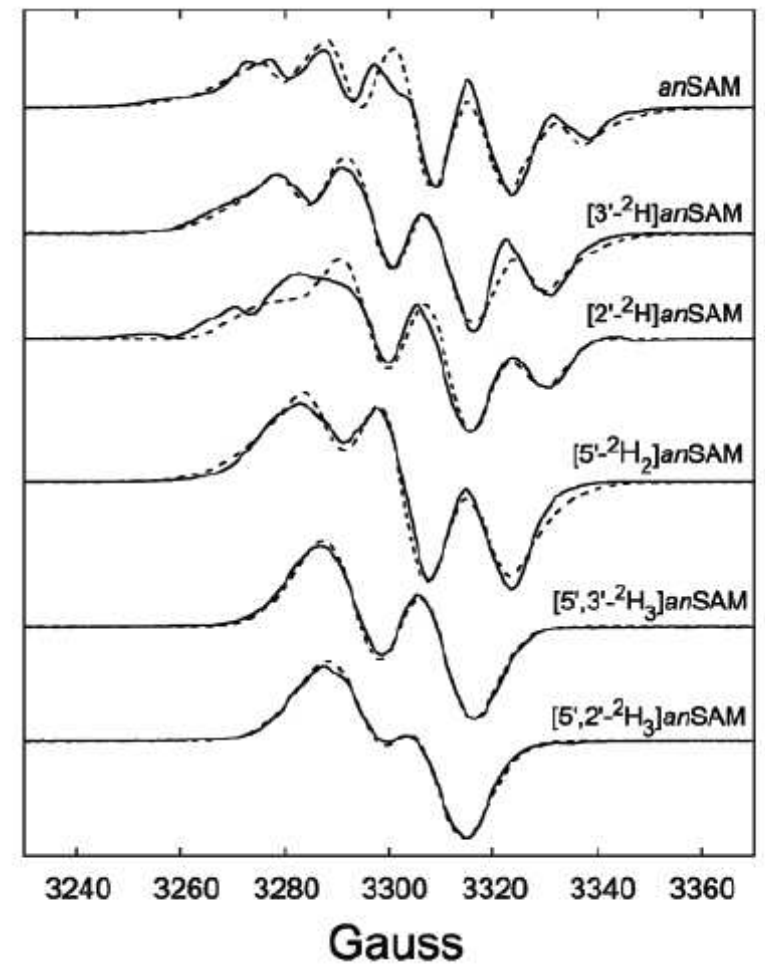
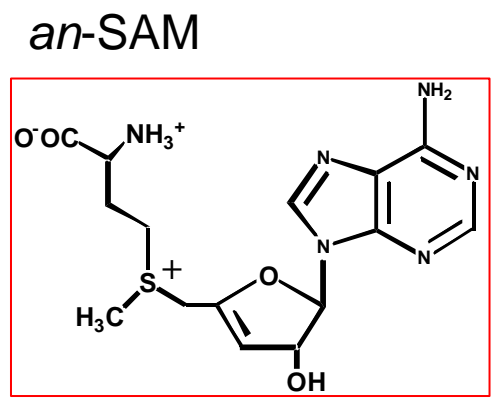
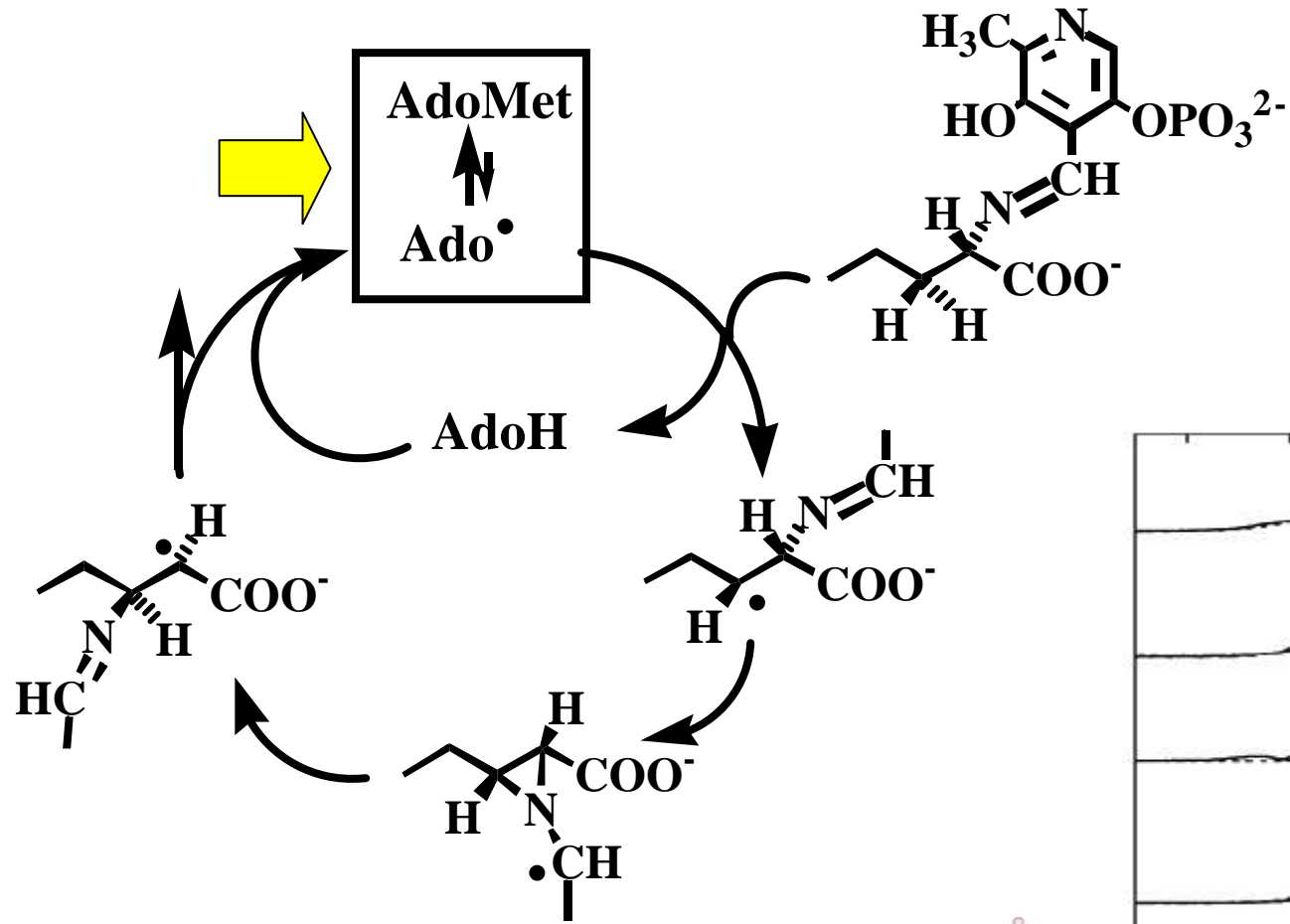




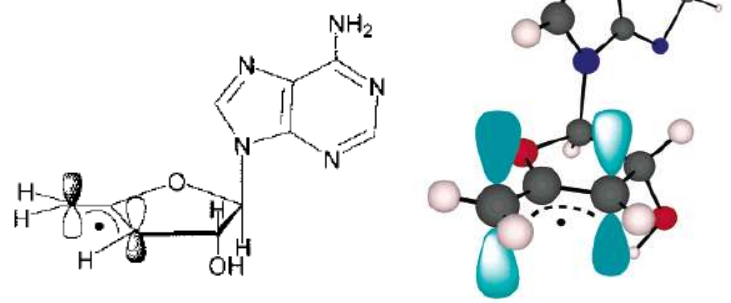


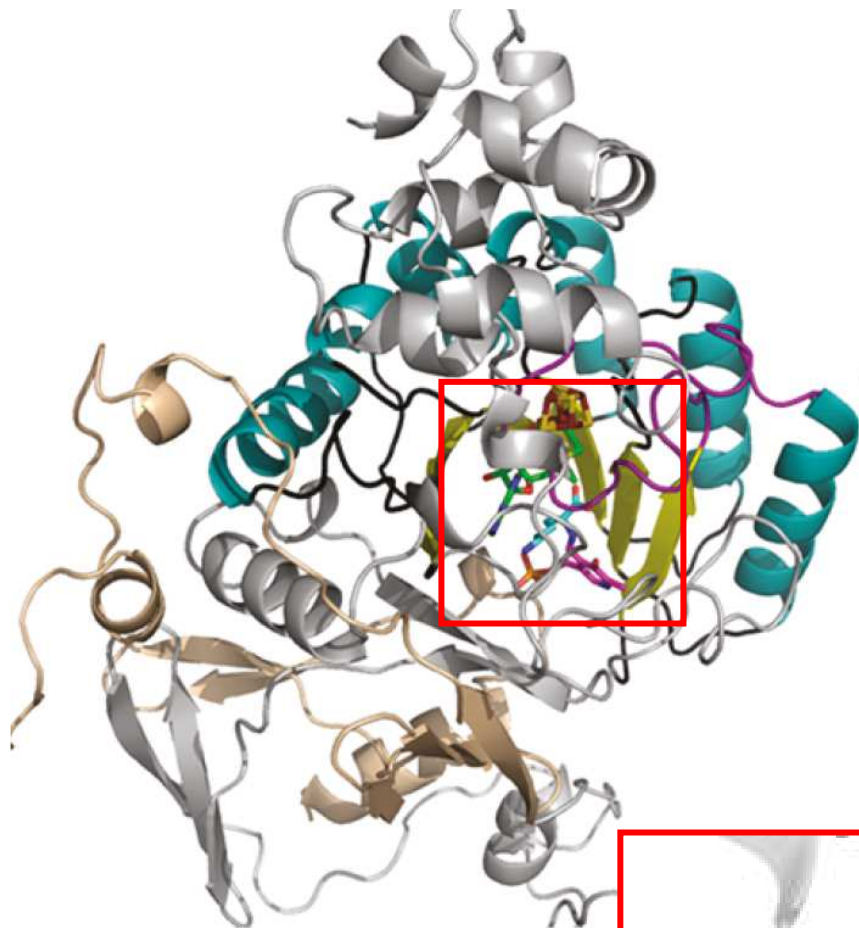
Analogues de substrat 2:  
**4,5-dehydrolysine**, un inhibiteur  
 Un autre intermédiaire radicalaire  
 P. Frey Biochemistry 2000





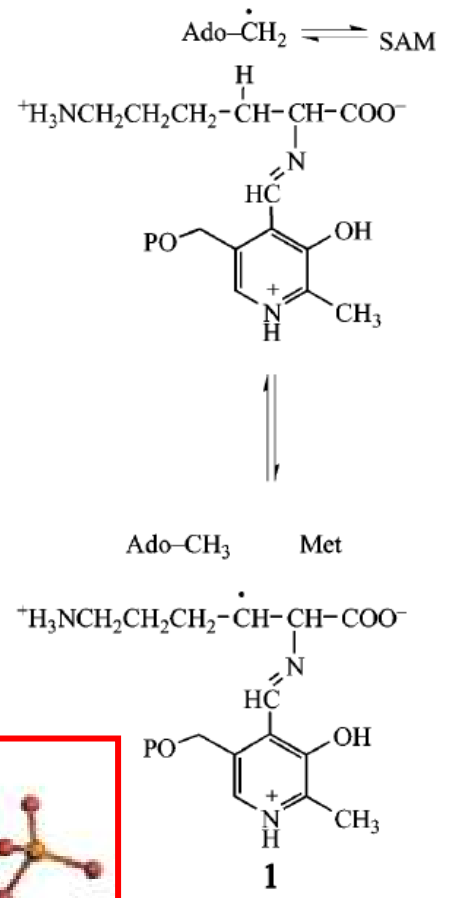
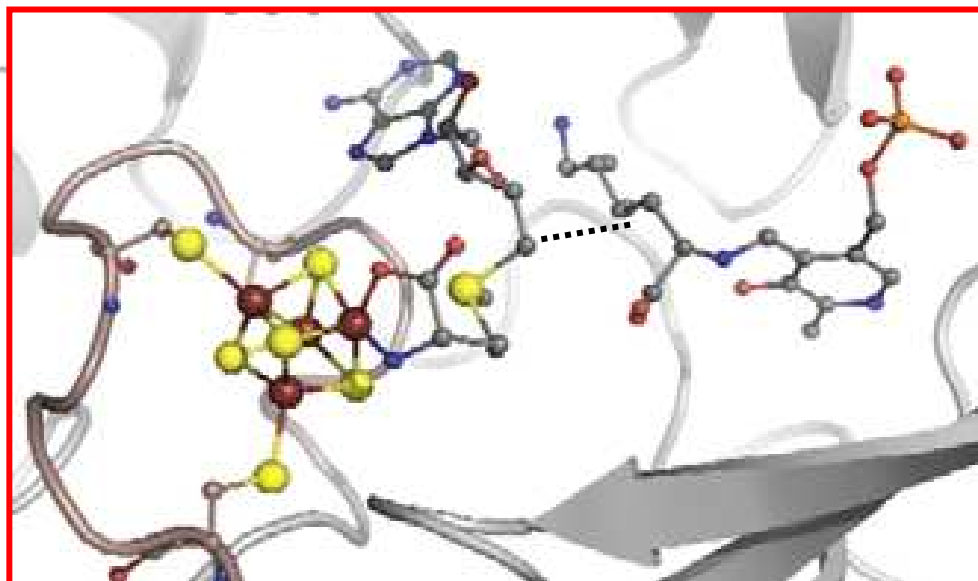
Analogue de SAM:  
**Le radical « Ado° »!!**  
 P. Frey Biochemistry 2001

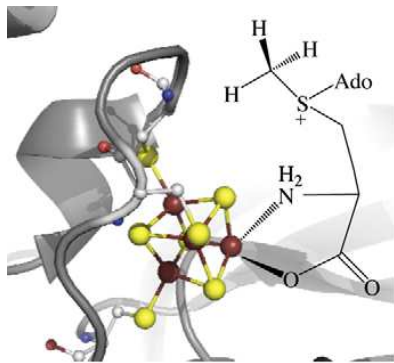




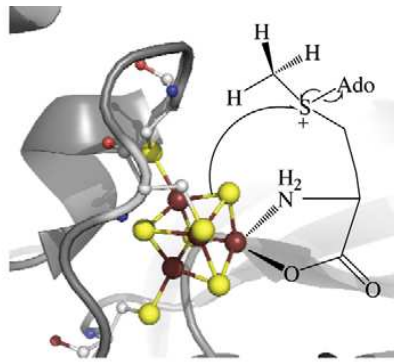
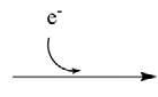
Structure de LAM

P. Frey PNAS 2005

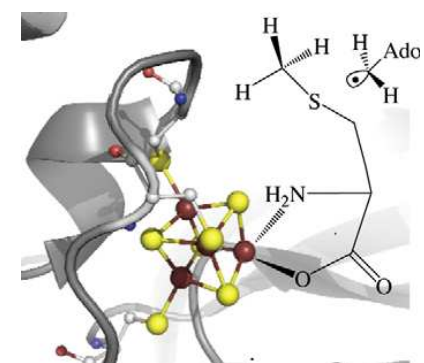
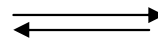




[4Fe-4S]<sup>2+</sup> - SAM

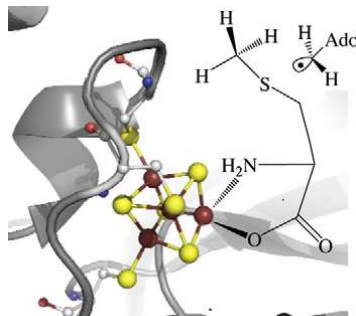


[4Fe-4S]<sup>1+</sup> - SAM



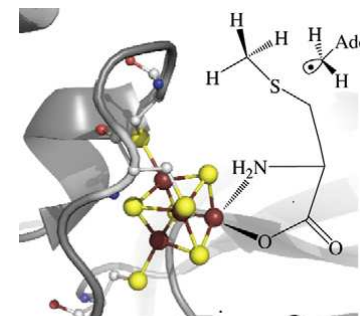
[4Fe-4S]<sup>2+</sup> - Met + dAdo<sup>•</sup>

RNR

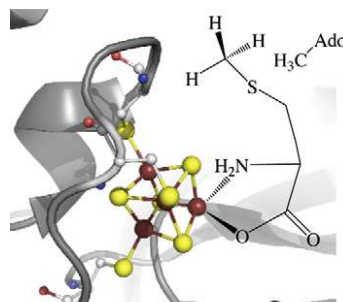
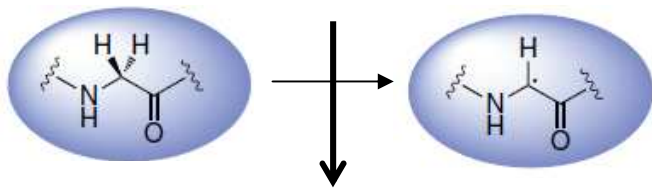


[4Fe-4S]<sup>2+</sup> - Met + dAdo<sup>•</sup>

LAM

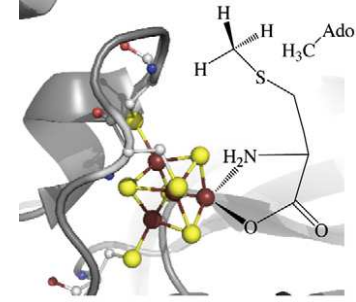
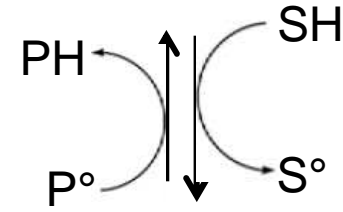


[4Fe-4S]<sup>2+</sup> - Met + dAdo<sup>•</sup>

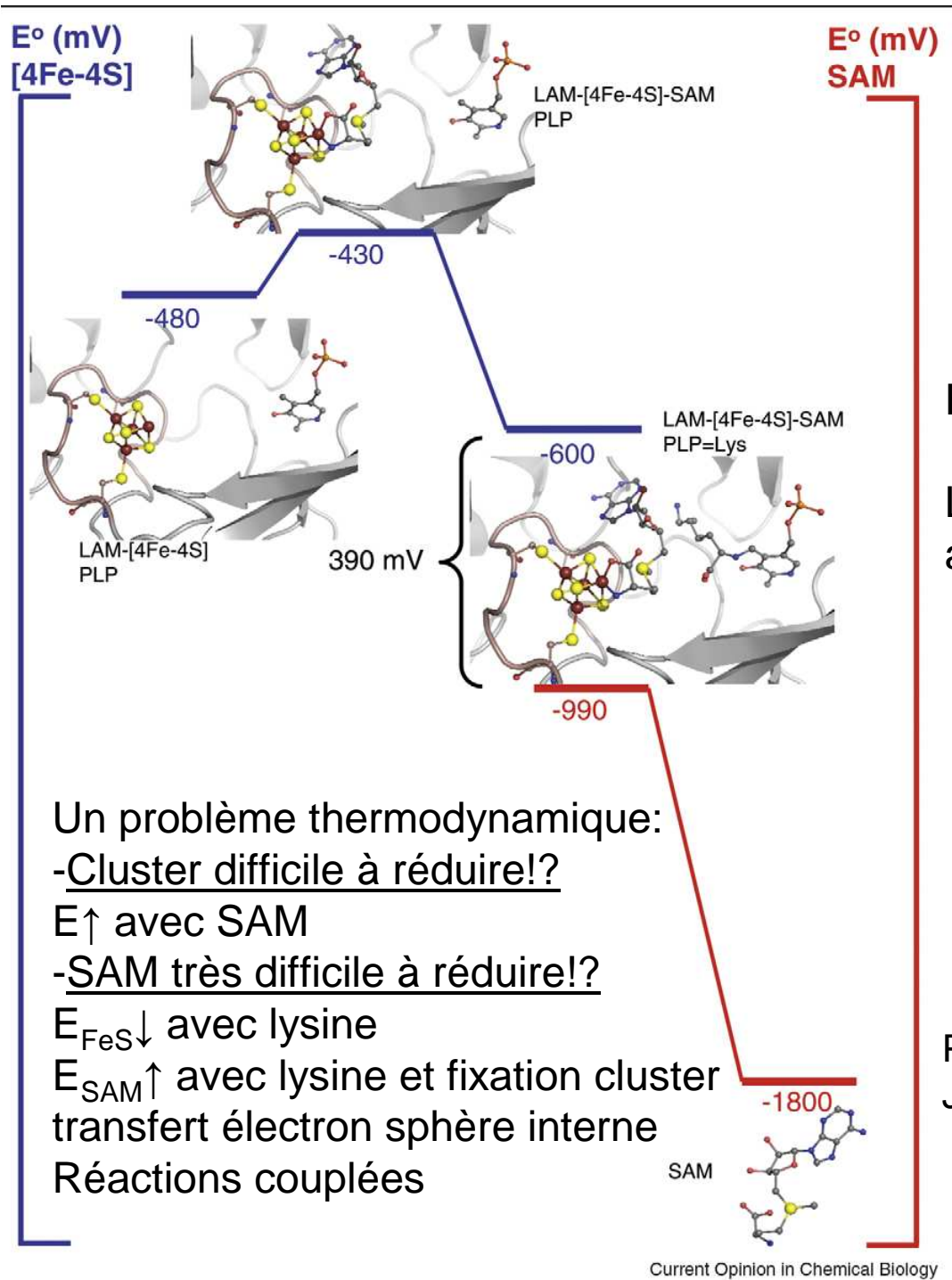


[4Fe-4S]<sup>2+</sup> - Met + dAdo

AdoH  
Met



[4Fe-4S]<sup>2+</sup> - Met + dAdo



Eviter le clivage de SAM sans substrat

Lever les contraintes thermodynamiques avec substrat

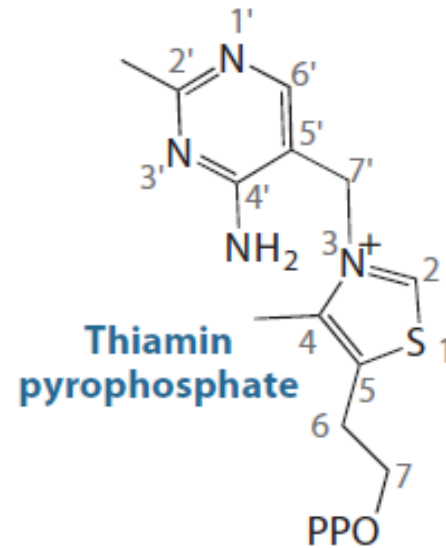
P. Frey Biochemistry 2007

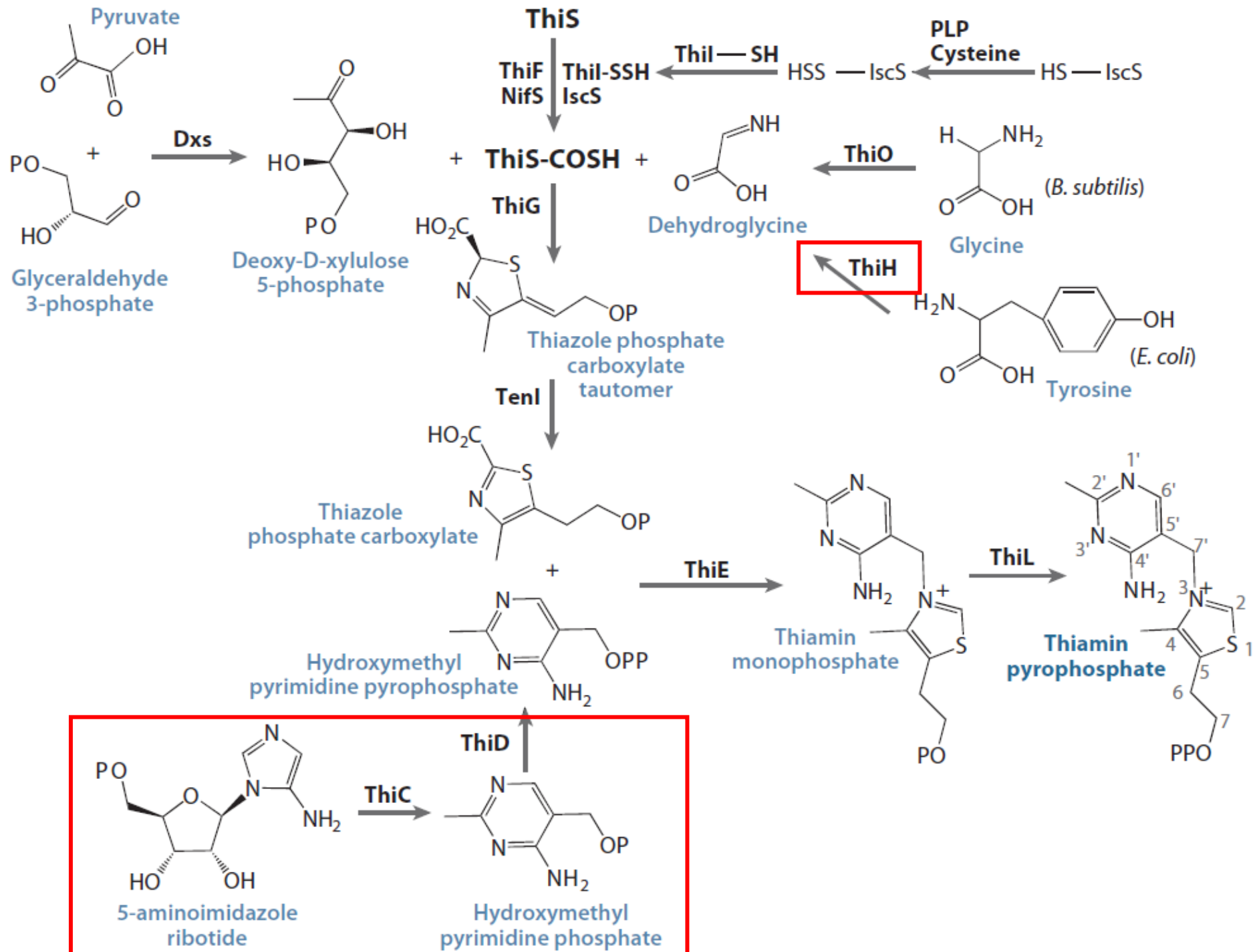
J Broderick Curr Op Chem Biol 2010

## Biosynthèse de la Thiamine

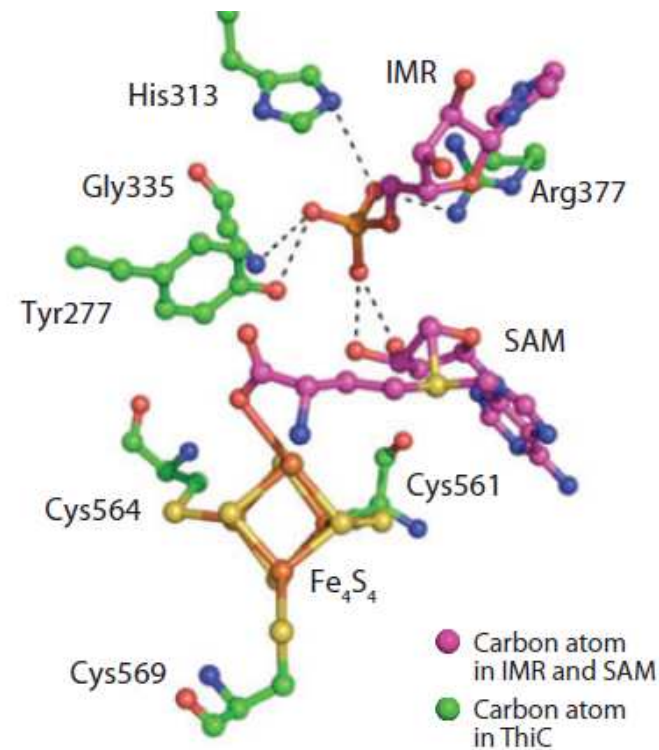
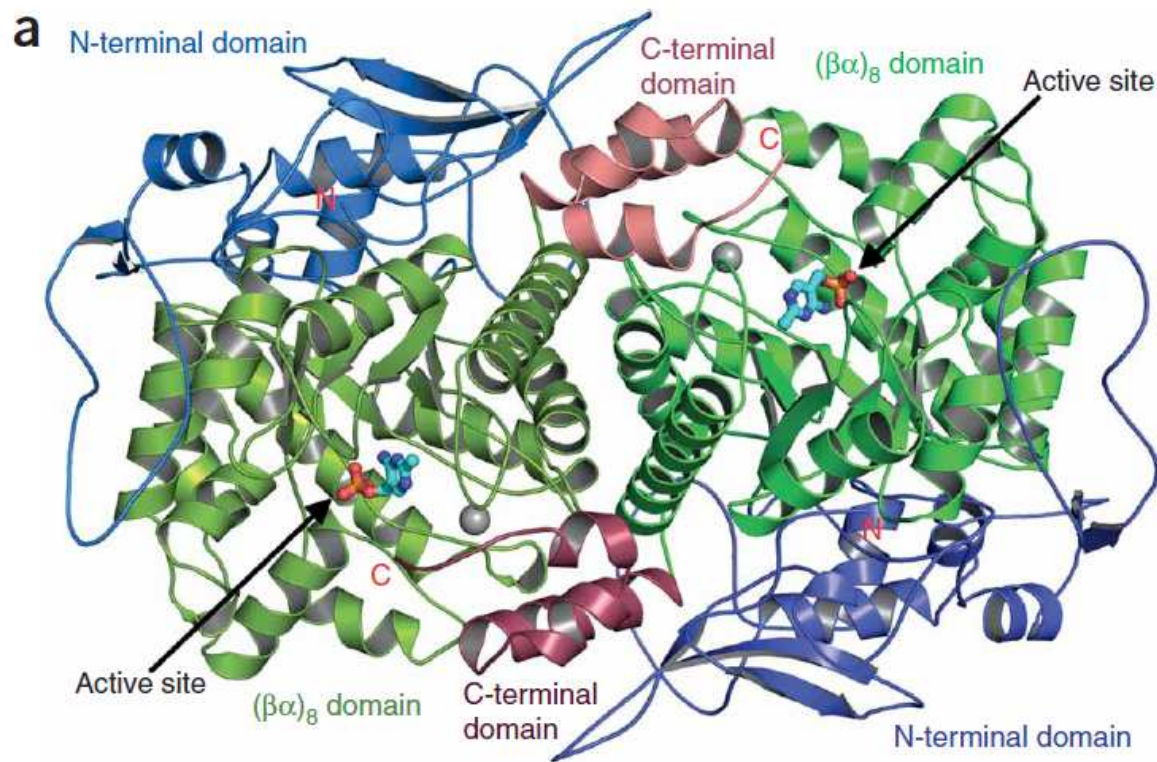
C. Eijkman, Prix Nobel 1929

T. Begley, Cornell University, New York





ThiC: une enzyme « Radical-SAM »

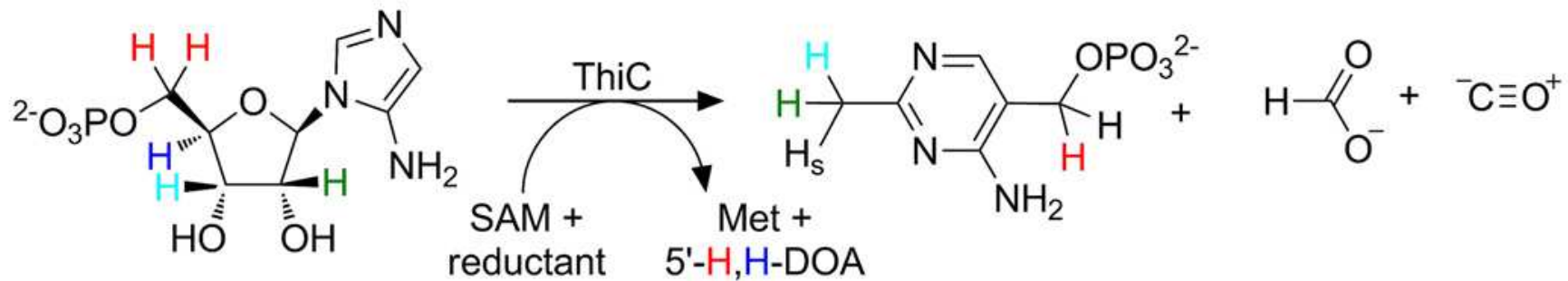
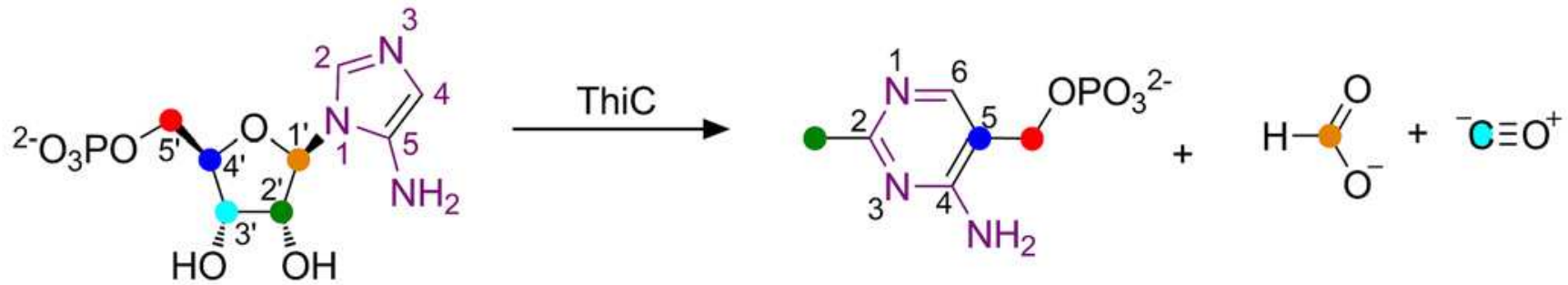


**Cys-X-X-Cys-X-X-X-X-Cys**

T. Begley, S. Ealick Nature ChemBiol 2008 4 758



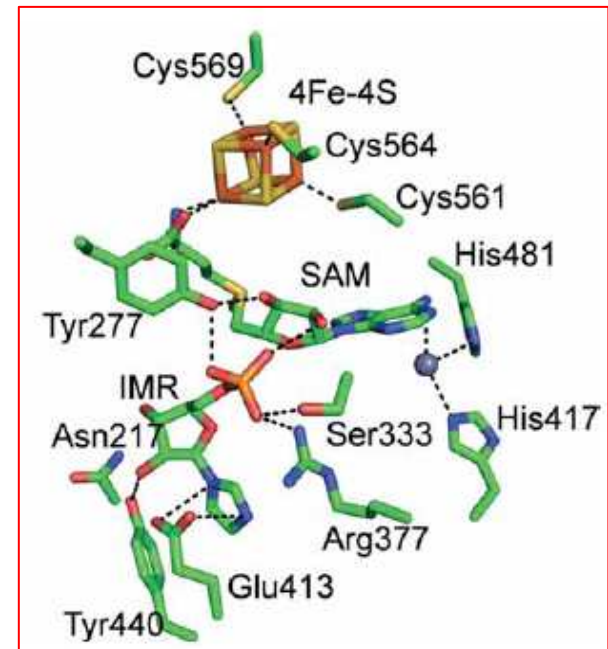
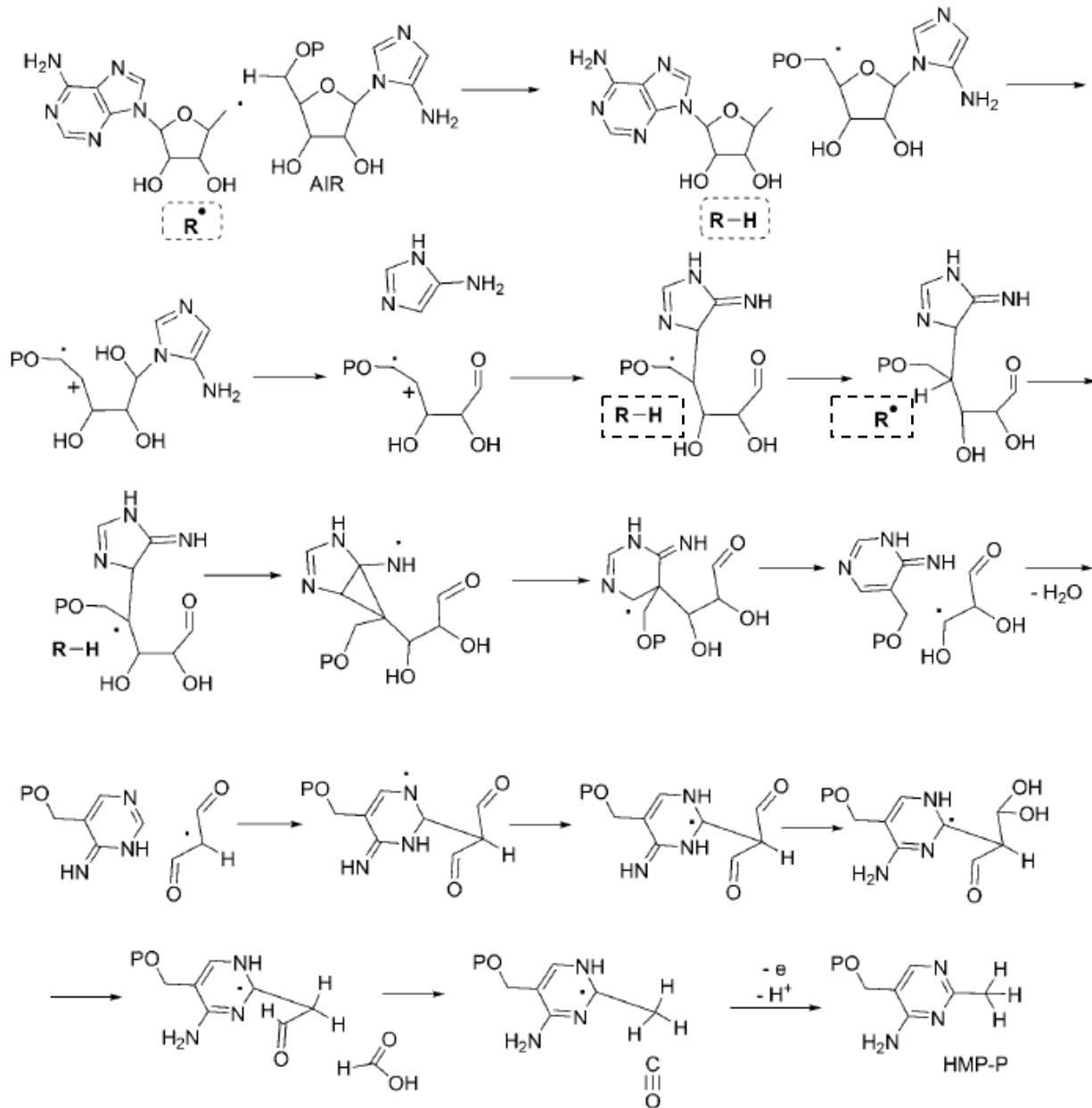
# ThiC: expériences de marquage isotopique

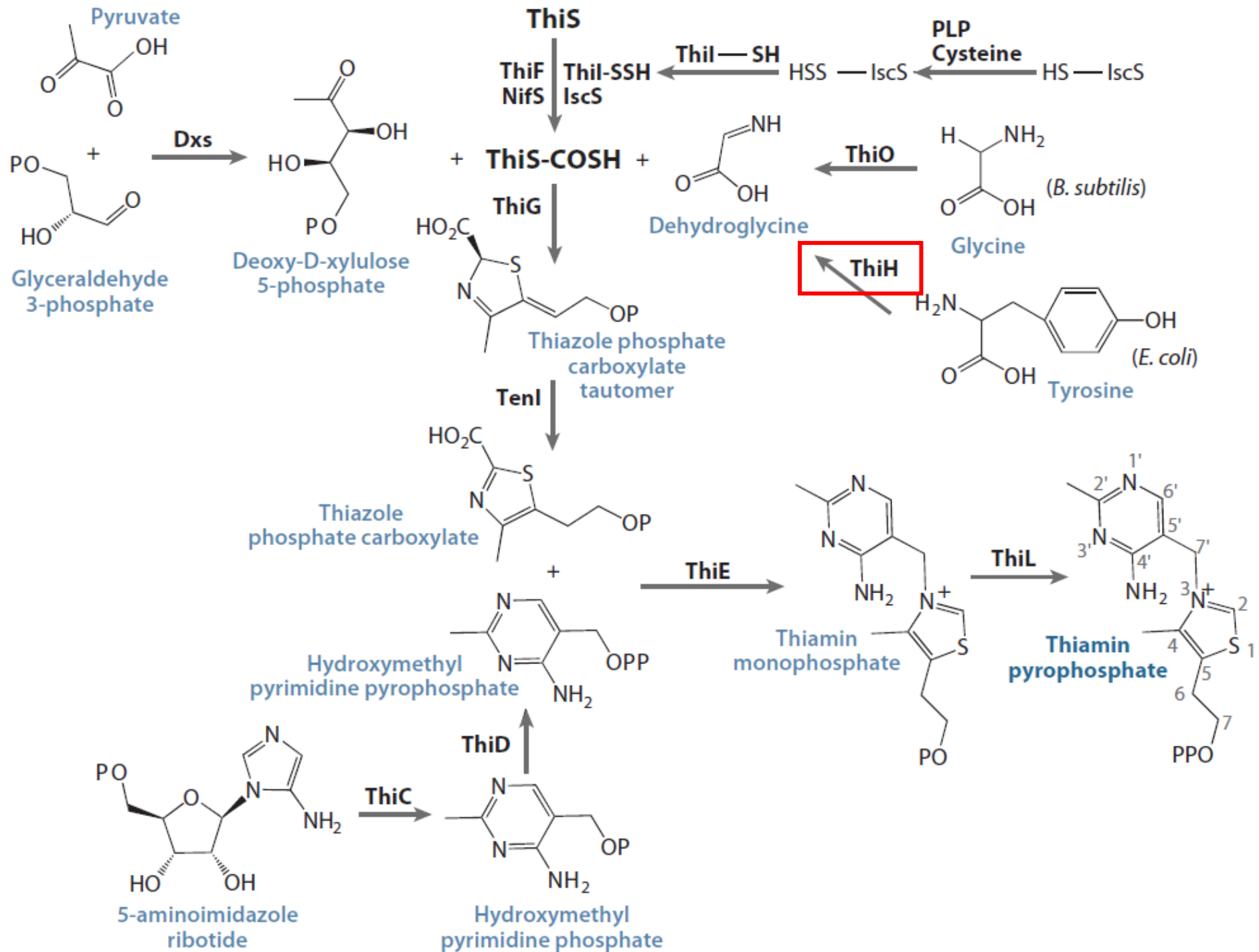


120 11/12/2016 10:25:10 9/23/16

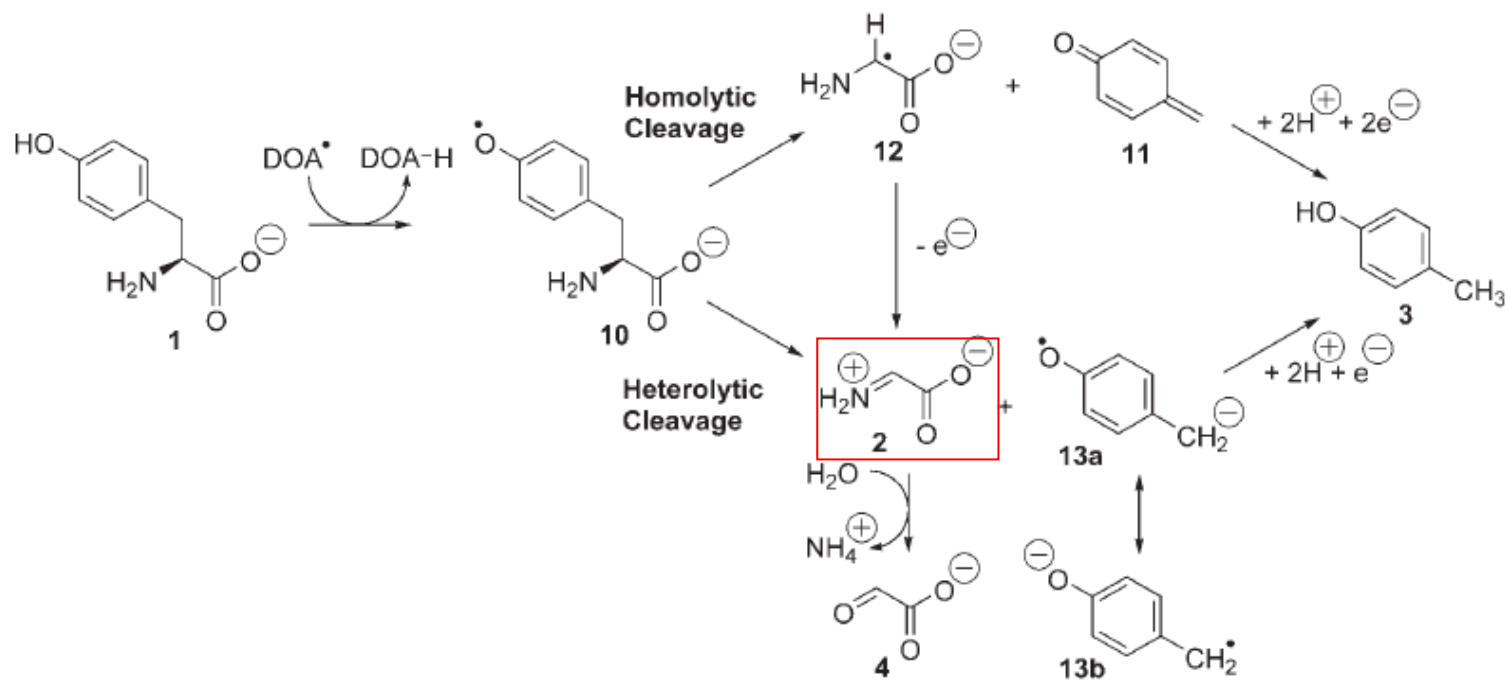
T. Begley, Annu Rev Biochem 2009, Angew Chem 2010

# Mécanisme ?

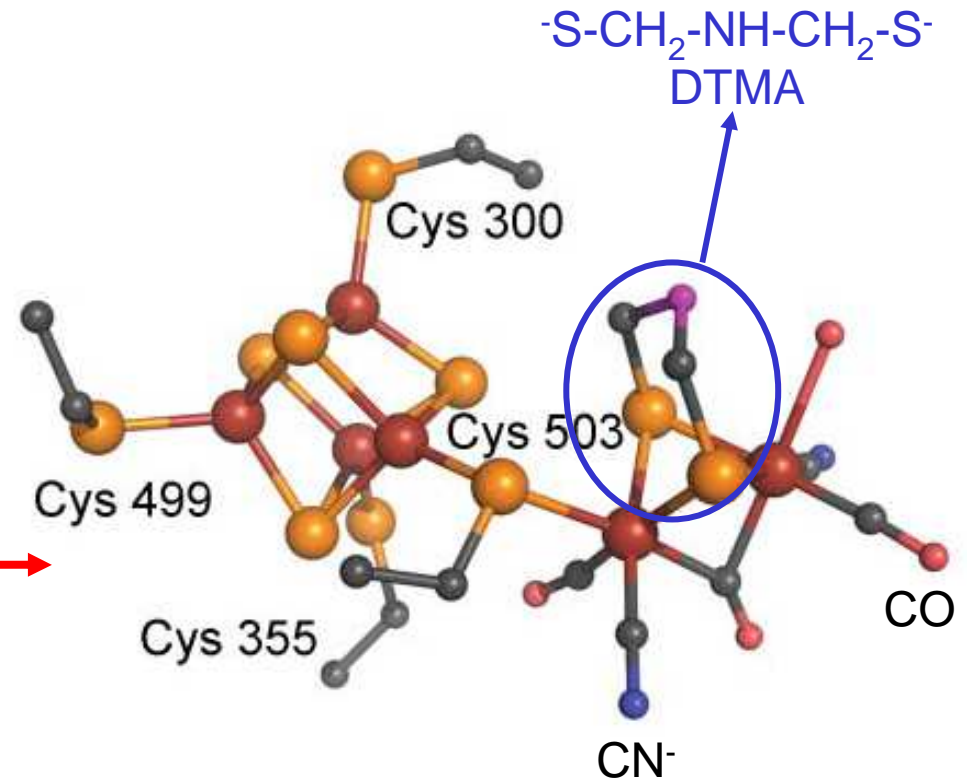
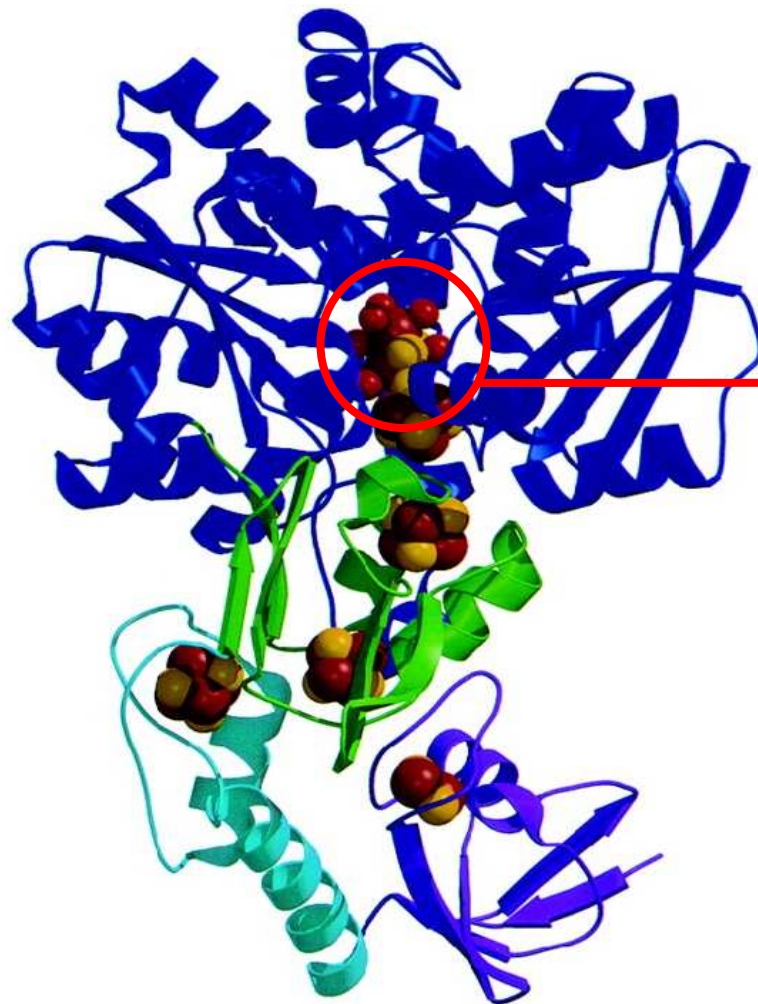




# ThiH: une enzyme « Radical-SAM »



# Maturation des Fe-hydrogénases

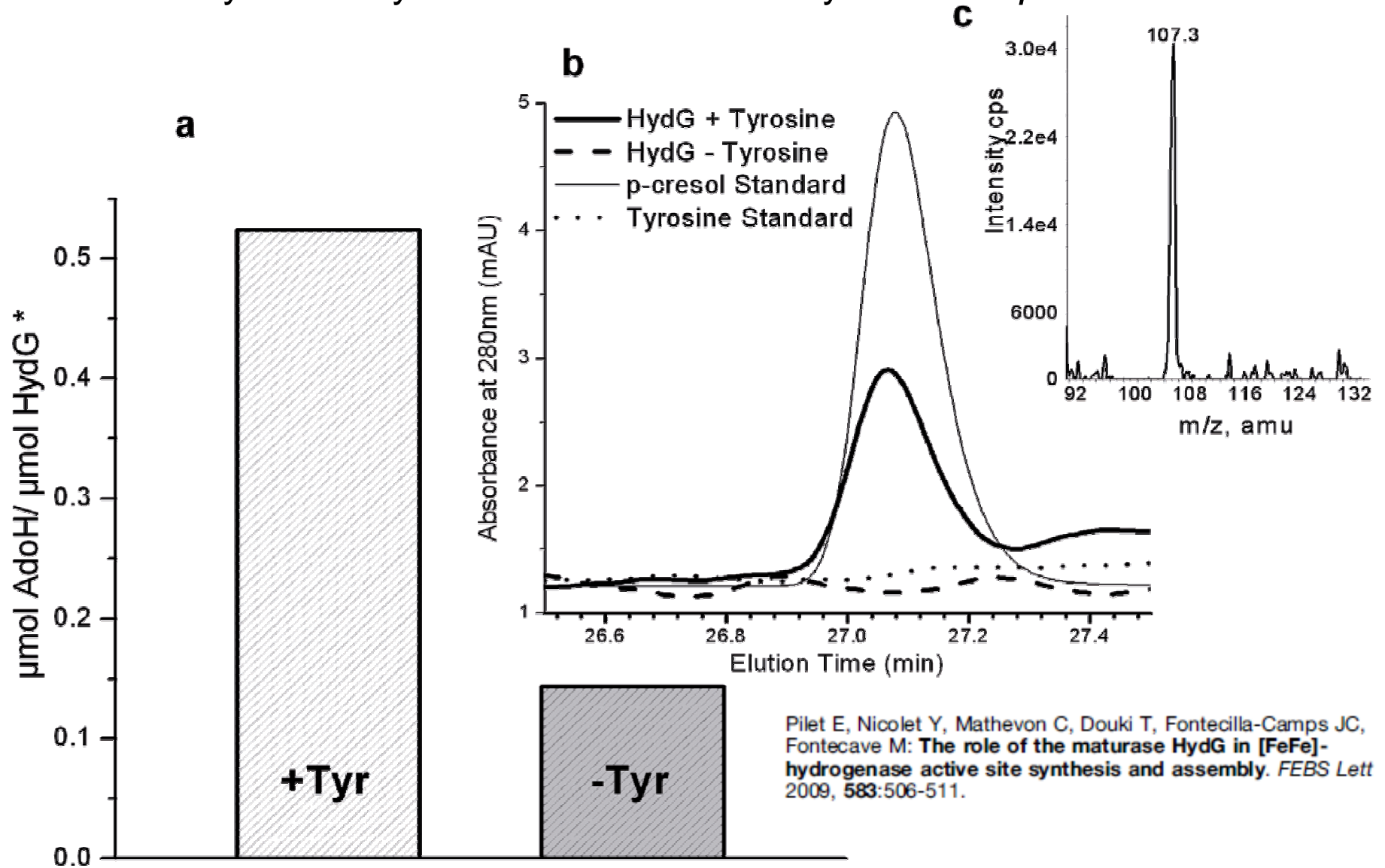


Enzymes de maturation: HydG, HydE, HydF

JW Peters et al *Science* (1998) **282**, 1853  
Y Nicolet et al *Structure* (1999) **7**, 13

# HydG et ThiH: homologues de séquence

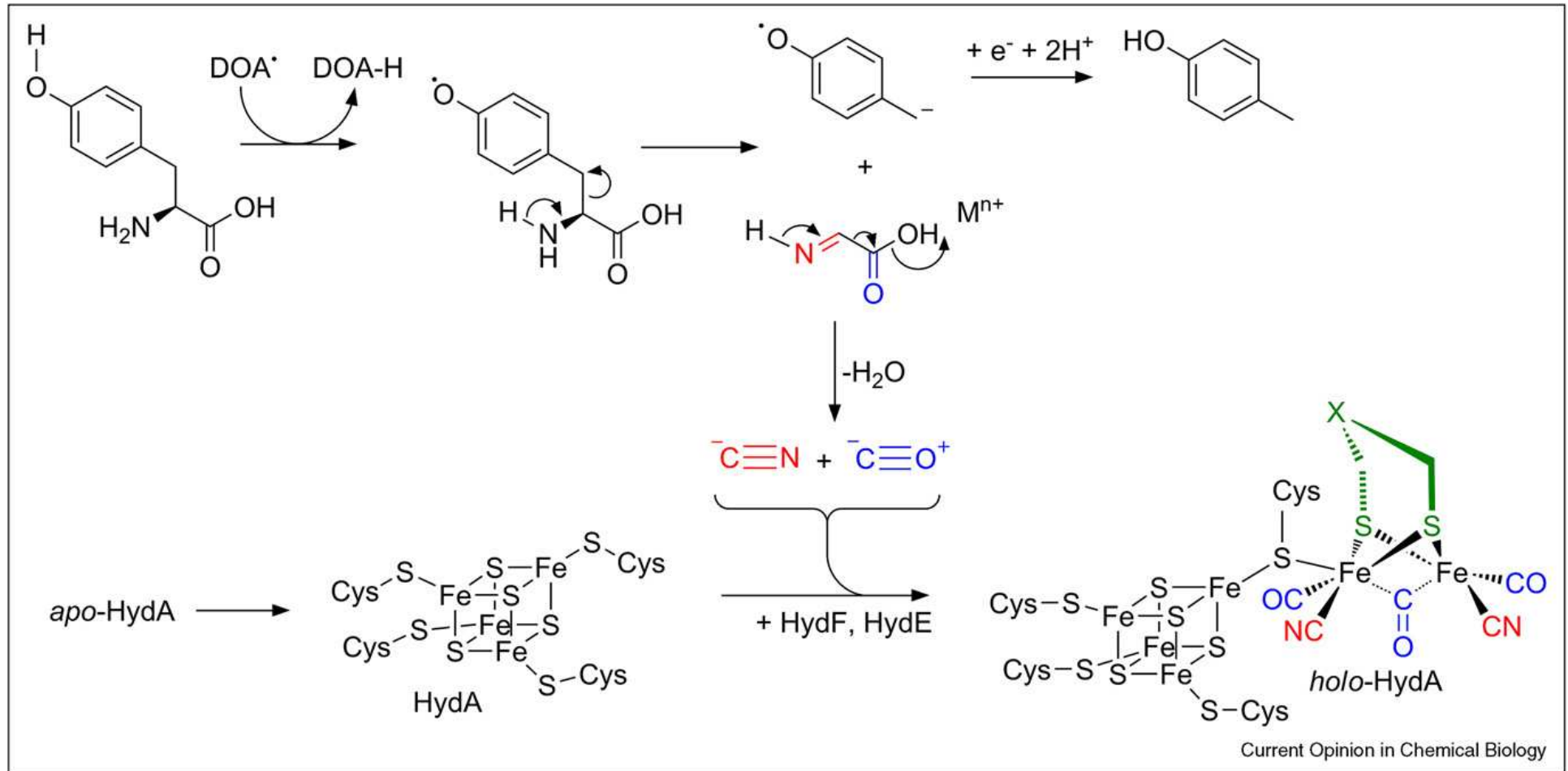
## HydG catalyse la conversion de la tyrosine en *p*-crésol



40  $\mu\text{M}$  holoHydG, 2mM SAM, 2 mM Dithionite, + tyrosine, 37°C, pH 8, anaerobic conditions

Pilet E, Nicolet Y, Mathevon C, Douki T, Fontecilla-Camps JC, Fontecave M: The role of the maturase HydG in [FeFe]-hydrogenase active site synthesis and assembly. *FEBS Lett* 2009, 583:506-511.

## HydG catalyse la transformation de la tyrosine en CO et CN<sup>-</sup>



Driesener RC, Challand MR, McGlynn SE, Shepard EM, Boyd ES, Broderick JB, Peters JW, Roach PL: **[FeFe]-hydrogenase cyanide ligands derived from S-adenosylmethionine-dependent cleavage of tyrosine.** *Angew Chem Int Ed Engl* 2010, **49**:1687-1690.

Shepard EM, Duffus BR, George SJ, McGlynn SE, Challand MR, Swanson KD, Roach PL, Cramer SP, Peters JW, Broderick JB: **[FeFe]-hydrogenase maturation: HydG-catalyzed synthesis of carbon monoxide.** *J Am Chem Soc* 2010, **132**:9247-9249.



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DE FRANCE  
—1530—

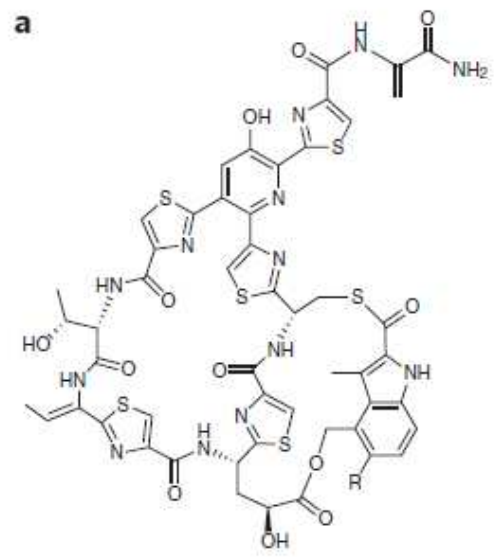
**Une découverte récente en enzymologie :  
la famille des métalloenzymes  
« Radical-SAM (S-AdénosylMéthionine) »**

**Marc Fontecave**

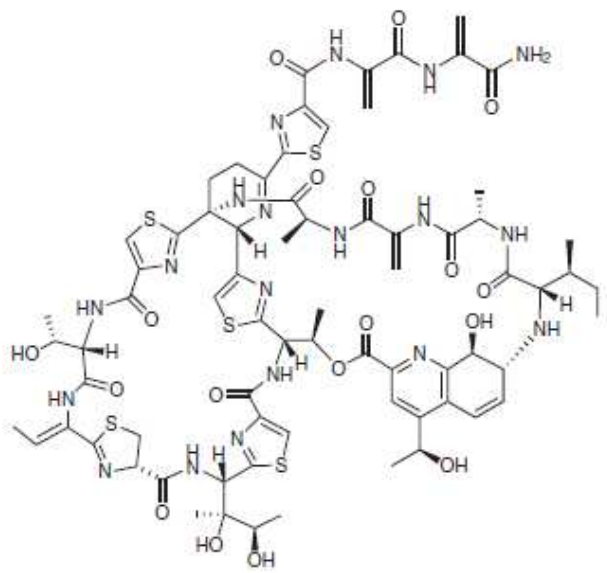
*Laboratoire de Chimie et Biologie des Métaux, Université Joseph Fourier, CNRS, CEA/DSV/iRTSV  
CEA-Grenoble 17 rue des martyrs 38054 Grenoble cedex 9, France  
mfontecave@cea.fr; Phone: (0033)438789103 ; Fax: (0033)438789124*

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





R = H, Nosiheptide (NOS);  
 R = F, 5'-fluoro-NOS (**27**)



Thiostrepton

