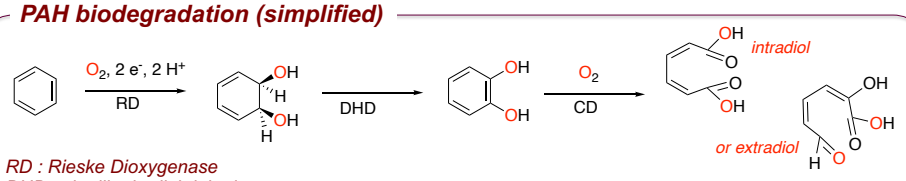
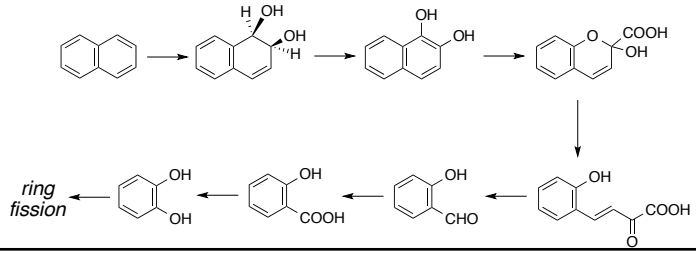
Dioxygénases de Rieske

PAH biodegradation (simplified)



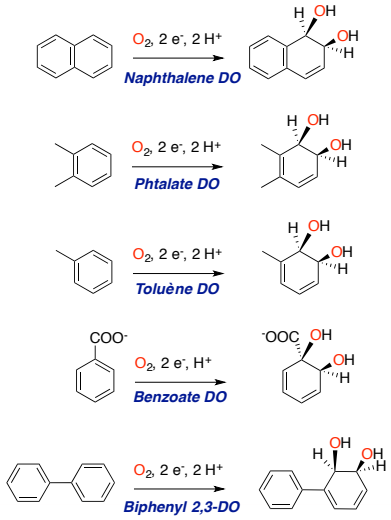
RD : Rieske Dioxygenase
DHD : cis-dihydrodiol dehydrogenase
CD : Catechol Dioxygenase

many bacteria use PAH such as naphthalene as sole source of C and energy



2

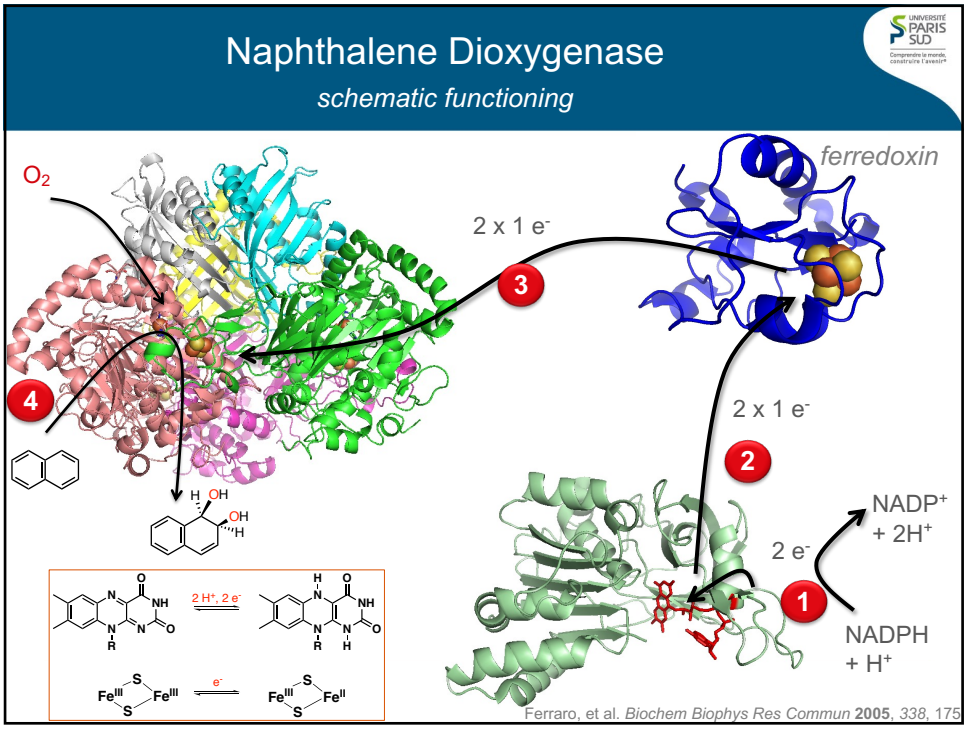
Some Rieske Dioxygenases



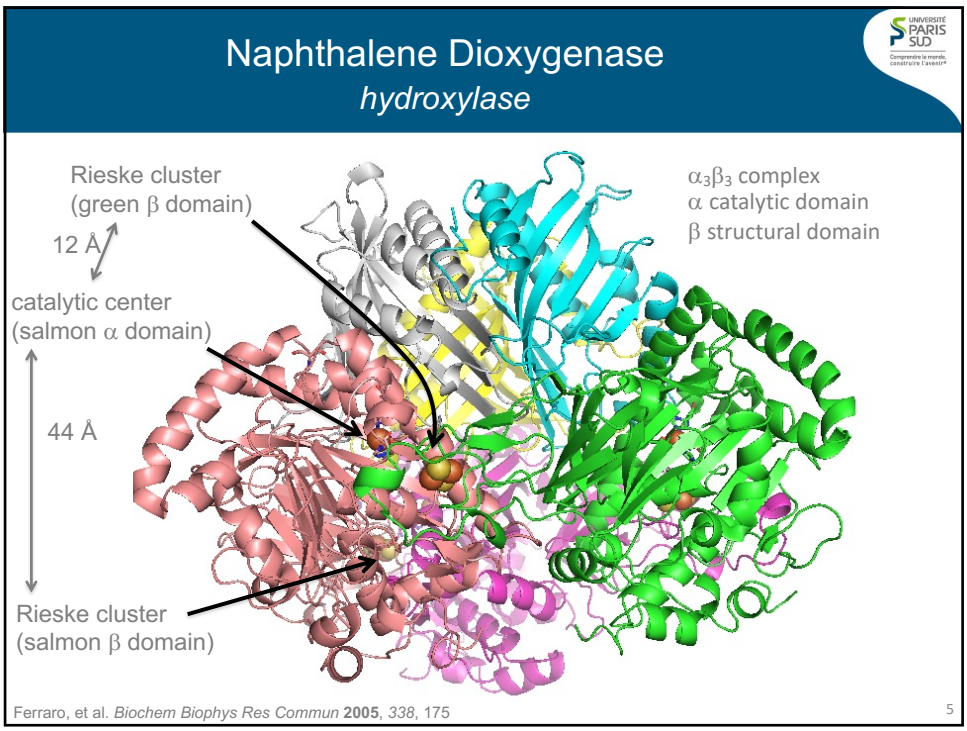
the best characterized

3

3

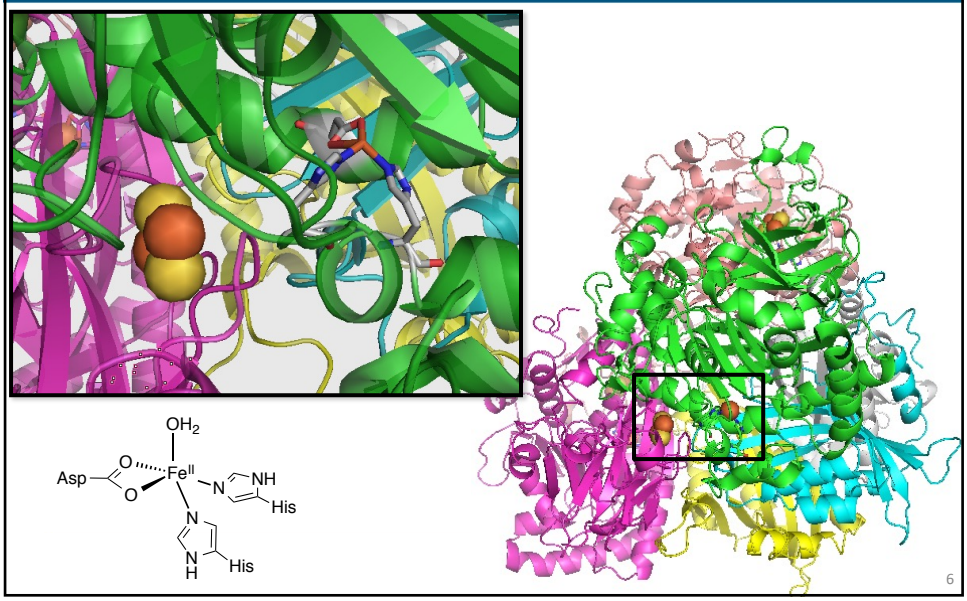

Naphthalene Dioxygenase
schematic functioning
O_2
ferredoxin
2 x 1 e⁻
3
4
2 x 1 e⁻
2
2 e⁻
1
NADP⁺ + 2H⁺
NADPH + H⁺
2 H⁺, 2 e⁻
e⁻
Ferraro, et al. Biochem Biophys Res Commun 2005, 338, 175

4


Naphthalene Dioxygenase
hydroxylase
Rieske cluster
(green β domain)
12 Å
catalytic center
(salmon α domain)
44 Å
Rieske cluster
(salmon β domain)
$\alpha_3\beta_3$ complex
α catalytic domain
β structural domain
Ferraro, et al. Biochem Biophys Res Commun 2005, 338, 175
5

5

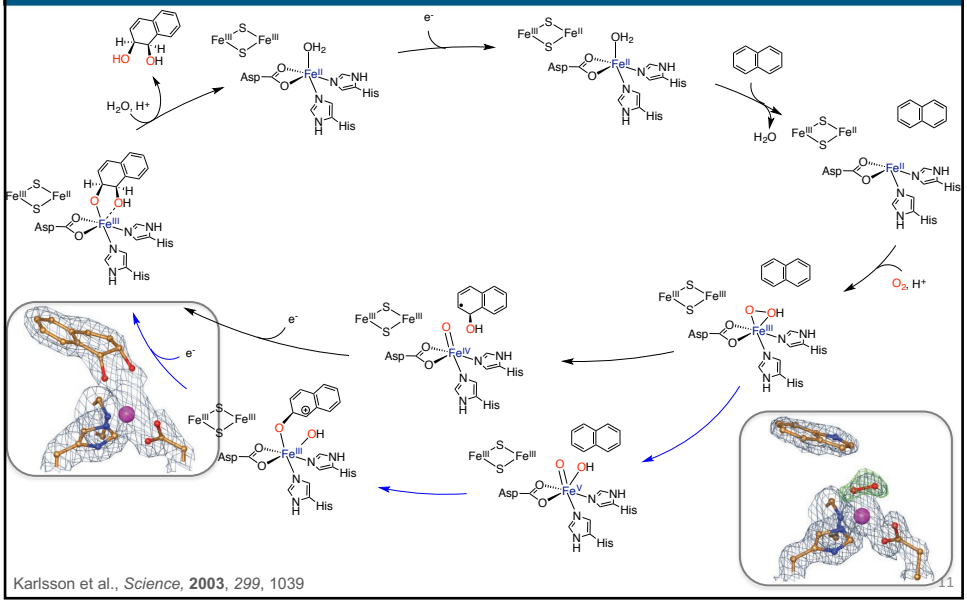
Naphthalene Dioxygenase
active site



6

6

Naphthalene Dioxygenase
proposed catalytic cycle



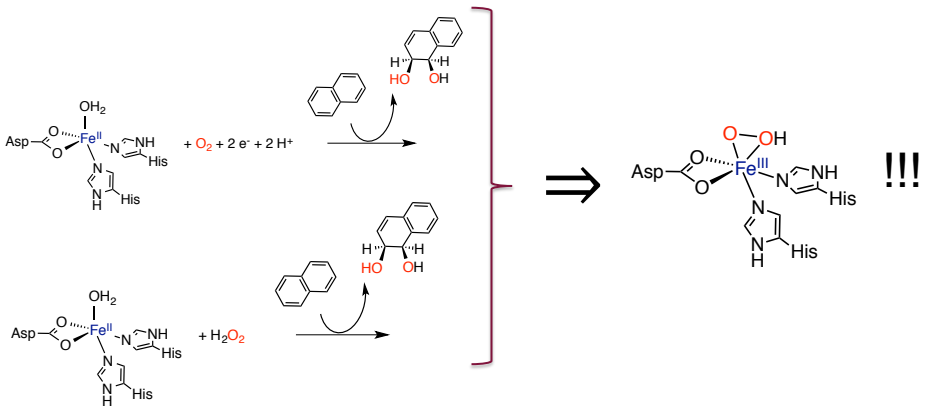
Karlsson et al., *Science*, **2003**, *299*, 1039

11

11

Naphthalene Dioxygenase

mechanistic considerations



13

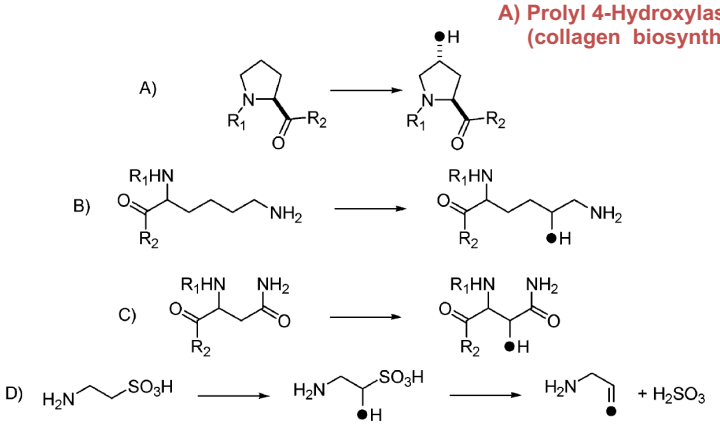
13

A vast family of enzymes

the largest and most diverse family of NH mononuclear enzymes

found in microorganisms, plants, animals

A) Prolyl 4-Hydroxylase (P4H) (collagen biosynthesis)



D) Taurine Dioxygenase (TauD) (amino acid derivative released during physical efforts to minimize toxins effects)

Costas et al. Chem Rev 2004, 104, 953

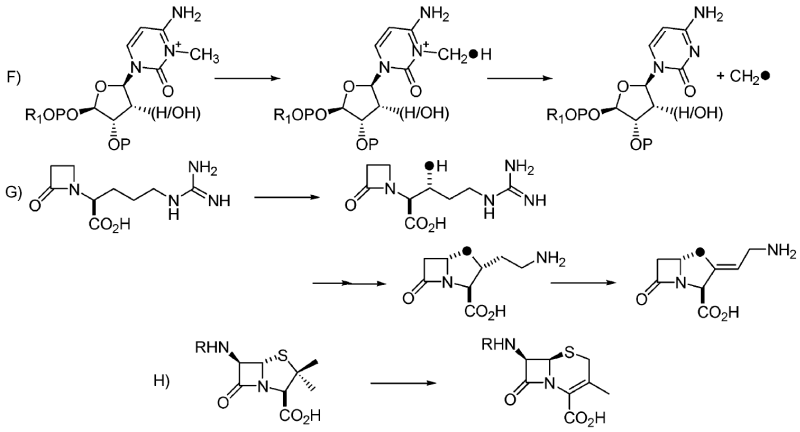
15

15

A vast family of enzymes

the largest and most diverse family of NH mononuclear enzymes

G) Clavaminate Synthase (CAS)
(part of the clavulanic acid biosynthesis)



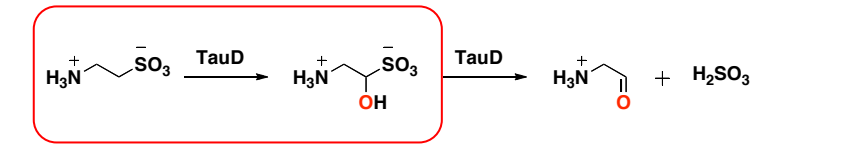
H) Deacetoxycephalosporin C synthase (DAOCS)
(part of the biosynthesis of Deacetoxycephalosporin C from penicillin N; intermediate of cephalosporin C)

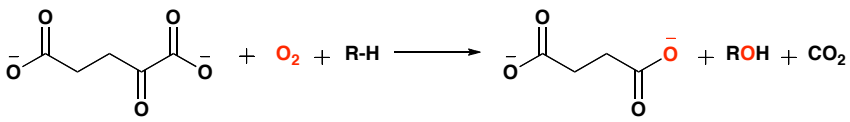
Costas et al. Chem Rev 2004, 104, 953

16

16

Taurine : α-ketoglutarate Dioxygenase (TauD)

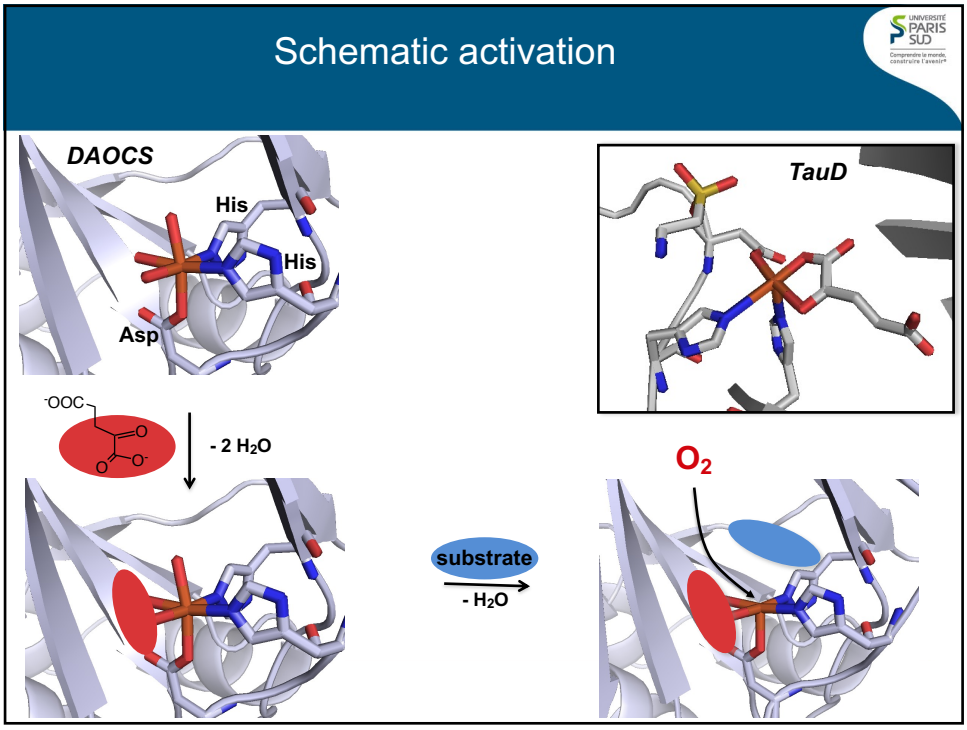




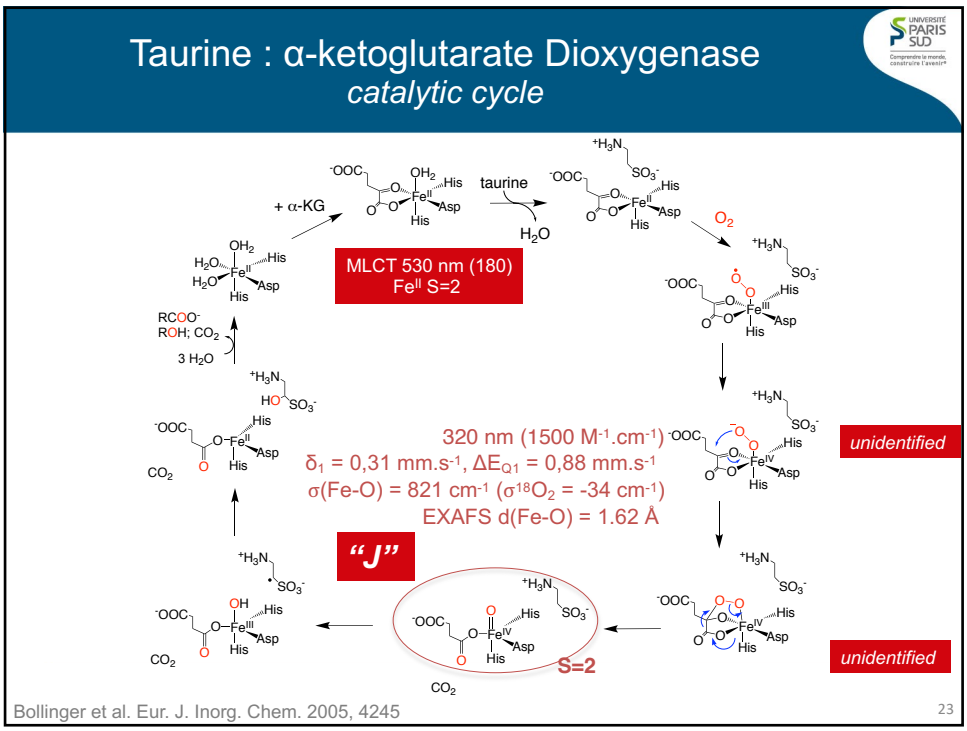
complete reduction of O_2 : 2 e^- from the substrate & 2 e^- from the cofactor

17

17


Schematic activation
DAOCS
His
His
Asp
TauD
-OOC
- 2 H2O
O2
substrate
- H2O

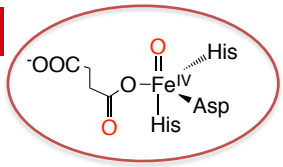
19


Taurine : α-ketoglutarate Dioxygenase
catalytic cycle
+ α-KG
taurine
H2O
O2
MLCT 530 nm (180)
FeII S=2
RCOO-
ROH; CO2
3 H2O
320 nm (1500 M-1.cm-1)
δ1 = 0,31 mm.s-1, ΔEQ1 = 0,88 mm.s-1
σ(Fe-O) = 821 cm-1 (σ18O2 = -34 cm-1)
EXAFS d(Fe-O) = 1.62 Å
"J"
unidentified
unidentified
S=2
Bollinger et al. Eur. J. Inorg. Chem. 2005, 4245
23

23

Taurine : α-ketoglutarate Dioxygenase
vibrational characteristics of "J"

"J"



320 nm (1500 $M^{-1}.cm^{-1}$)
δ_1 = 0,31 $mm.s^{-1}$, ΔE_{Q1} = 0,88 $mm.s^{-1}$
σ(Fe-O) = 821 cm^{-1} ($\sigma^{18}O_2$ = -34 cm^{-1})
EXAFS d(Fe-O) = 1.62 Å

$$\nu_{Fe-O} = 2\pi c\sqrt{\frac{k_{Fe-O}}{\mu_{Fe-O}}}$$

$$\frac{\nu_{Fe-^{16}O}}{\nu_{Fe-^{18}O}} = \sqrt{\frac{\mu_{Fe-^{18}O}}{\mu_{Fe-^{16}O}}} = \sqrt{\frac{\frac{56\times18}{56+18}}{\frac{56\times16}{56+16}}} = 1.046$$

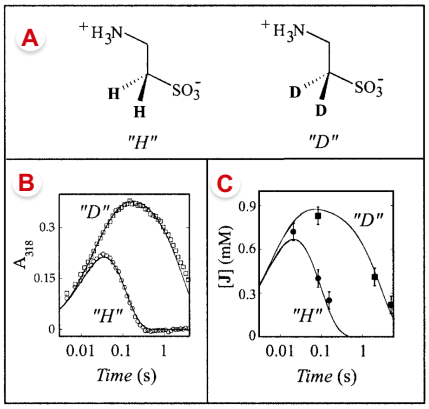
$$\Rightarrow \nu_{Fe-^{18}O} = 0.956\times\nu_{Fe-^{16}O}$$

$$\Rightarrow \nu_{Fe-^{18}O} = 0.956\times821 = 785\ cm^{-1}\ (-36)$$

24

24

Reactivity of "J"

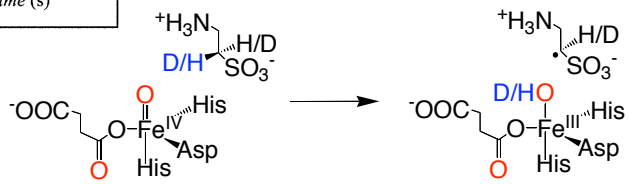


(A) Structures of 1,1-[^{1}H]2-taurine **"H"** and 1,1-[^{2}H]2-taurine **"D"**.

Kinetics of ***J*** monitored by **(B)** stopped-flow absorption and **(C)** RFQ Mössbauer.
The circles and squares represent the experimental data obtained using ***"H"*** and ***"D"***, respectively.
The solid lines are simulations for ***J*** according to the catalytic cycle.

→ $k_H / k_D \approx 37$

rebound mechanism



Bollinger et al. Eur. J. Inorg. Chem. 2005, 4245

26

26

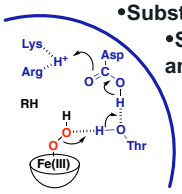
en résumé…

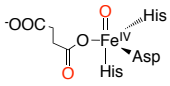
monooxygenases $S + O_2 + 2\,e^- + 2\,H^+ \rightarrow SO + H_2O$

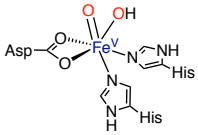
reductase dependent dioxygenases $S + O_2 + 2\,e^- + 2\,H^+ \rightarrow S(OH)_2$

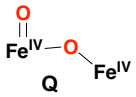
cofactor dependent dioxygenases and hydroxylases $S + O_2 + \text{cofactor} \rightarrow SO + \text{cofactor}^{ox}$

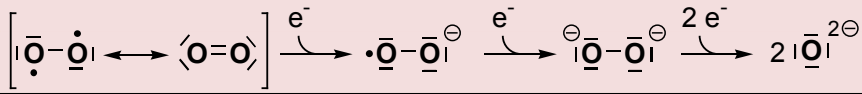
- **1st coordination sphere**
- Substrate binding and preorganization
- Substrate triggered reactivity and 2nd sphere interactions











27

41

41