



**La lyase du photoproduit des spores (SPL):
Une métalloenzyme “Radical-SAM” de
réparation de l’ADN.**

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- Structure de l'ADN.
- Lésions de l'ADN et systèmes de réparation.
NER, BER, MMR

Une lésion ou un dommage de l'ADN correspond à toute modification chimique non physiologique de l'ADN qui perturbera la configuration de la double hélice et/ou ses propriétés biologiques.

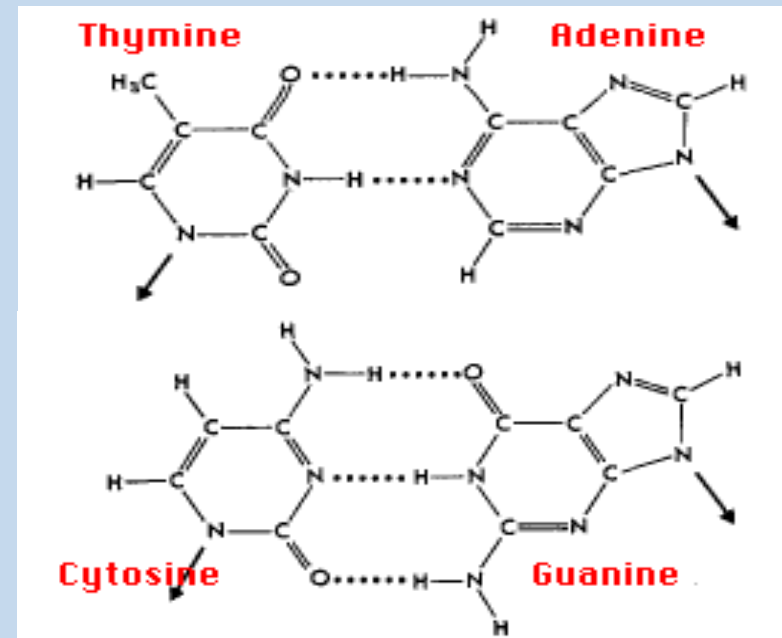
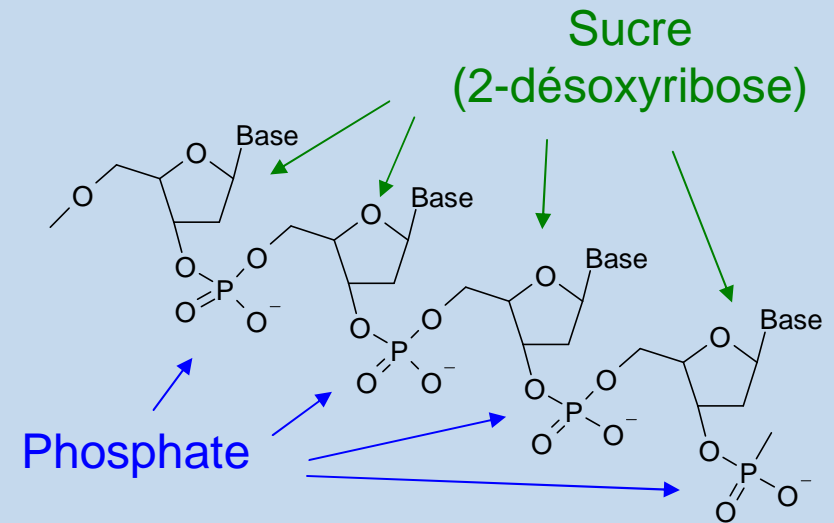
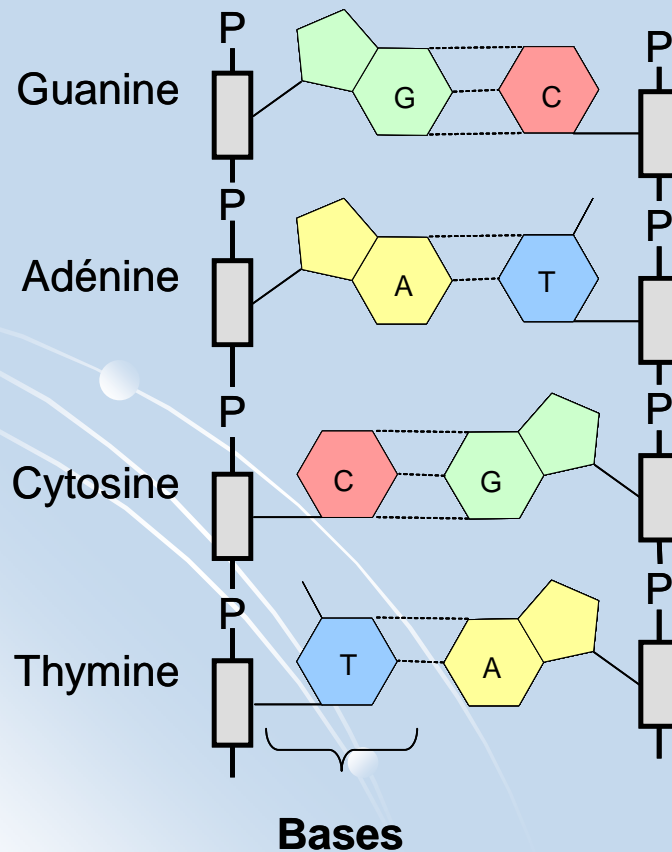
- Lésions UV –induites de l'ADN et réparation.
- Ce qui se passe dans les spores de bactéries (SP).
- La Lyase du photoproduit des spores (SPL).

Structure de l'ADN et complémentarité des bases

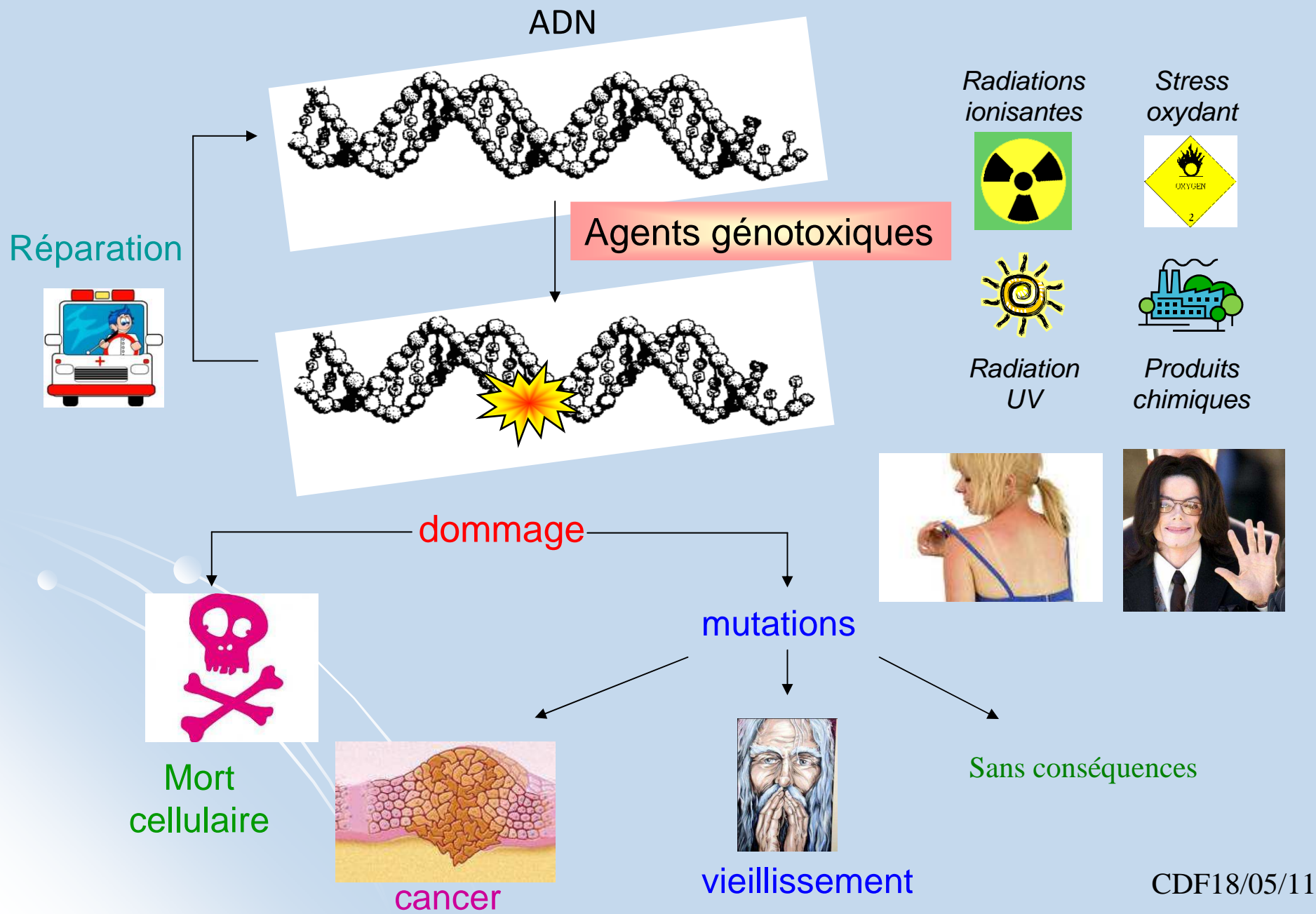
Deux rôles majeurs dans la cellule :

- Dépositaire de **l'information génétique.**
- Support de **l'hérédité.**

**Polymère
sucre-phosphate**



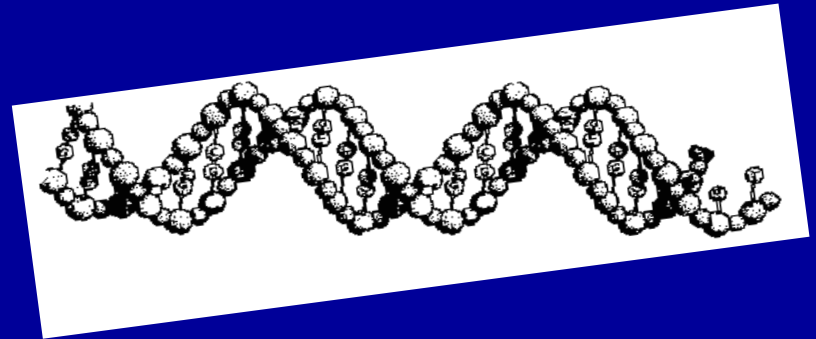
Conséquences des dommages de l'ADN





Source	Type de lésions	Nombre d'adduits induits/cellules
Bain de soleil (1 heure)	dimères de thymine	60 000 à 80 000
Tabagisme (20 cigarettes/jour)	Adduits sur l'ADN (hydrocarbures polycycliques)	100 à 200
Bruit de fond des radiations naturelles (2,4 à 40mSv/an)	Ruptures simple brin	2/an
Température corporelle (37°C)	Ruptures simples Site apuriniques Sites apyrimidiques Déamination	20 000 - 40 000/ par jour 10 000 500 100-300
Radicaux libres	Thymine glycol 5-hydroxométhyluracile 8-hydroxométhylguanosine Ruptures simples, doubles, pontages	27 620 168 ?

La nécessité du maintien de l'intégrité du génome a conduit les organismes à développer de nombreux mécanismes de sauvegarde et de réparation de l'ADN

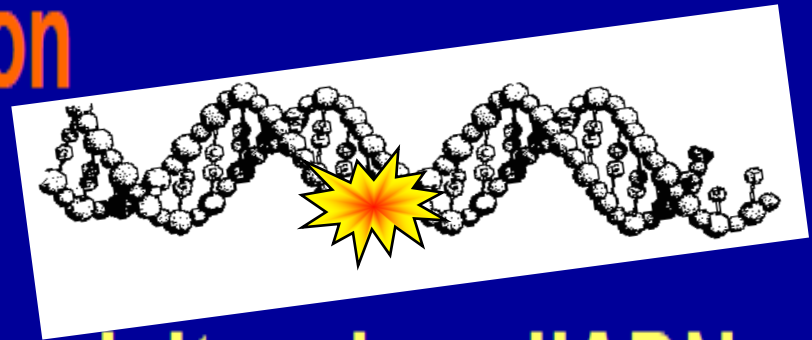


1) Système de sauvegarde

→ Convertir les agents toxiques ou mutagènes en molécules inoffensives

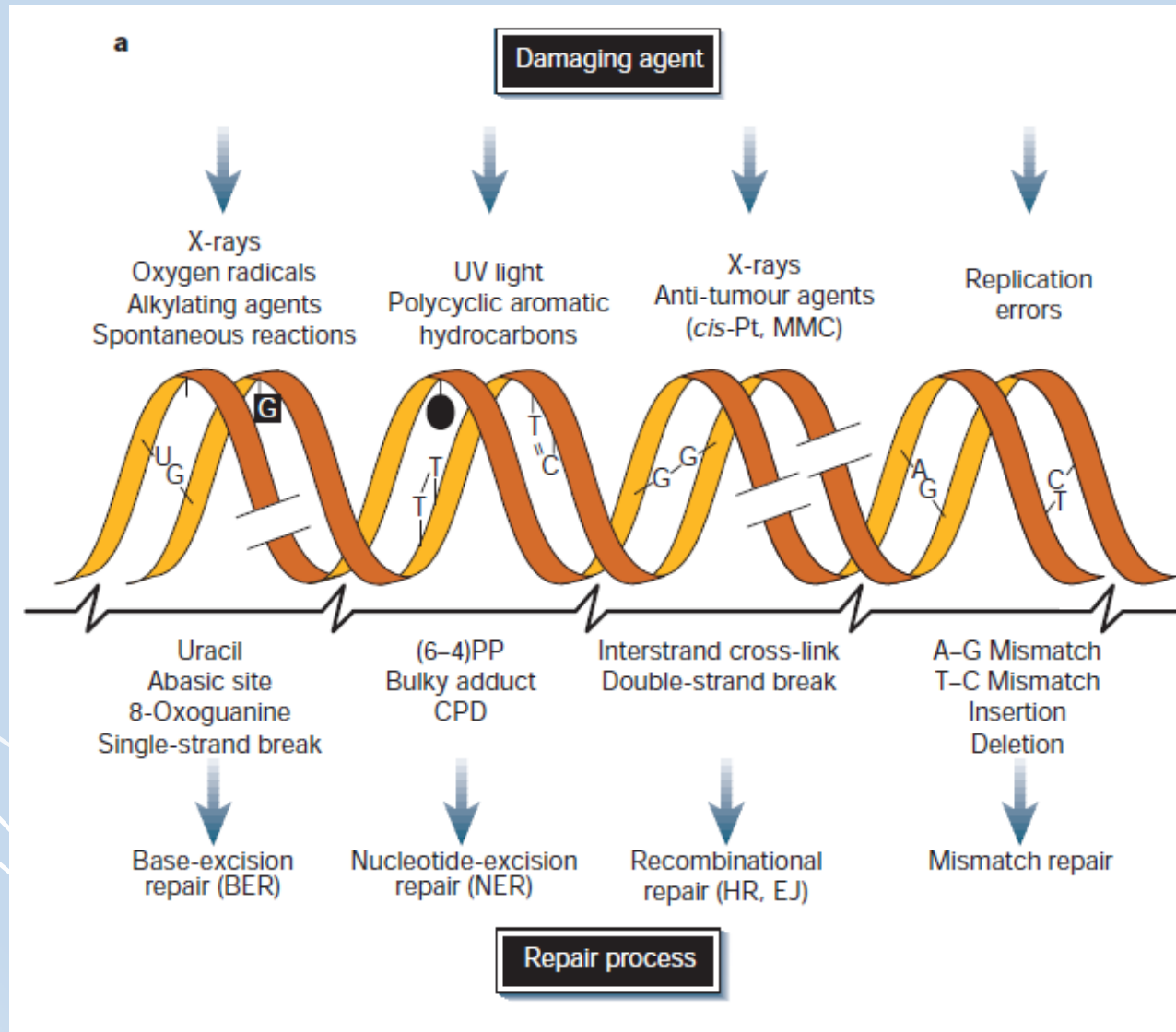
→ Système de détoxification des agents Xénobiotiques, centrés sur les enzymes cytoplasmiques, (les cytochromes P450)

2) Systèmes de réparation



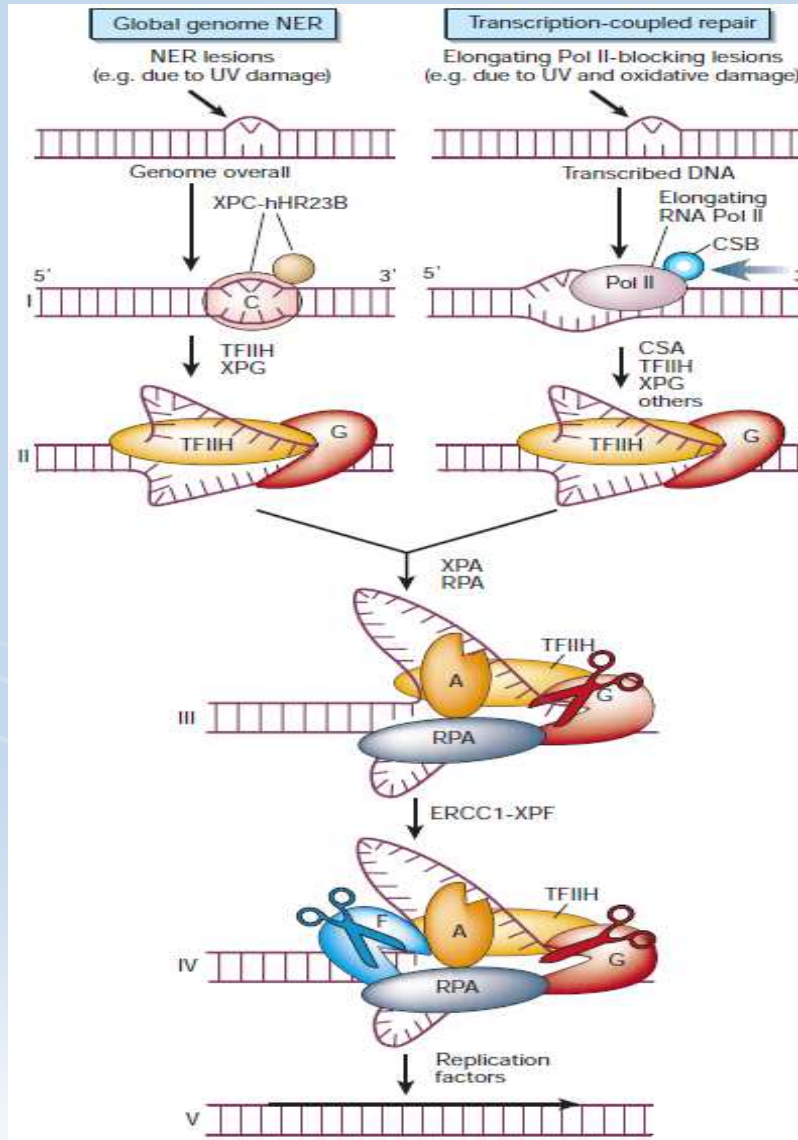
→ corriger les lésions produites dans l'ADN par les agents toxiques

plusieurs systèmes de batterie d'enzymes et de protéines (nombreuses) agissant de concert



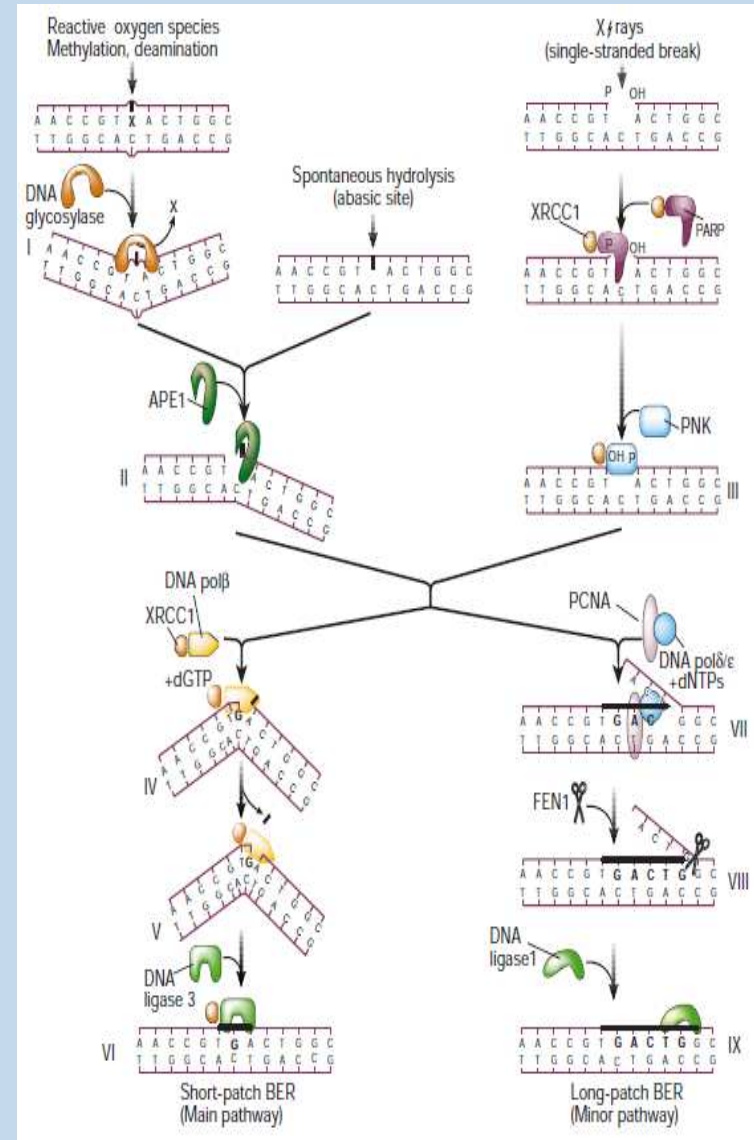
Réparation par Excision de Nucleotides

NER



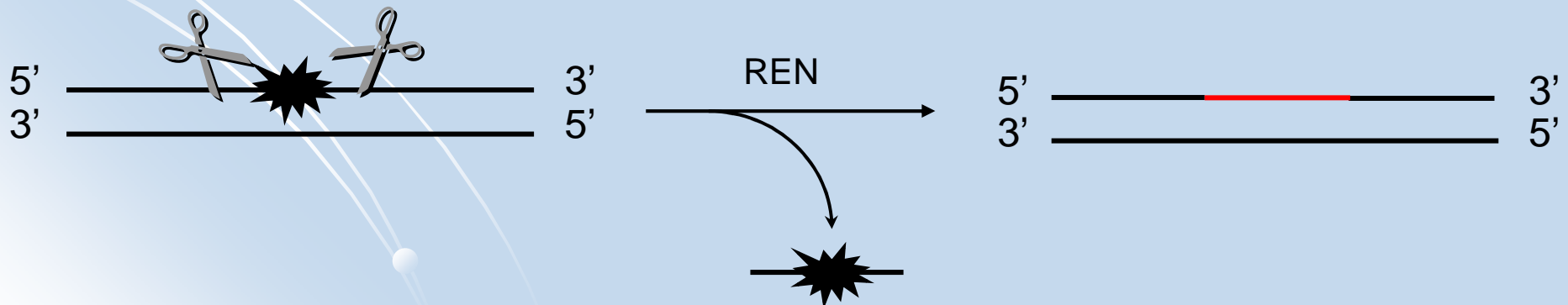
Réparation par Excision de Bases

BER



Réparation par excision de: *nucléotides *bases

- Chez tous les organismes vivants
- Excision d'un oligonucléotide de 12-13 bases
- 3 étapes :
 - Reconnaissance du dommage.
 - Excision de l'oligonucléotide ou de base.
 - Re-synthèse et ligation.



ADN et Irradiation UV

Types de lésions

Lésions de l'ADN induites par les UV

- Irradiation UV = Agent mutagène bien connu.
- **UV-C et UV-B** : absorption directe : dimérisation des bases pyrimidiques (T,C) – lésions très létales et mutagènes.
- Réparées par “Réparation par excision de nucléotides” (mécanisme non spécifique) et enzymes photoactivables (Photolyase)

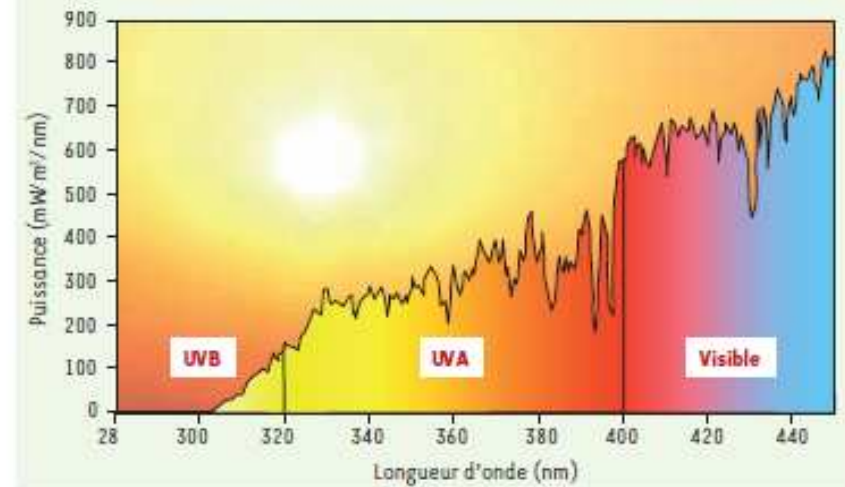
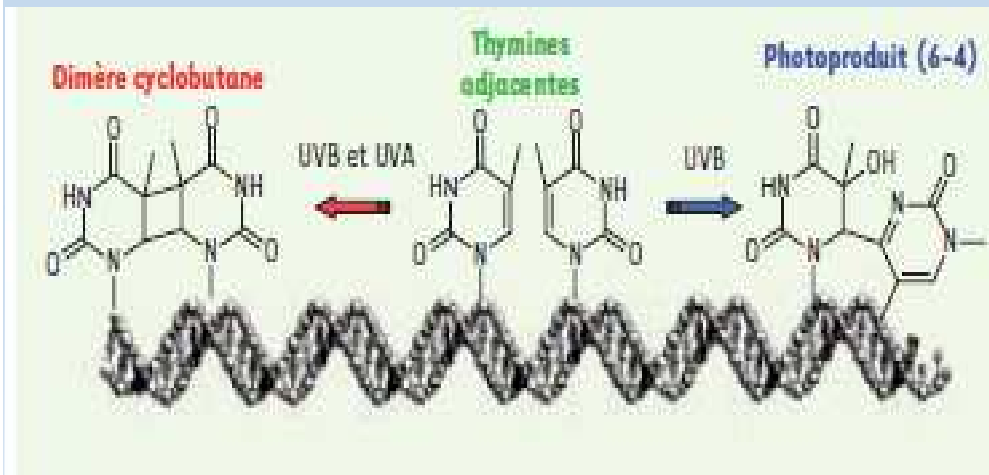
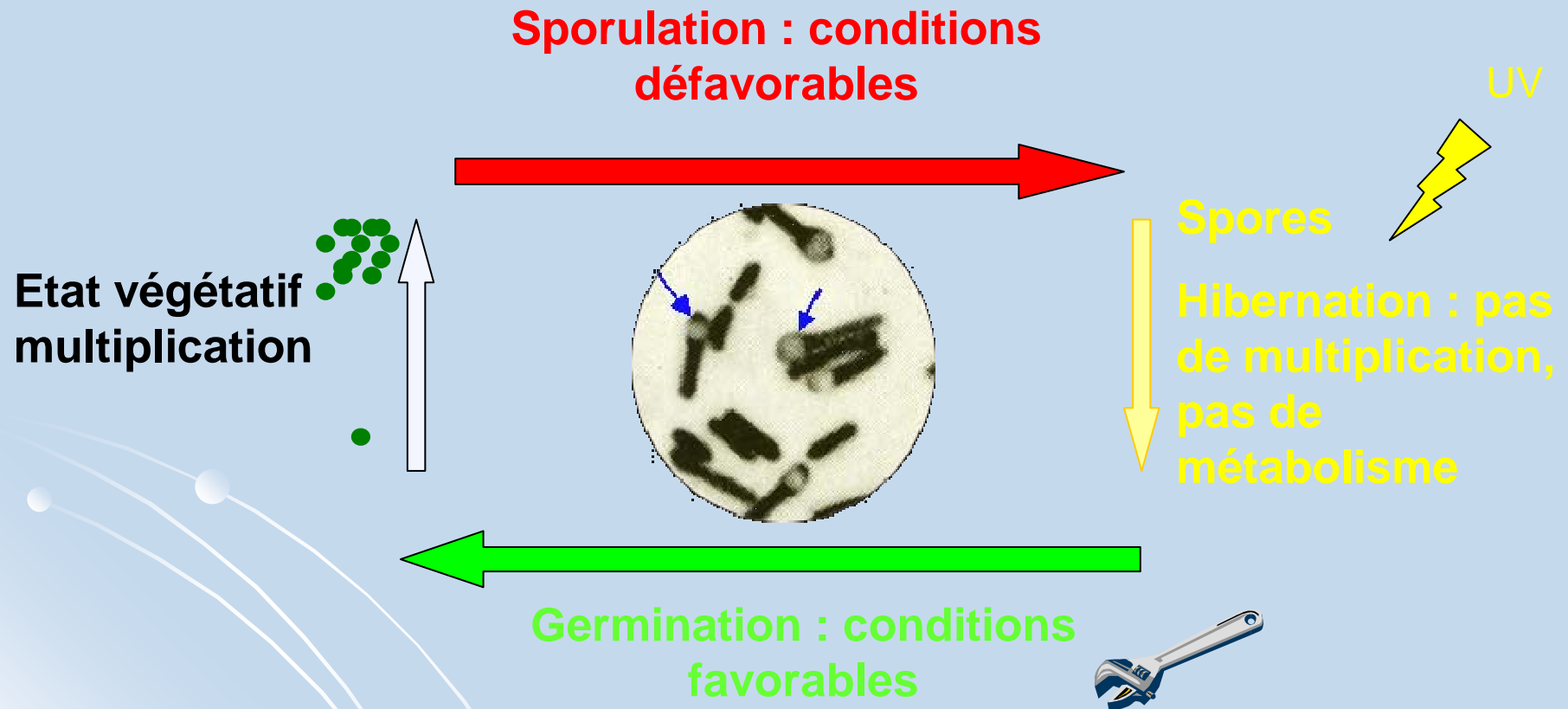


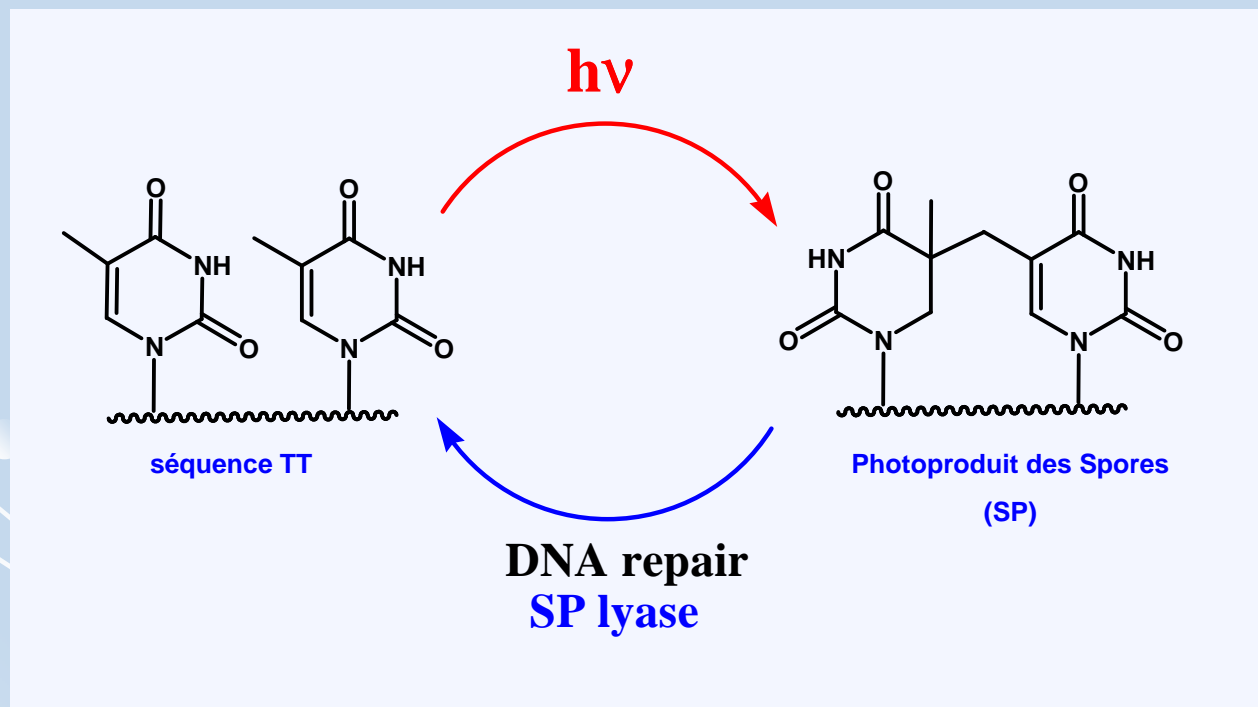
Figure 1. Spectre de la lumière solaire à la surface terrestre. La portion située entre 280 et 320 nm correspond au rayonnement UVB et celle entre 320 et 400 nm à l'UVA. Seuls les UVB sont significativement absorbés par l'ADN des cellules.

Cycle de vie de *B. subtilis*. Spores sont très résistants?



Le Photoproduit des Spores

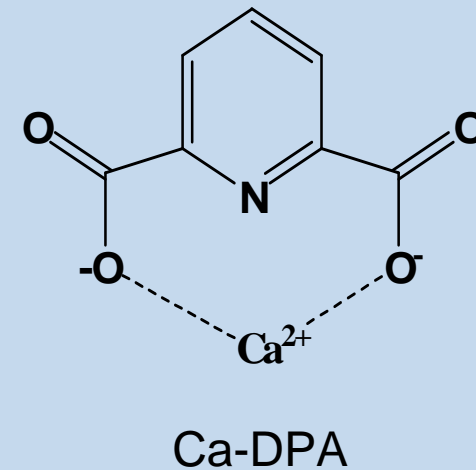
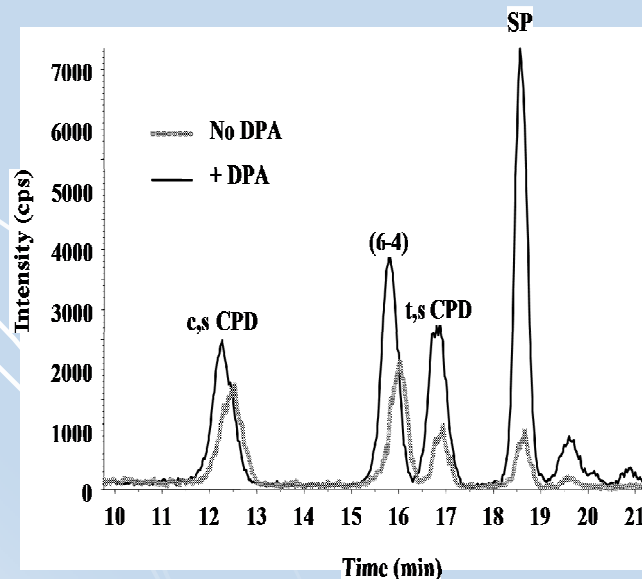
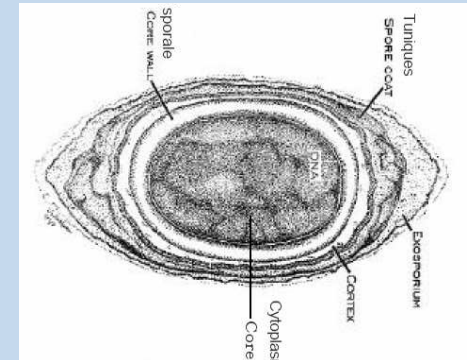
- Chez les **spores** : exposition UV → **un seul photoproduit**
- Découvert par Donnellan et Setlow en 1965



5-thyminy-5,6-dihydrothymine

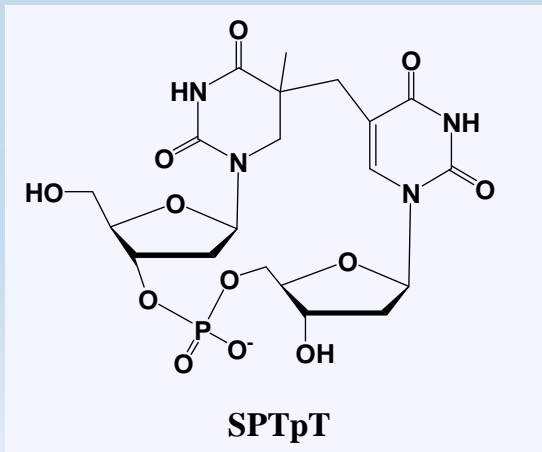
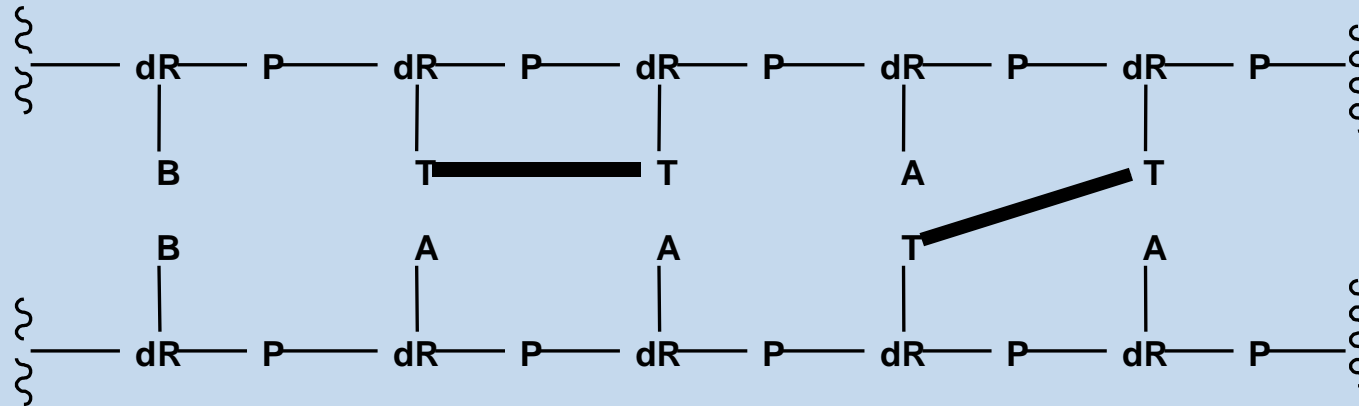
Le Photoproduit des Spores

- Formation de cette lésion spécifique expliquée par les caractéristiques spécifiques des spores :
 - Complexation de l'ADN aux Small acid-soluble proteins (SASP)
 - Déshydratation
 - Conformation de l'ADN (forme A)
 - Présence d'acide dipicolinique dans les spores

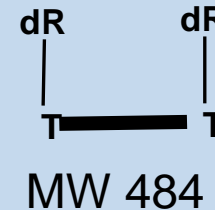
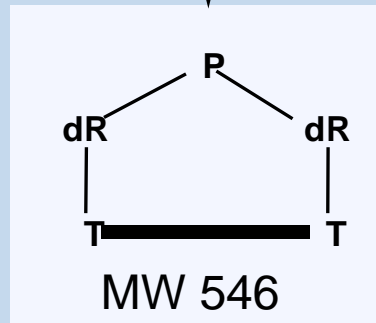


Le Photoproduit des Spores

- Principalement une lésion intra-brin

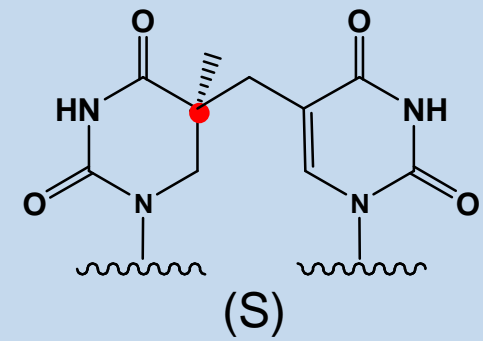
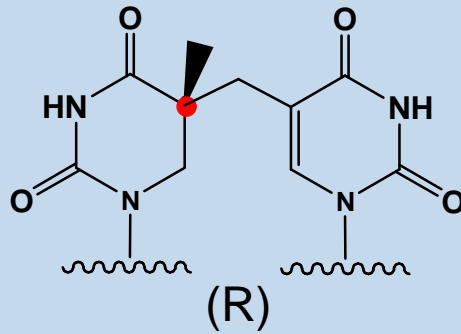


Hydrolyse enzymatique

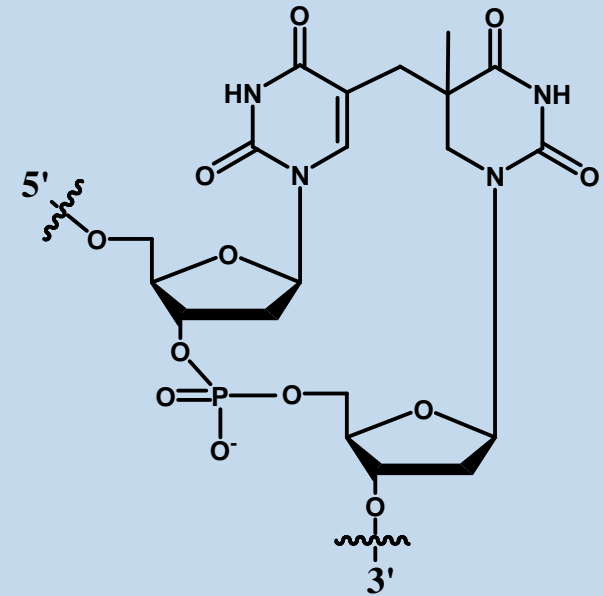
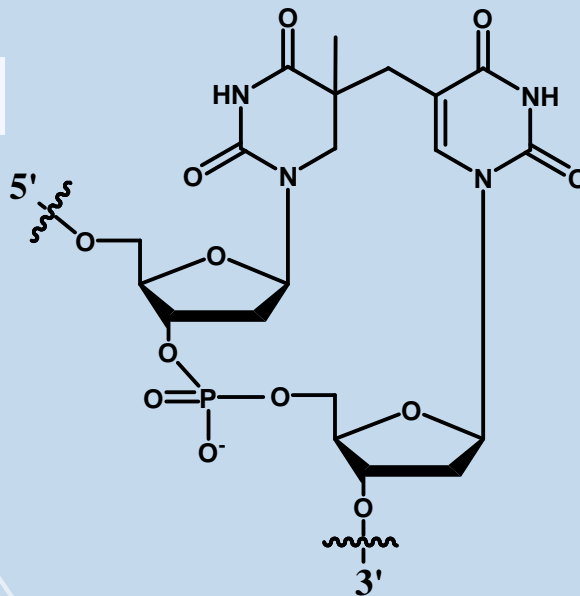


Questions: structure de photoproduit des spores?

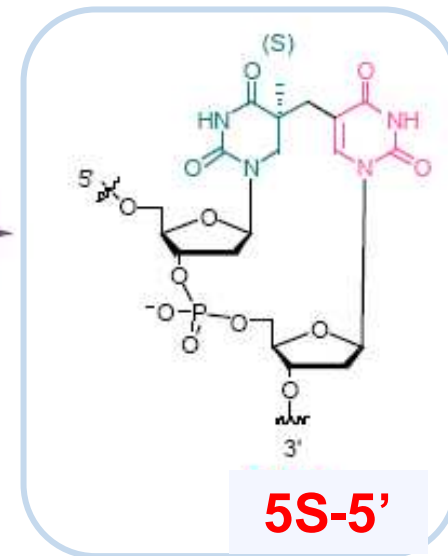
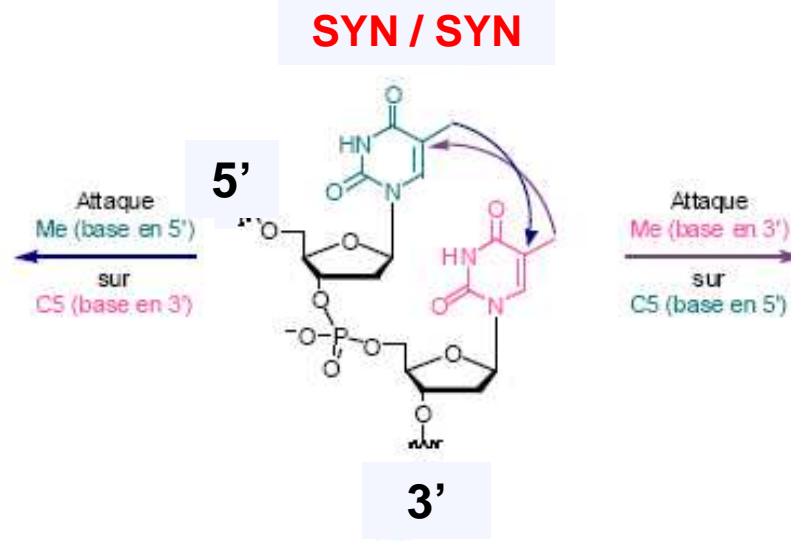
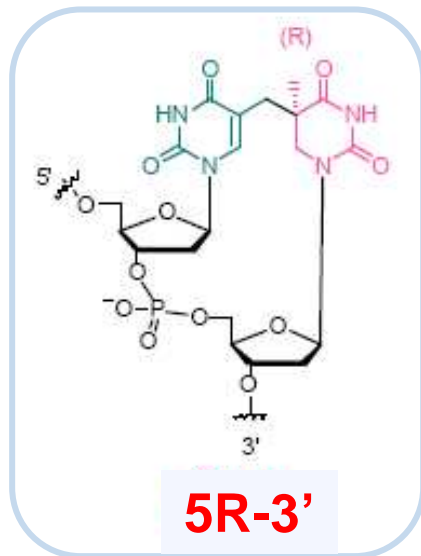
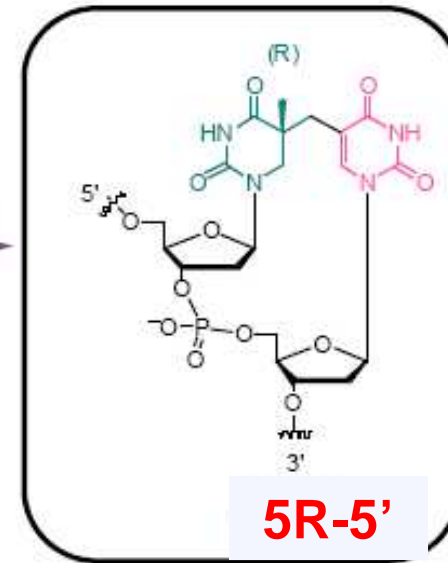
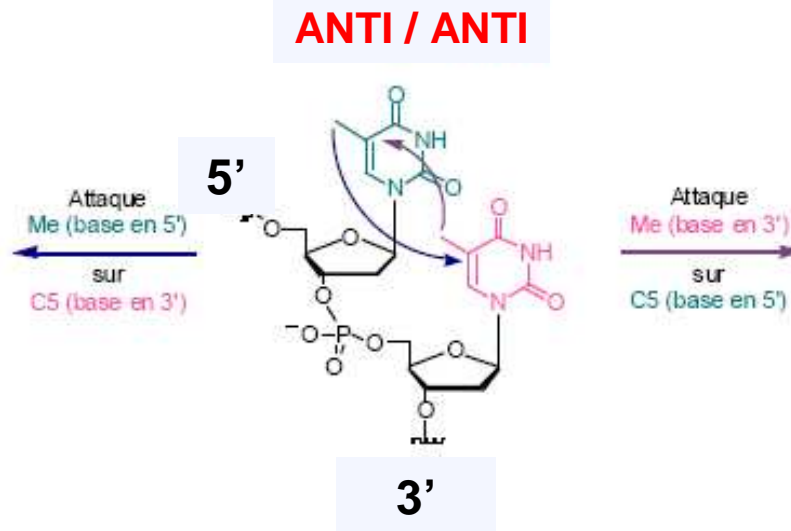
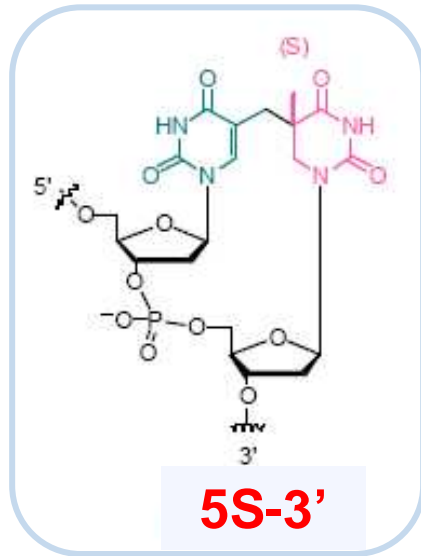
Stéréochimie ?



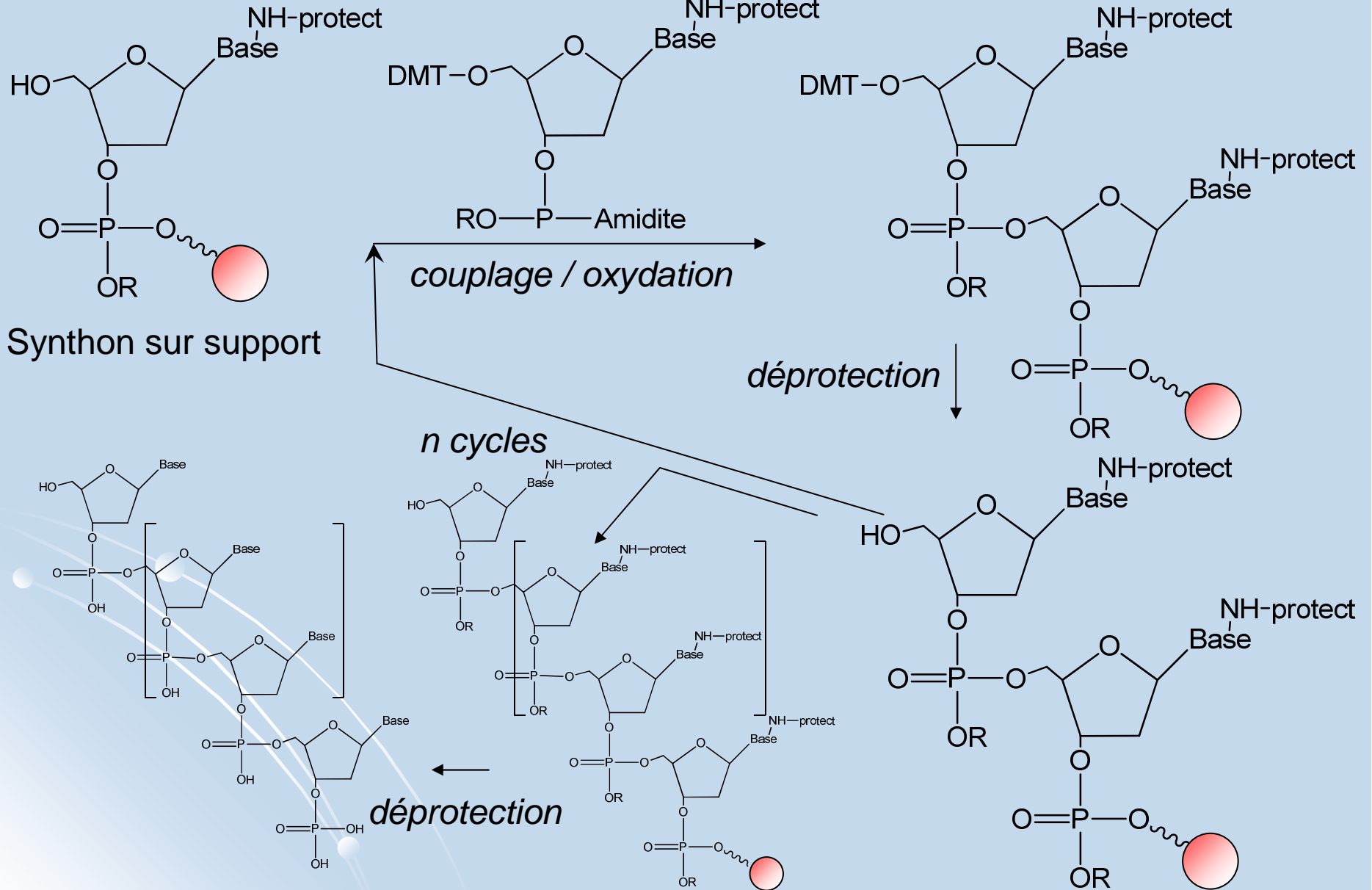
5'-Me ou 3'-Me ?



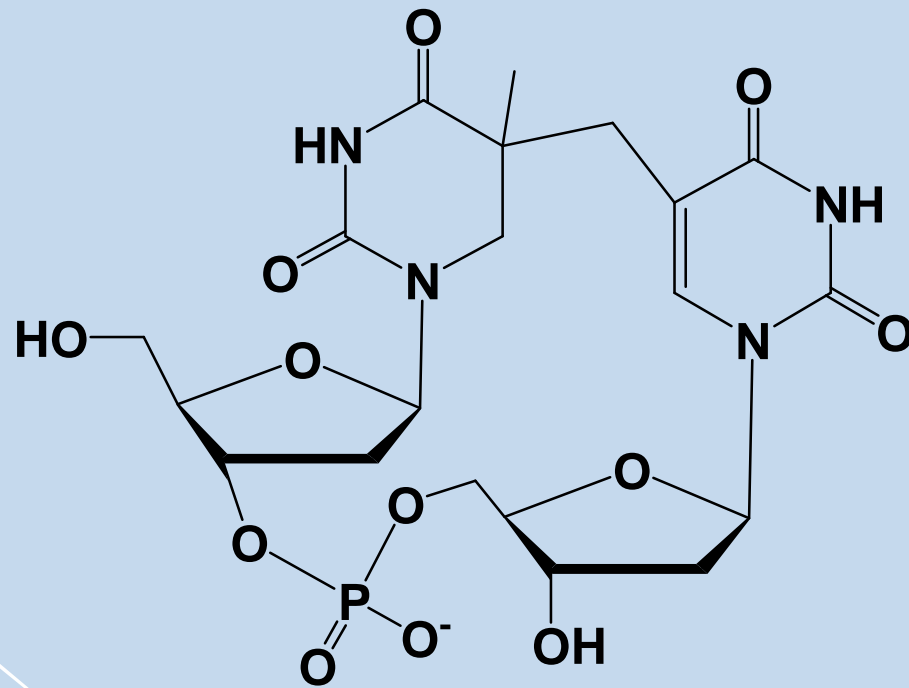
Stéréochimie



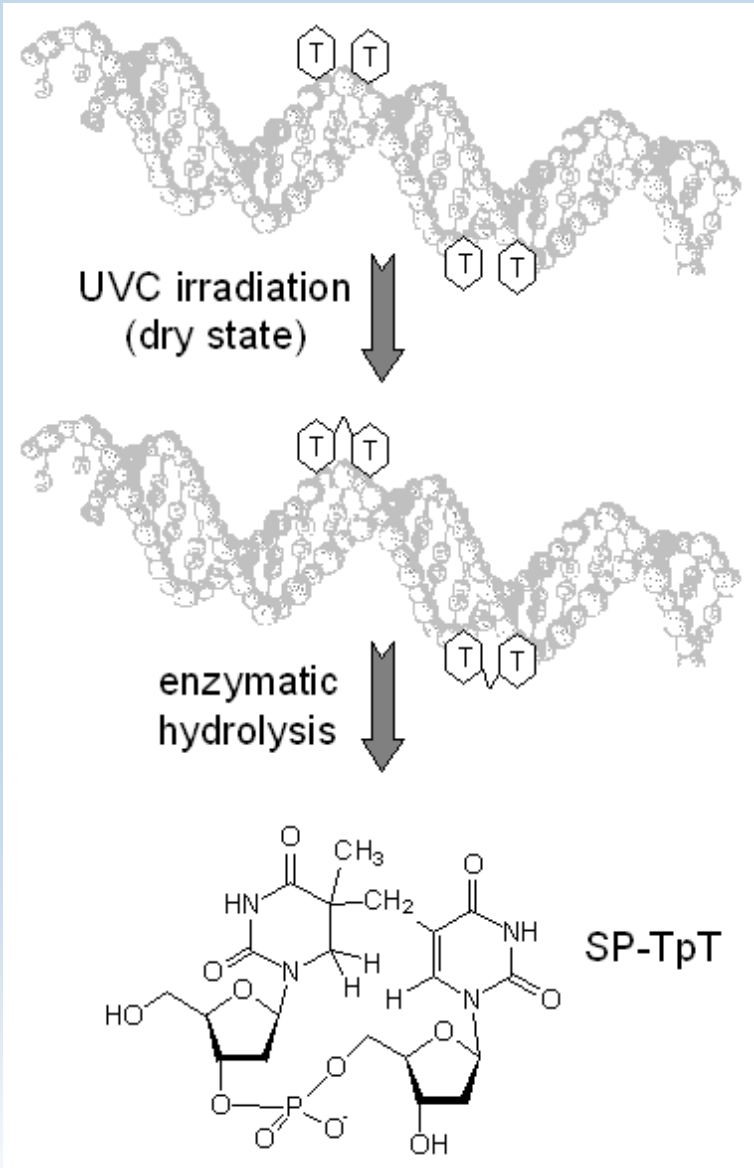
Synthèse d'ADN



Obtention du substrat SPTpT

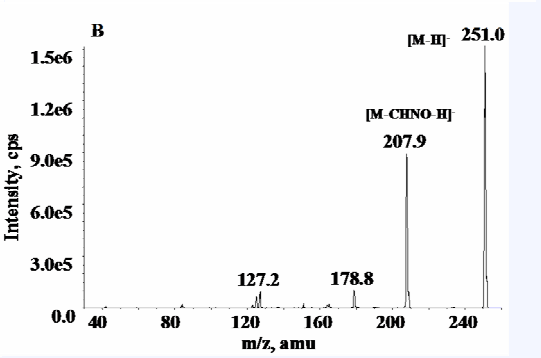
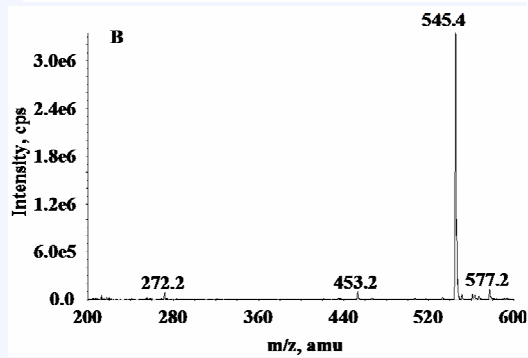
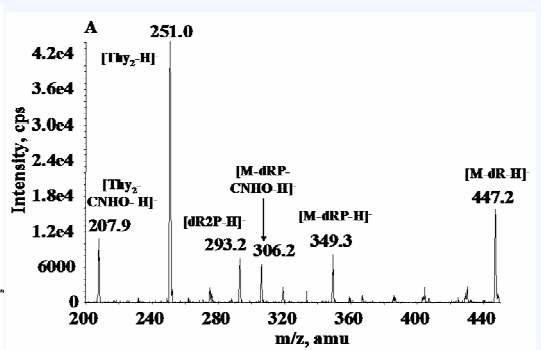
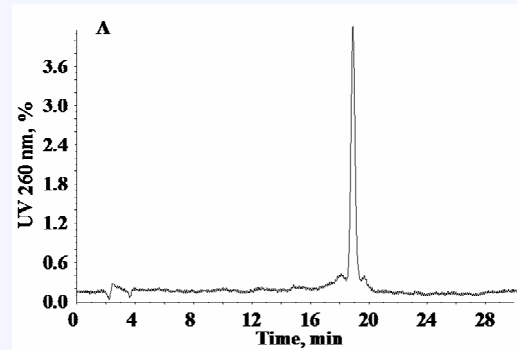


SPTpT

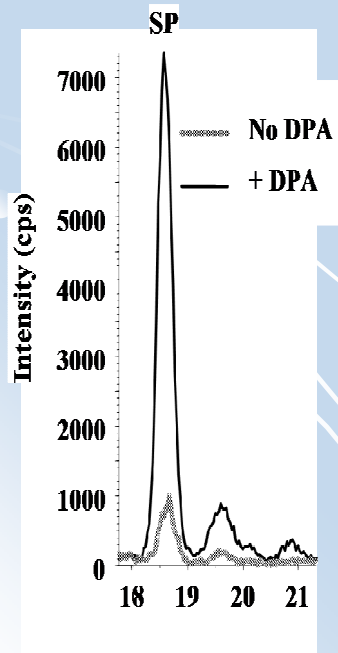
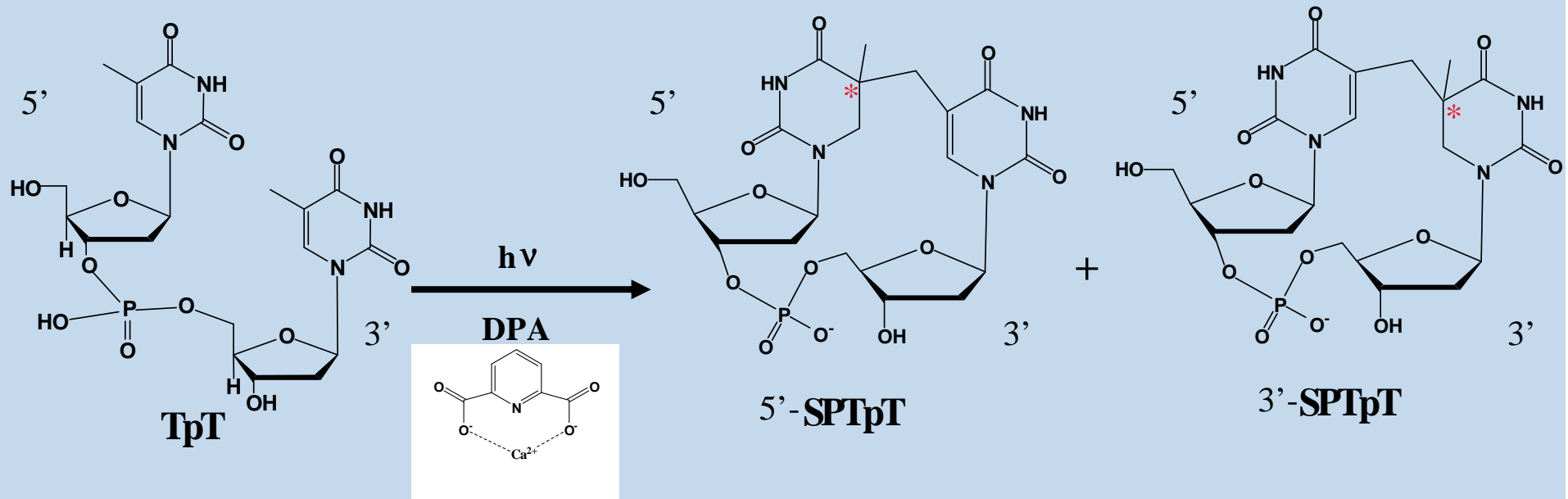


HPLC/MS

MS/MS



Strategie : irradiation en présence de DPA

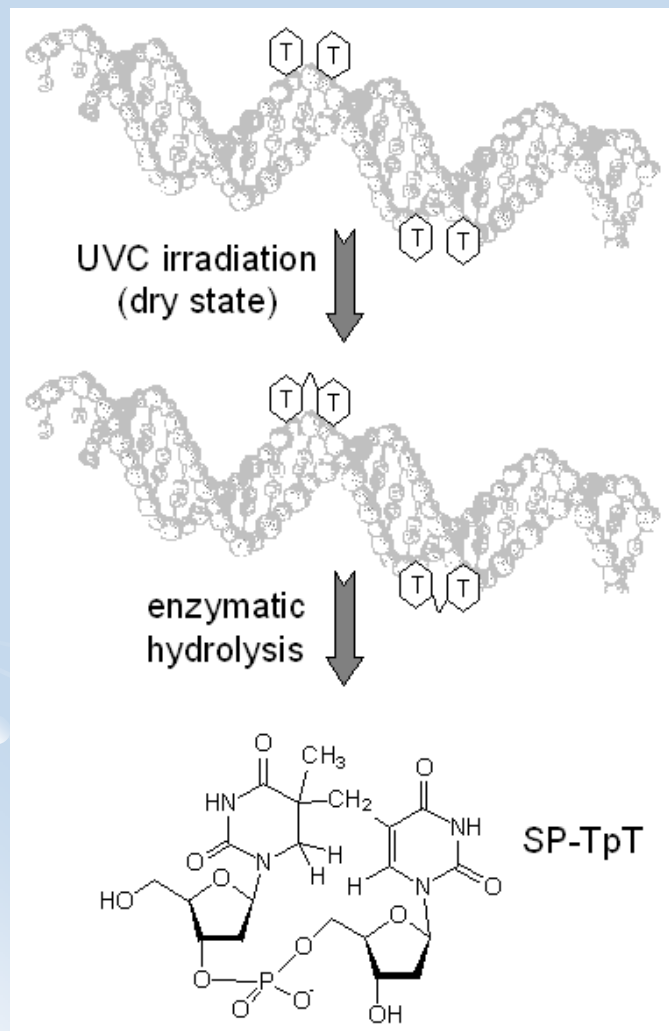


- Un seul pic HPLC de même temps de rétention que le SPTpT des spores.
- Le spectre de fragmentation MS/MS est identique à SPTpT des spores.



Le dimère de thymine obtenu par irradiation d'un TpT conduit à l'obtention du SPTpT identique à celui obtenu à partir des spores irradiées.

Structure du SPTpT ?



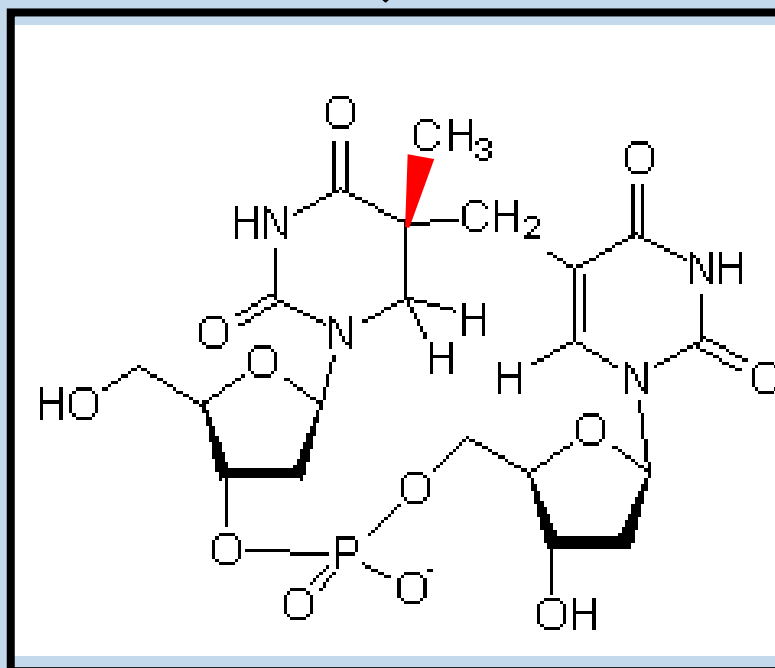
T. Douki, M. Atta, M. Fontecave *et al.*
J. Am. Chem. Soc. 2008, 130, 16978-16984.

NMR measurements:

2D-NMR experiments 1H-1H COSY, TOCSY, 1H-13C HSQC,

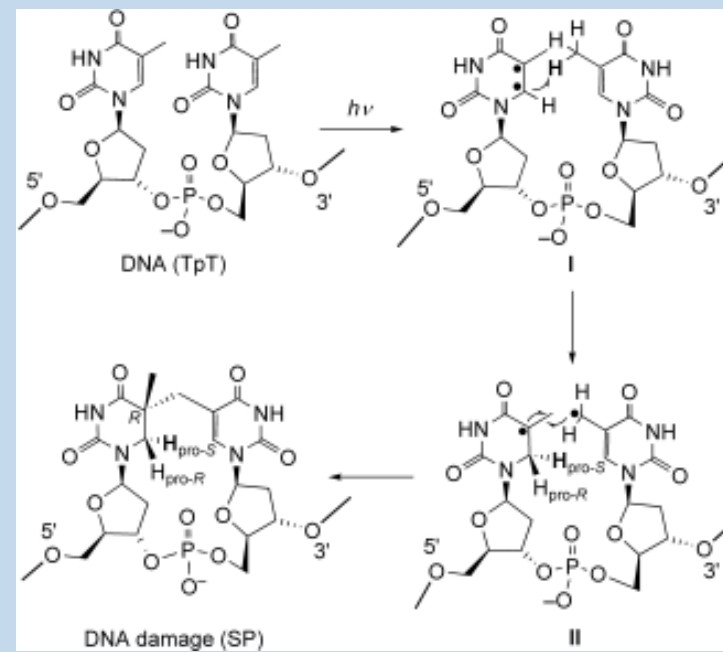
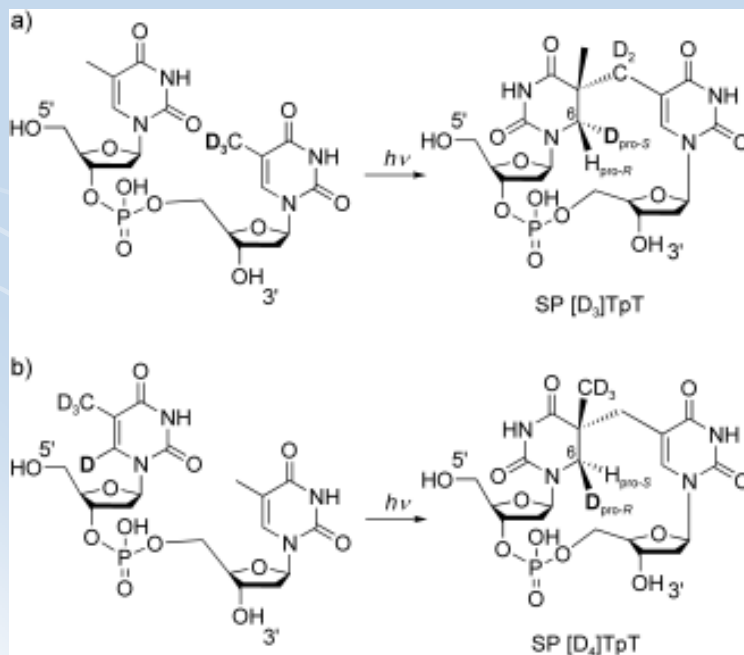
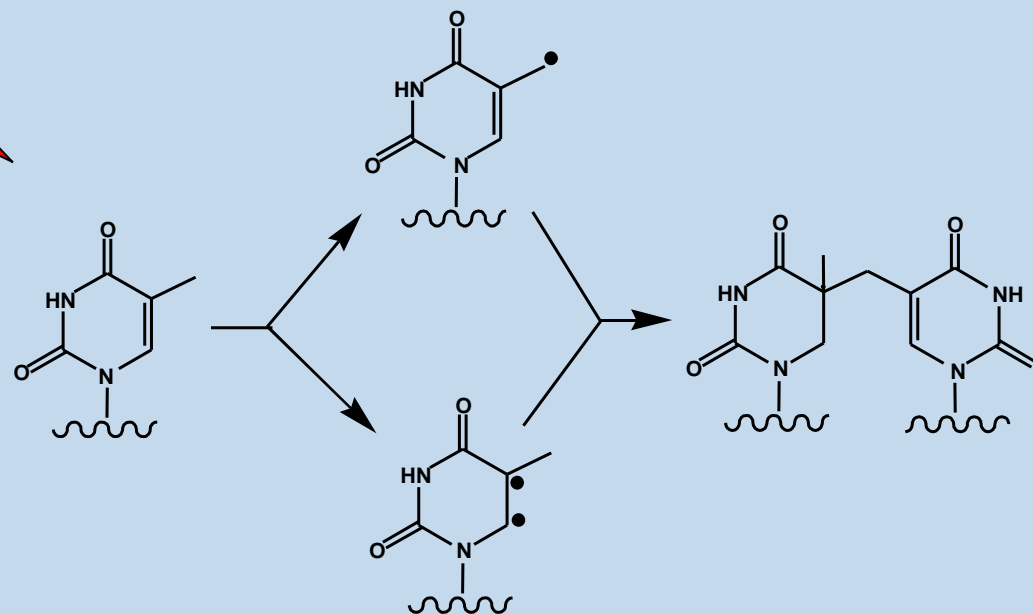
1H-13C HMBC, 1H-31P HMBC, NOESY, ROESY

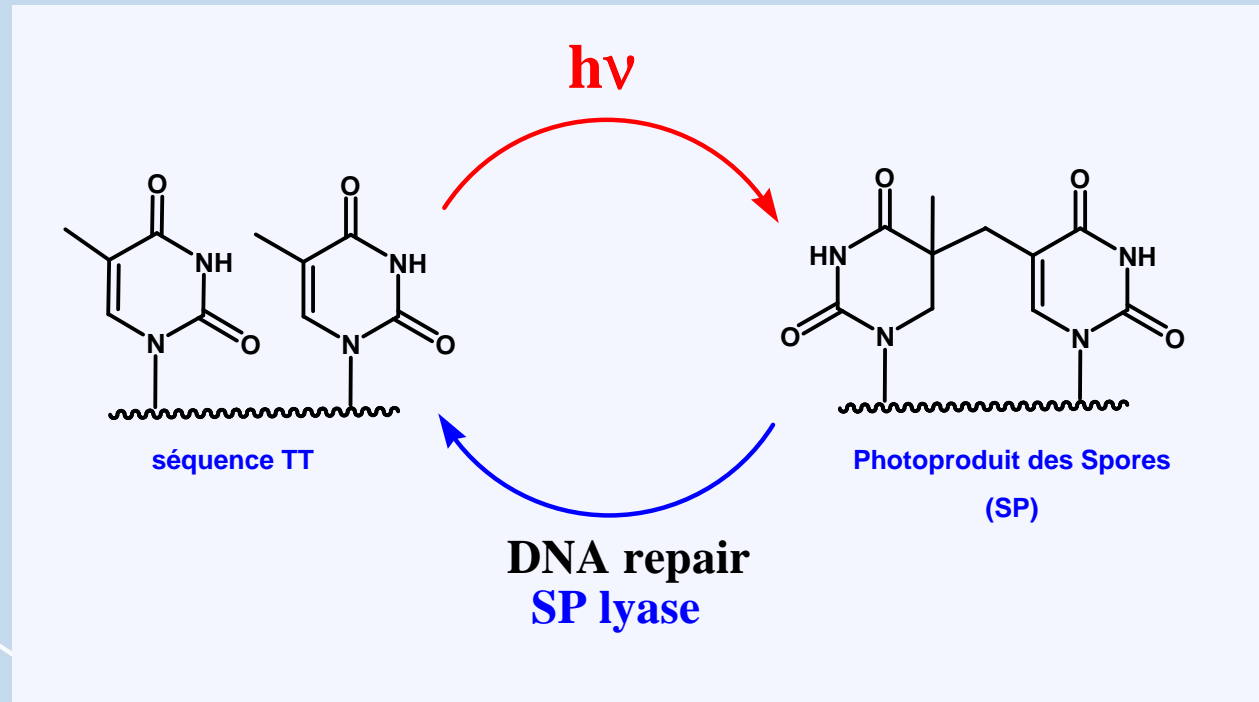
Theoretical DFT calculations



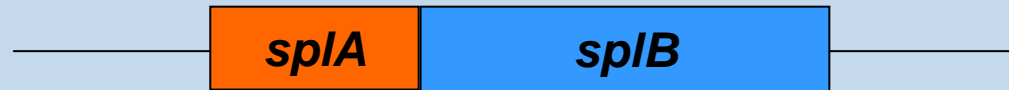
C₅ est sur la base 5'
Avec la configuration R

Mécanisme ?



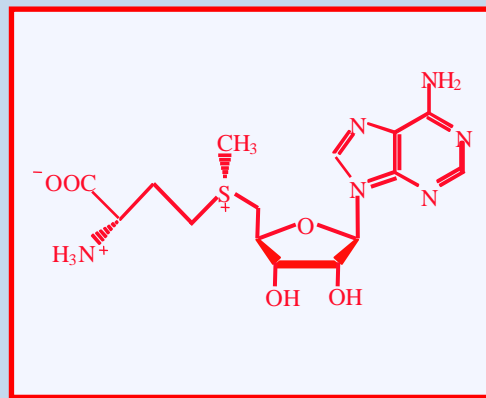
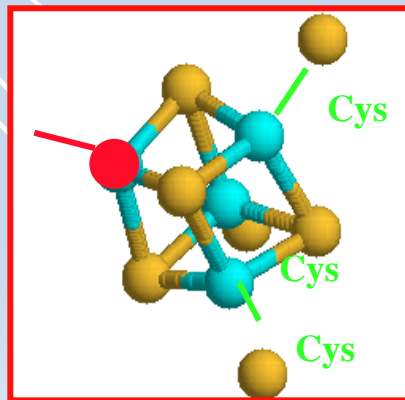


La lyase du photoproduit des spores



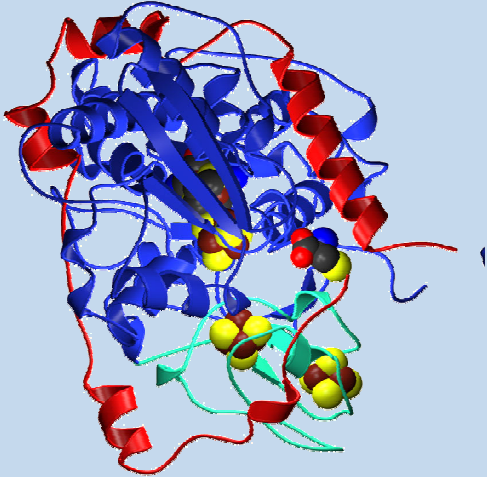
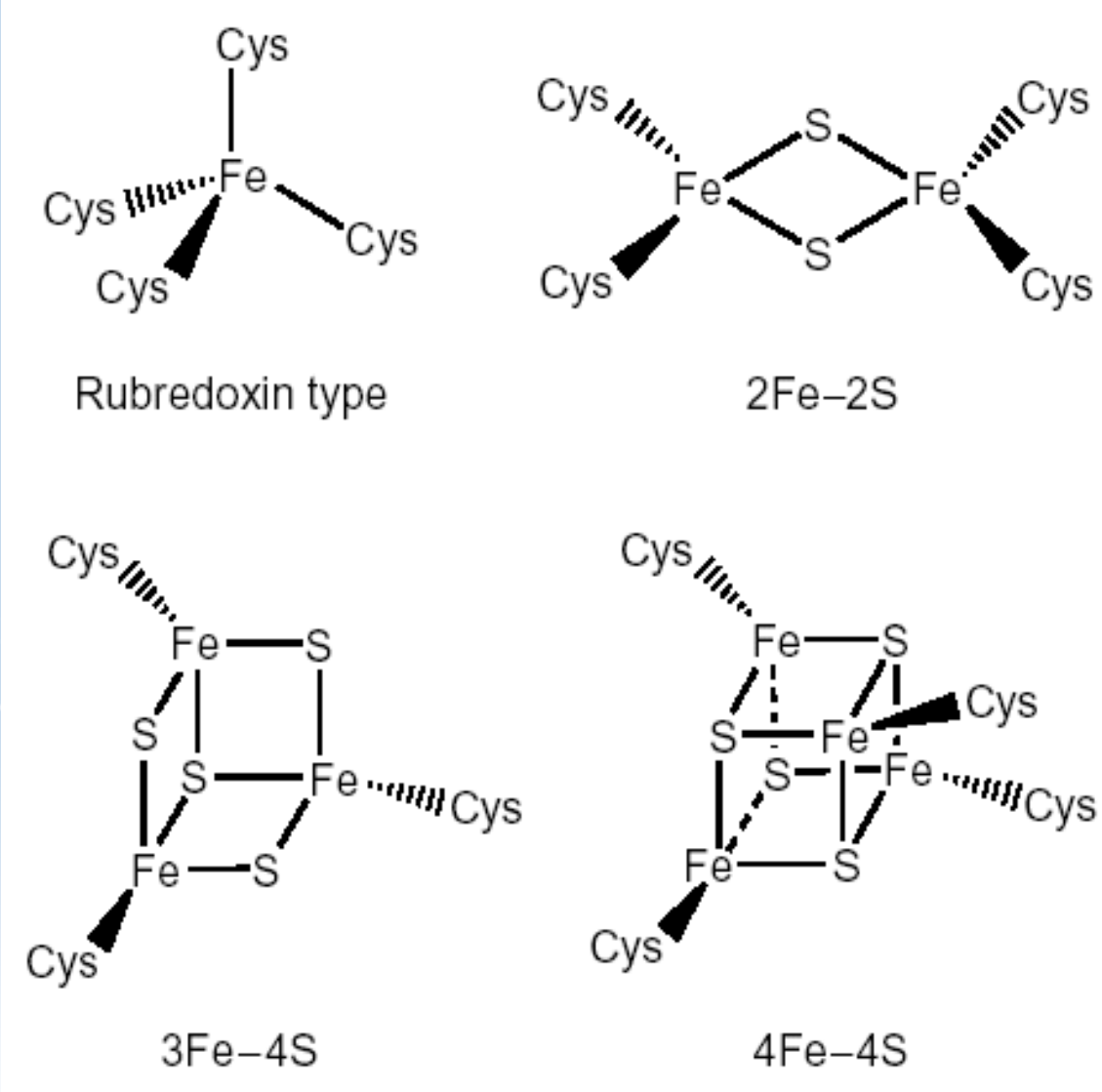
- *splB* → SPL de *B. Subtilis* : 342 aa, 40 kDa.
- 3 cysteines, **CxxxCxxC.**
- *SPL B. Subtilis* **GCMGHCHYCY**
- *SPL C. Perfringens* **GCVGNCQYCY**
- *SPL C. Acetobutylicum* **GCAAMCEYCY**
- SPL : est la seule enzyme de réparation de l'ADN de la famille « Radical-SAM ».

[4Fe-4S]

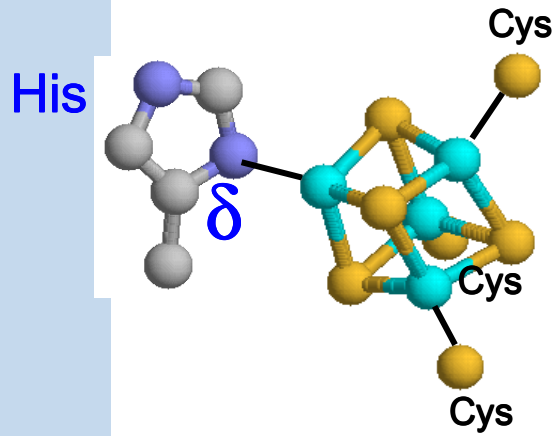


S-Adenosylmethionine (SAM)

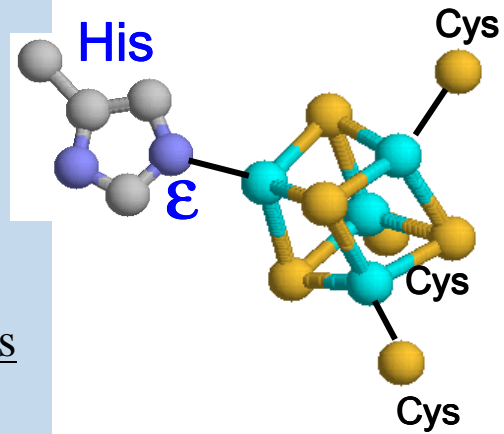
Les centres Fe-S



[4Fe-4S]



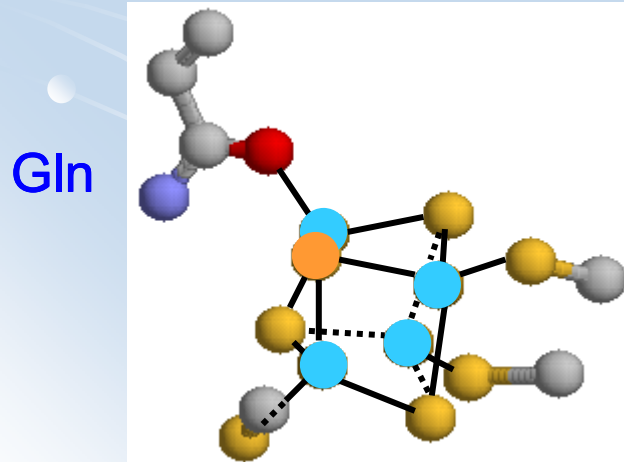
Volbeda et al (1995) *Nature* 373, 580



Peters et al (1998) *Science* 282, 1853

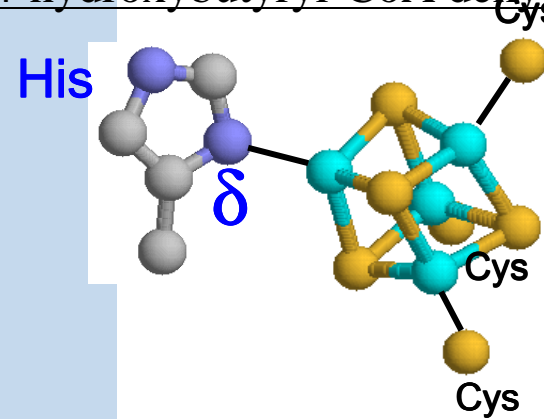
Hydrogenases

Dihydropyrimidine deshydrogenase



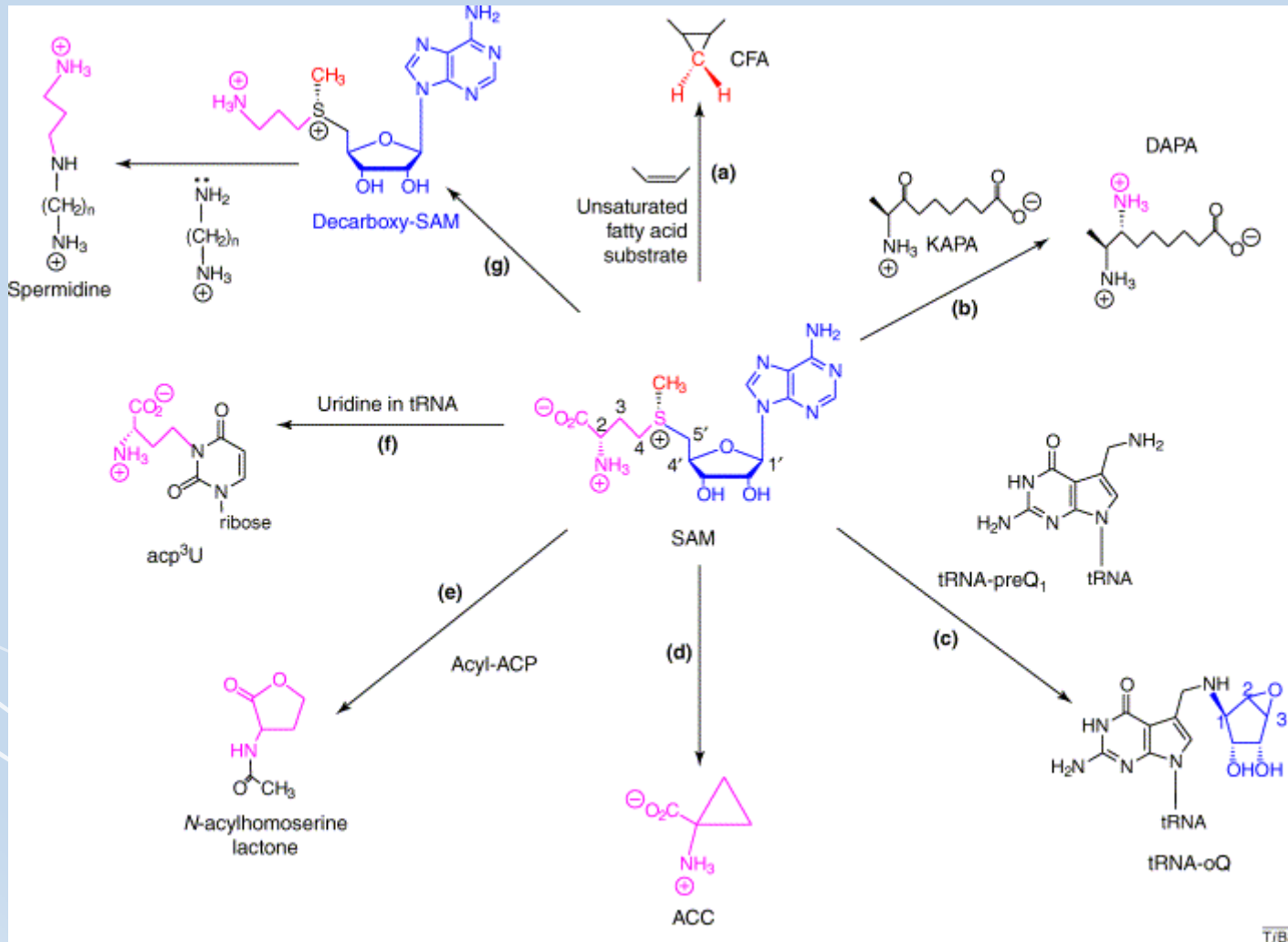
Dobritzsch et al (2001) *EmboJ* 20, 650

4-hydroxybutyryl-CoA dehydratase



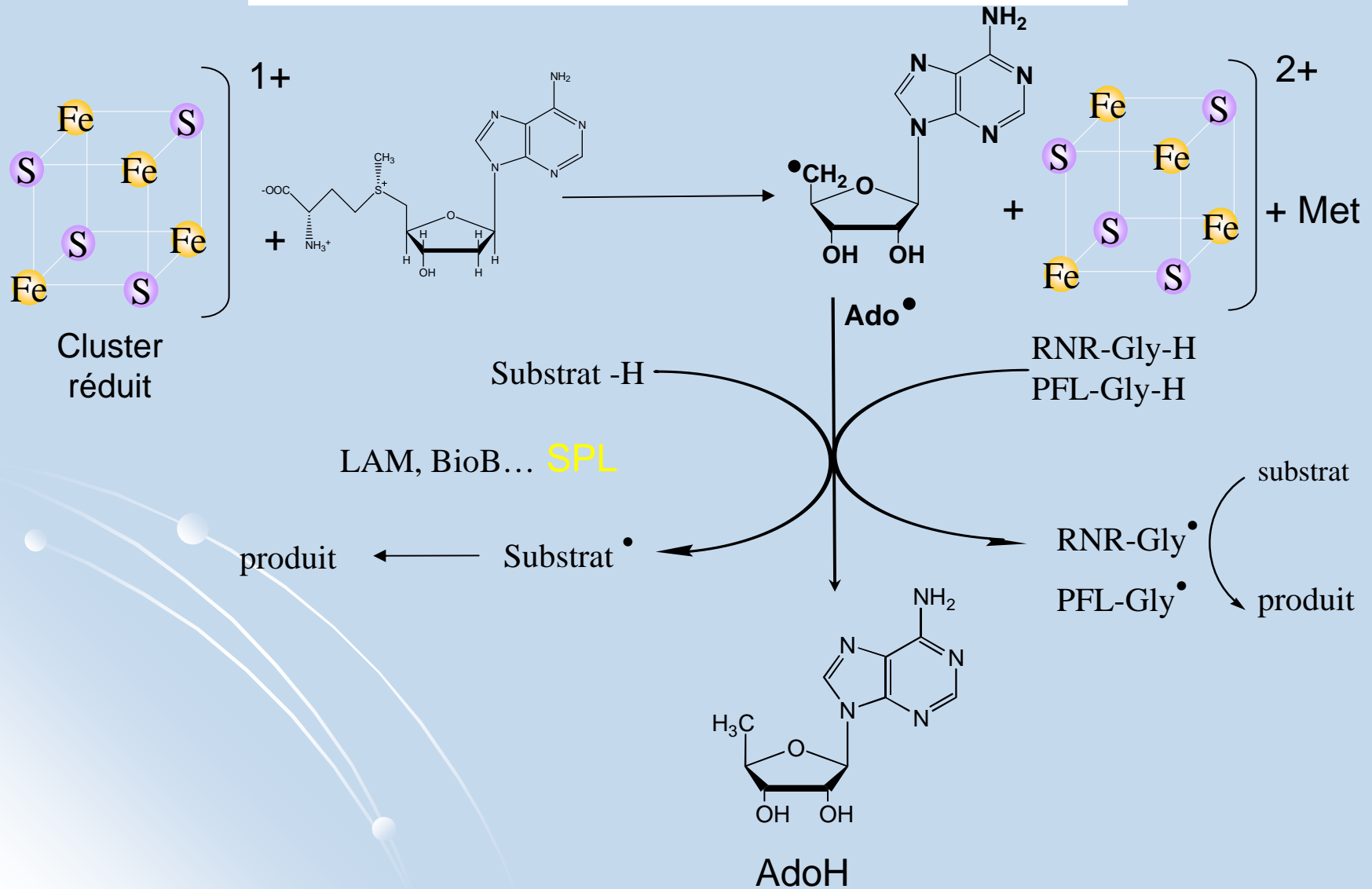
Martins B.M et al (2004) *PNAS* 101, 15645

S-adenosylmethionine: Each chemical group appended to the S atom is used

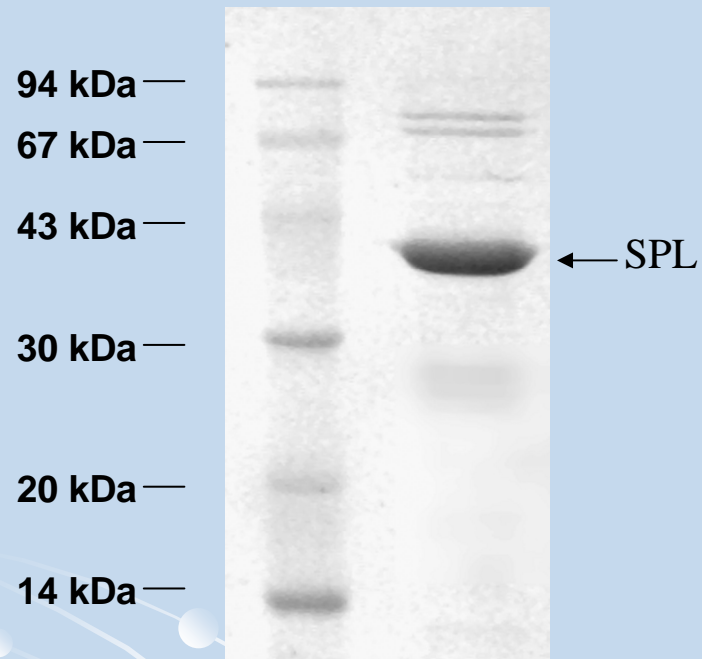


Fontcave M. Atta M. Mulliez E. *Trends Biochem Sci.* 2004, (5):243-949

Mécanisme des "Radical-SAM"

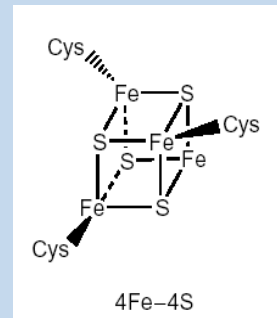


Caractérisation biochimique



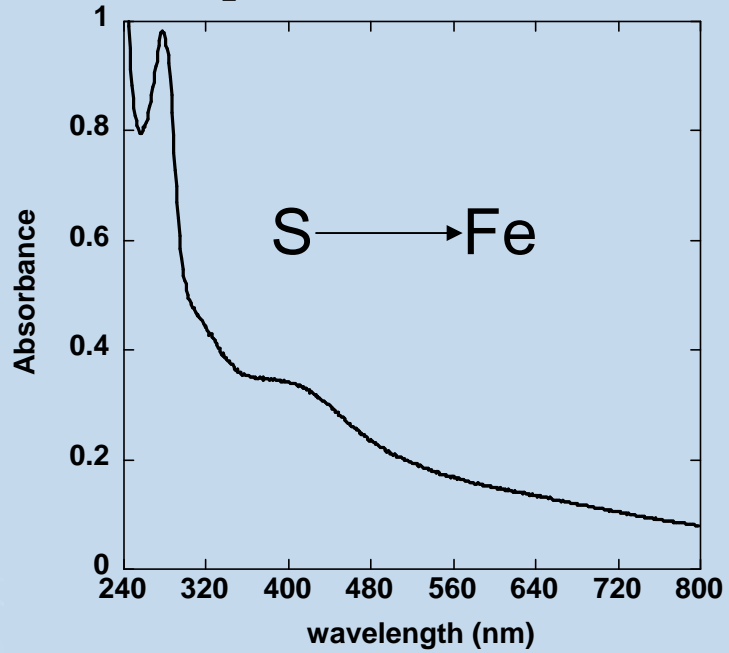
SDS-PAGE (12%) analysis

- SPL sous forme apoprotéine.
- Reconstitution chimique du centre [4Fe-4S].
- 3.9 Fe/monomer
- 4 S/monomer
- SPL est dimérique .

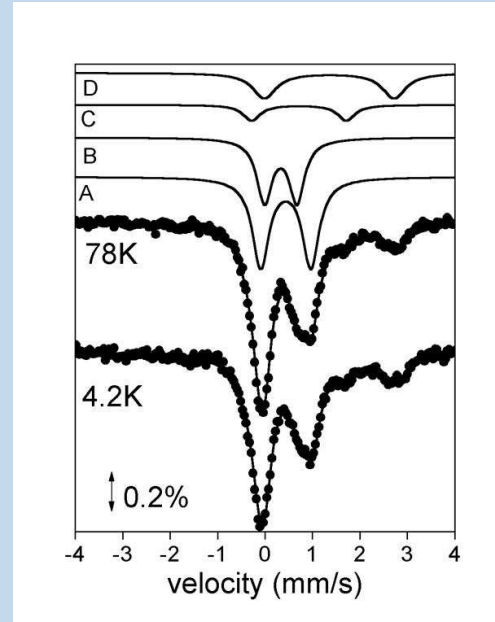


Caractérisation spectroscopique de l'Holo-SPL

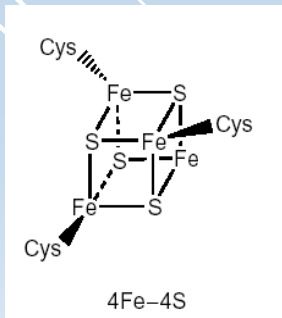
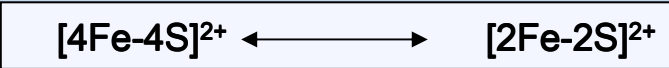
Spectre UV-visible.



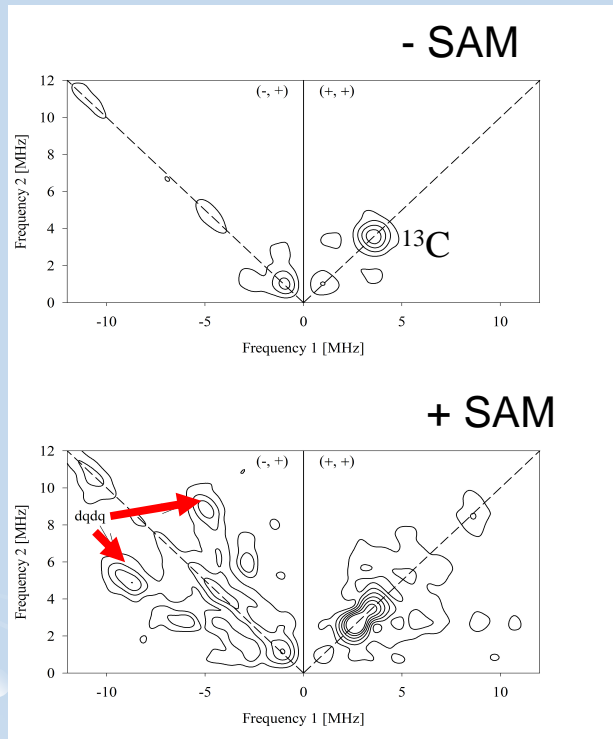
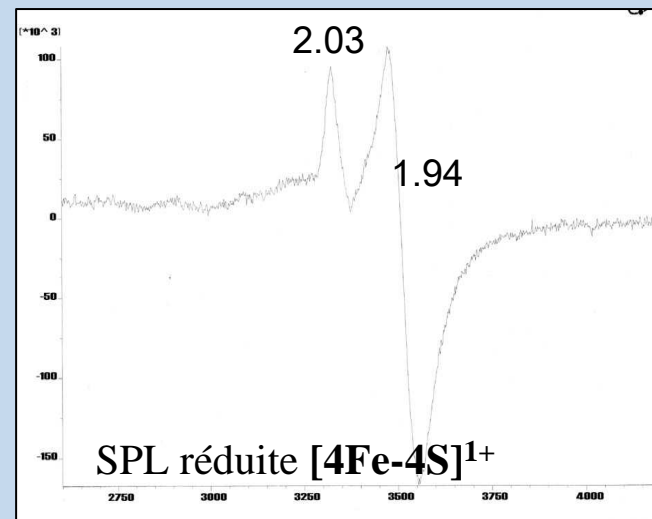
Spectroscopie Mössbauer



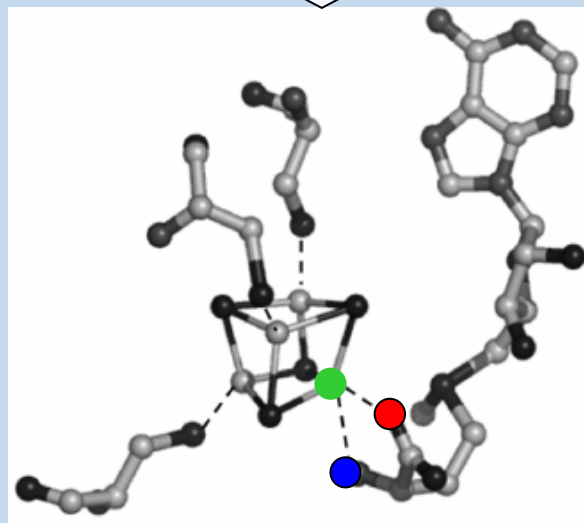
SITE	Attribution
A (40%)	$[4\text{Fe-4S}]^{2+}$ S=0
B (27%)	$[2\text{Fe-2S}]^{2+}$ S=0
C (12%)	Fe^{2+}
D (21%)	Fe^{2+} (S=2)



**SAM est en interaction avec [4Fe-4S]⁺ (S=1/2):
Spectroscopie HYSCORE
(Hyperfine Sublevel Correlation)**



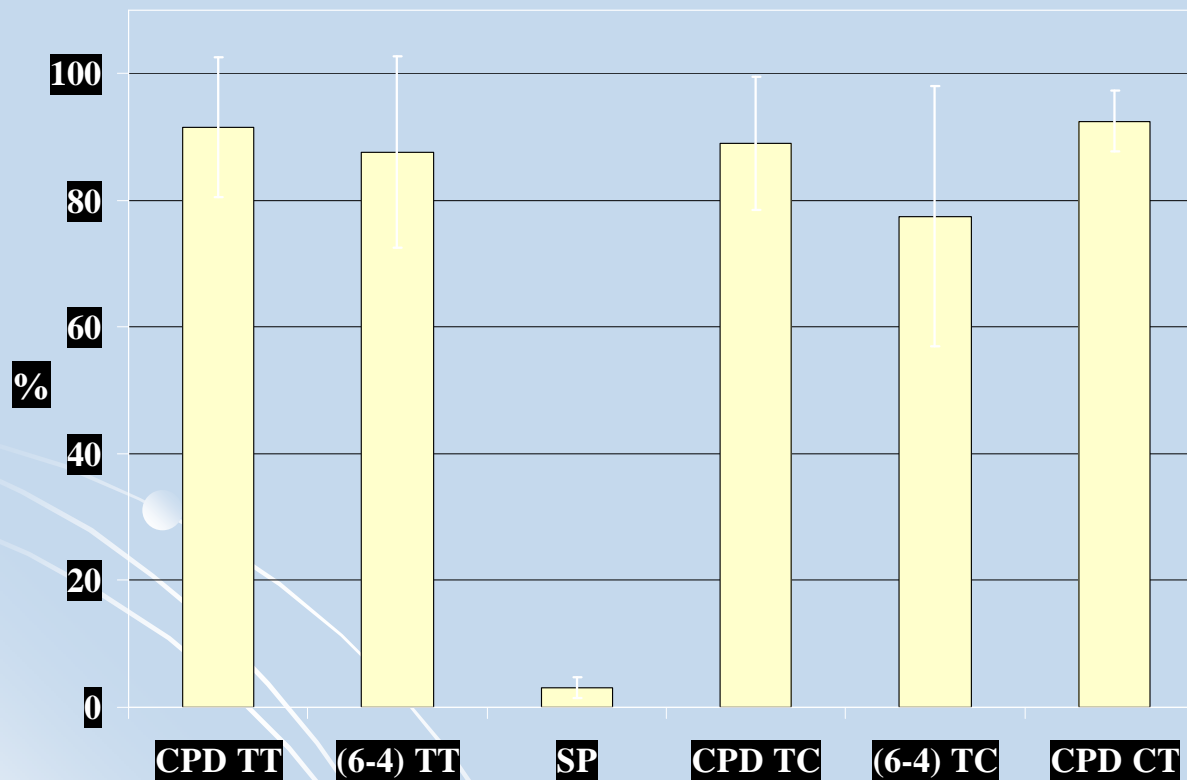
Un atome d'azote
 $a_N = 9.1$ MHz



Ribonucleotide Reductase:
Gambarelli S, Luttringer F, Padovani D, Mulliez E Fontecave M.
(2005) *ChemBioChem* 6:1-3
SPL:
A.Chandor, M. Atta, M. Fontecave et al
Comptes Rendus de l'académie des Sciences 2007, 10, 756-765

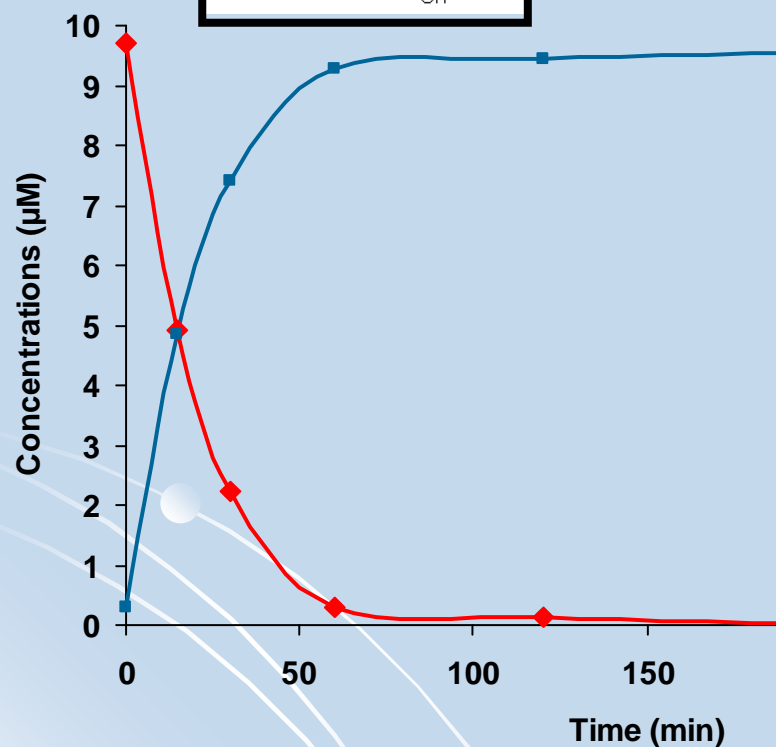
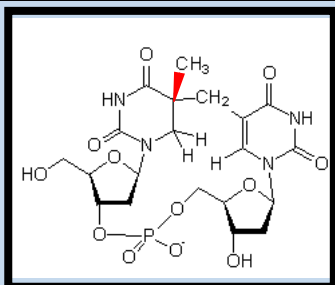
SPL et les autres photoproduits

Le mélange réactionnel:
SPL: 50 μ M
SAM: 1 mM
dithionite: 3 mM
Photoproduits dans l'ADN
en anérobiose.



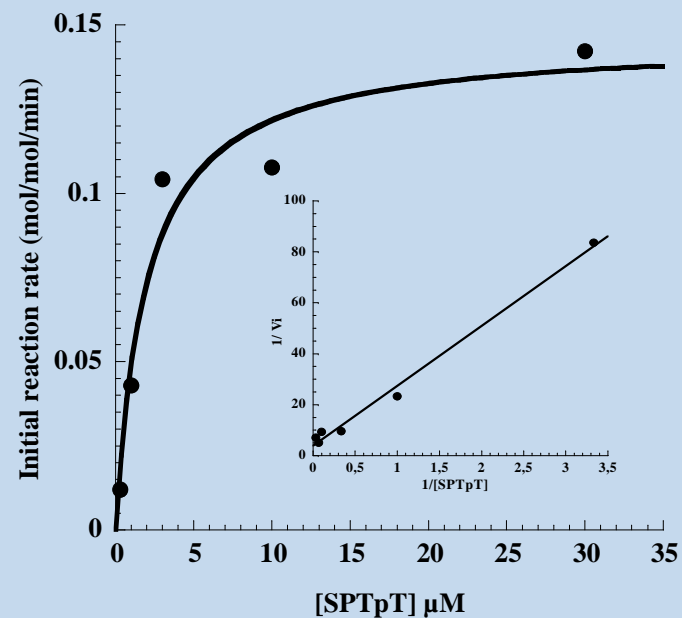
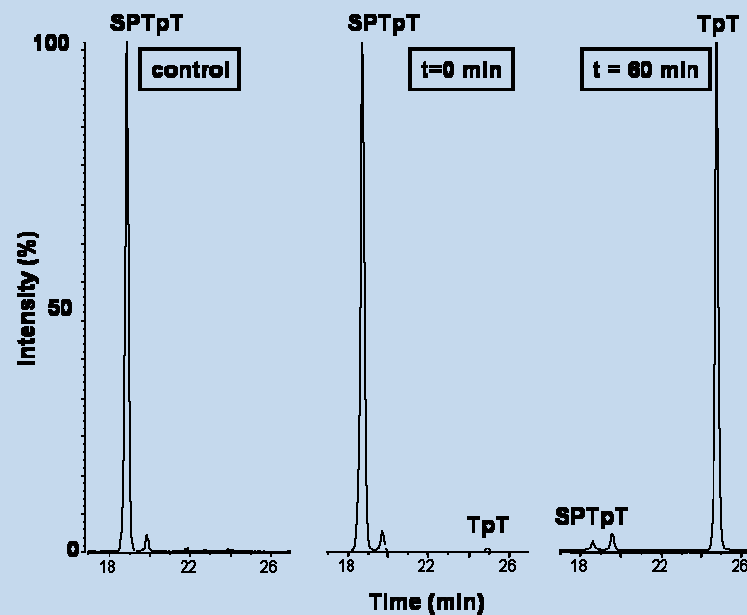
La proportion du photoproduit après 30 min d'incubation avec la SPL

5R-SPTpT est substrat de la SPL



SP-Lyase (1 μM), 5R-SPTpT (10 μM),
SAM (1.5 mM), dithionite (2 mM), DTT (2 mM),
dans 100 mM Tris-Cl pH 8, 0.2 M KCl.

La réaction s'effectue en anéarobiose



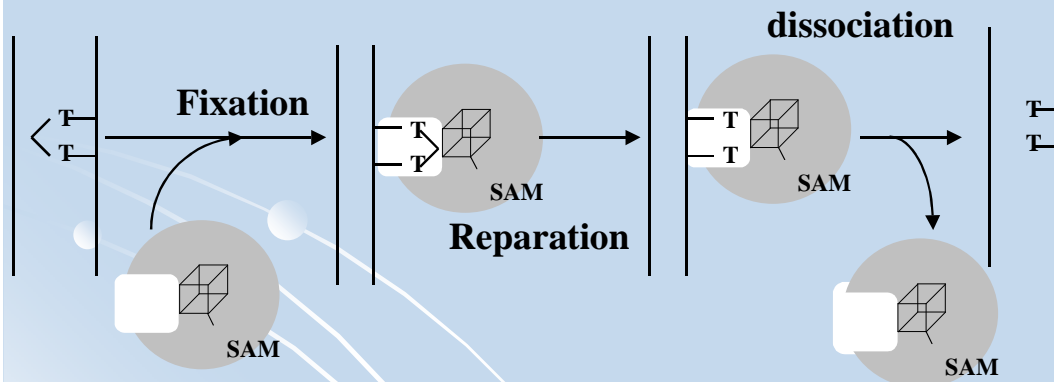
$V_{max} = 0.4 \text{ mol/mol/min}$ $K_m = \sim 6 \mu\text{M}$

CDF18/05/11

Conclusions

1. SP Lyase : est une enzyme « Radical-SAM ».
Fixe un centre $[4Fe-4S]^{2+/1+}$.
Le centre $[4Fe-4S]^{2+/1+}$ interagit avec la SAM
La SAM est réduite en AdoH + méthionine même en absence du substrat.

2. 5R-SPTpT est un substrat pour la SPL.
Pas besoin d'une séquence d'ADN

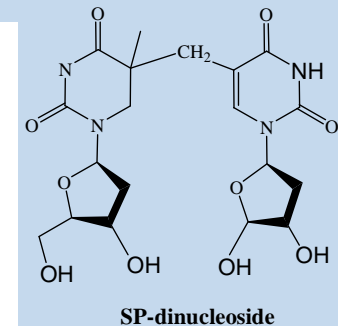
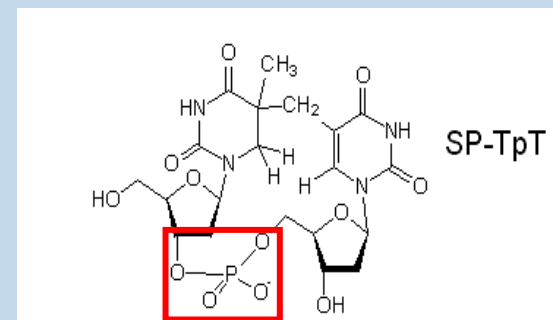
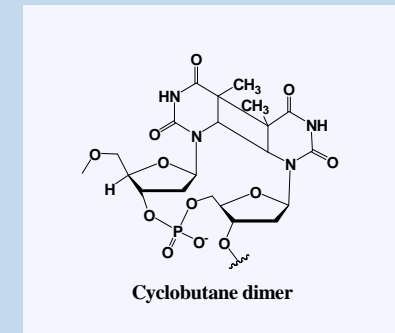
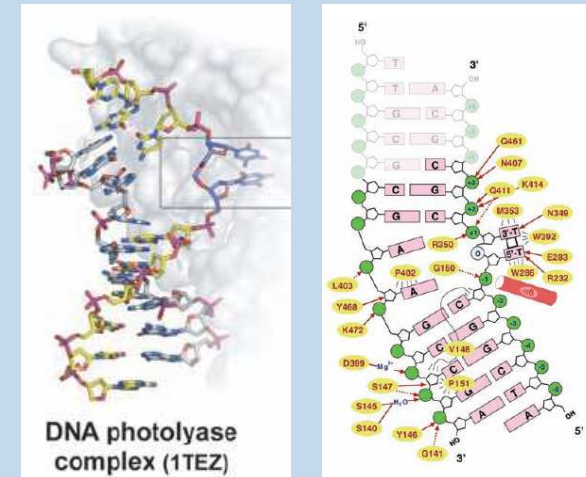


3. SP dinucleoside est un mauvais substrat
0.004 vs 0.4 mol/mol/min (SP-TpT)
Importance du pont phosphate

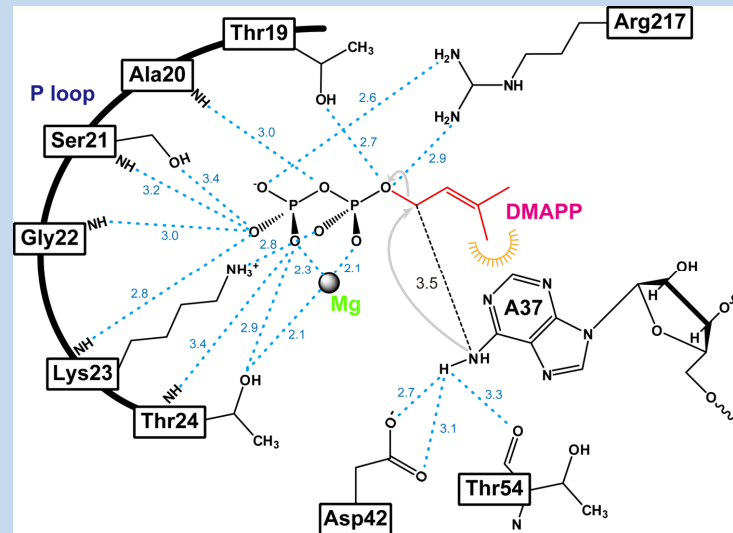
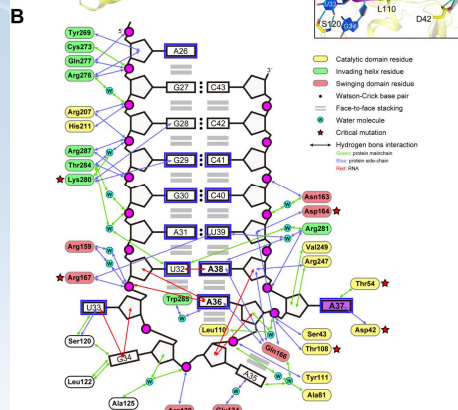
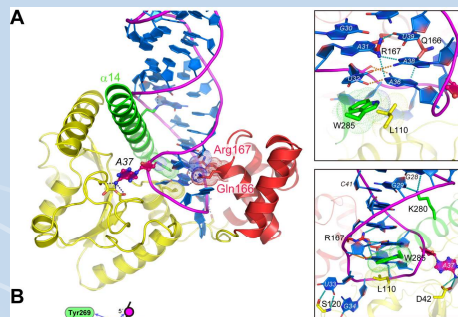
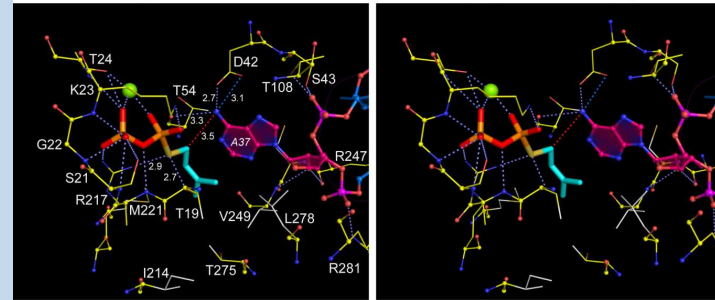
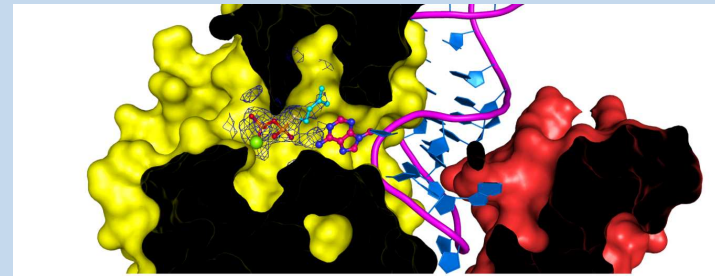
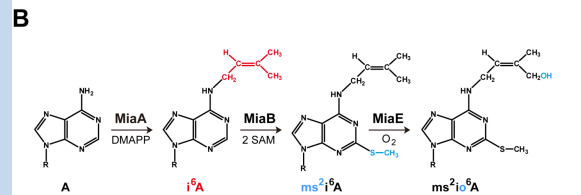
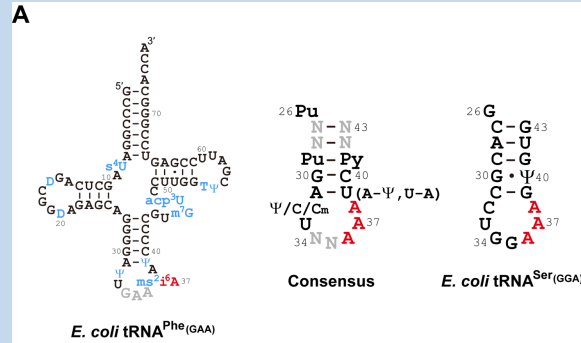
T Carell, M. M. Atta, Fontecave *et al*:
Chem Commun 2006, 4, 445-447

Mécanisme de réparation par « base-flipping »

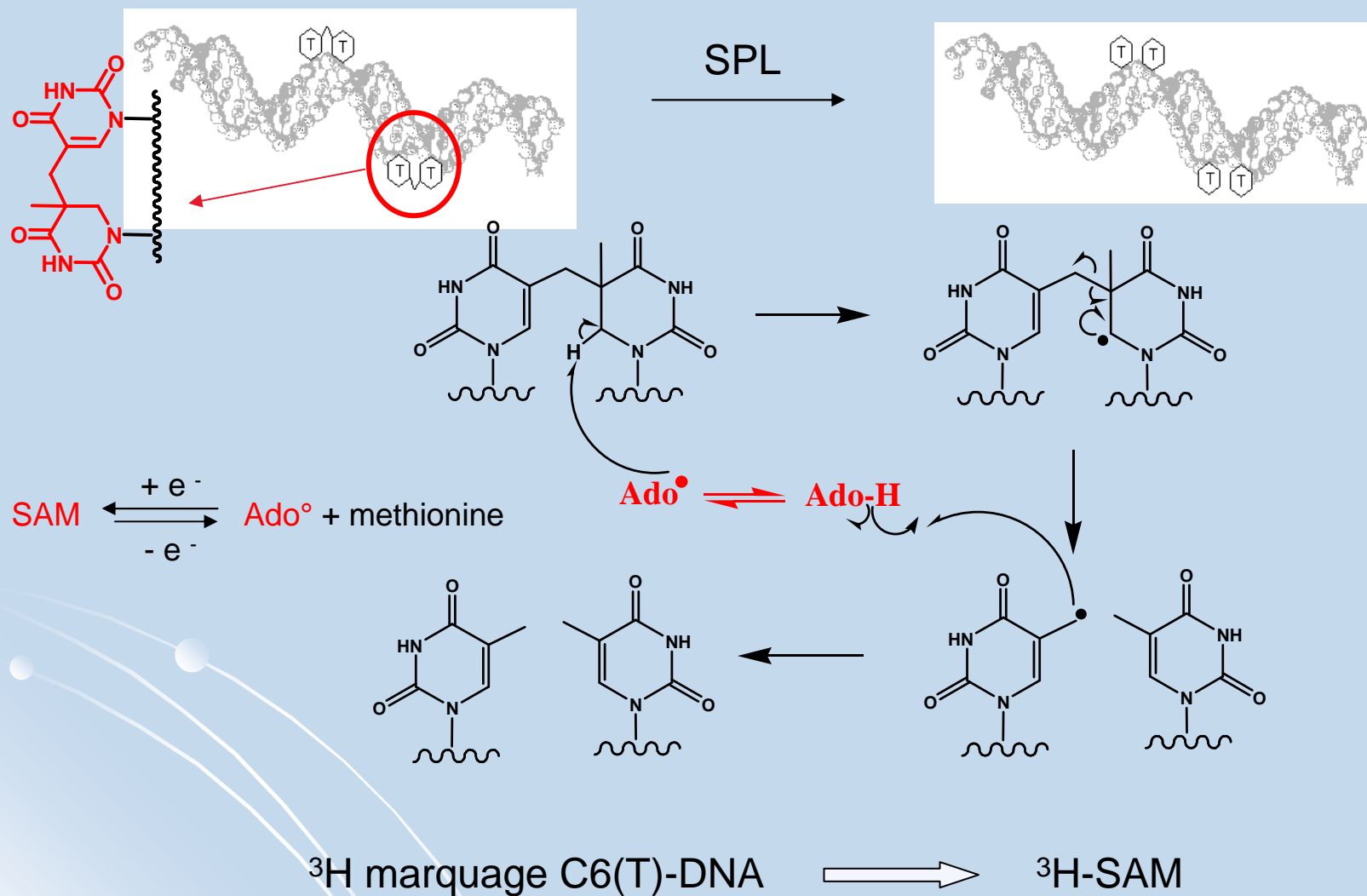
Carell, T., and Essen, L. O. (2004) *Science* 306, 1789-1793



Snapshots of dynamics in synthesizing N6-isopentenyladenosine at tRNA anticodon, M. Atta, M. Fontecave, J-F. Hunt, and I. Tanaka. *Biochemistry*. (2009), 48, 5057–5065.



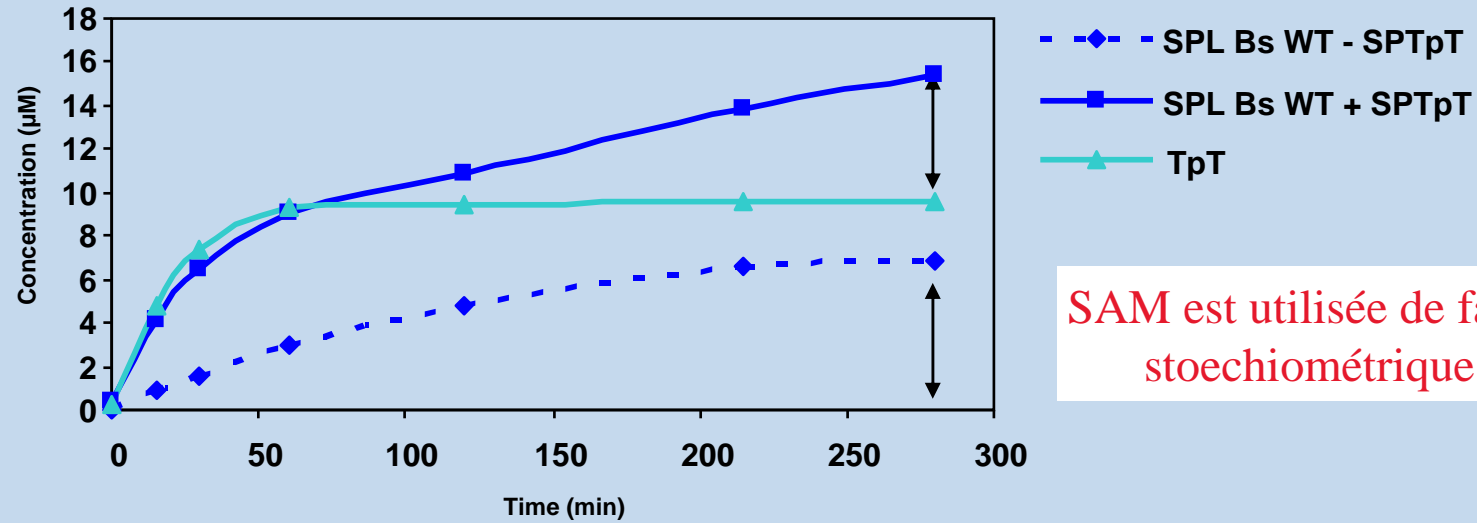
Mécanisme proposé par J.B. Broderick



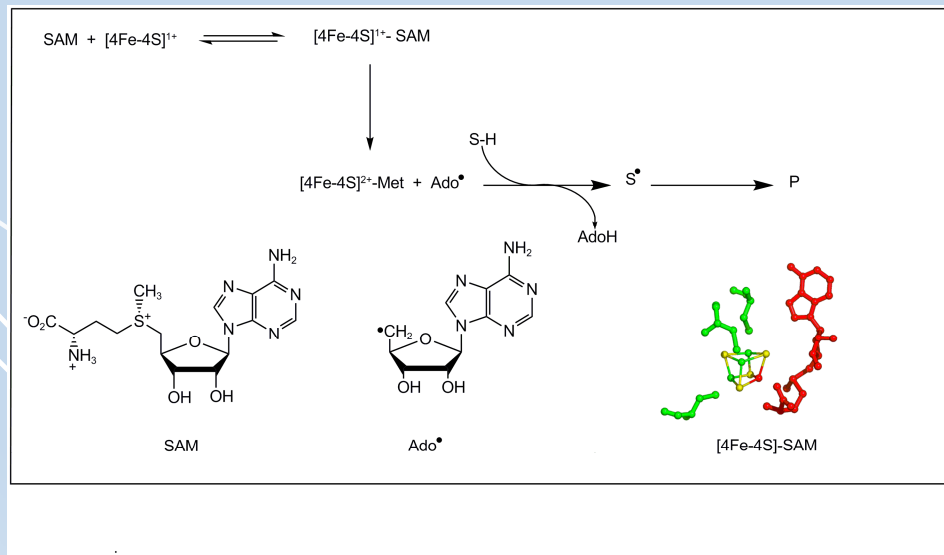
J. B. Broderick, *J. Am. Chem. Soc.*, 2002, **124**, 2860. *J. Biol. Chem.*, 2006, **281**, 25994

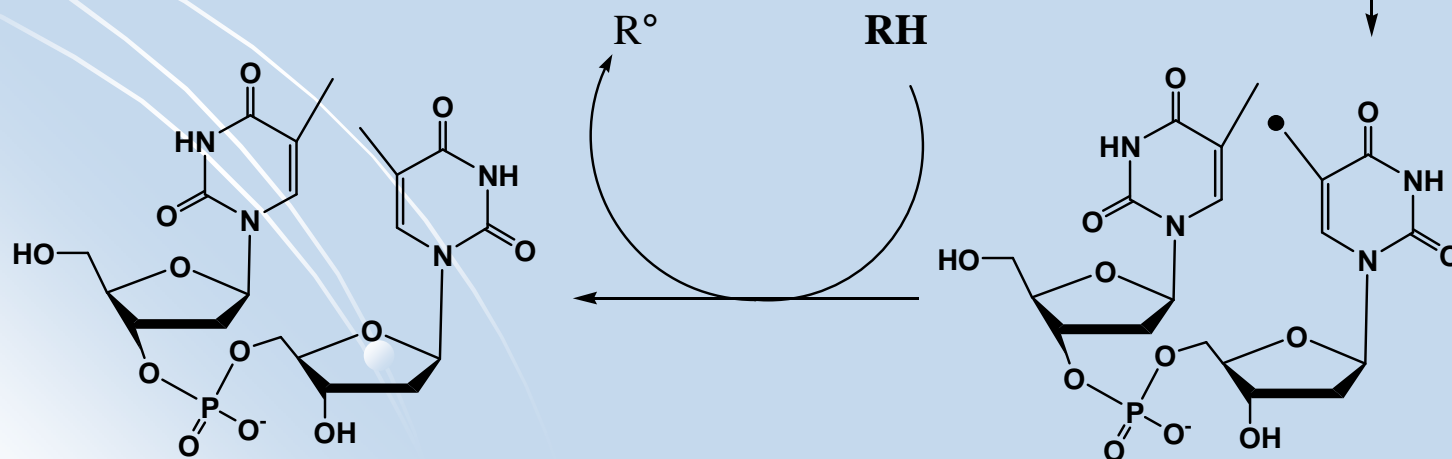
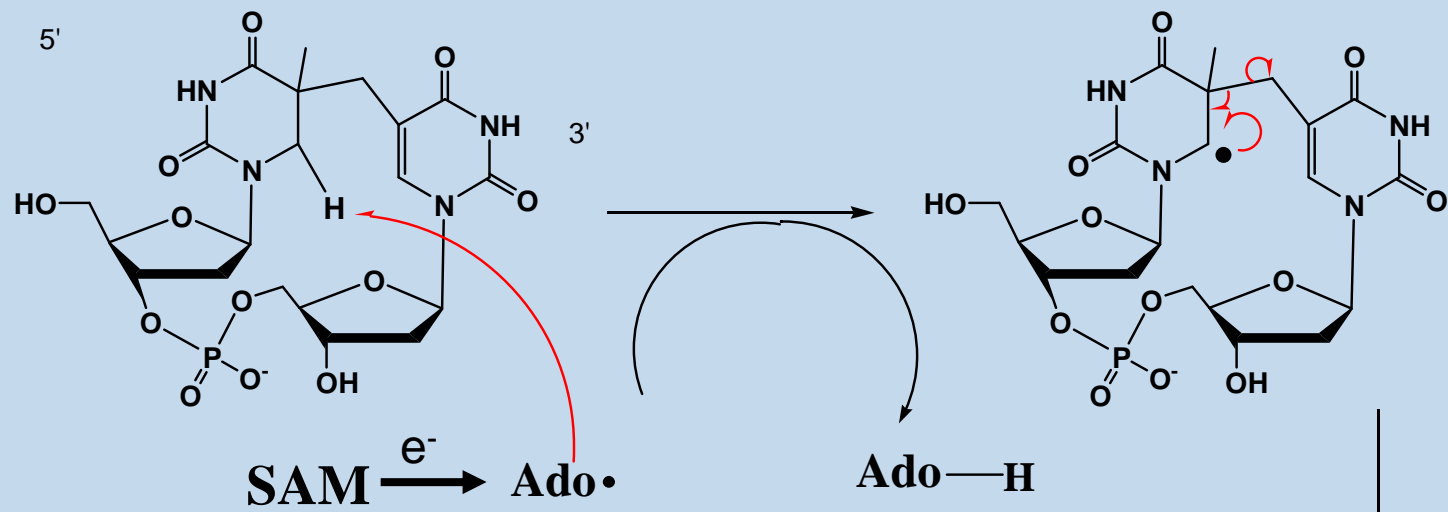
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Analyse des produits de la réaction.



SAM est utilisée de façon stoechiométrique.



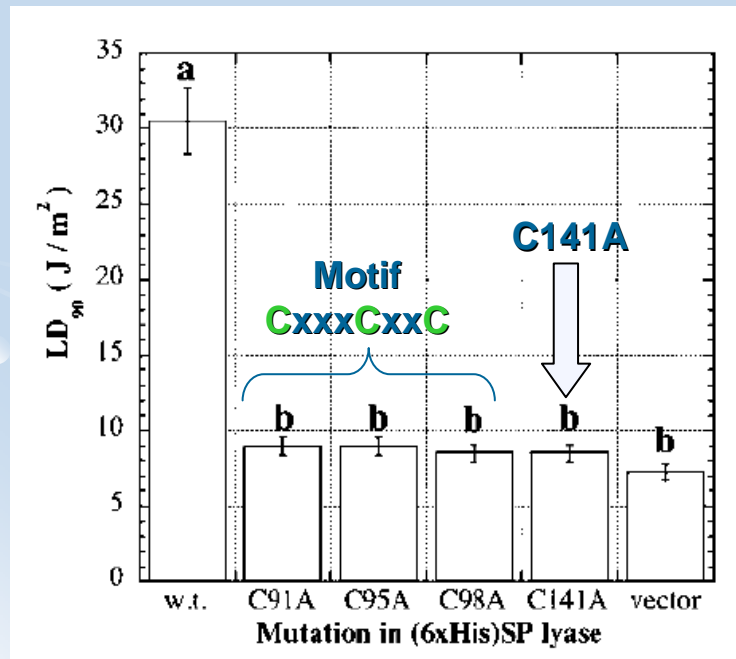


Rôle de la cystéine-141?

B. subtilis
B. cereus
B. Halodurans
B. thurigiensis
B. anthracis

```

SSKPSAEYAI PFATGCMGHCHYCYLQTTMGSKPYIRTYVNVEEILDQADKYMKERAPEFTRFEASCTSD
TSKPSAEYAI PFATGCMGCHYCYLQTTMGSKPYIRTYVNVEEILGAADKYMEEERAPELTRFEASCTSD
TSKPSAEYAI PLATGCMGCHYCYLQTTMGSKPYIRTYVNLEEIFAADQYIHEREPEITRFEAACTSD
TSKPSAEYAI PLATGCMGCHYCYLQTTMGSKPYVVRVYVNLDEIFEKAKQYMDERAPEITRFEAACTSD
TSKPSAEYAI PLATGCMGHCHYCYLQTTMGSKPYVVRVYVNLDEIFEKAQQYMDERAPEITRFEAACTSD
: .*****. : **: **: * . * .*****: * .:***: : .*::*: * :*: :* * : * **.:* **
  
```



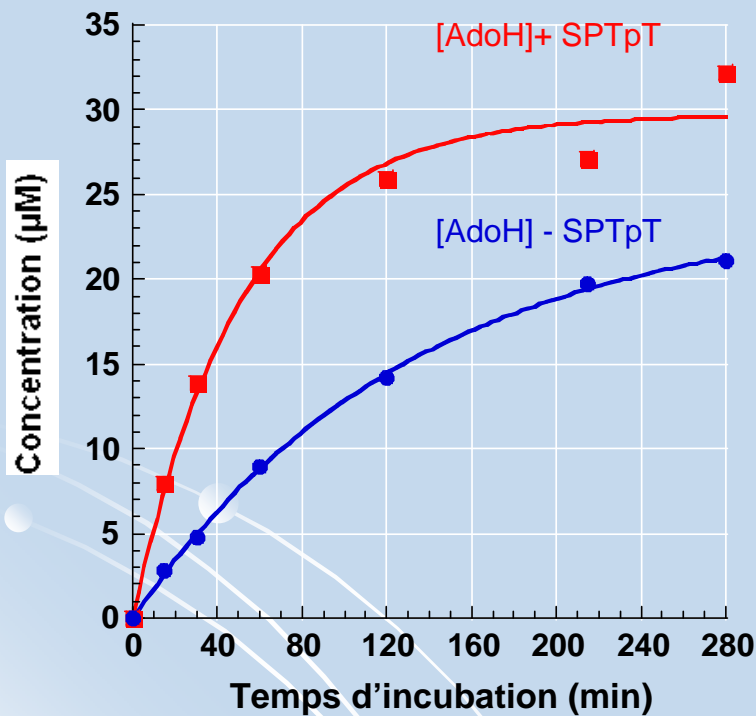
Cys 141 :

N'est pas impliquée dans la coordination du centre [Fe-S]

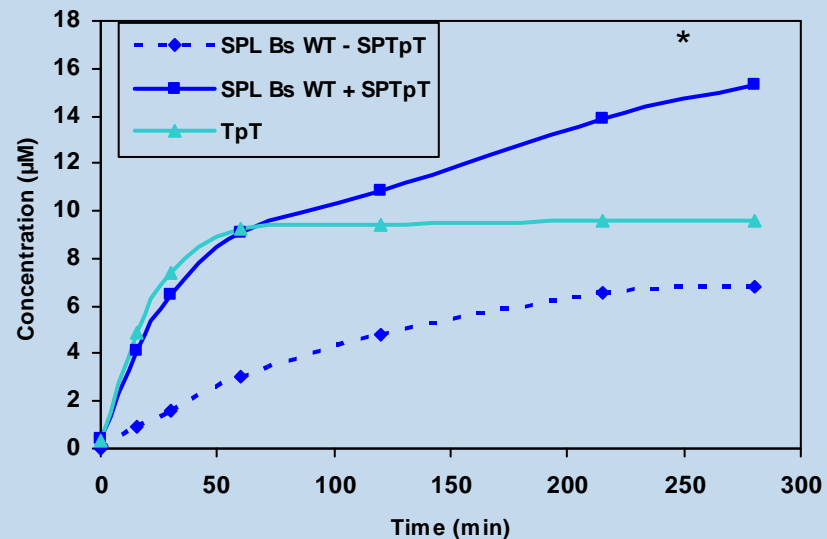
Absolument nécessaire à l'activité de l'enzyme

<i>B. subtilis</i>	SSKPSAEYAI PFATG CMGH CHY CYL QTTMGSKPYIRTYVNVEEILDQADKYMKERAPEFTRFEASCTSD
<i>B. cereus</i>	TSKPSAEYAI PFATG CMGH CHY CYL QTTMGSKPYIRTYVNVEEILGAADKYMEEERAPELTRFEASCTSD
<i>B. Halodurans</i>	TSKPSAEYAI PLATG CMGH CHY CYL QTTLGSKPYIRTYVNLEEIFAAADQYIHEREPEITRFEAACTSD
<i>B. thurigiensis</i>	TSKPSAEYAI PLATG CMGH CHY CYL QTTLGSKPYVRVYVNLDEIFEKAKQYMDERAPEITRFEAACTSD
<i>B. anthracis</i>	TSKPSAEYAI PLATG CMGH CHY CYL QTTLGSKPYVRVYVNLDEIFEKAQQYMDERAPEITRFEAACTSD
	: . * * * * . : : * : : * * . * . * * * * : * : . * * * * : . * * * * : * : * : . * * : * * * . : . * *

SPLC141A



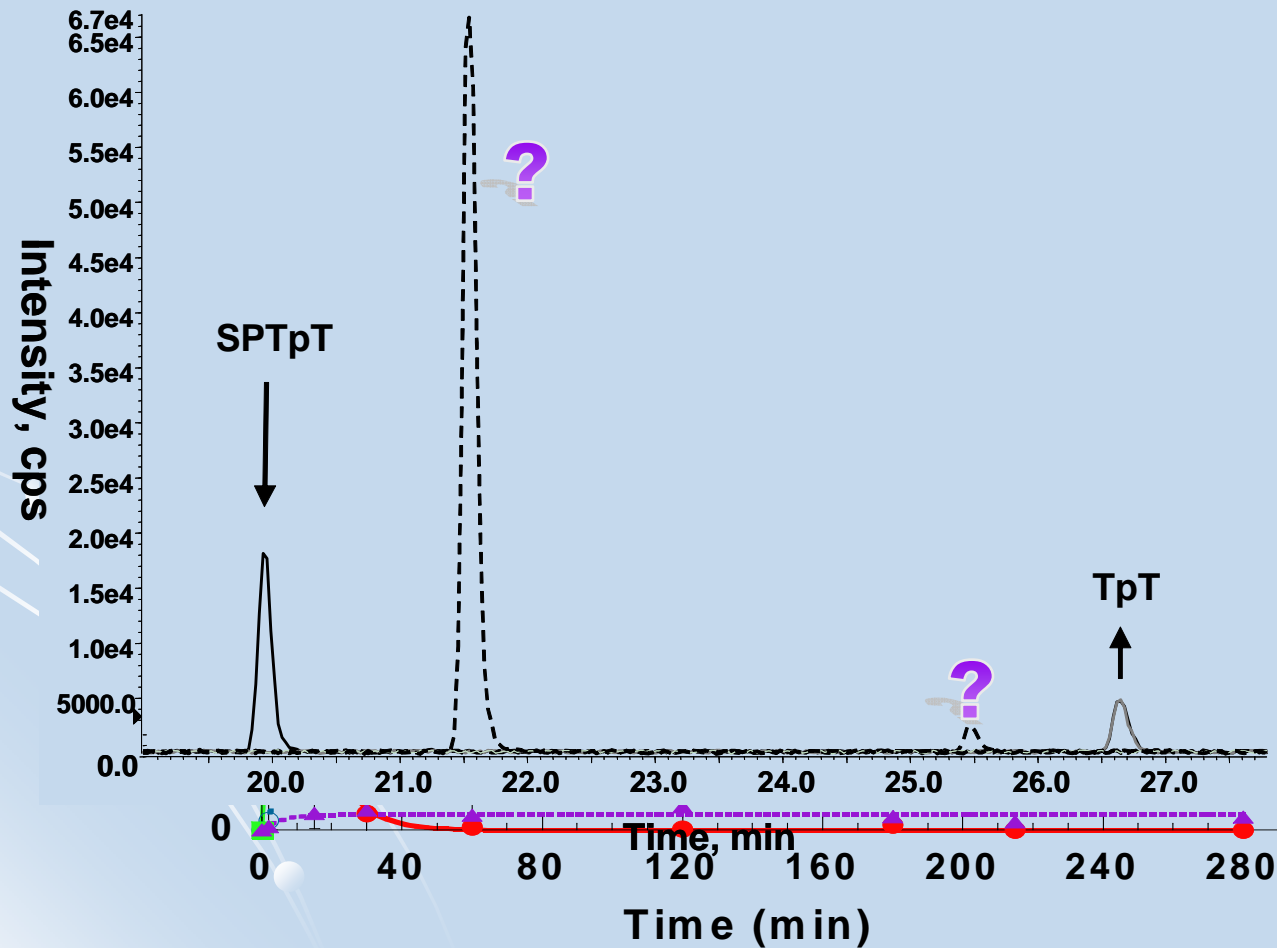
W.T SPL



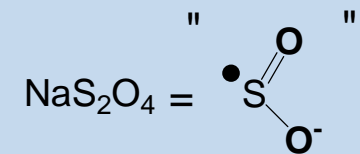
La SPL sauvage et la SPLC141A catalyse la réductolyse de la SAM

The SPLC141A mutant

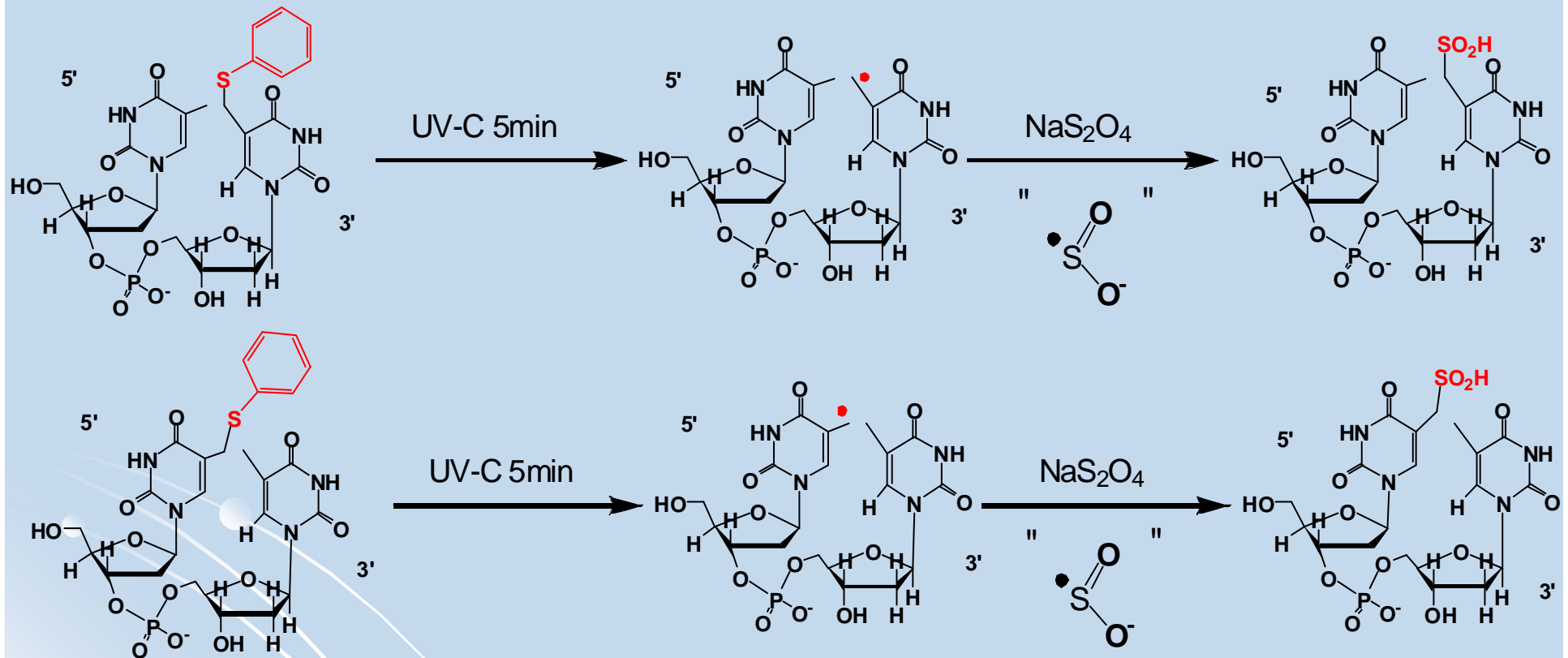
- Les caractéristiques biochimiques et spectroscopiques de la protéine SPLC141A et SPL sauvage sont semblables .



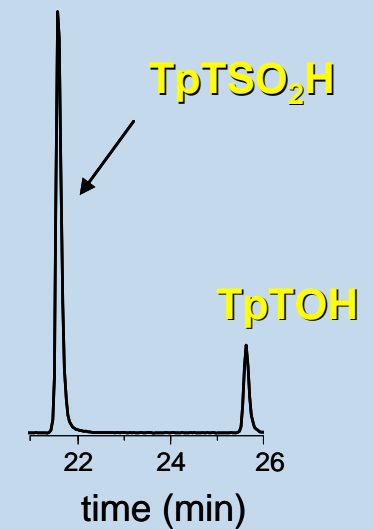
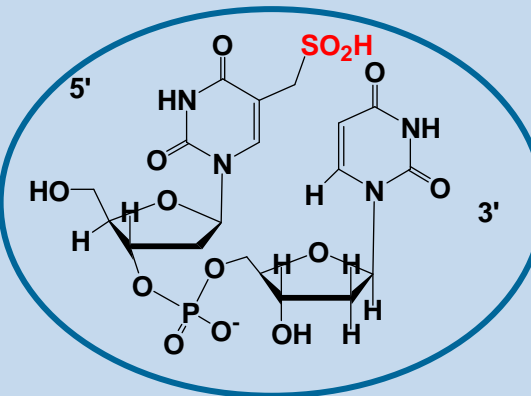
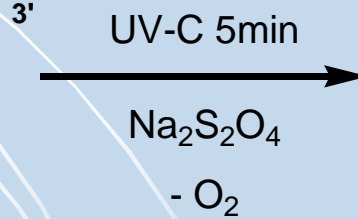
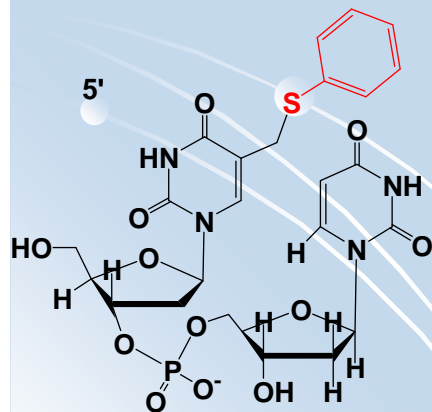
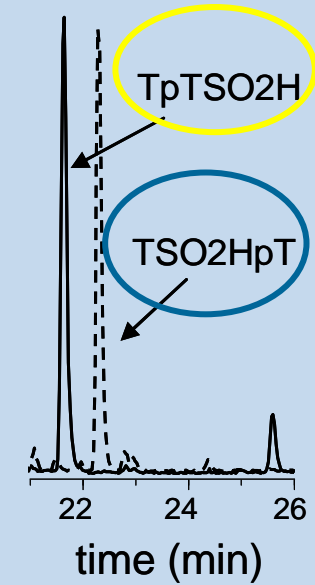
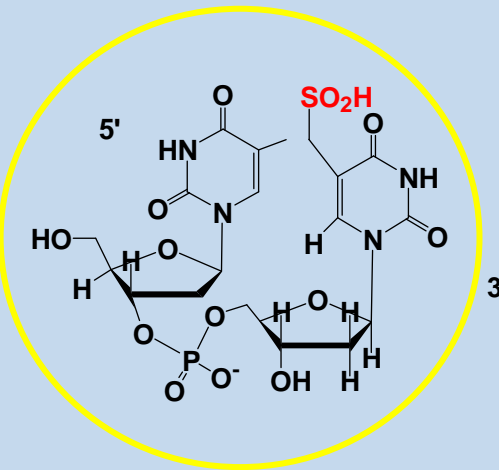
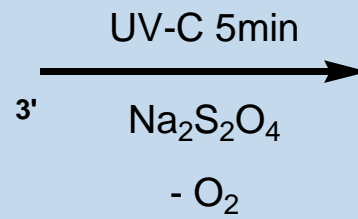
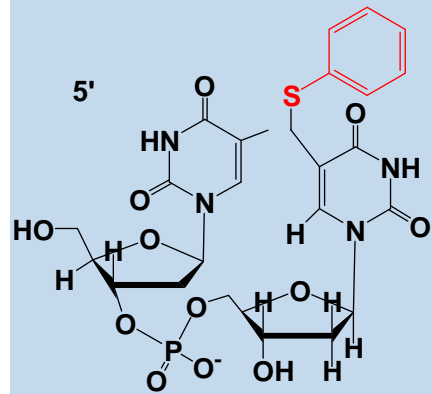
Adduct SO_2H

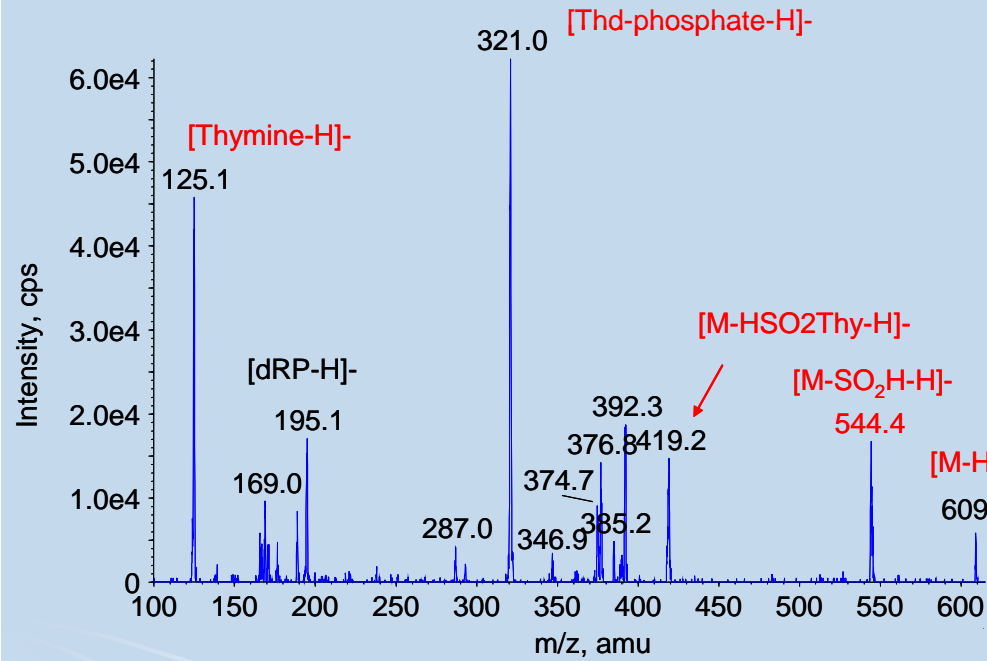


Caratérisation de l'adduit avec le dithionite.

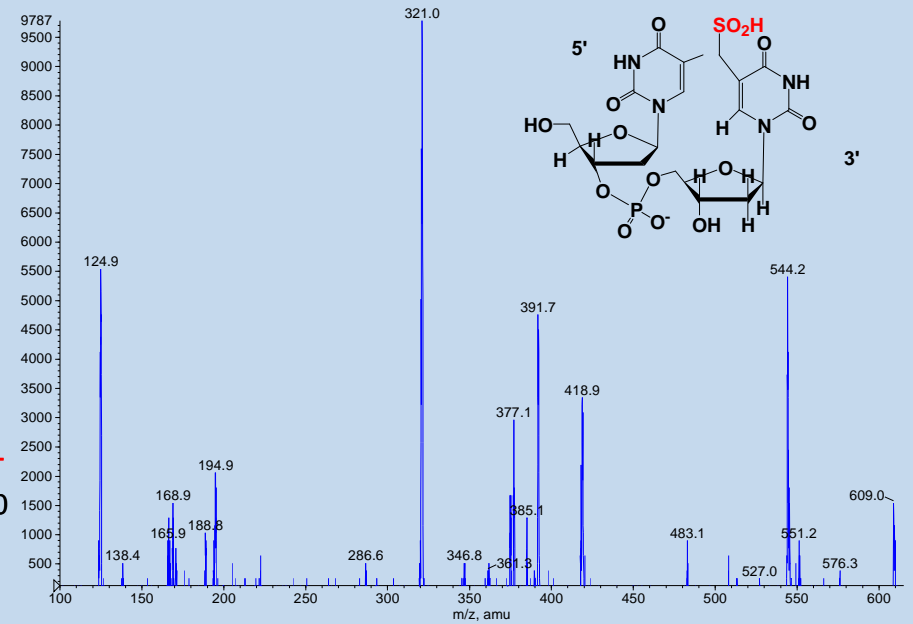


Caratérisation par HPLC

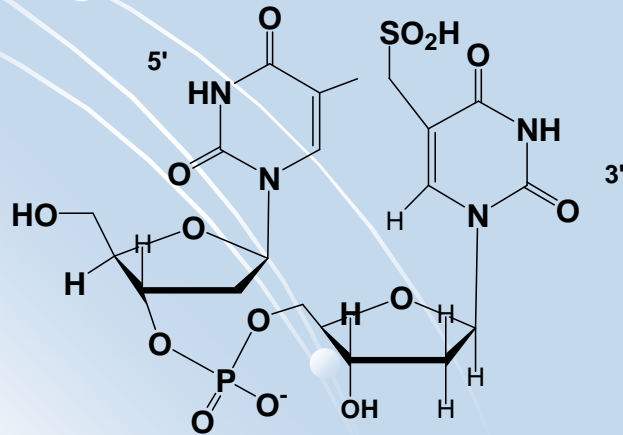
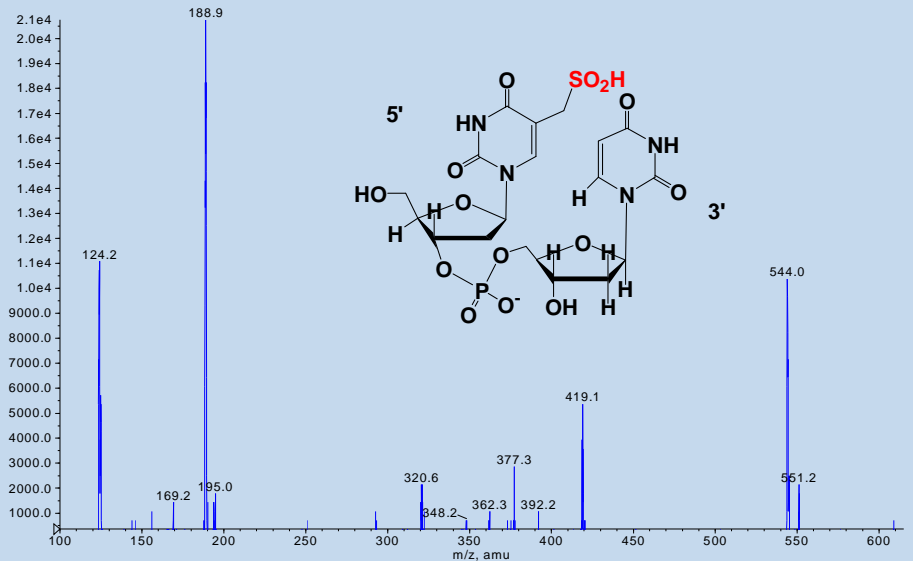


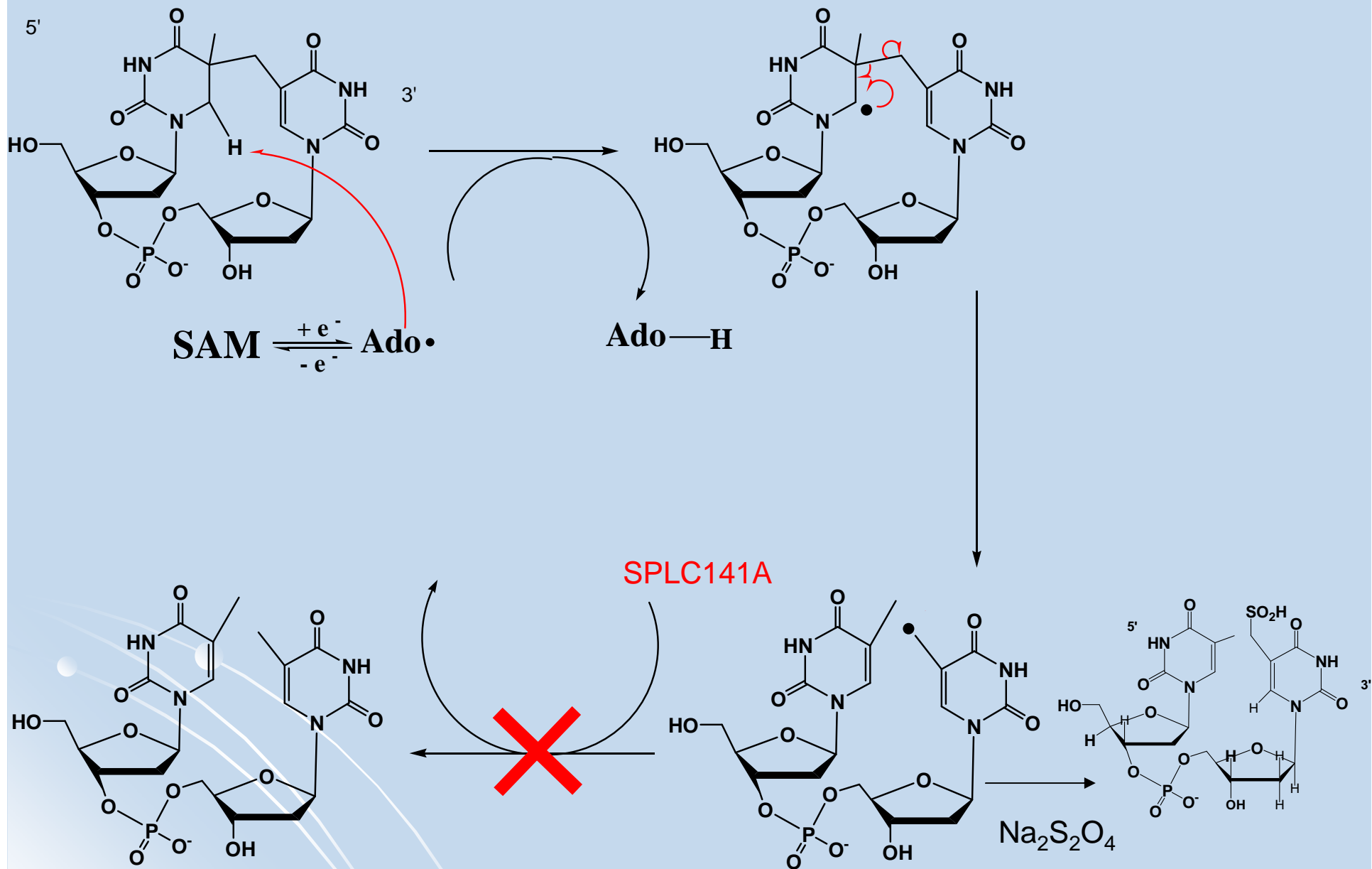


■ -MS2 (609.00) CE (-40): Exp 2, 17.515 to 18.845 min from Sample 2 (TTSPH 5min dithio cc) o... Max. 9786.9 cps.



■ -MS2 (609.00) CE (-40): Exp 2, 19.224 to 19.686 min from Sample 6 (TSPHT 5min dithio cc) of ... Max. 2.1e4 cps.







PSU; 2008

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- Olivier Berteau
- Sandrine Ollagnier-de-Choudens

CEA-Grenoble

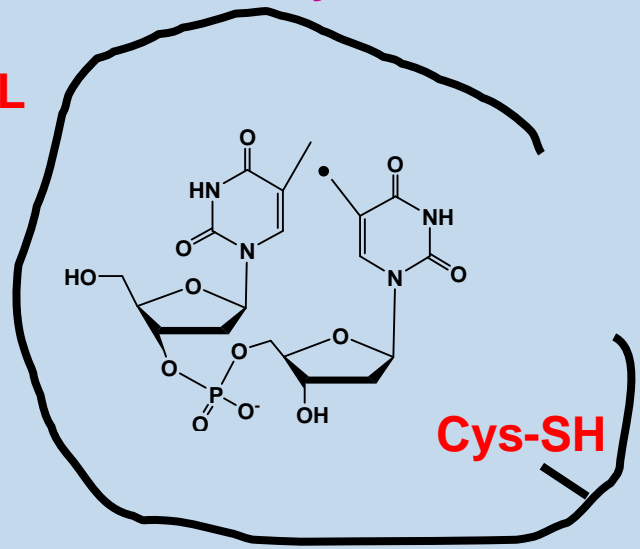
- T. Douki- HPLC, mass spectrometry
- D. Gasparutto- Synthesis
- S. Gambarelli- Hyscore
- M. Bardet, C. Mantel-NMR
- J.M. Mouesca-DFT

- T. Carell (Munich, Germany)- Synthesis
- Y. Sanakis (Greece)- Mössbauer spectroscopy

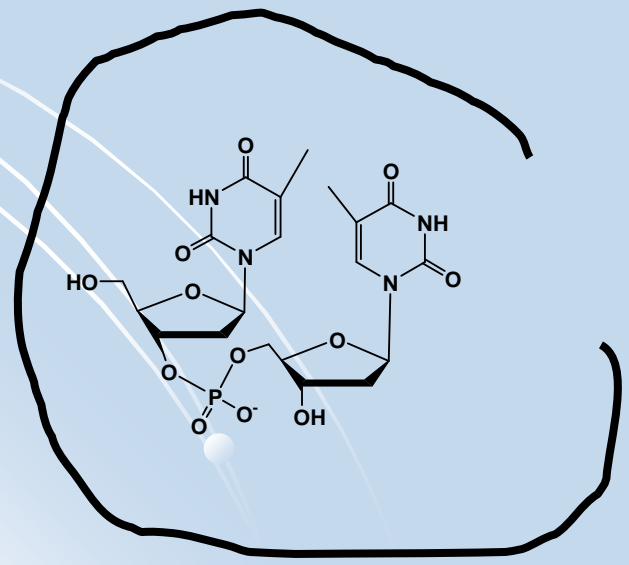


Function of Cysteine 141: a structural effector ?

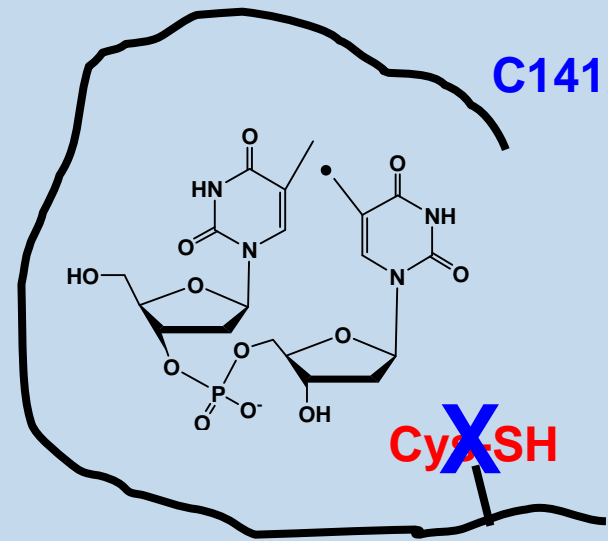
wt SPL



RH



C141A-SPL



SO₂⁻

