Métallo-Enzymes Artificielles

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13397 Marseille cedex 20

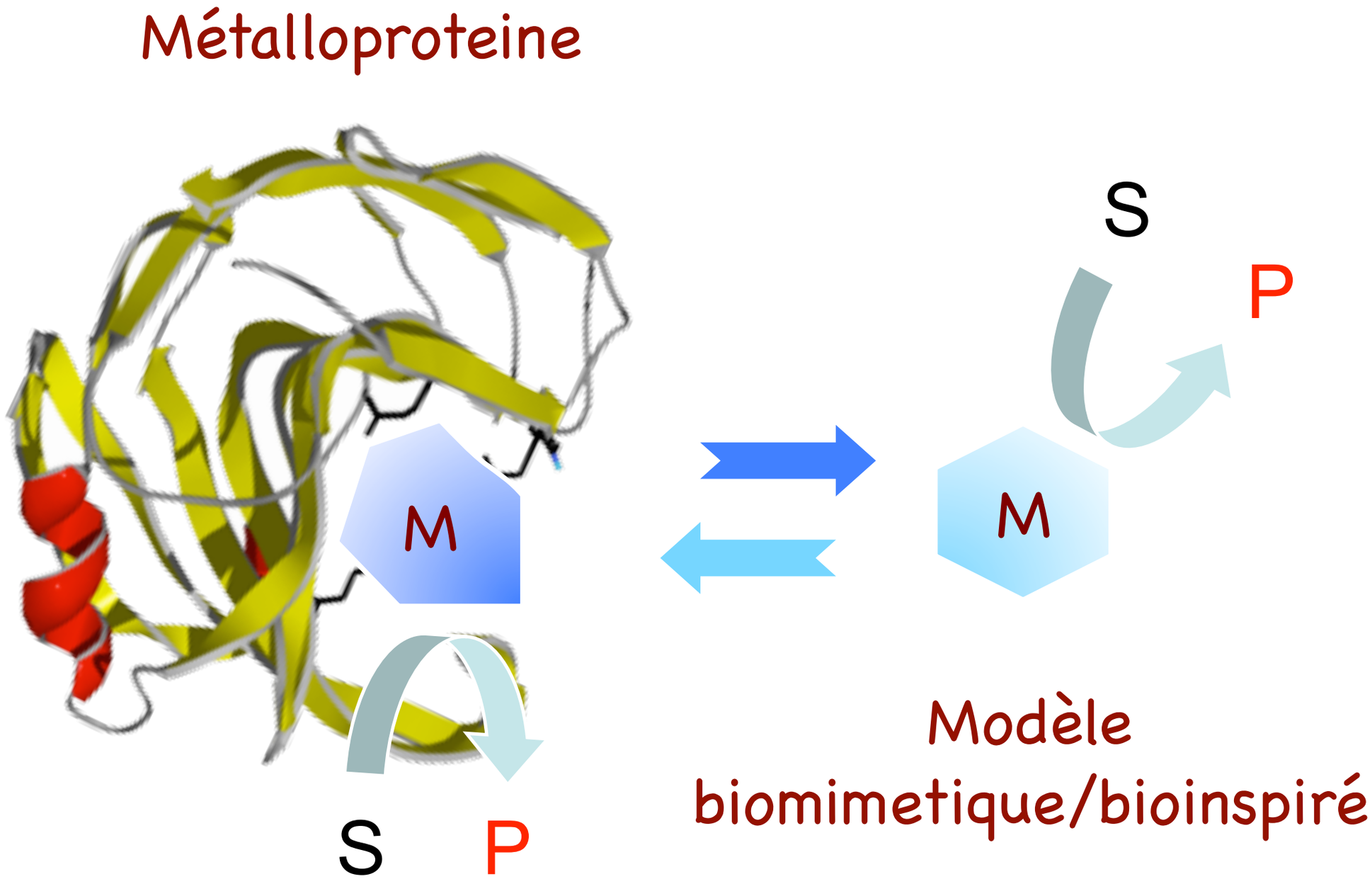
Biologie Synthétique

Synthetic biology is the design and construction of new biological entities such as enzymes, genetic circuits, and cells **or the redesign of existing biological systems**.

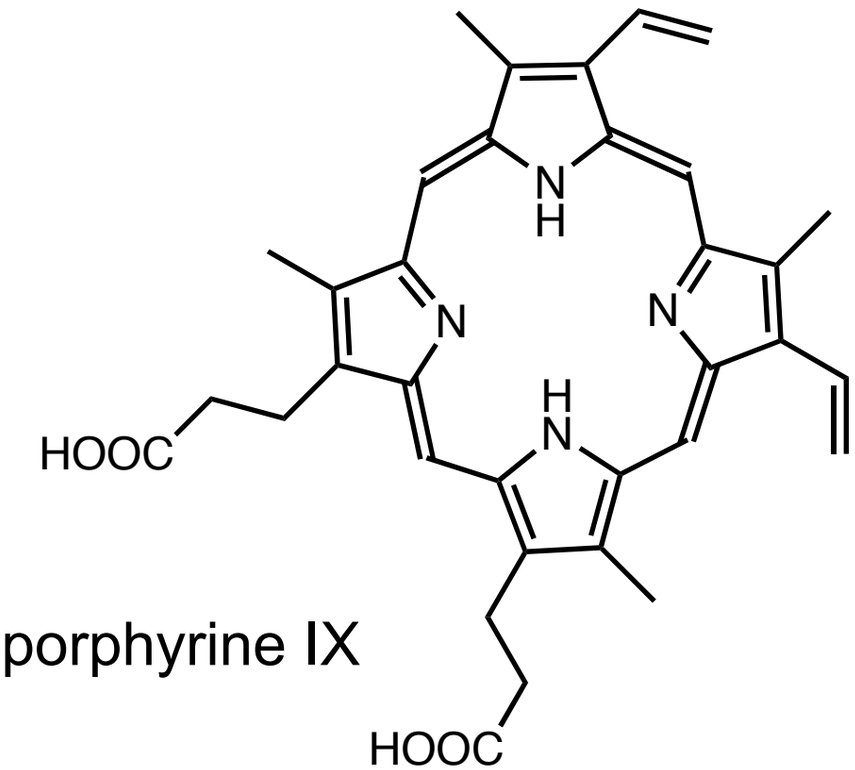
Synthetic biology builds on the advances in molecular, cell, and systems biology and **seeks to transform biology in the same way that synthesis transformed chemistry** and integrated circuit design transformed computing.

The element that distinguishes synthetic biology from traditional molecular and cellular biology **is the focus on the design and construction of core components** (parts of enzymes, genetic circuits, metabolic pathways, etc.) **that can be modeled, understood, and tuned to meet specific performance criteria**, and the assembly of these smaller parts and devices into larger integrated systems that solve specific problems.

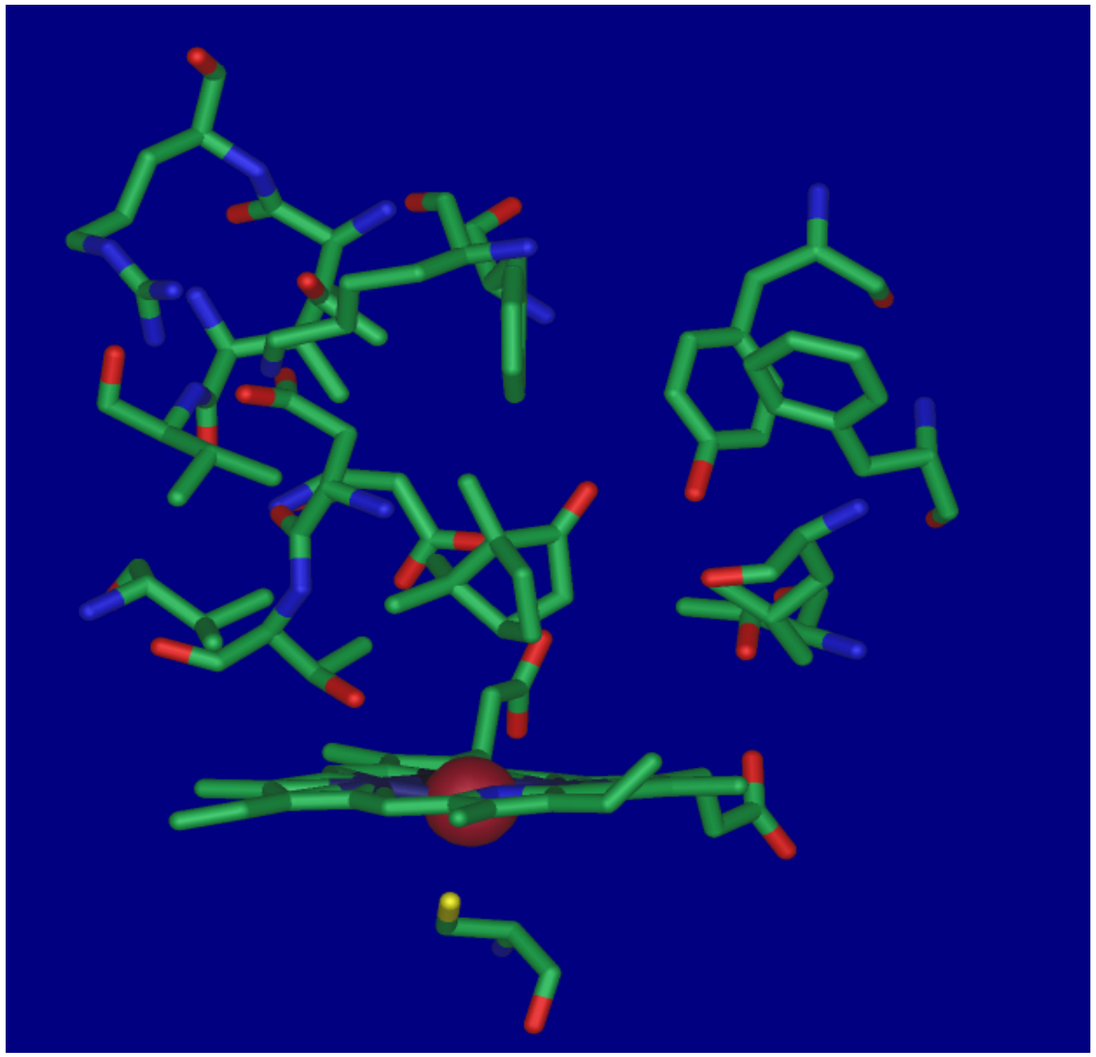
Club Métallo:
Des enzymes aux modèles et *vice versa*

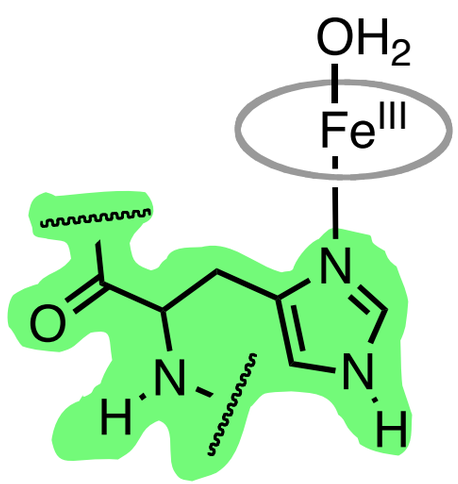


Hémoprotéines

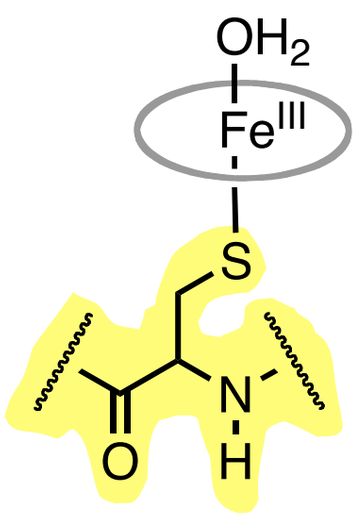


protoporphyrine IX

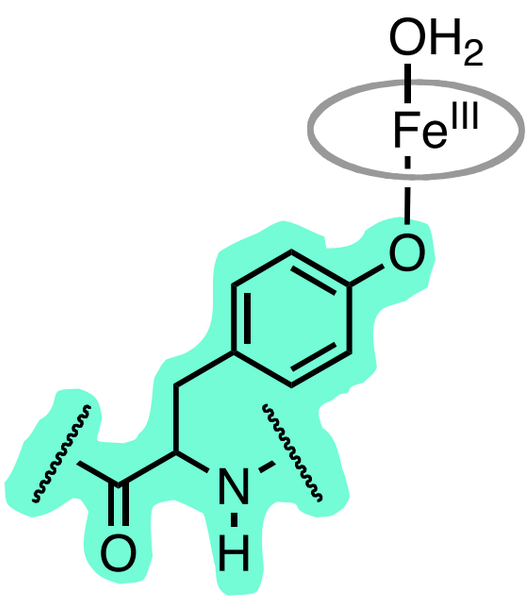




Hb, Mb, Peroxydase
SOD

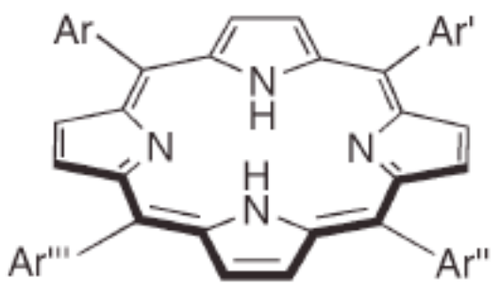


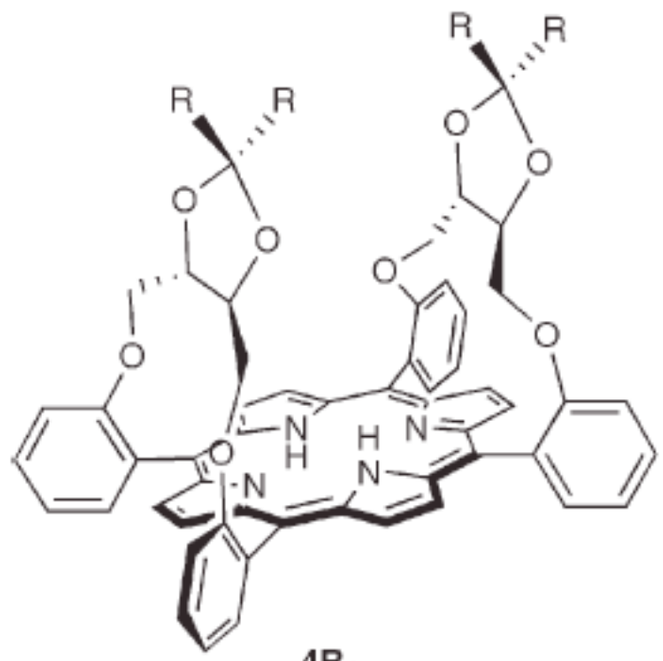
CP450

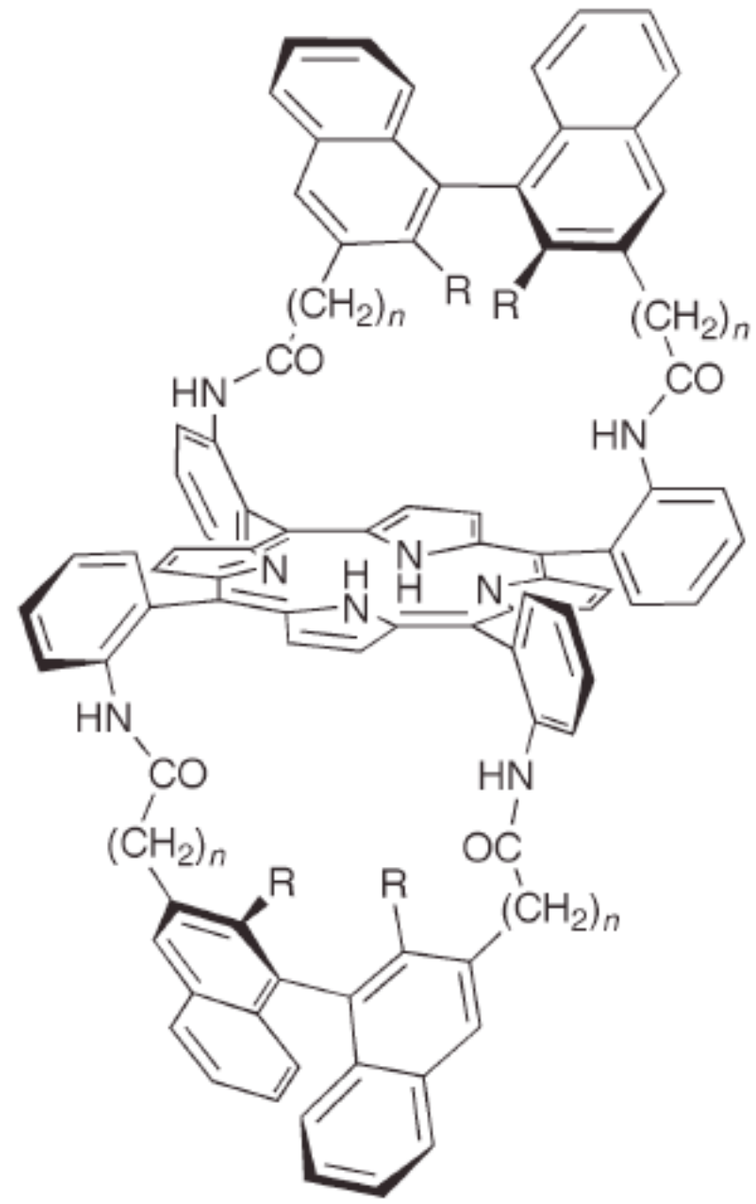


Catalase

Elles ont tout d'une grande







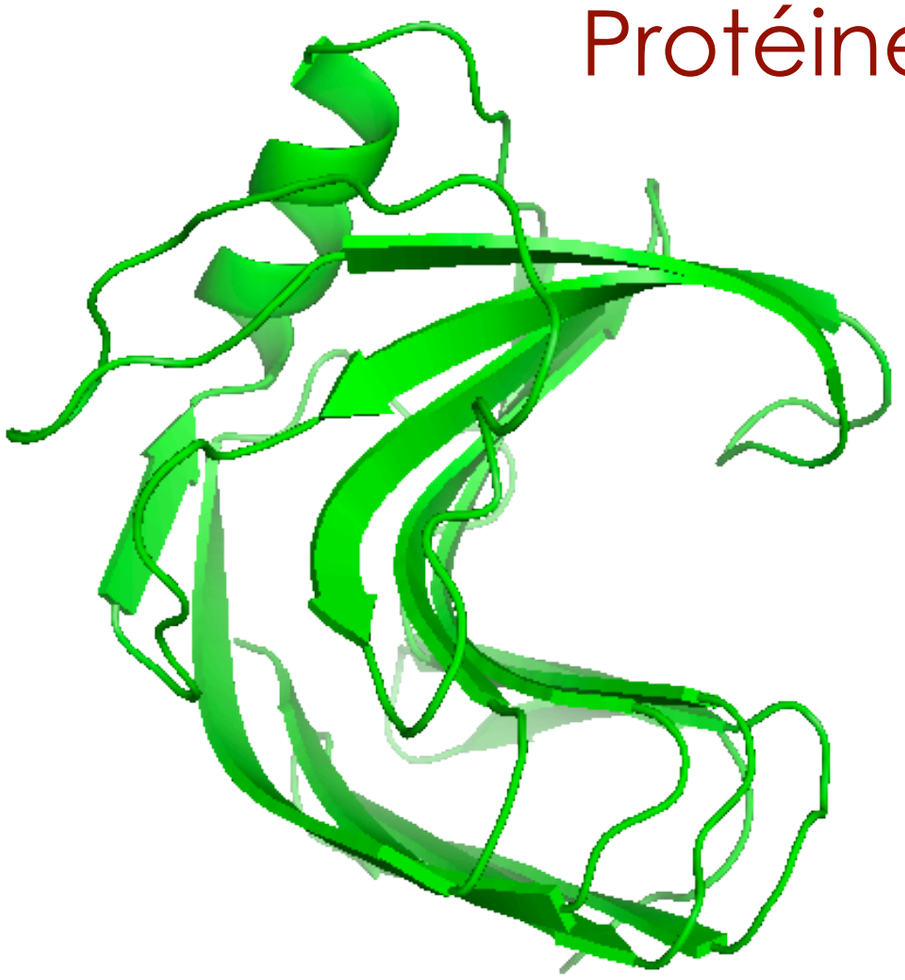
Chimio, régio et enantio-selectivités

Nombre d'étapes et difficulté de synthèse

E Rose, B Andrioletti, S Zrig, M Quelquejeu-Ethève *Chem Soc Rev* **2005**, *34*, 573-583

Des enzymes et des modèles aux Artzymes

Protéines

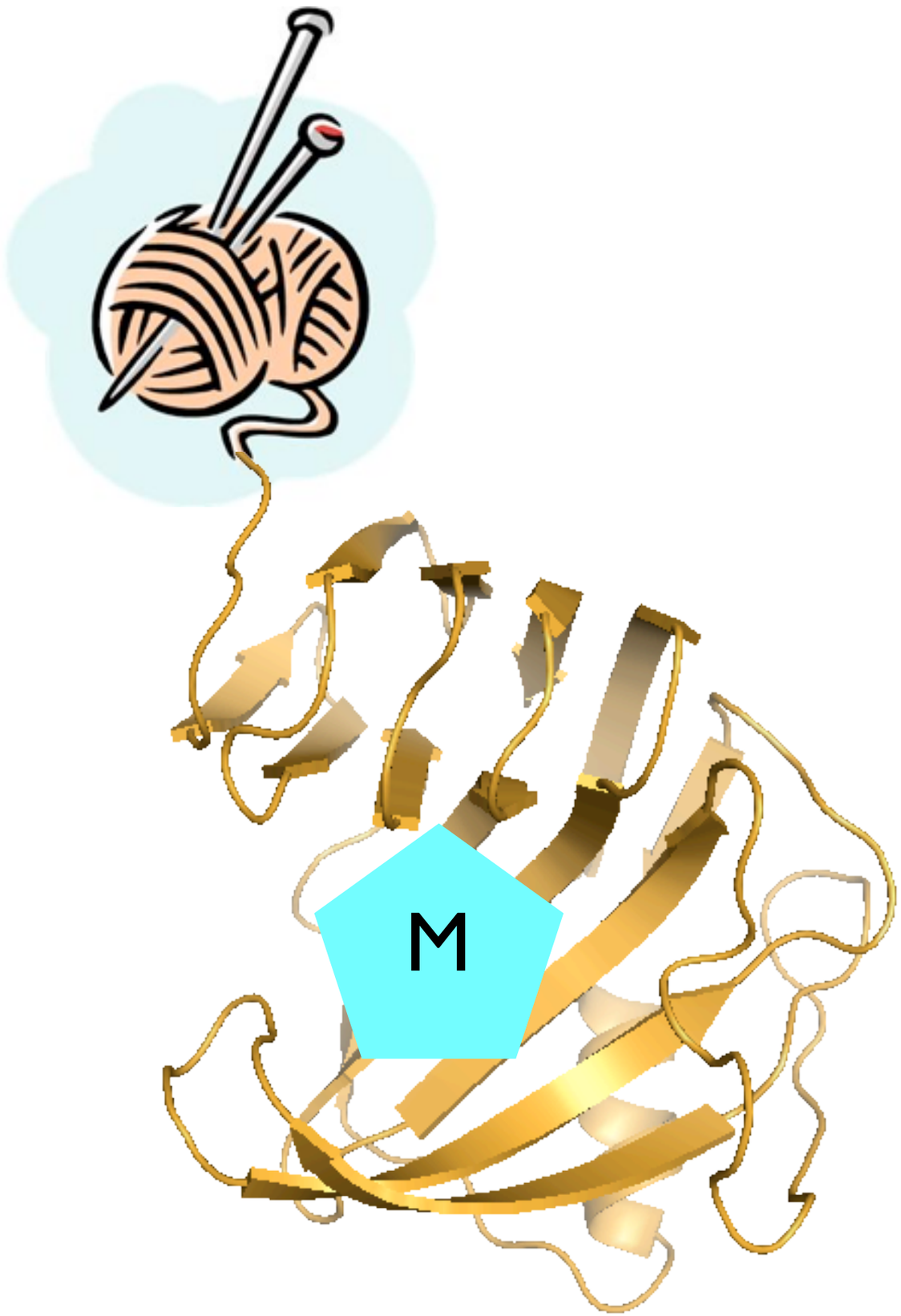


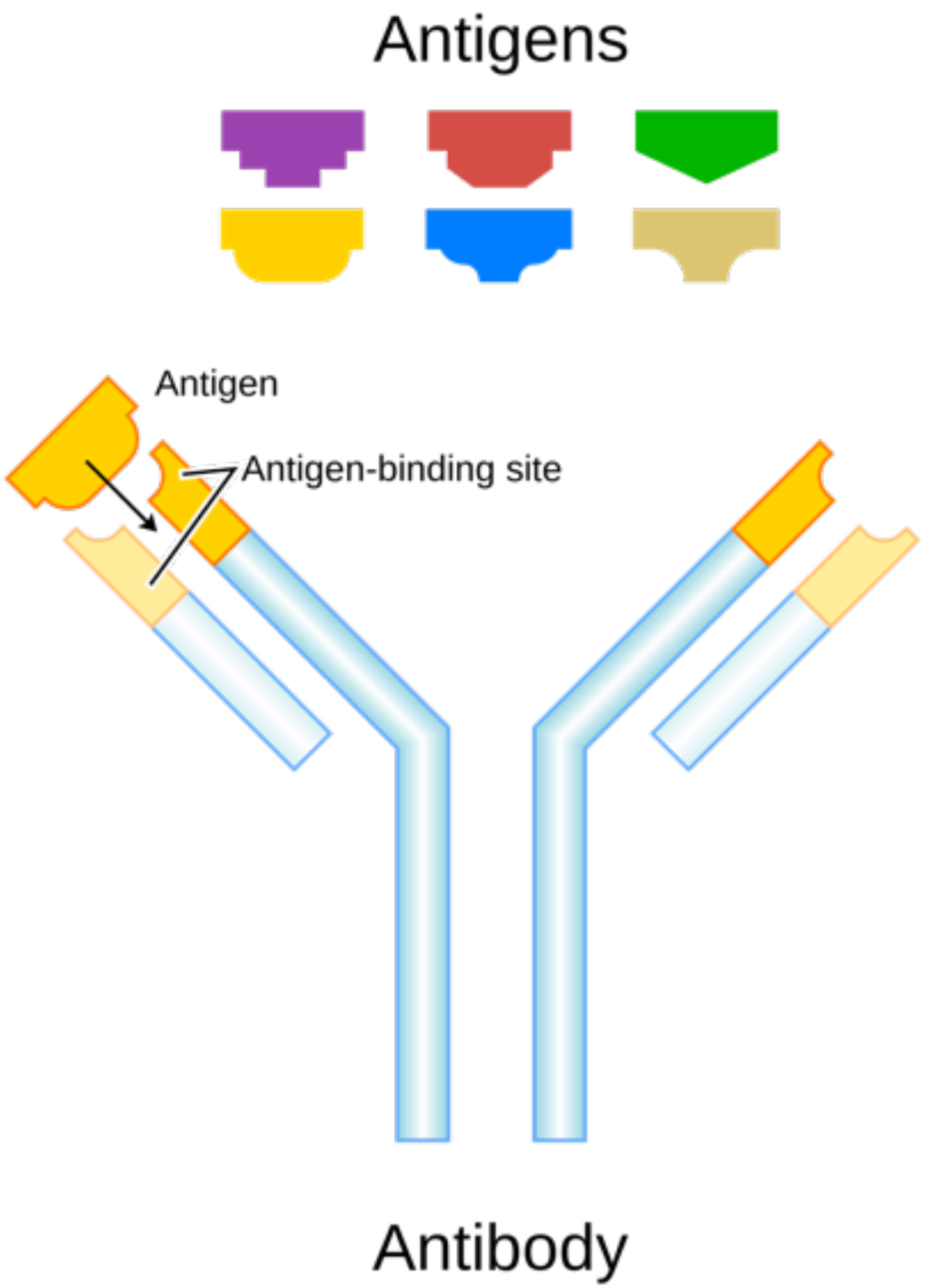
Complexes

3 approches

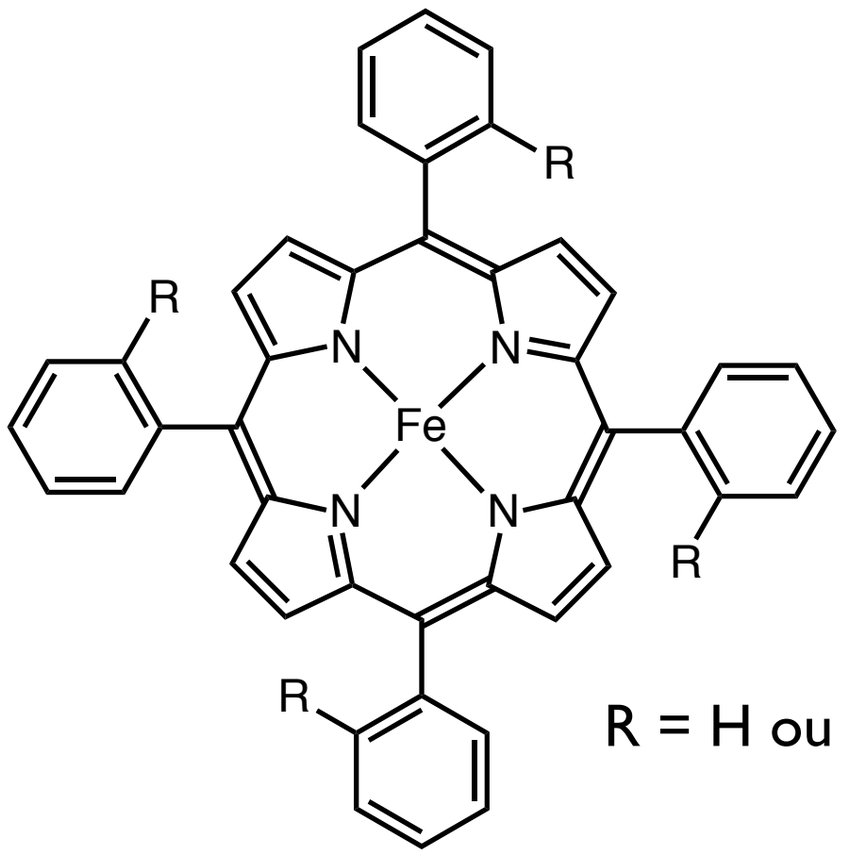
(*i*) création; (*ii*) adaptation et (*iii*) modification d'un environnement protéique autours d'un centre ou complexe métallique

1) Création : Abzymes, du Tricot Moléculaire



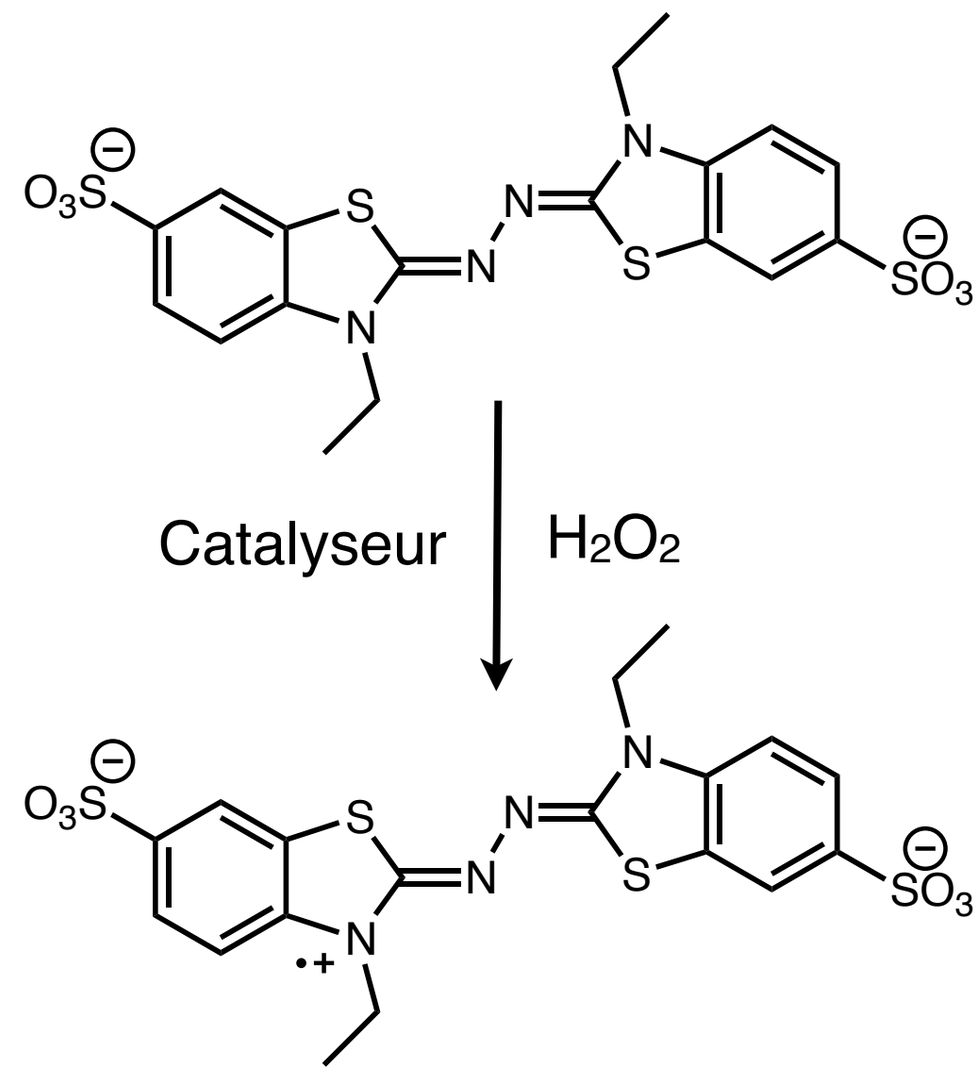


Anticorps anti-porphyrine à activité peroxydase



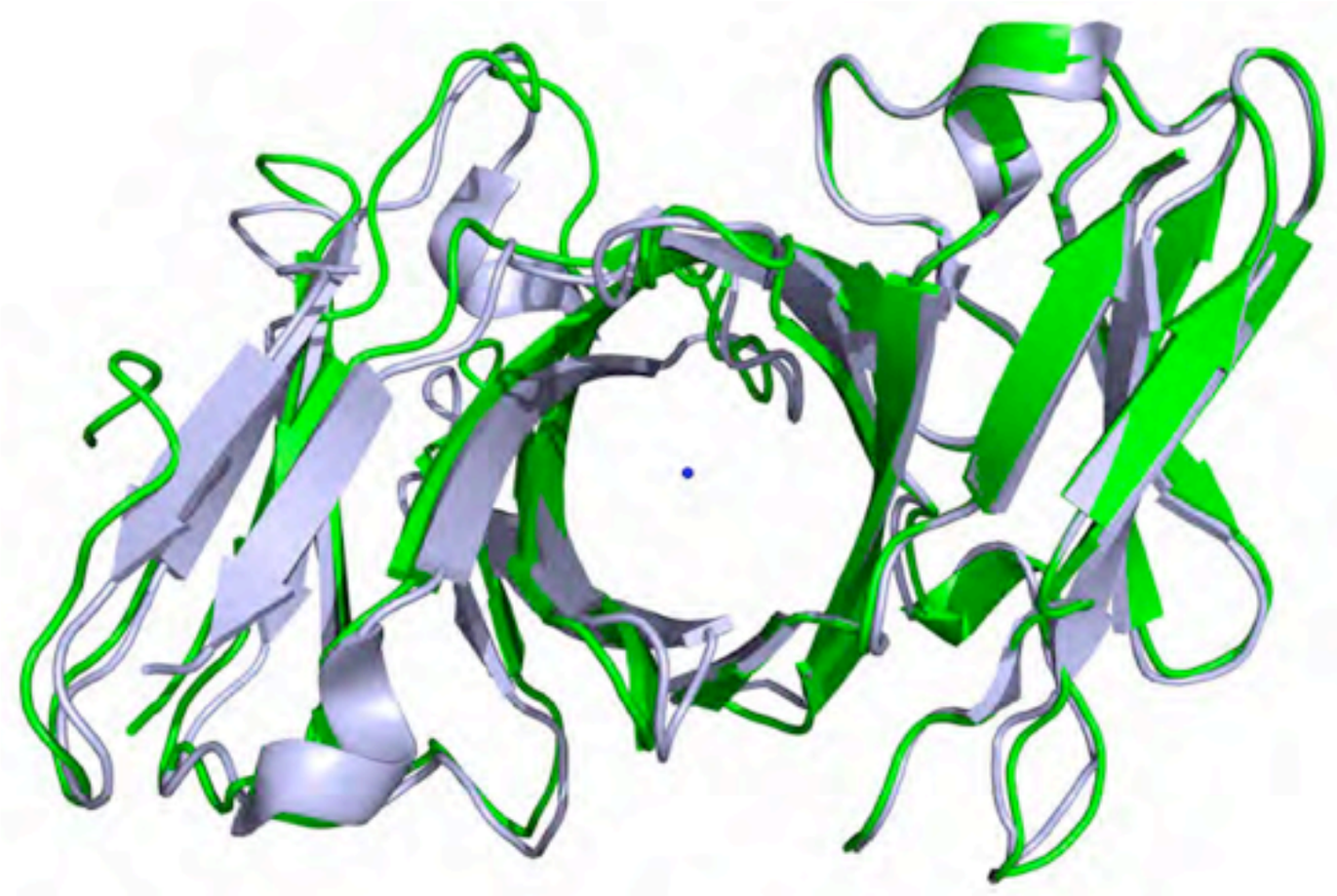
R = H ou COOH

Fe(MoCPP)
13G10/Fe(MoCPP)
14H7/Fe(MoCPP)



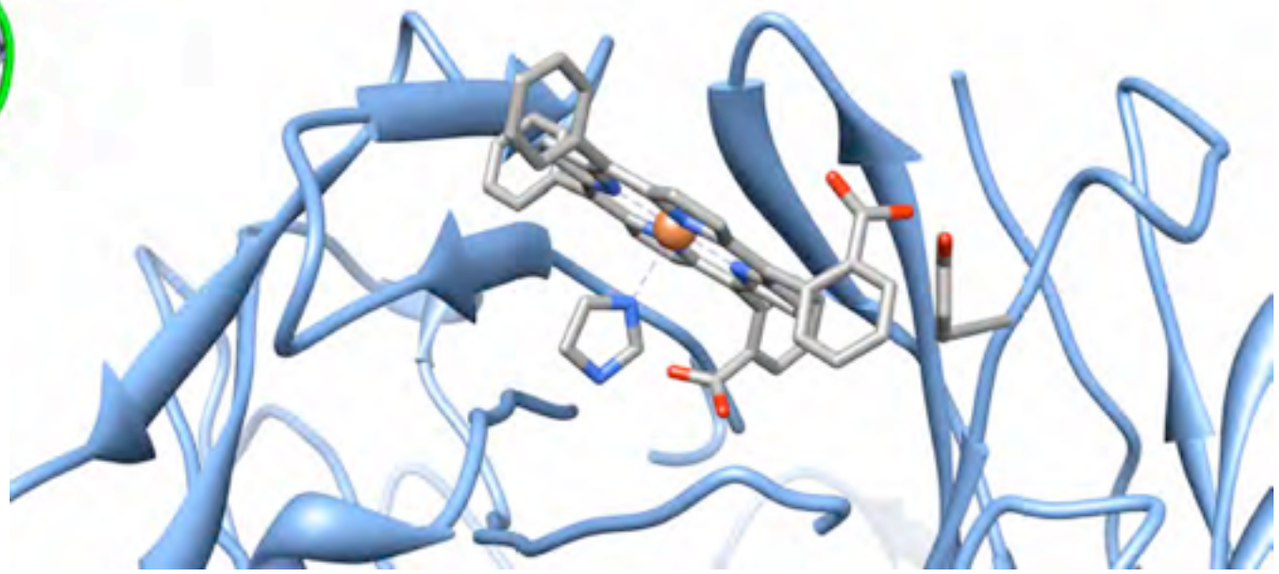
S de Lauzon, B Desfosses, D Mansuy, JP Mahy *FEBS Lett* **1999**, *443*, 229-234.

Structure d'un anticorps anti-porphyrine



13G10
14H7

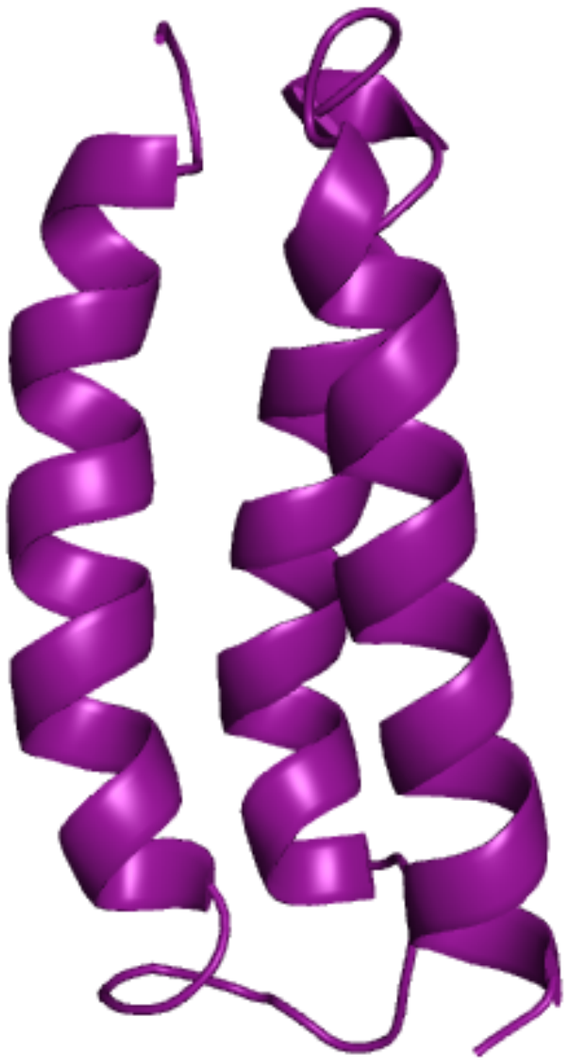
13G10/Fe(MoCPP)

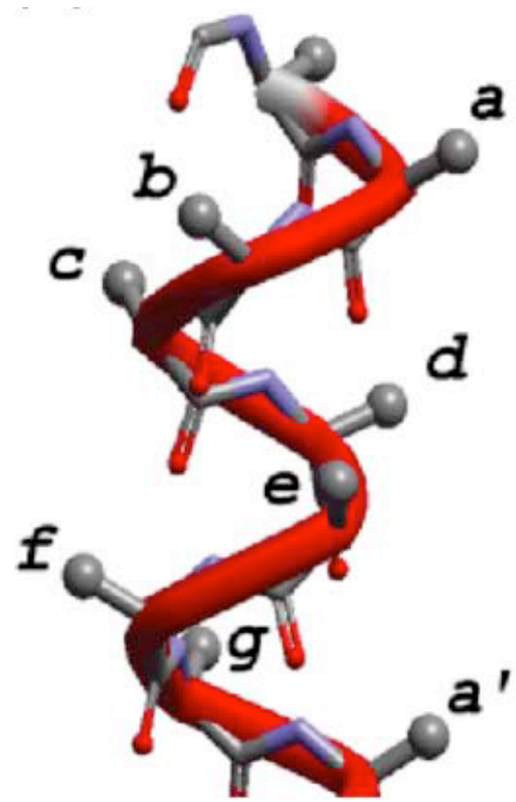


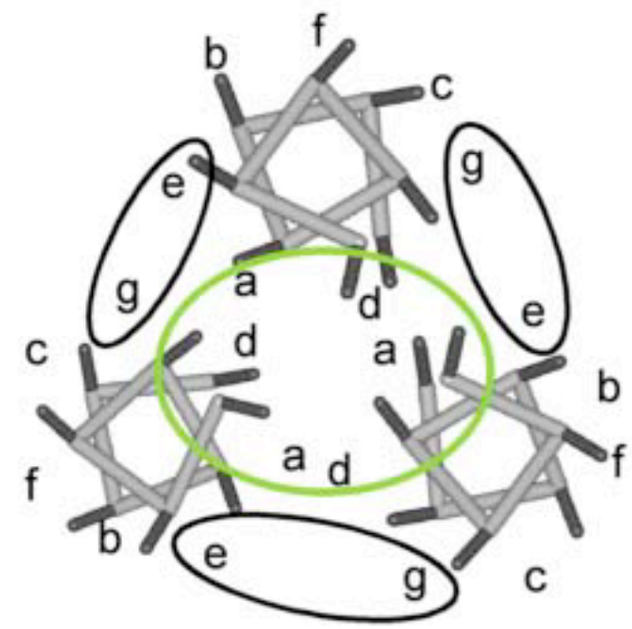
V Muñoz Robles, JD Maréchal, A Bahloul, MA Sari, JP Mahy, B Golinelli-Pimpaneau
PLoS One **2012**, sous presse

1) Création : Construction *de novo*

3 helix bundle





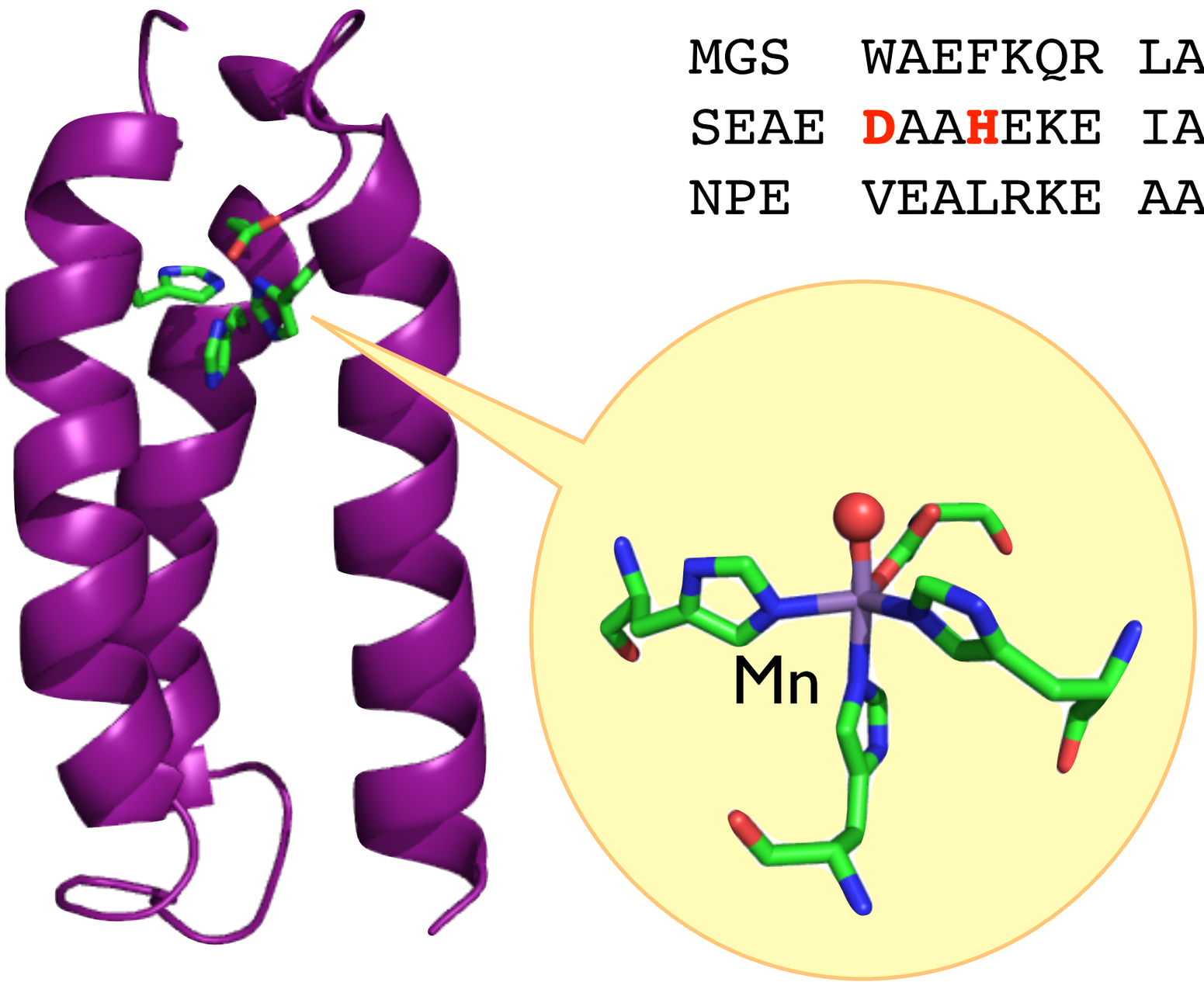


	abcdefg	*abcdefg*	*abcdefg*
MGS	WAEFKQR	LAAIKTR	LQAL
SEAE	LAAFEKE	IAAFESE	LQAY
NPE	VEALRKE	AAAIRDE	LQAYRHN

STR Walsh *et al.* *PNAS* **1999**, 96, 5486

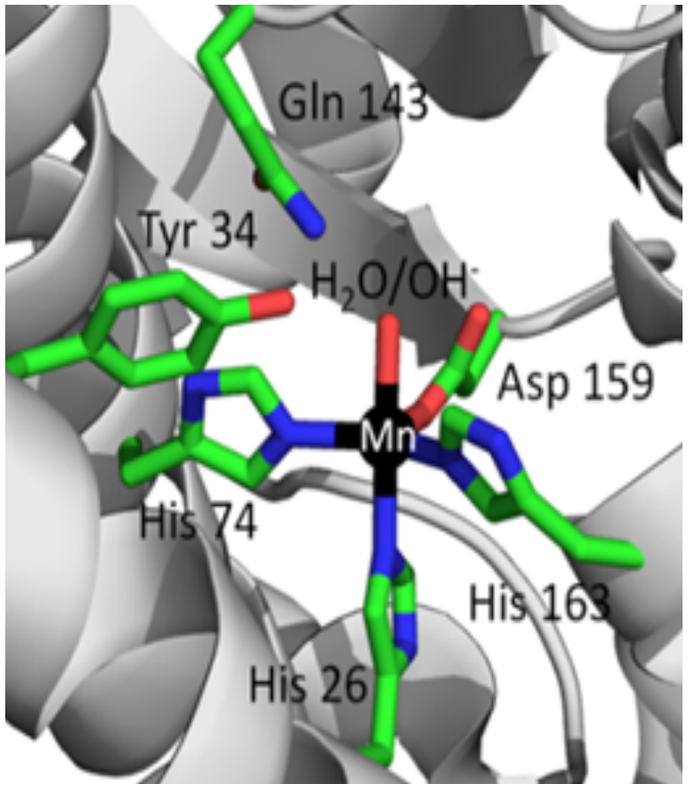
Superoxyde dismutase à Manganèse

$$2\ O_2^- + 2\ H^+ \longrightarrow O_2 + H_2O_2$$



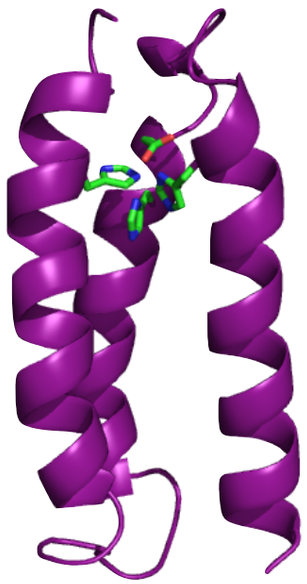

```
MGS   WAEFKQR LAAIKTR HQAL
SEAE  DAAHEKE IAAFESE LQAY
NPE   VEALRKE AAAIRDE HQAYRHN
```

Site actif Mn-SOD



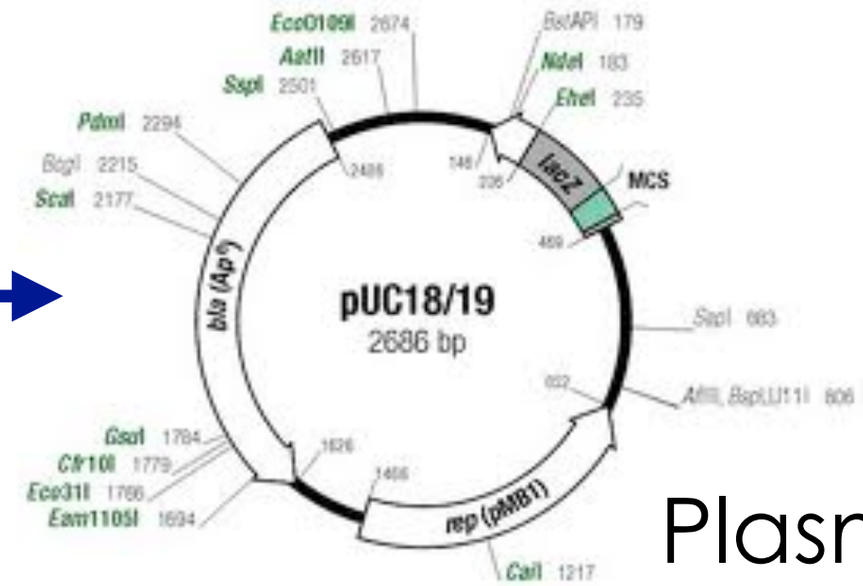
O Iranzo

Bactérie usine à production de ligands

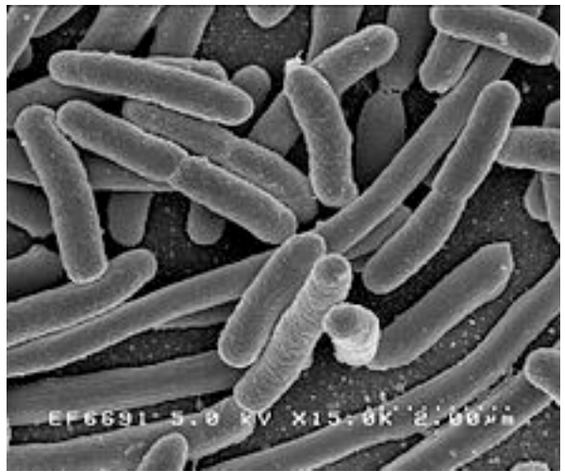


peptide

ADNc



Plasmide

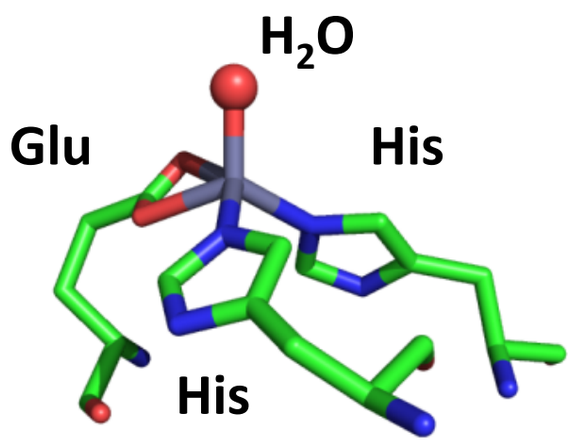


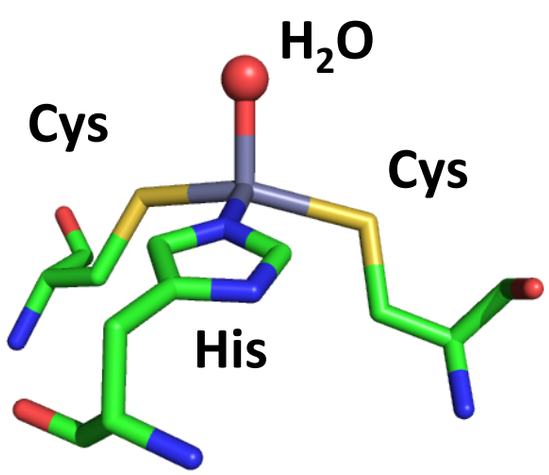
Bactérie

Production de peptides

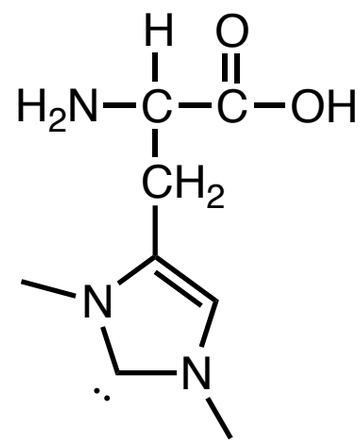
Accessibilité à une très grande variété de sites métalliques

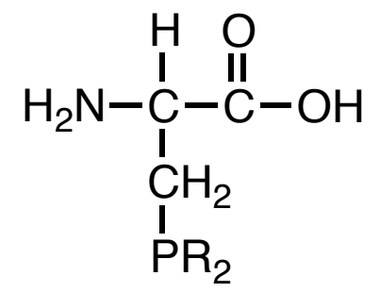
Les classiques

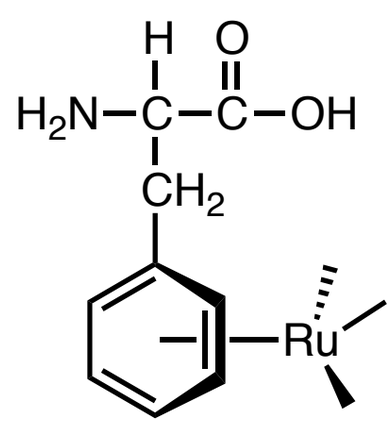


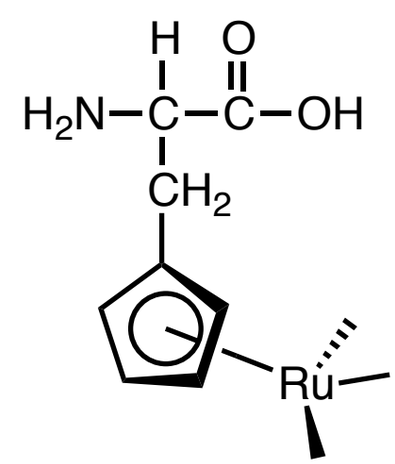


Les moins classiques





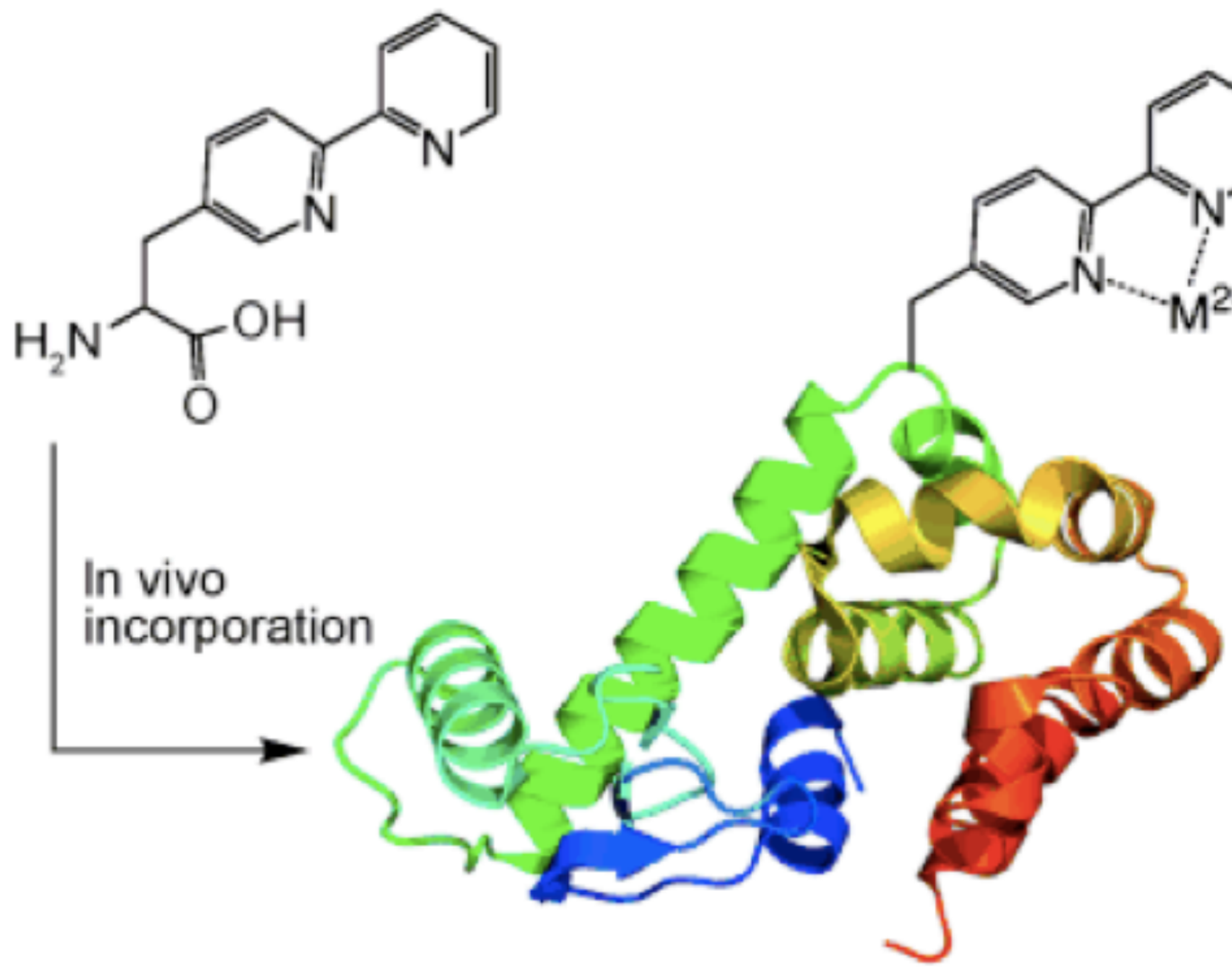




Fe, Cu, Co, Zn
Mo ...

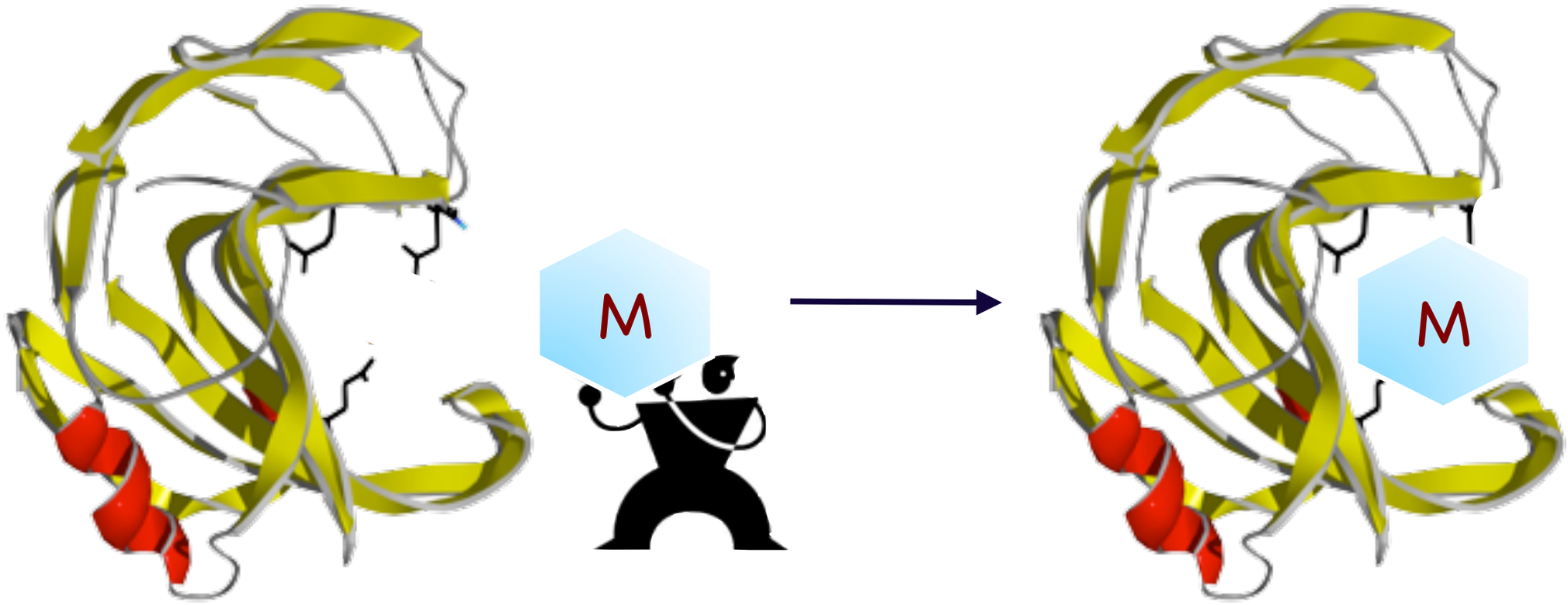
Pd, Pt, Ru ...

A Genetically Encoded Bidentate, Metal-Binding Amino Acid



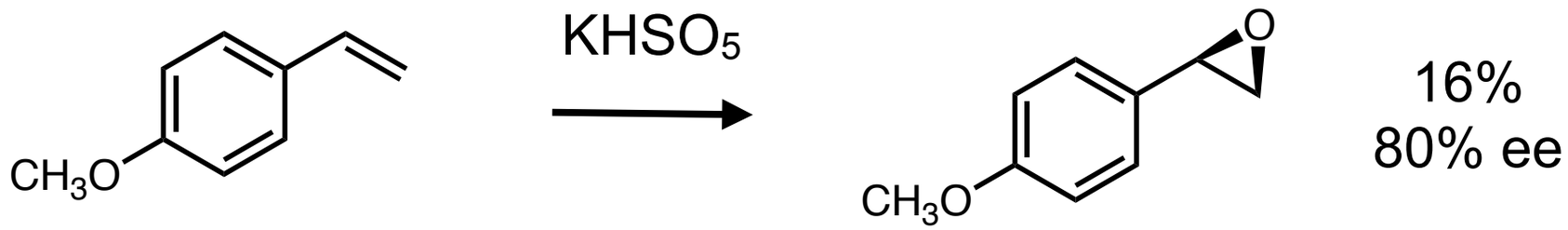
J Xie, W liu, PG Schultz *Angew Chem Int Ed Engl* **2007**, *46*, 9239-9242

2) Adaptation : Ajustage Moléculaire

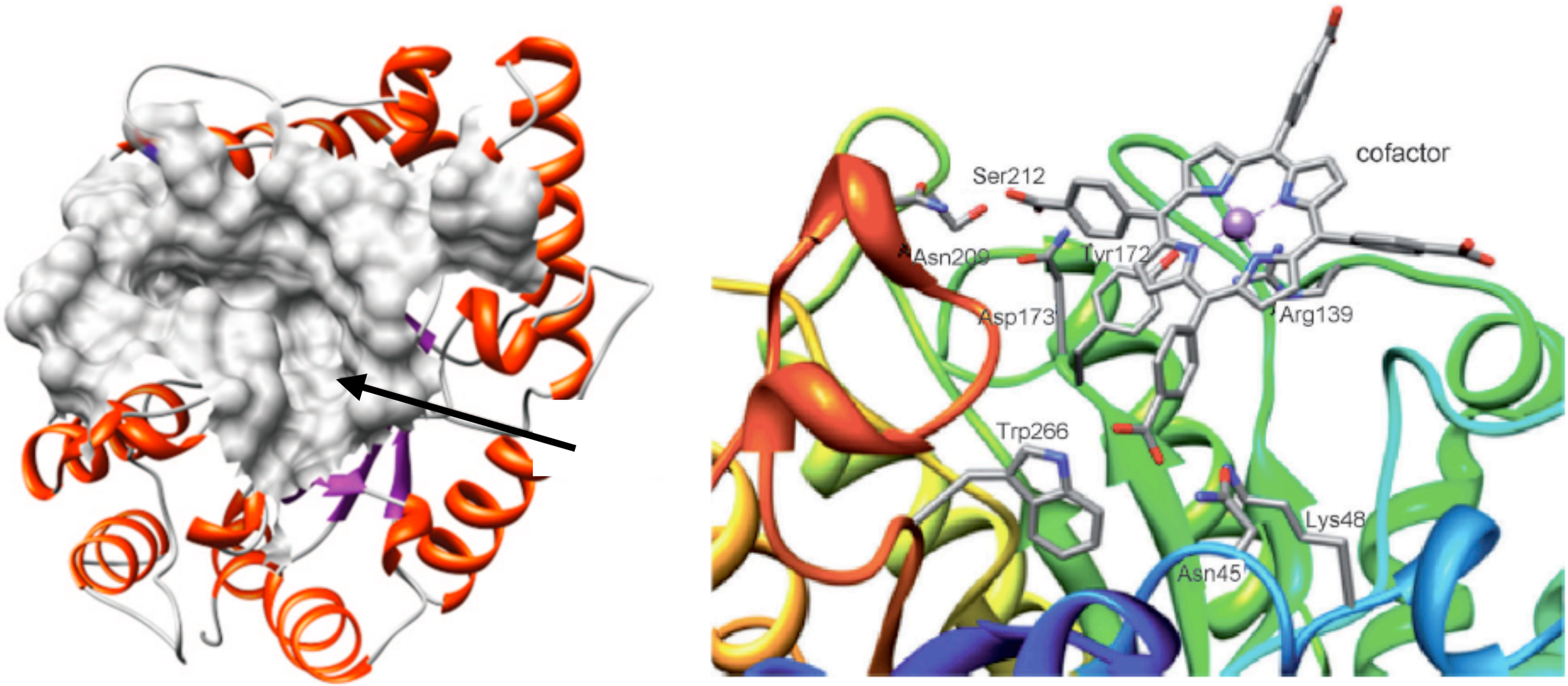


Protéine	Métal	Fonction	Auteur
Myoglobine	Cr- et Mn-salen	Oxydation	*Y Watanabe*
Avidine	Rh et Ru	Oxydation, réduction	*T Ward, M Salmain*
Albumine	Mn-salen	Oxydation	*S Ménage*
Papaïne	Arene-Ru	Réduction	*M Salmain*
Ferritine	Pd, Ag, CdS, Fe_3O_4 …	Catalyseur et matériaux	*Y Watanabe*
Xylanase GH10	Porphyrine de Fe et Mn	Oxydation	*JP Mahy*
NikA	Complexe de Fe	Oxydation	*S Ménage*

Xylanase GH10/Mn(TpCPP)

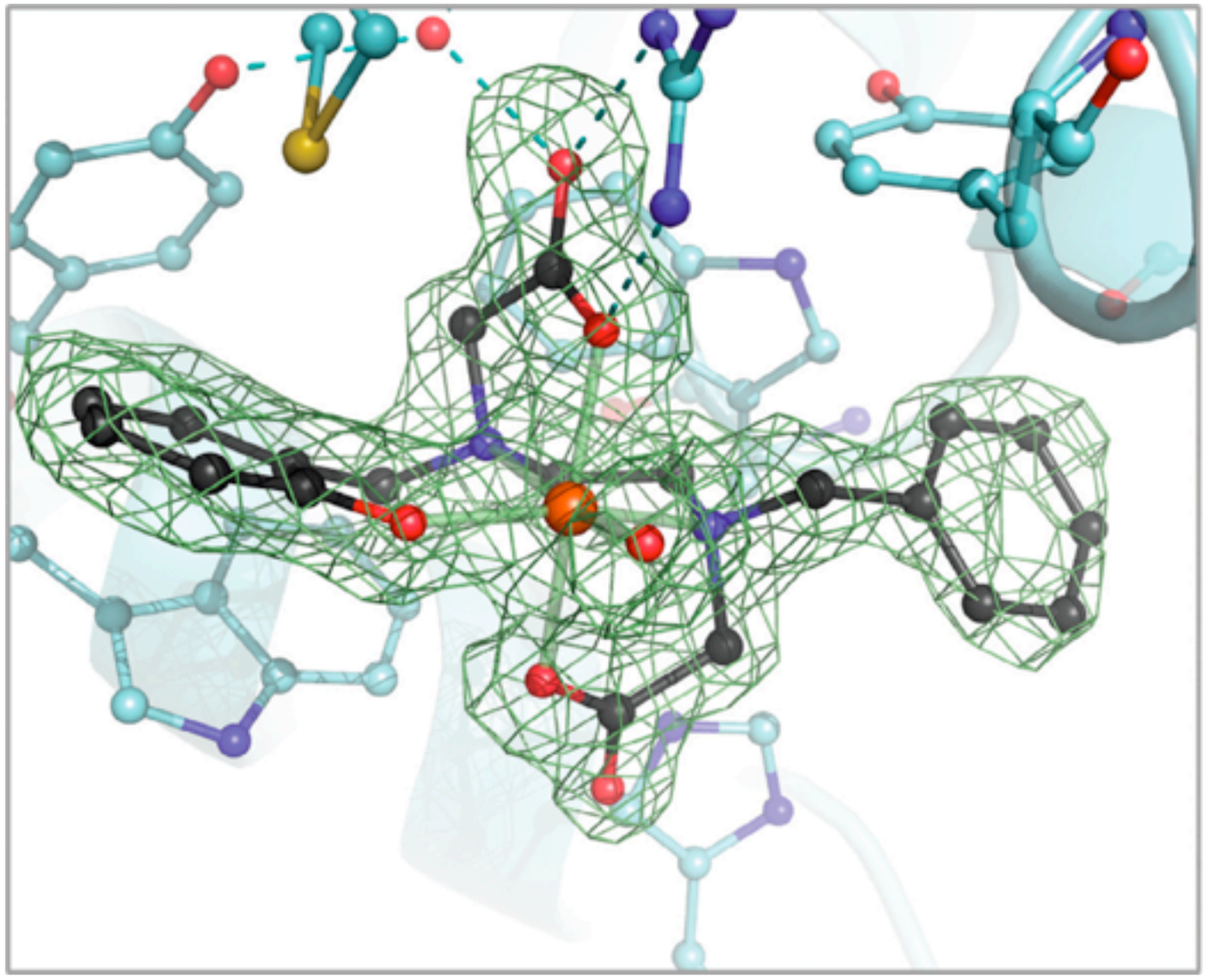


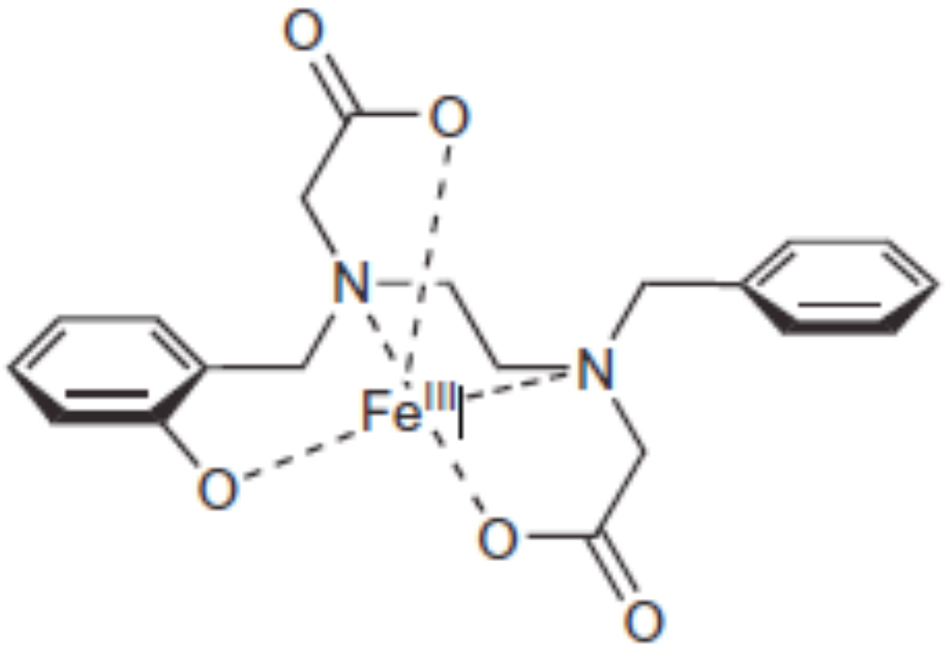
16%
80% ee



M Allard, C Dupont, V Muñoz Roble, N Doucet, A Lledós, JD Marchal, A Urvoas, JP Mahy, R Ricoux *ChemBioChem* **2012**, *13*, 240-251

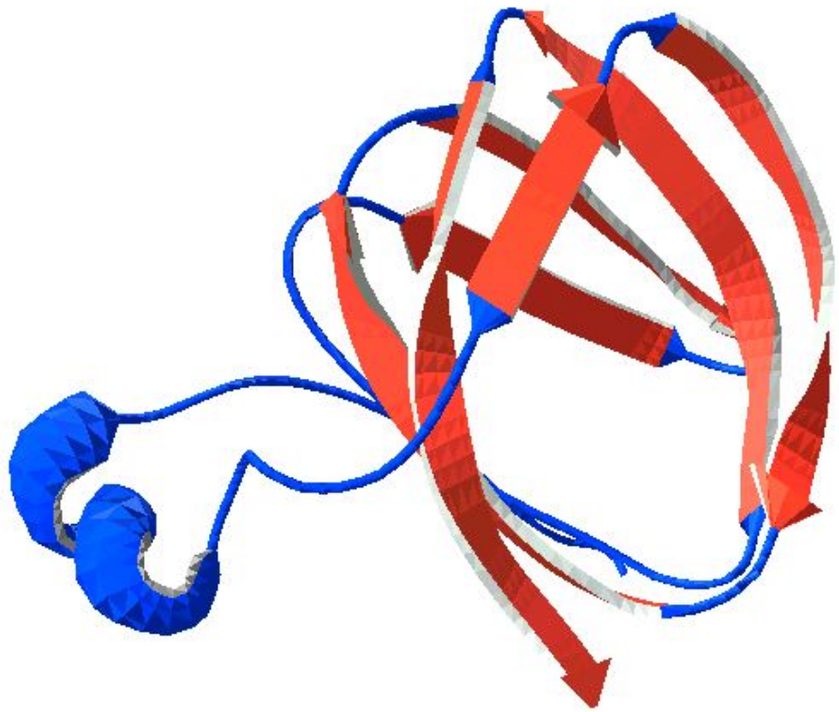
NikA/Fe(EDDA)





C Cavazza, C Bochot, P Rousselot-Pailley, P Carpentier, MV Cherrier, JL Martin, C Marchi-Delapierre, JC Fontecilla-Camps, S Ménage *Nat Chem* **2010**, *2*, 1069-1076

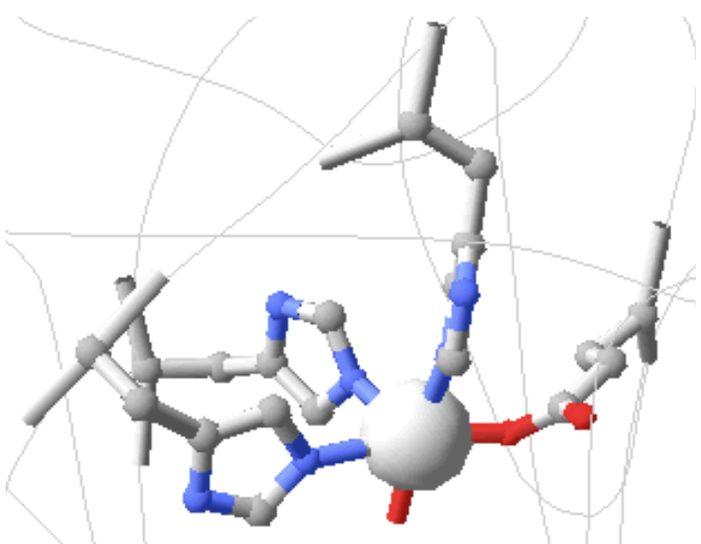
3) Modification : Les cupines, une famille formidable



Cupine
Double Stranded β-Helix β-Barrel (DSBH)
Jimonji

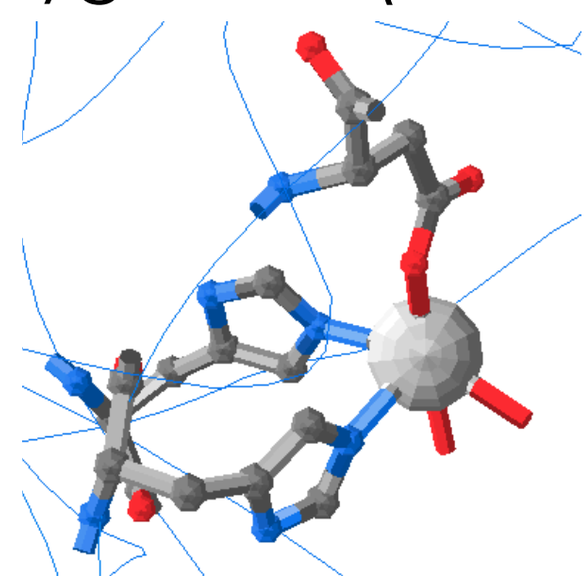
Métalloenzymes (Mn, Fe, Cu …)
Grande variété de fonction (hydrolase, oxygénase, oxydase …)

Germine (3 His + 1 Glu)



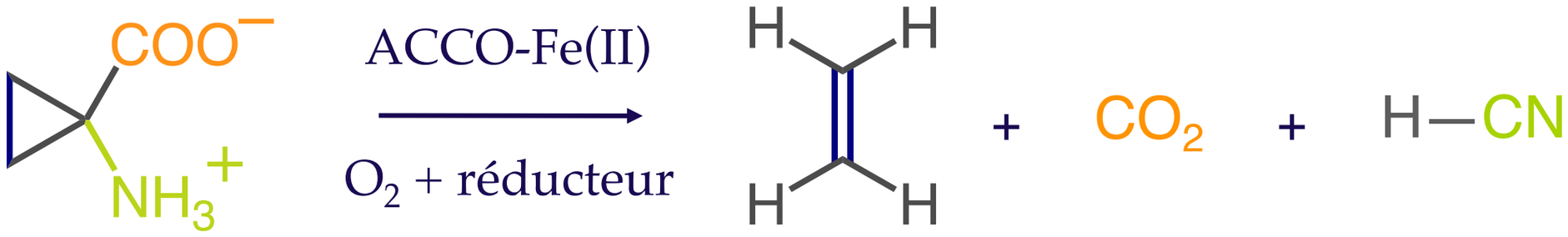
Mn, Cu, Fe

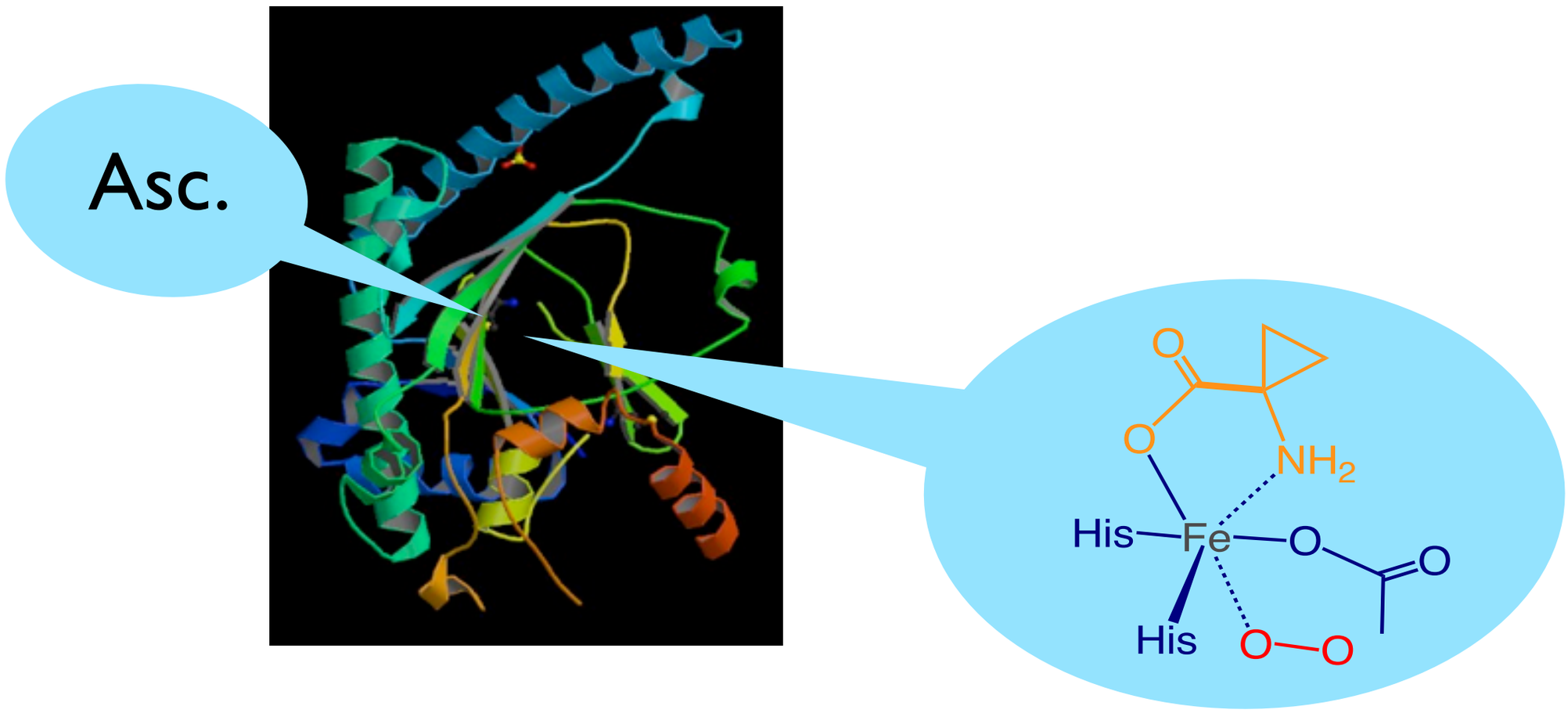
2-OG oxygénase (2 His + 1 Asp)



Fe

ACC oxydase (ACCO)

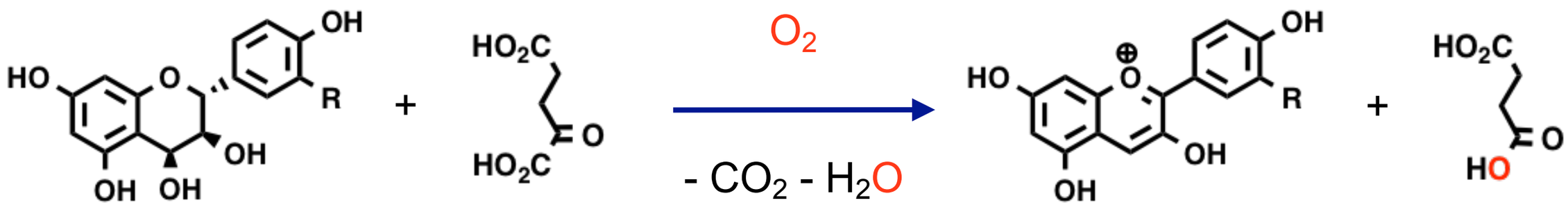


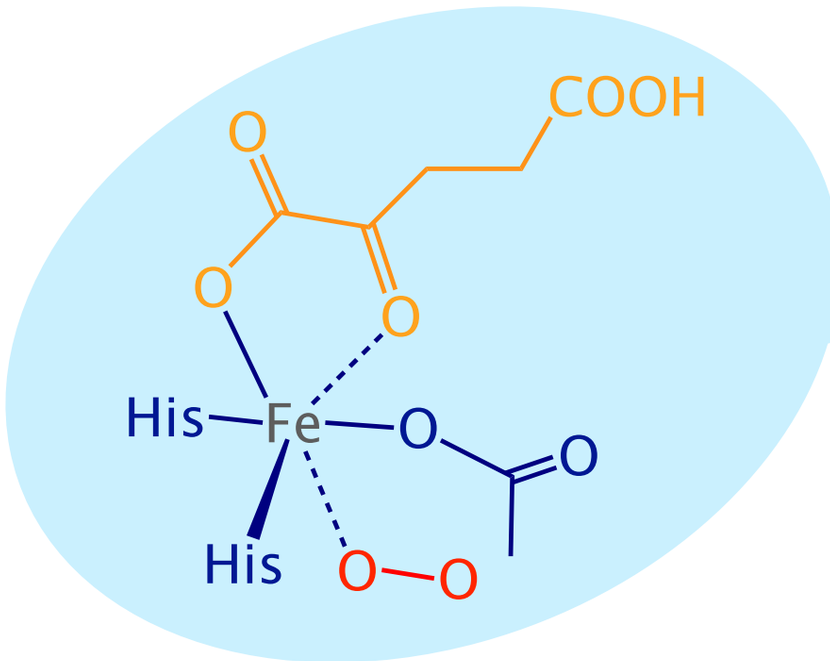


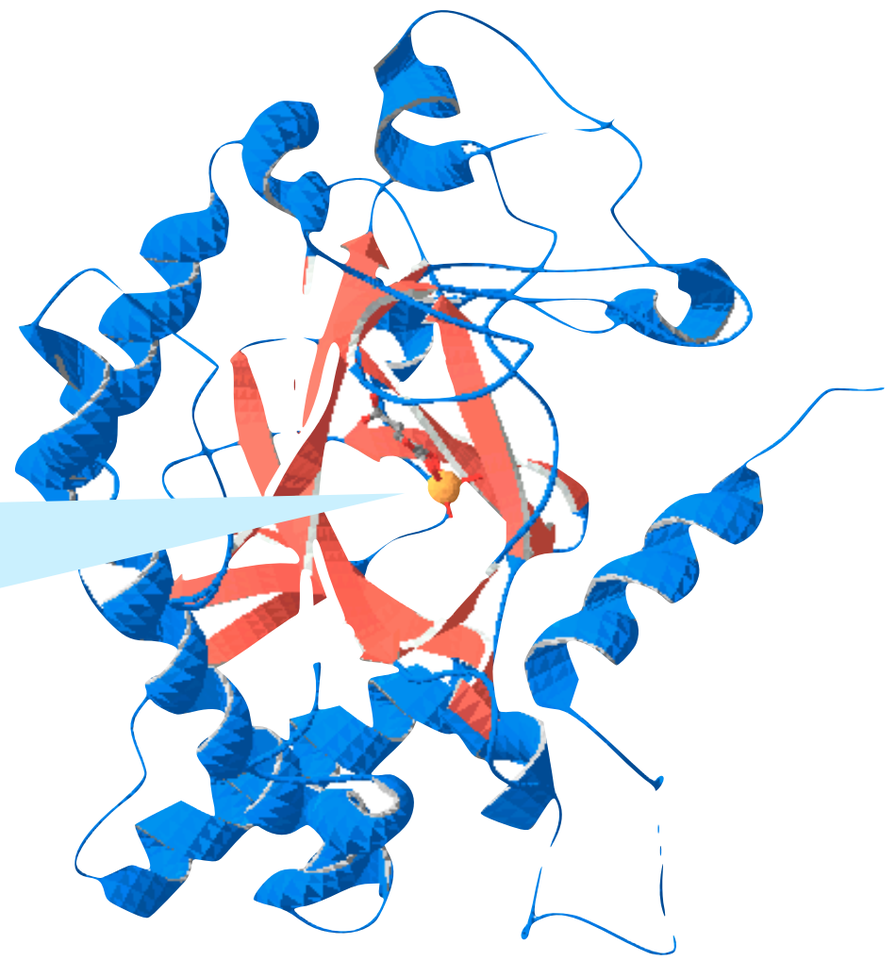
ACCO-PH IKGLRA**H**T**D**AGGIILLFQDDK VITNGKYKSVM**H**TVIA

J Simaan

Anthocyanidine Synthase (ANS)

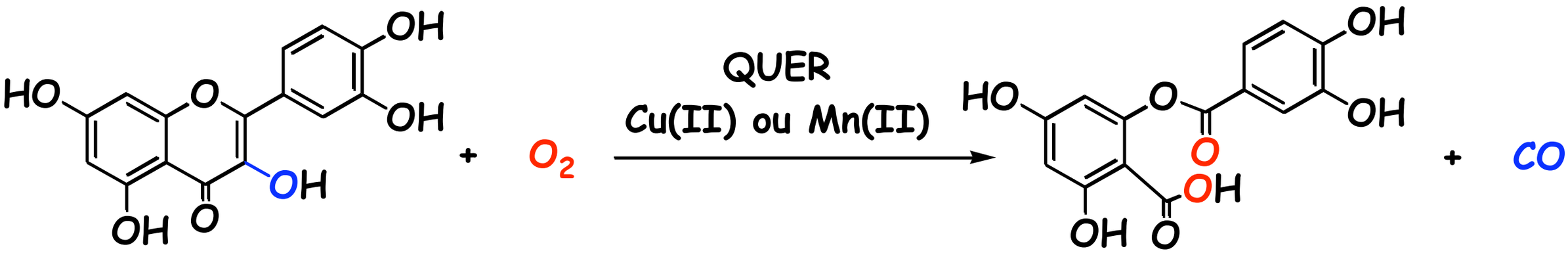




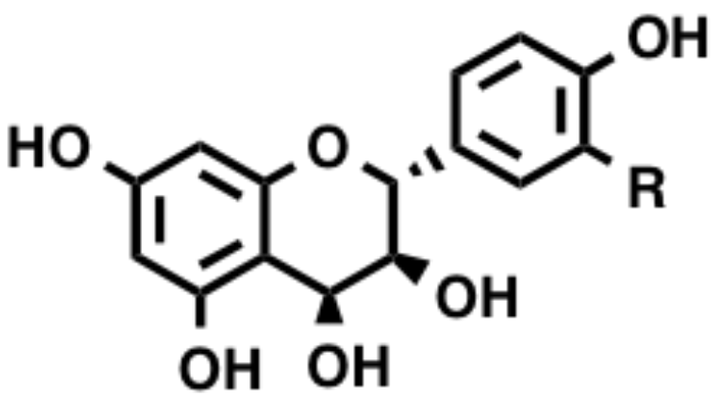


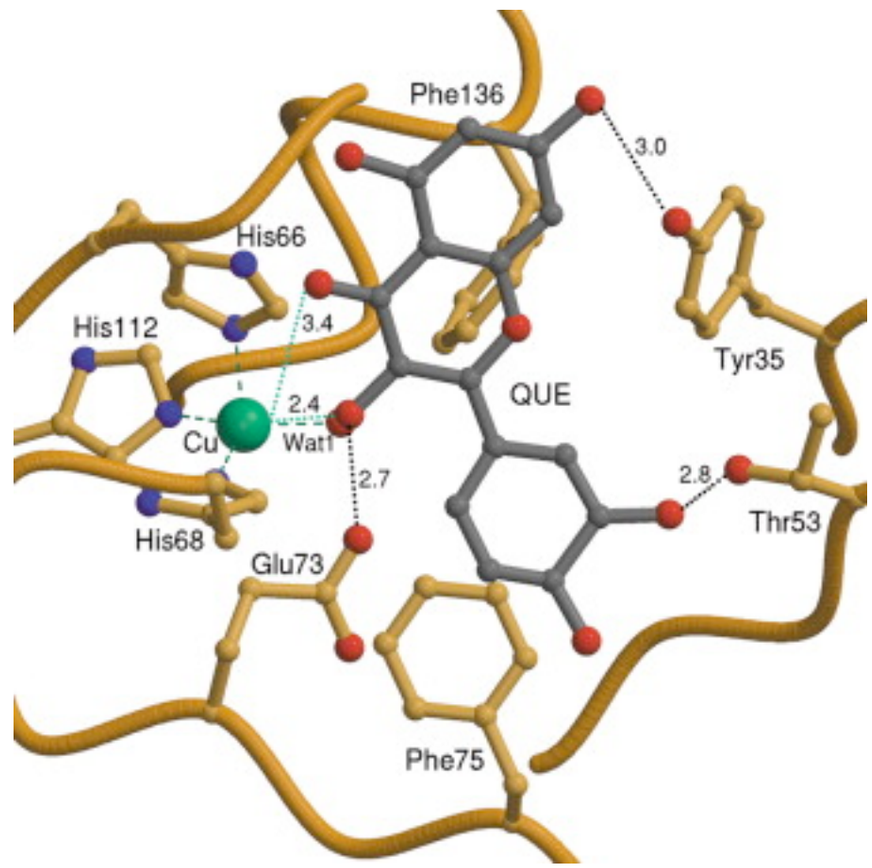
ACCO-PH	IKGLRA**H**T**D**AGGIILLFQDDK	VITNGKYKSVM**H**TVIA
ANS-AT	ALGVEA**H**T**D**VSALTFILHNM-	ILSNGKYKSIL**H**RGLV

Quercétinase (QUER)



Substrat de l'ANS





ACCO-PH	IKGLRA**H**T**D**AGGIILLFQDDK	VITNGKYKSVM**H**TVIA
ANS-AT	ALGVEA**H**T**D**VSALTFILHNM-	ILSNGKYKSIL**H**RGLV
QUER-BSa	GDAFPL**H**V**H**KDTH**E**GILVLDG	GDYANIPAGTP**H**SYRM

ANS/Quercétinase

			U/min
ANS-AT	ALGVEA**H**T**D**VSALTFILHNM-	ILSNGKYKSIL**H**RGLV	0
ANSD243H	ALGVEA**H**T**H**VSALTFILHNM-	ILSNGKYKSIL**H**RGLV	31
ANST239E	ALGVEA**H**T**D**VSAL**E**FILHNM-	ILSNGKYKSIL**H**RGLV	8
ANSD/T	ALGVEA**H**T**H**VSAL**E**FILHNM-	ILSNGKYKSIL**H**RGLV	106
QUER-BSa	GDAFPL**H**V**H**KDTH**E**GILVLDG	GDYANIPAGTP**H**SYRM	130

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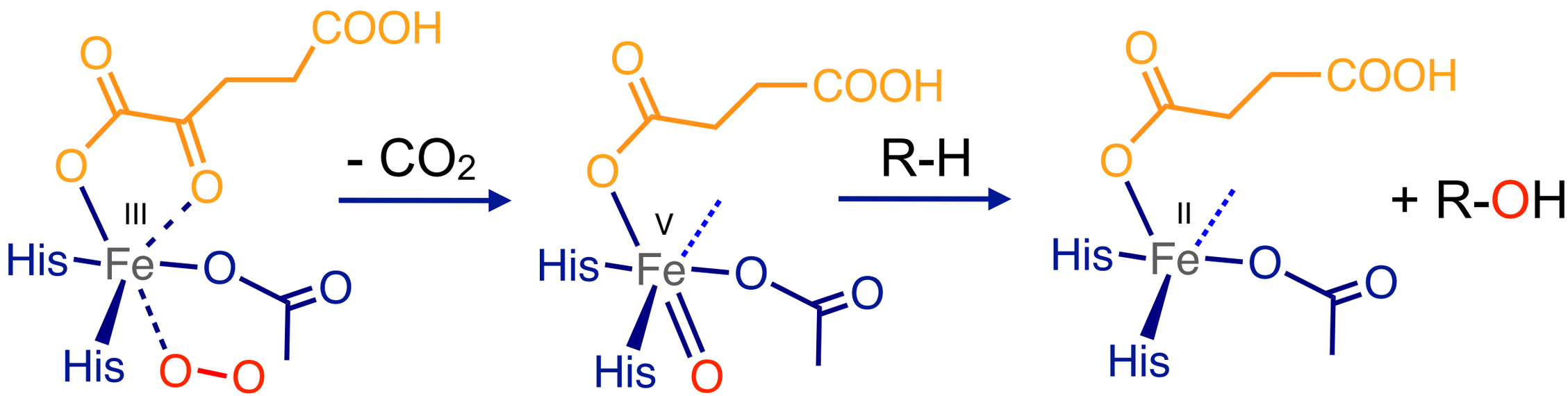
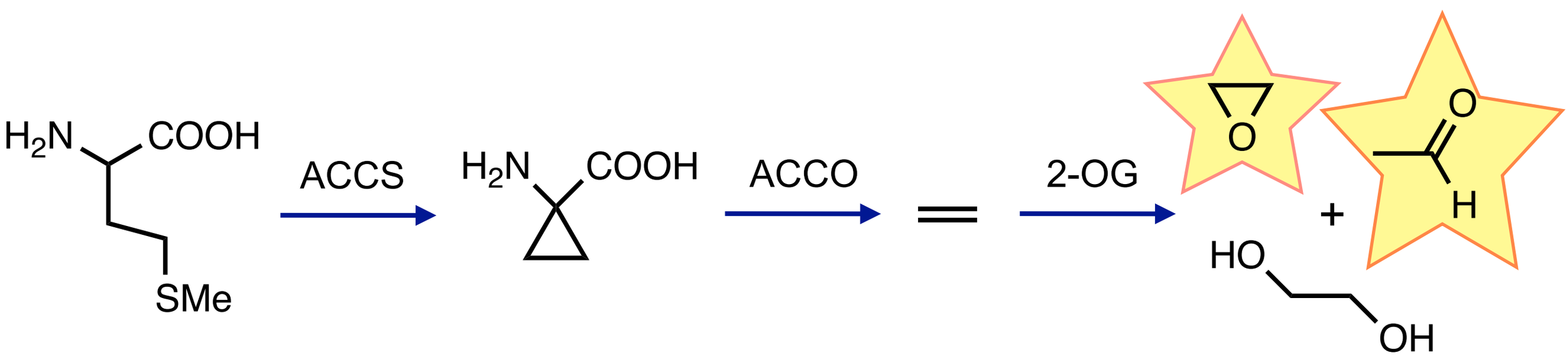
Vers la fin du vapocracker ?





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