



COLLÈGE
DE FRANCE
—1530—

Les hydrogénases: des biocatalyseurs pour la production et l'oxydation de l'hydrogène

Marc Fontecave

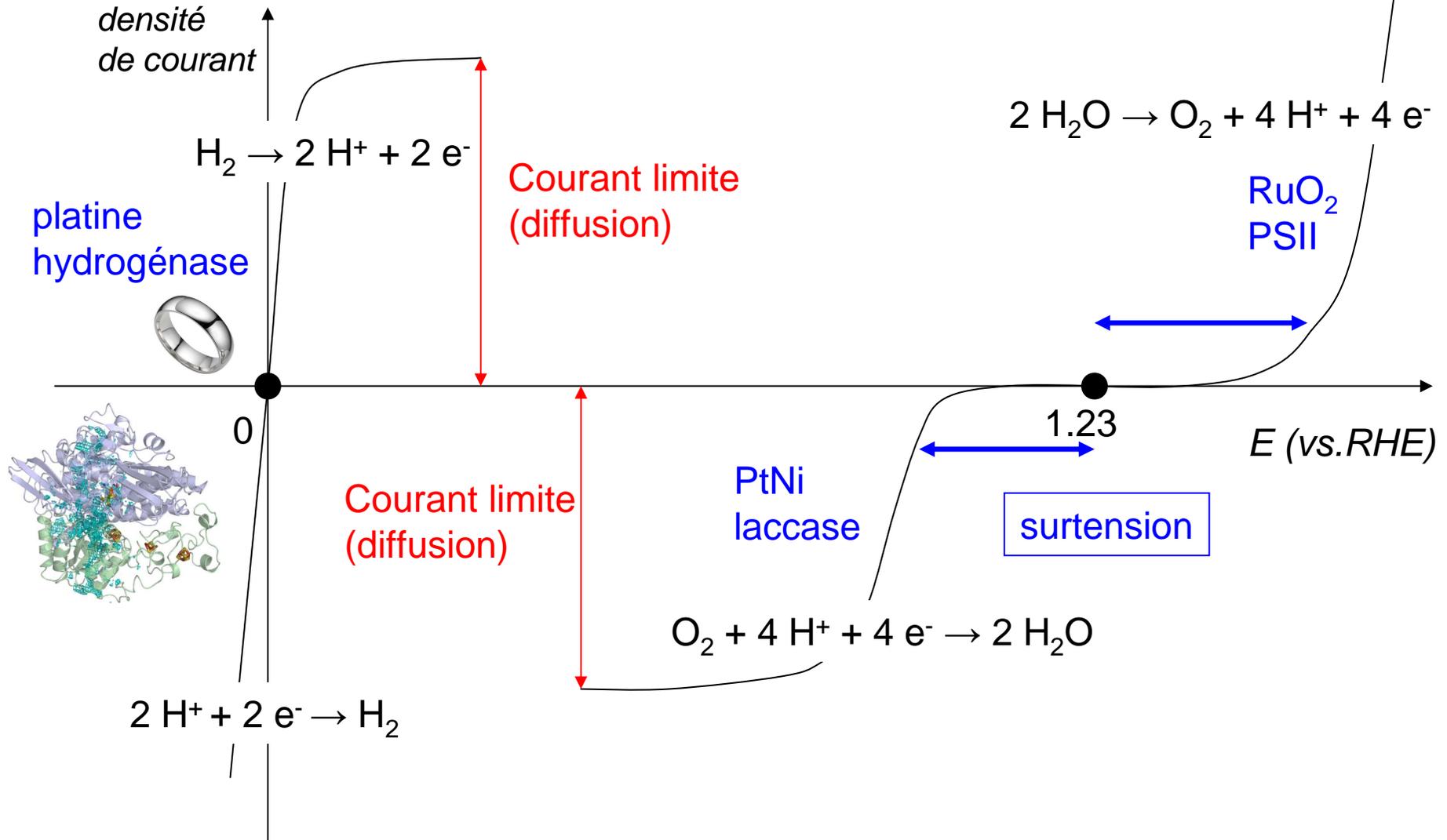
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Collège de France, 11 Place Marcelin Berthelot, 75231 Paris Cedex 05

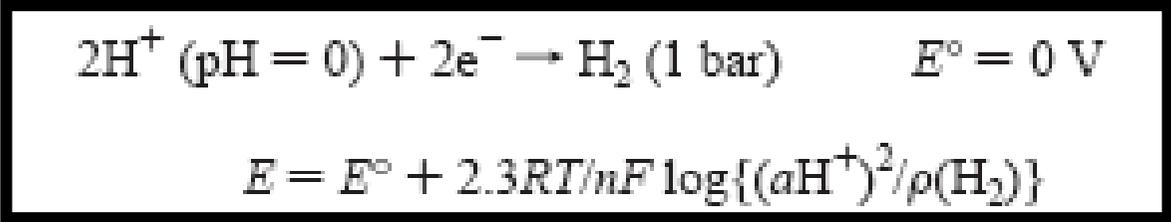
F. Armstrong: Chem Rev 2007 107, 4366,
Chem Rev 2008, 108, 2439

J. Fontecilla-Camps: Chem Rev 2007 107, 4273

Réactions rédox de l'eau



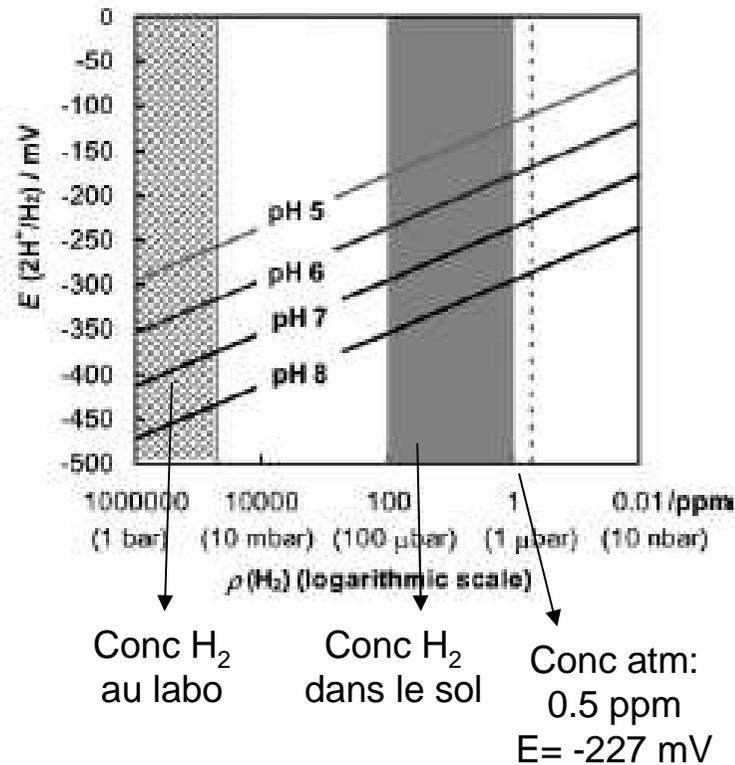
Thermodynamique



Électrode standard à H₂: pH=0; 25°C; 1 bar H₂

pH=7; 1 bar H₂ E'° = -413 mV

Conditions physiologiques ρ(H₂) <<1 bar; E'>>-413 mV



Cinétique ?

Réaction à deux électrons

2 réactions monoélectroniques

1 intermédiaire

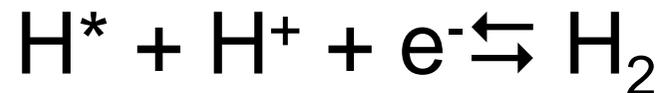
$$E^0 = 0 \text{ V}$$



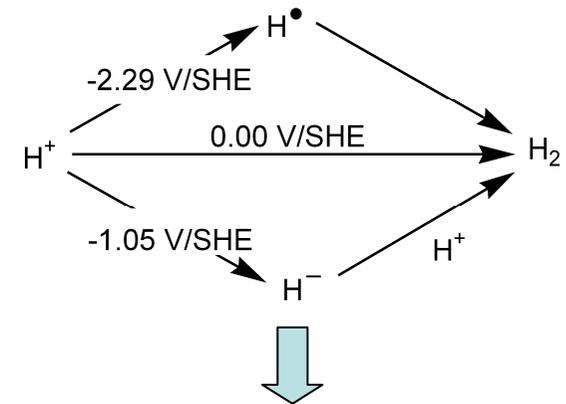
$$E_1^0 = - \Delta G (\text{H}^*)/e^0$$



$$E_2^0 = + \Delta G (\text{H}^*)/e^0$$



$$(E_1^0 + E_2^0)/2 = E^0$$

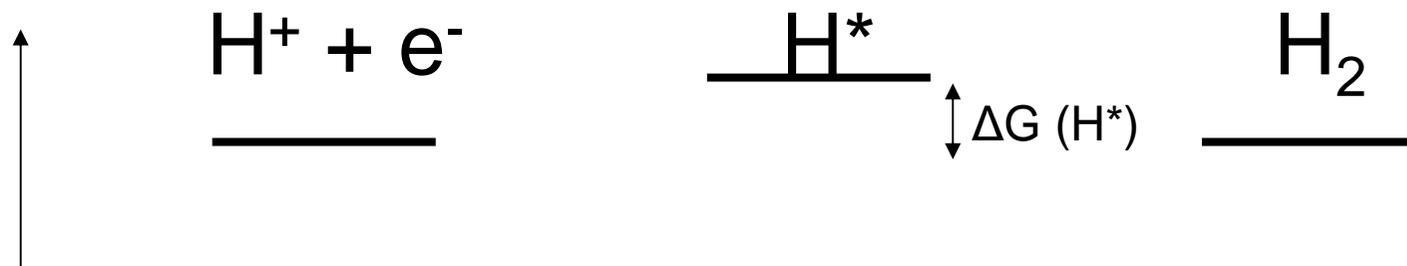


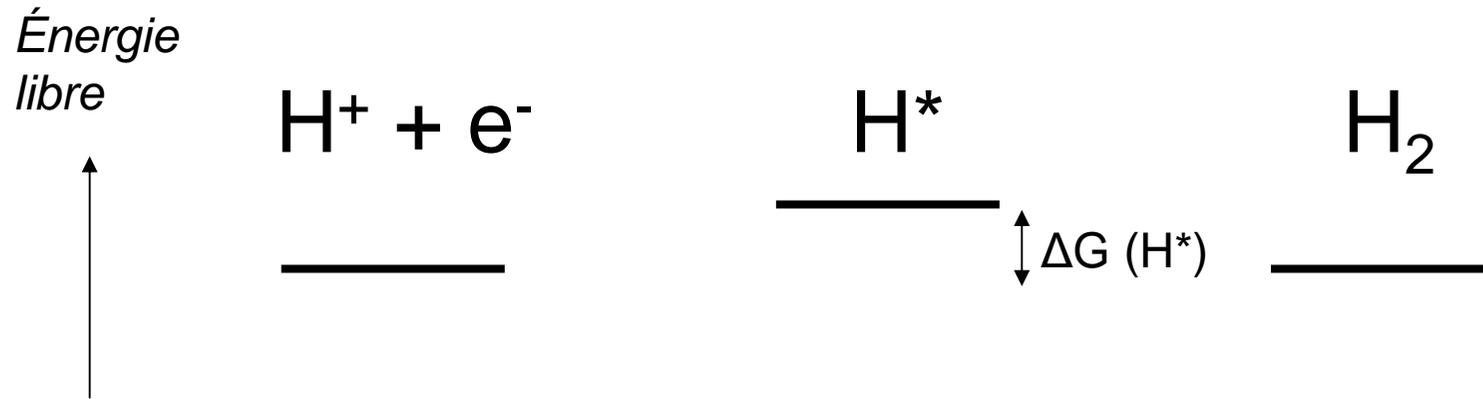
Des catalyseurs pour stabiliser H^* (ou H^-)



$$\Delta G = 0 \text{ kcal. mol}^{-1}$$

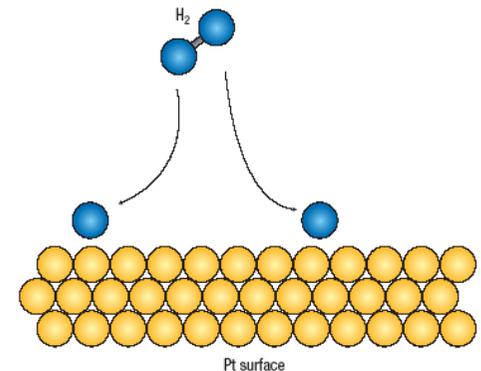
Énergie
libre



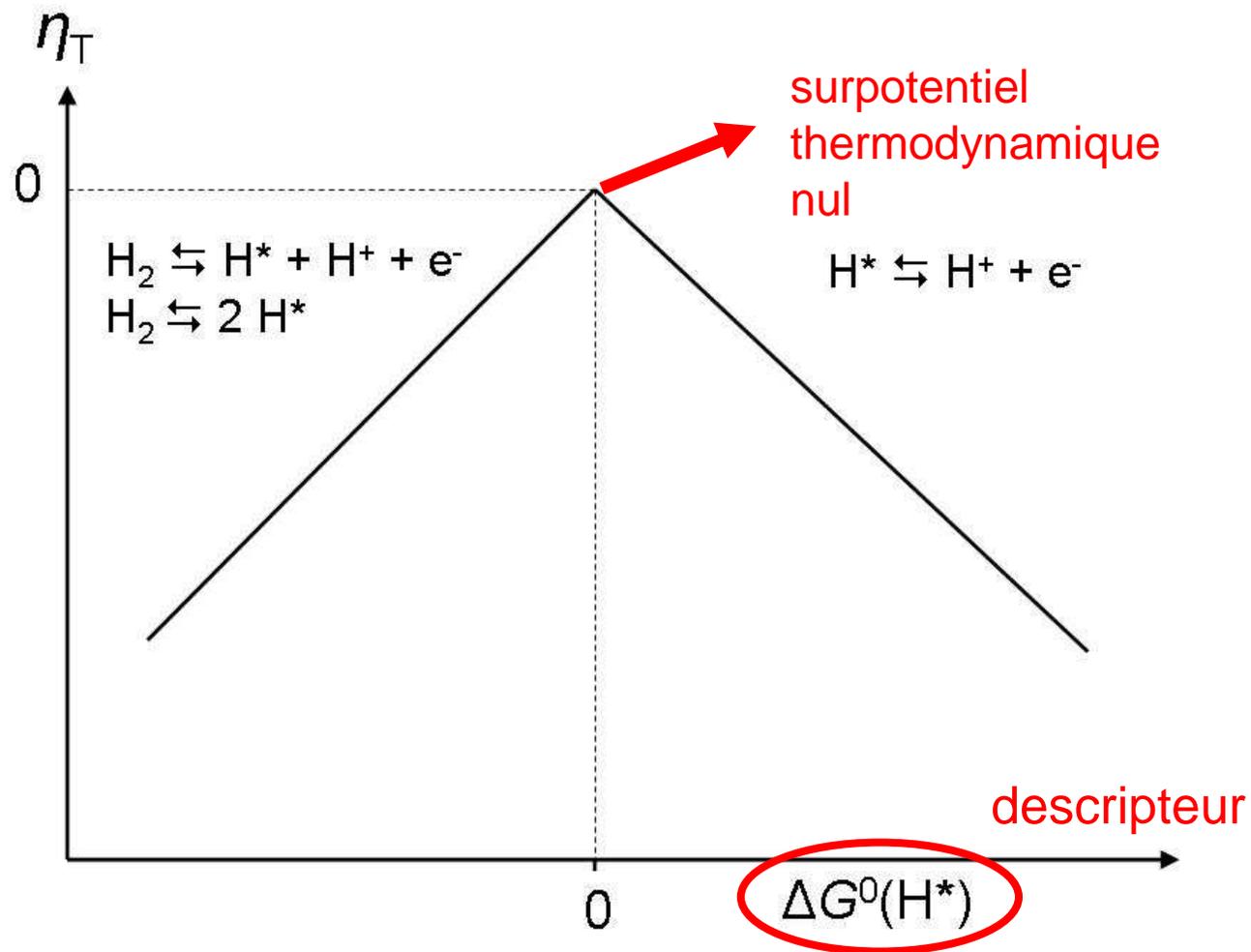


L'électrocatalyseur optimal est celui pour lequel chaque étape est thermodynamiquement neutre ($\Delta G (H^*)=0$).

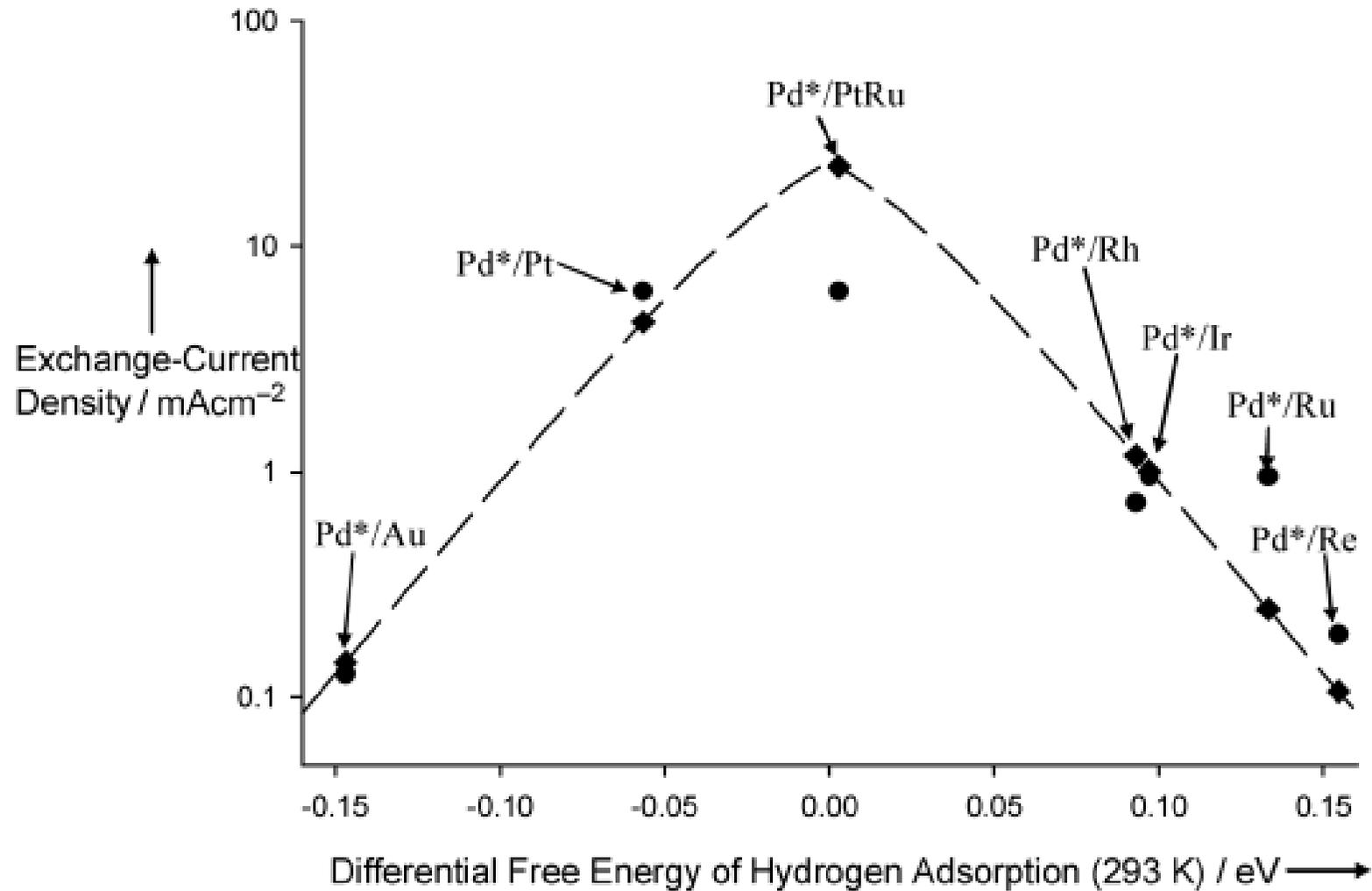
L'intermédiaire H^* doit se fixer au catalyseur avec une énergie de liaison = $\frac{1}{2} E(H-H)$.



Le surpotentiel thermodynamique η_T est la différence entre le potentiel de l'étape qui détermine le potentiel et le potentiel d'équilibre global η_T peut être nul !



Vérification expérimentale



Le **platine** métallique est le catalyseur idéal...
 L'**hydrogénase** aussi...
 (optimisation de la fixation de H*)

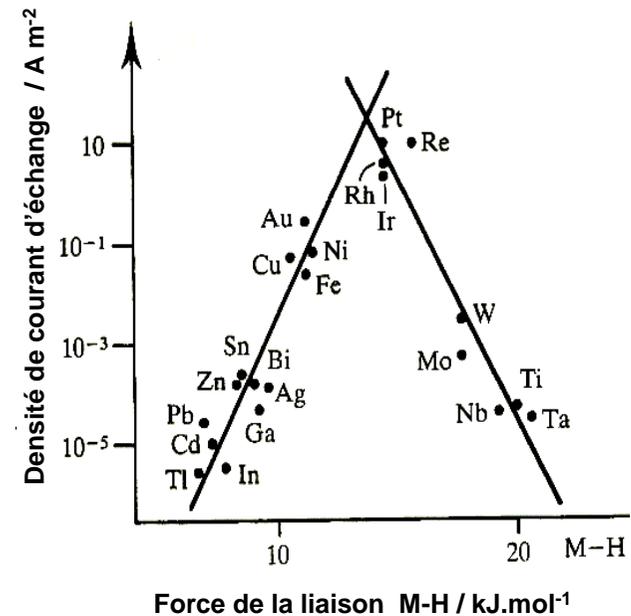
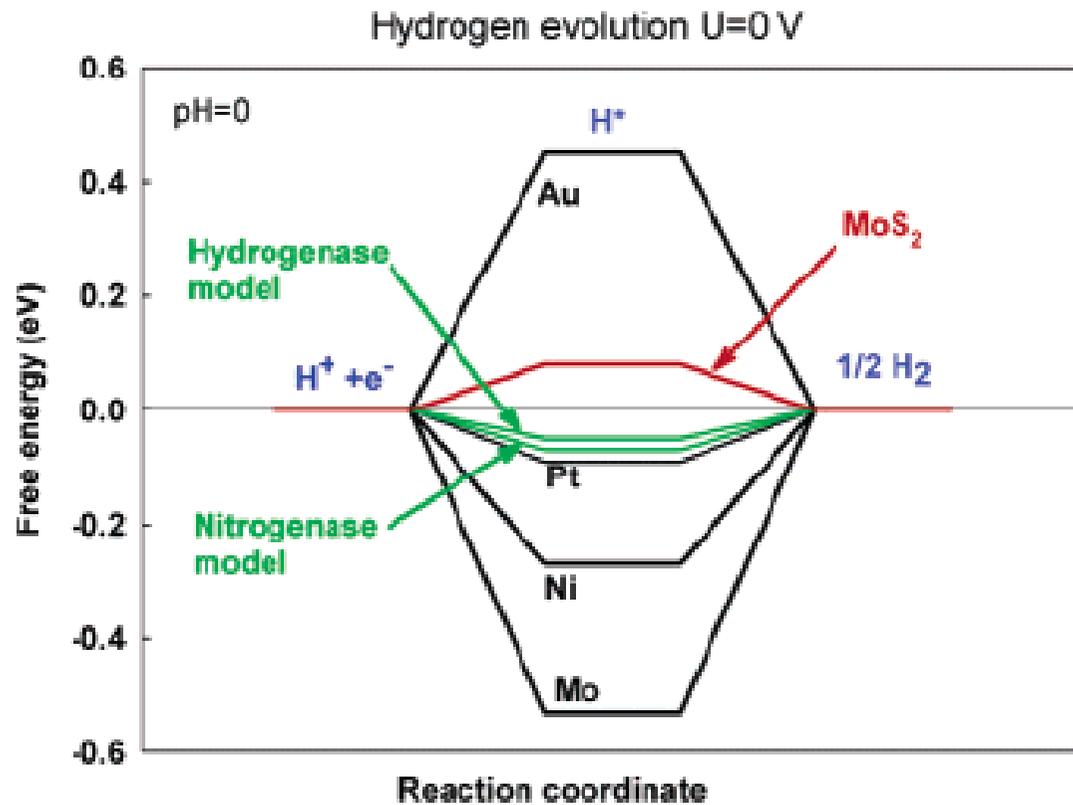
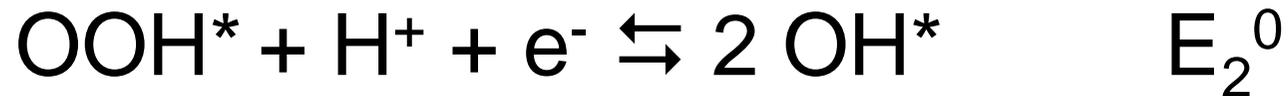
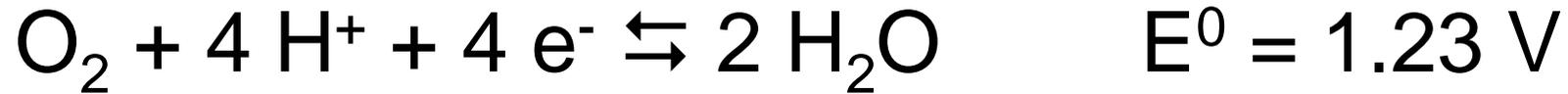


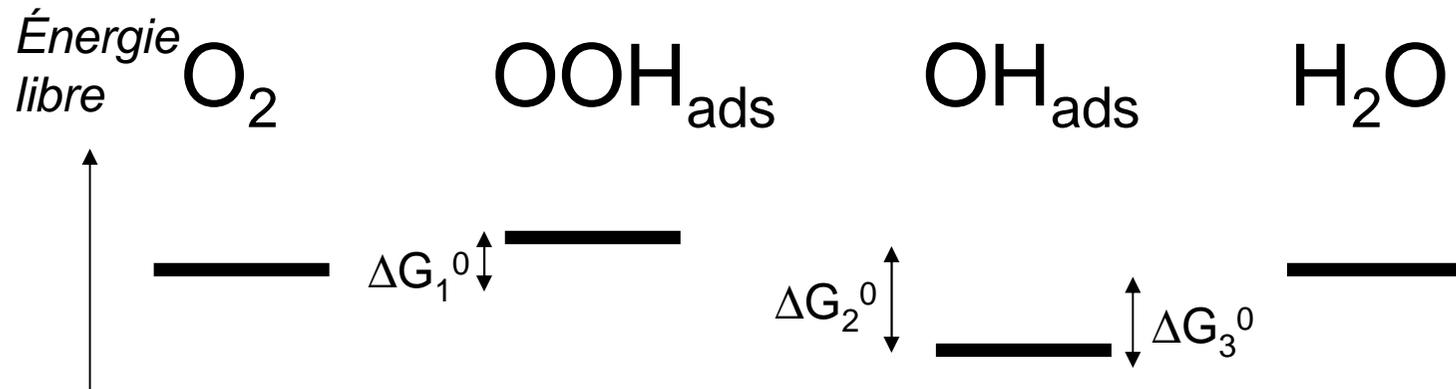
Figure 1. Calculated free energy diagram for hydrogen evolution at a potential $U = 0$ relative to the standard hydrogen electrode at $\text{pH} = 0$. The free energy of $\text{H}^+ + \text{e}^-$ is by definition the same as that of $1/2 \text{H}_2$ at standard conditions. The free energy of H atoms bound to different catalysts is then

Réactions avec plus de deux électrons,
plus d'un intermédiaire



Contrainte thermodynamique:

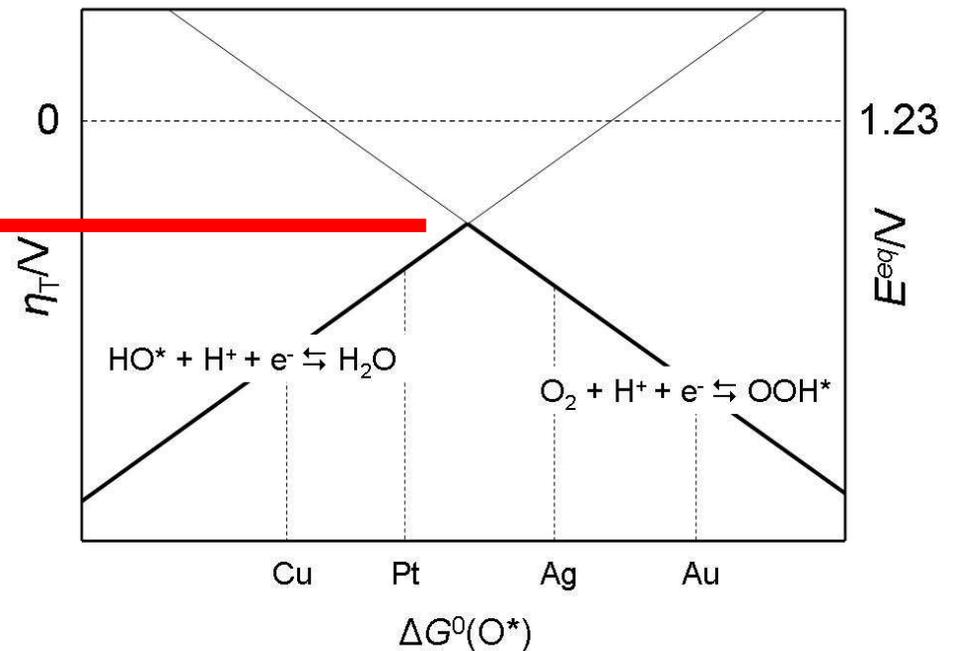
$$(E_1^0 + E_2^0 + 2 E_3^0)/4 = E^0$$



Le surpotentiel thermodynamique dépend des affinités du catalyseur pour différentes (>1) formes d'oxygène activé
 ηT ne peut pas être nul !

surtension thermodynamique non nulle

Les meilleurs catalyseurs (mais avec surtension non nulle)
 Pt, laccase



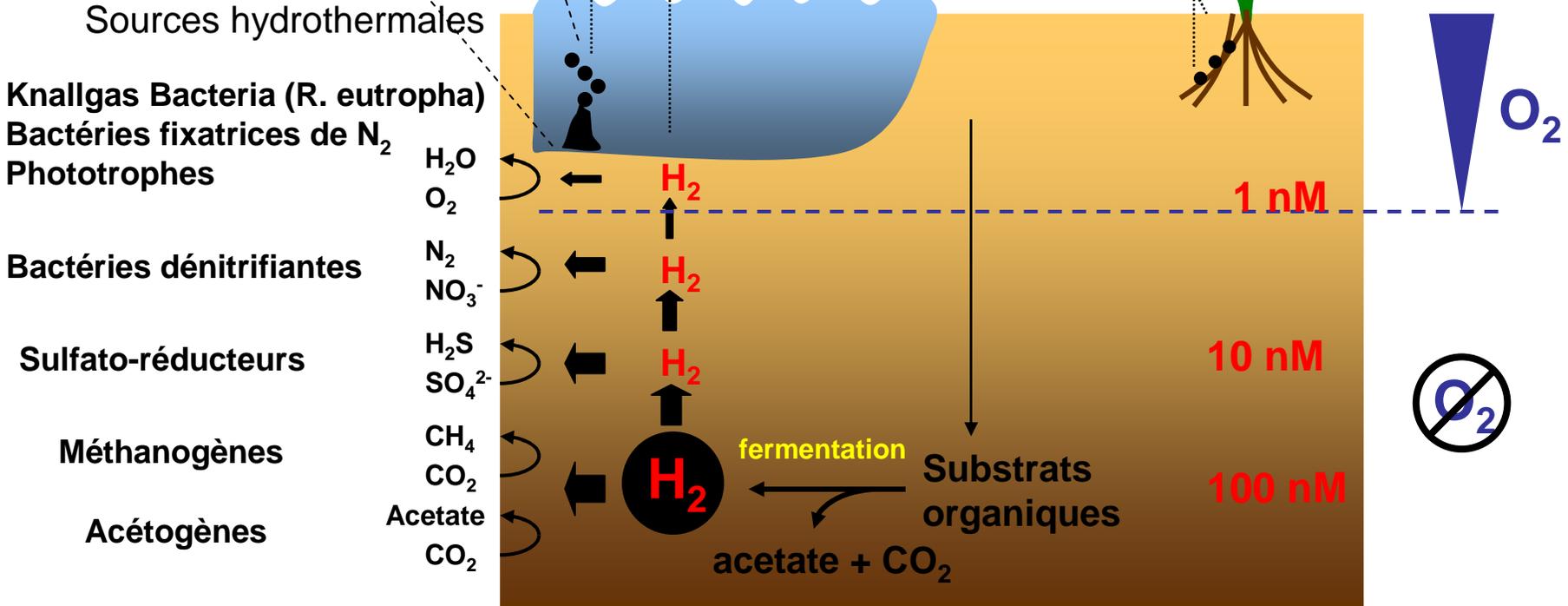
Hydrogène dans l'environnement



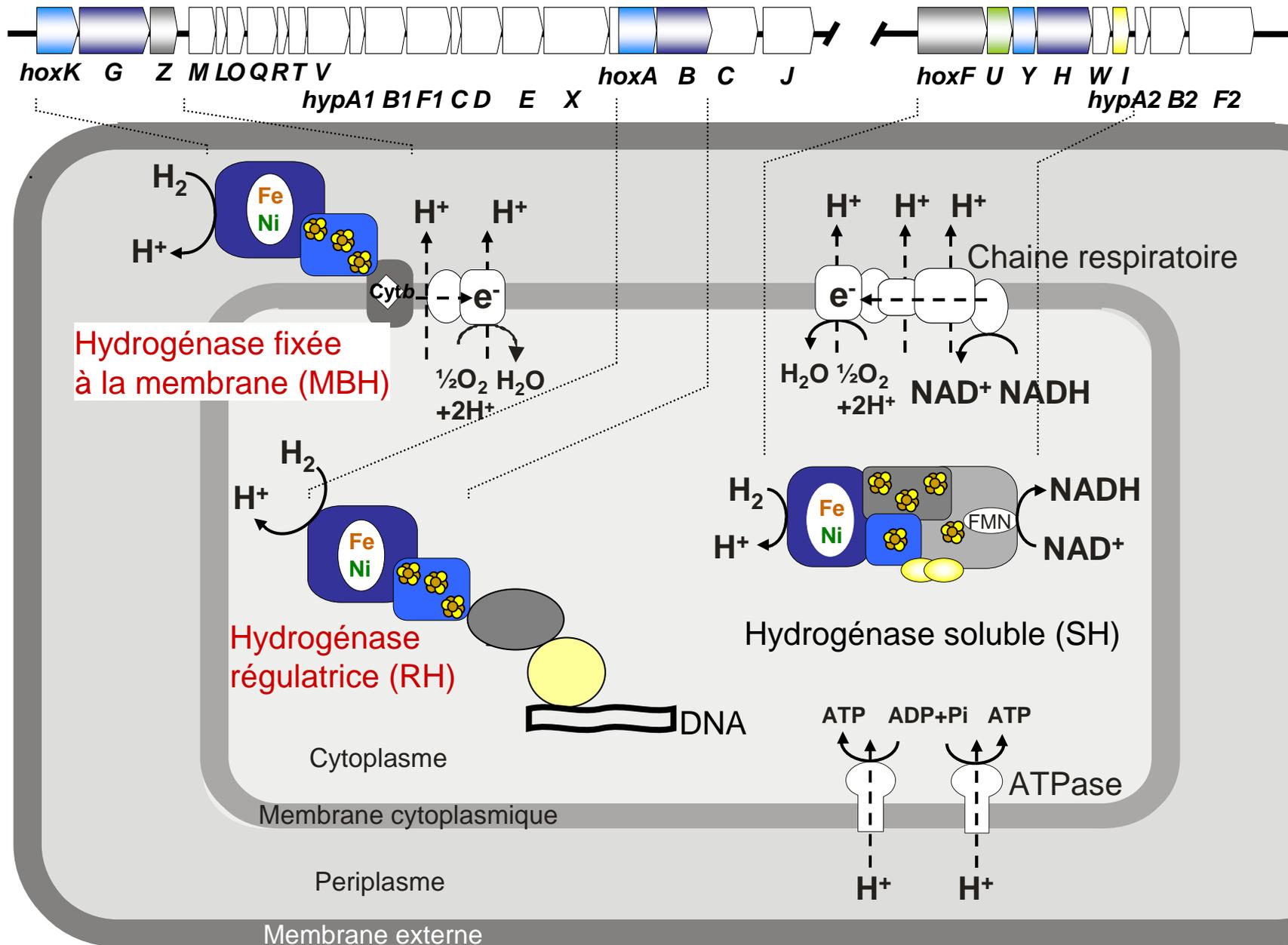
Robert D. Ballard



John Boman



Hydrogénases (*Ralstonia eutropha*) et chaînes de transfert d'électrons



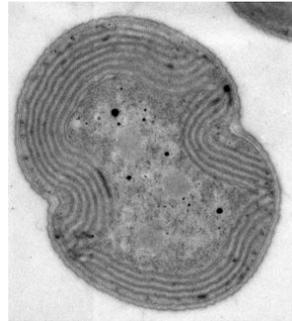
Autres environnements :

- **Volcans** et organismes thermophiles et hyperthermophiles
- **Intestin** des mammifères et méthanogènes
et bactéries sulfato-réductrices (fermentation des carbohydrates)
- *Helicobacter pylori*, pathogène gastrique (**estomac**)
- microalgues et cyanobactéries (photosynthèse)

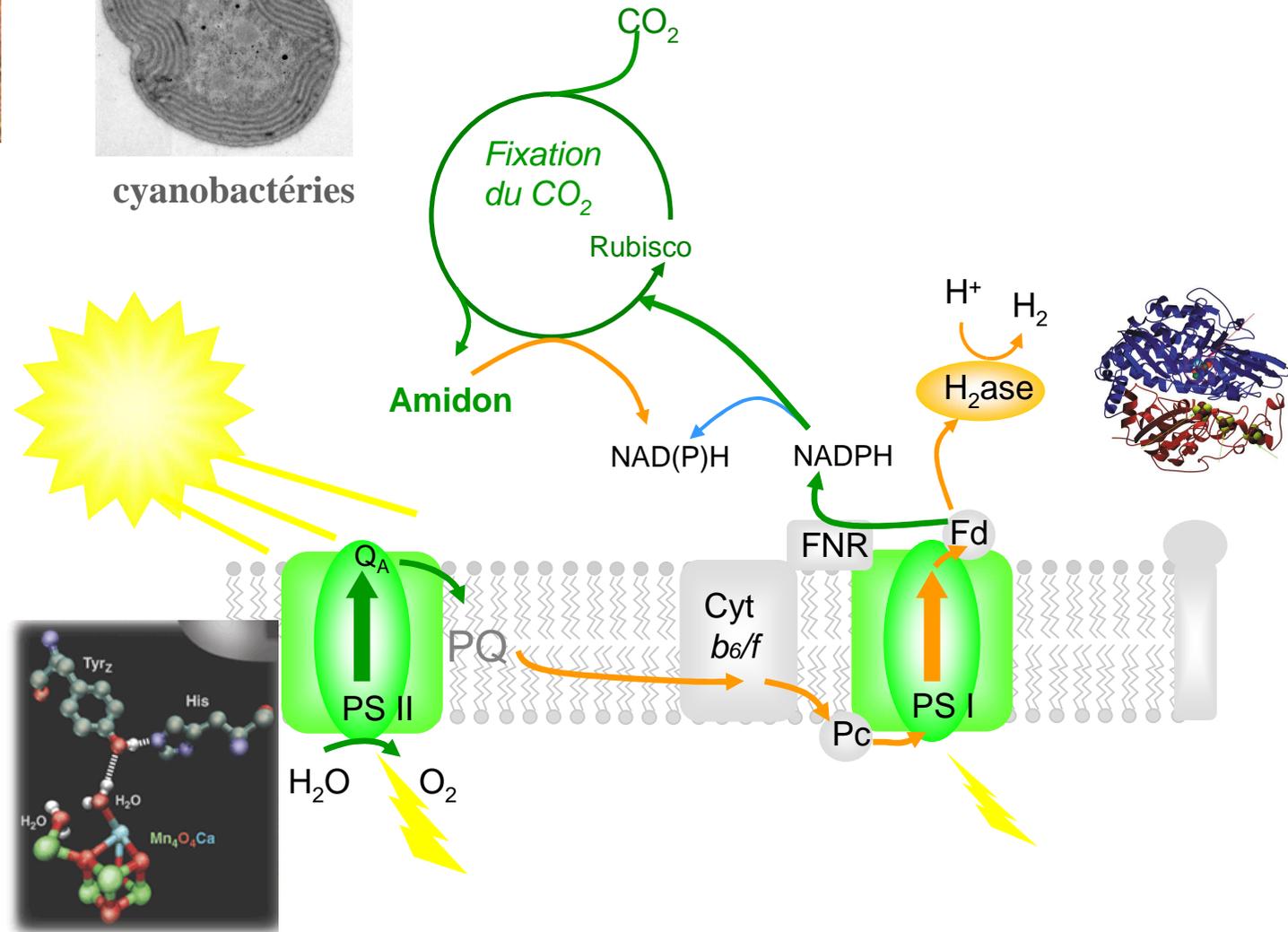
microorganismes photosynthétiques



microalgues



cyanobactéries

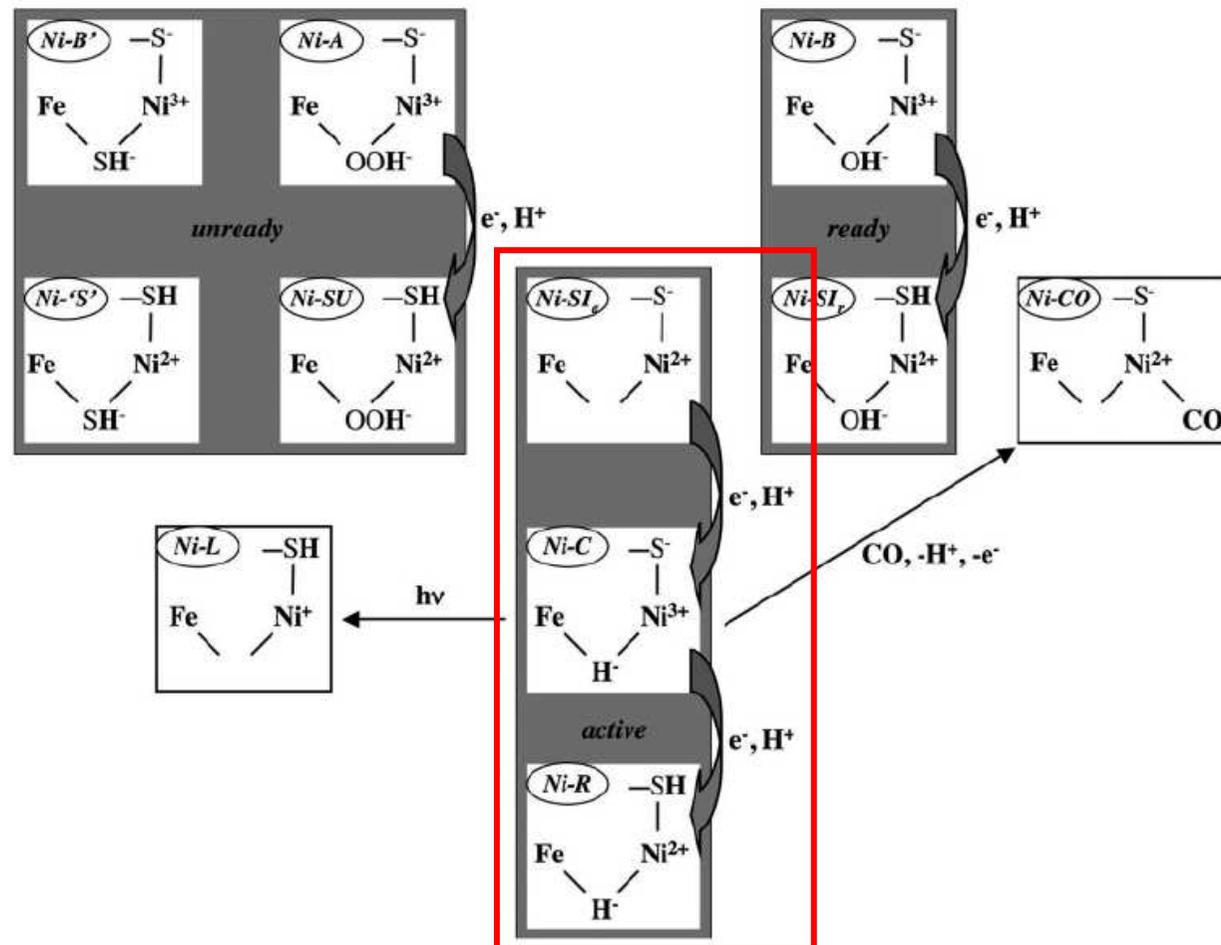


Historique des hydrogénases: la lumière vient des cristaux

1931: Stephenson et Stickland découvrent les hydrogénases

1982: présence de Ni

1982-1994: une grande variété de formes



Historique: la lumière vient des cristaux

1931: Stephenson et Stickland découvrent les hydrogénases

1982: présence de Ni

1982-1994: une grande variété de formes

1993: hydrogénase de *D. gigas* (structure 5Å résolution)

1994: présence de clusters fer-soufre

1995: Structure à 2,8 Å de résolution (Nature 373 580)

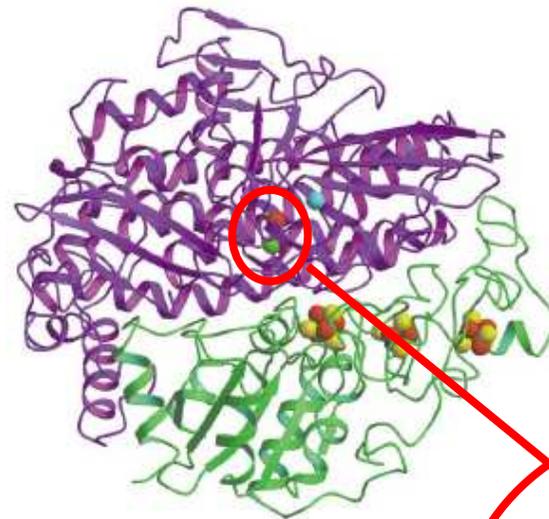
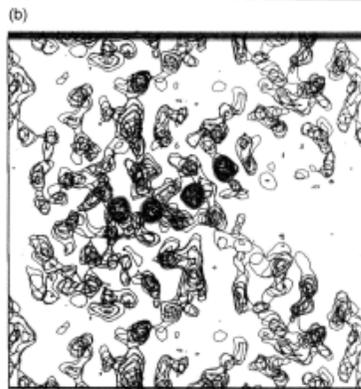
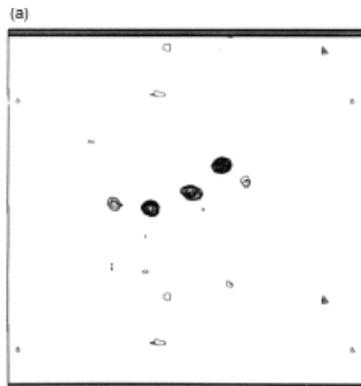


J. Fontecilla-Camps

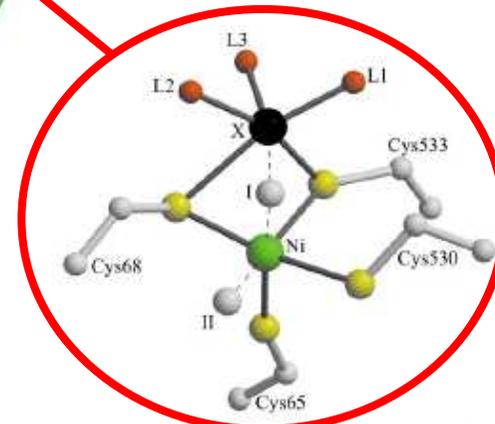
IBS, Grenoble

Chem Rev 2007

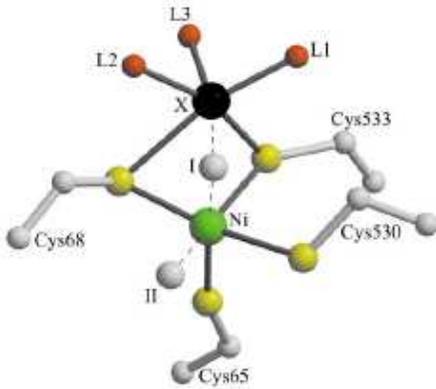
Dalton 2003



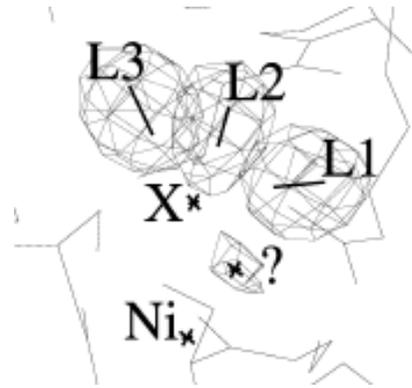
X ?
L ?
I ?



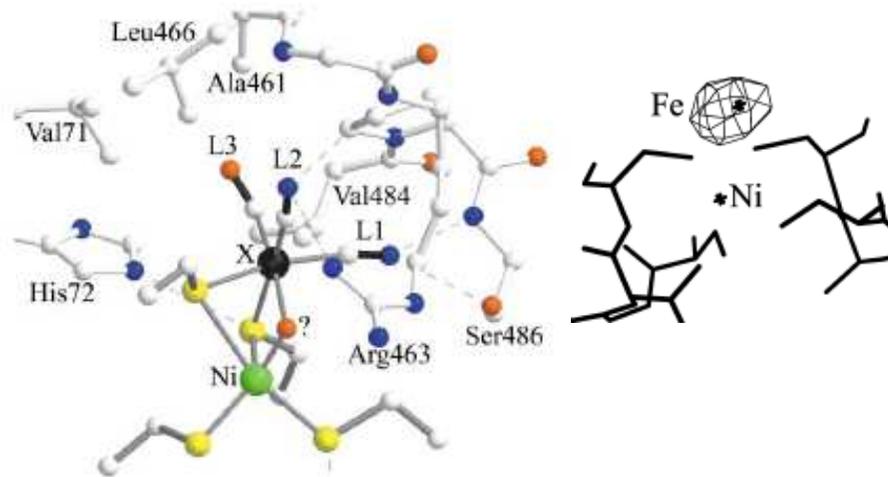
Historique: la lumière vient des cristaux



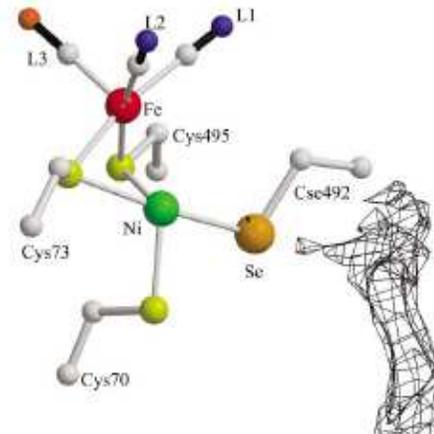
1995 Nature 373 580



1996 JACS 118 12989



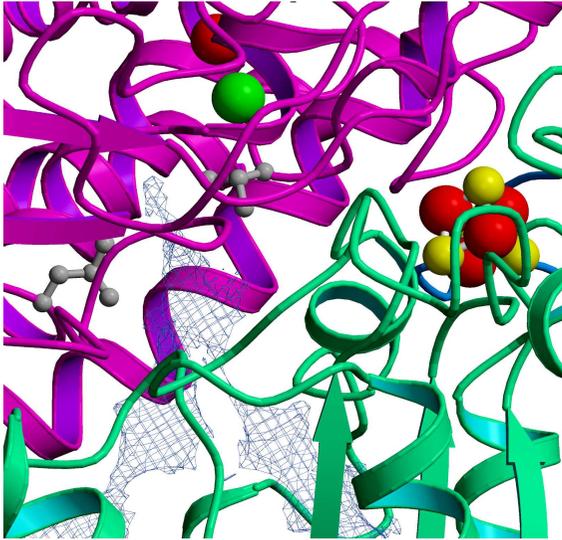
1997 JACS 119 7181
(IR S. Albracht Amsterdam)



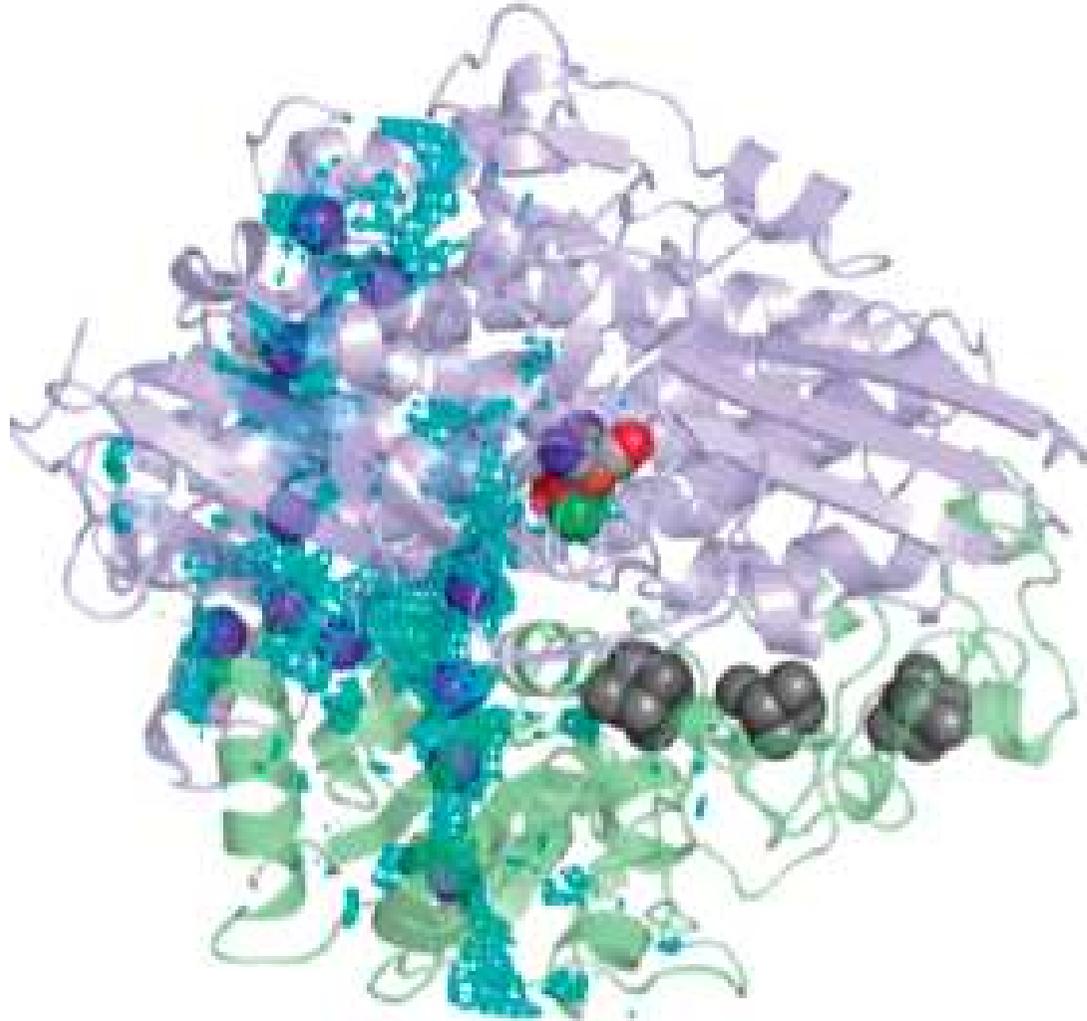
1999 Structure 7 557
Forme réduite sans pont

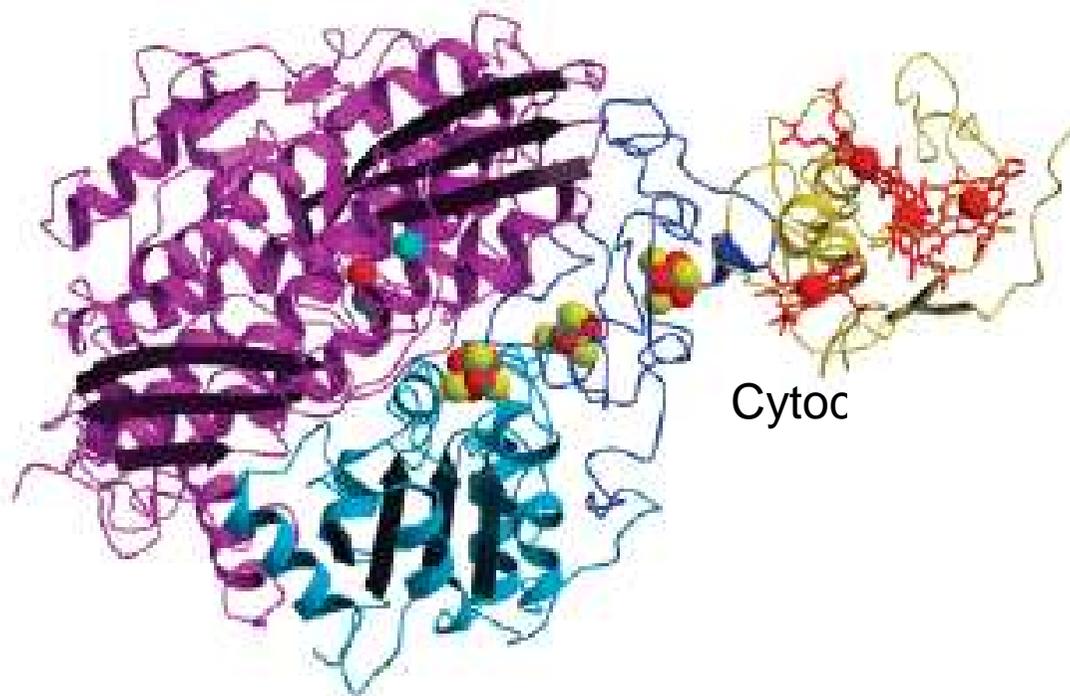
Historique: la lumière vient des cristaux

1997 Nature Str. Biol. 4 523
Des canaux hydrophobes pour
le transport des gazs

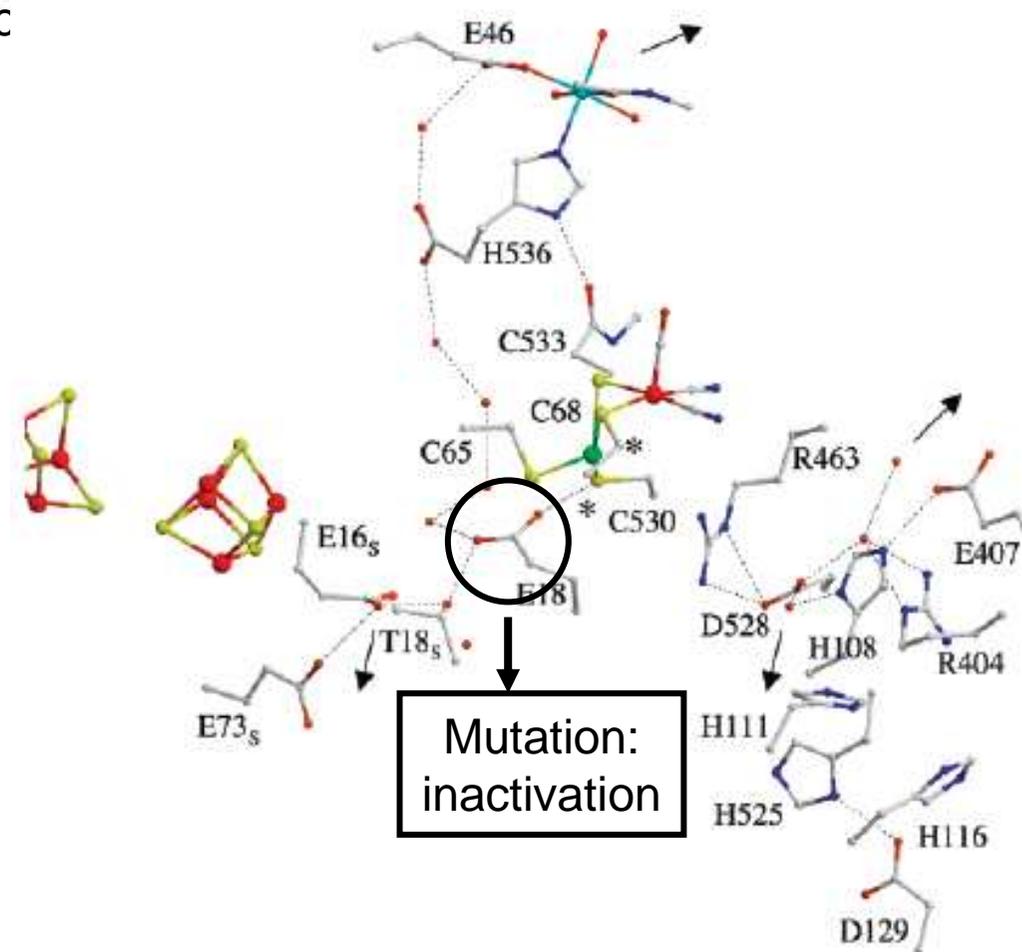


? Ni site d'interaction
avec H₂





Des chaines de transferts de:
-Électrons
-Protons

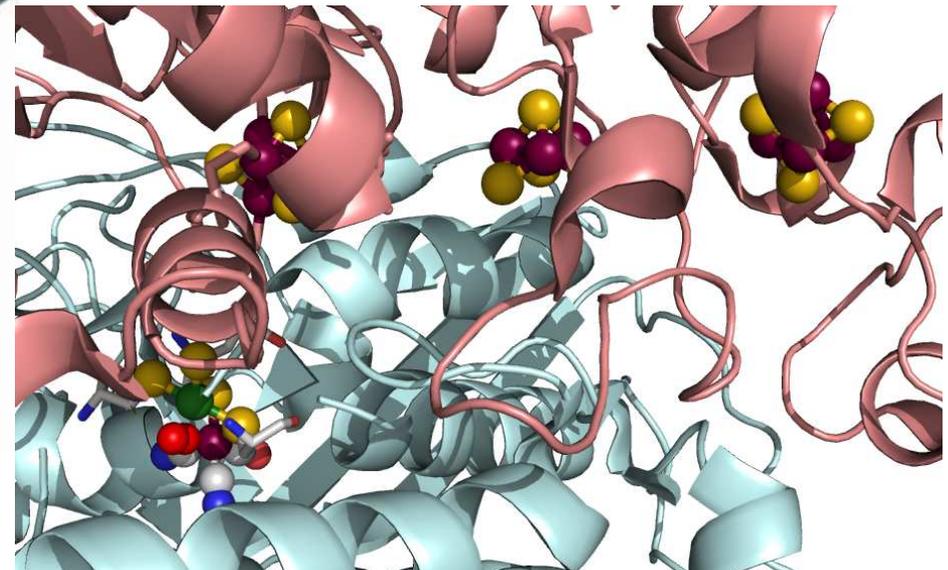
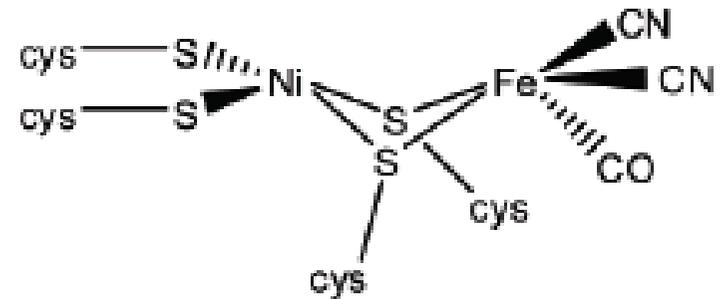
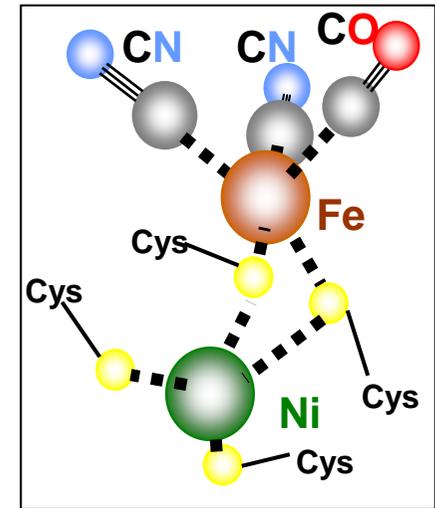
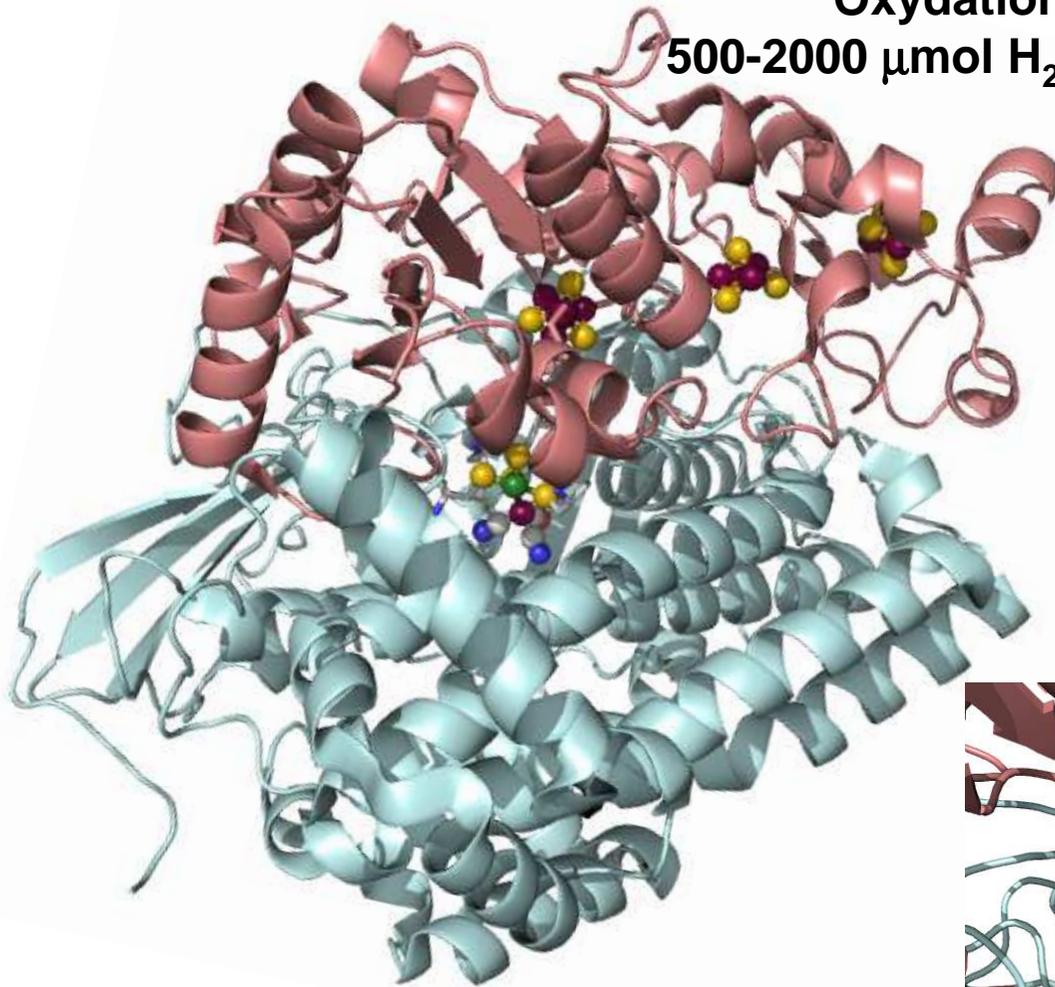


Deux classes d'hydrogénases:

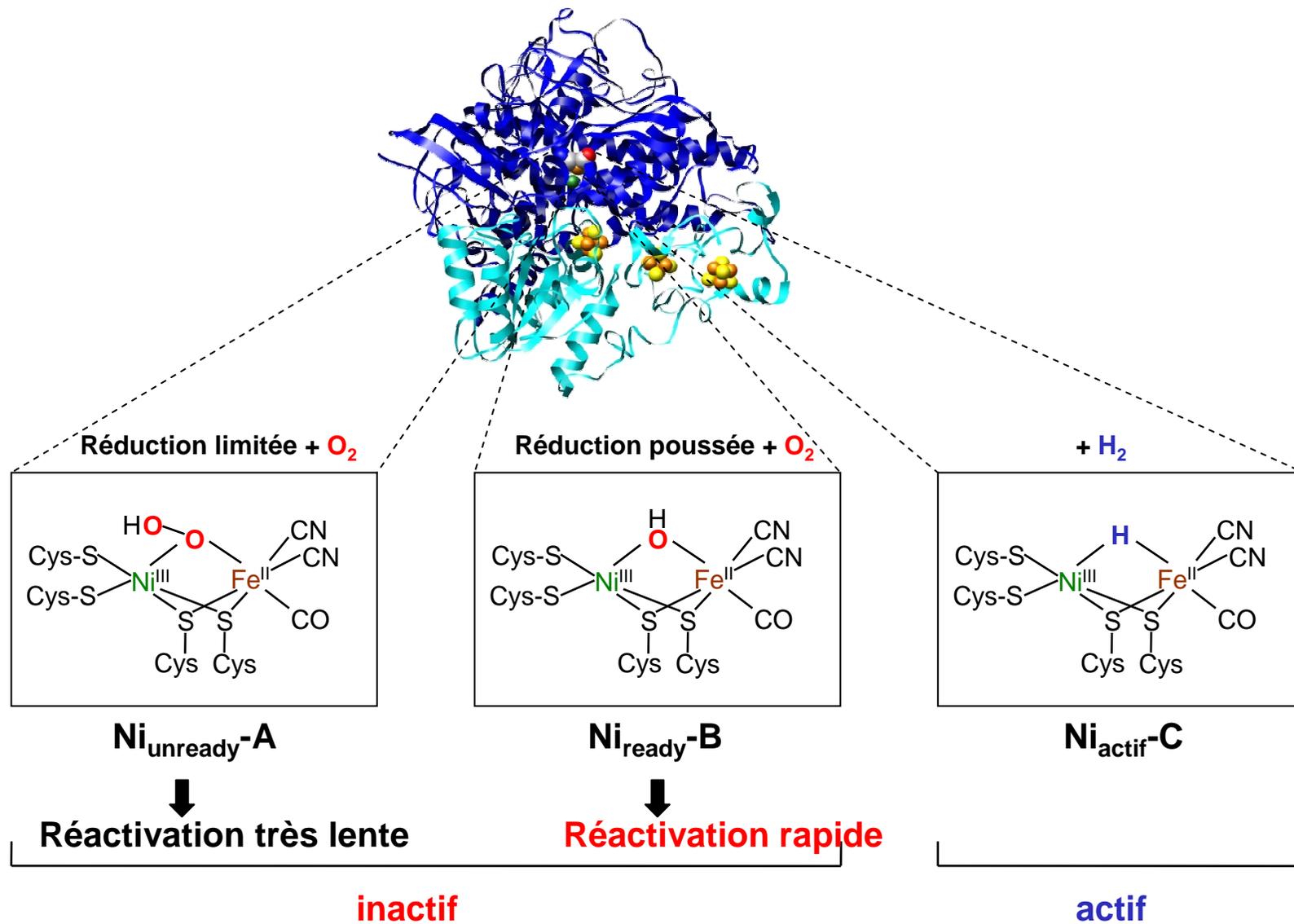
1. [Ni-Fe]-H₂ase

Oxydation H₂

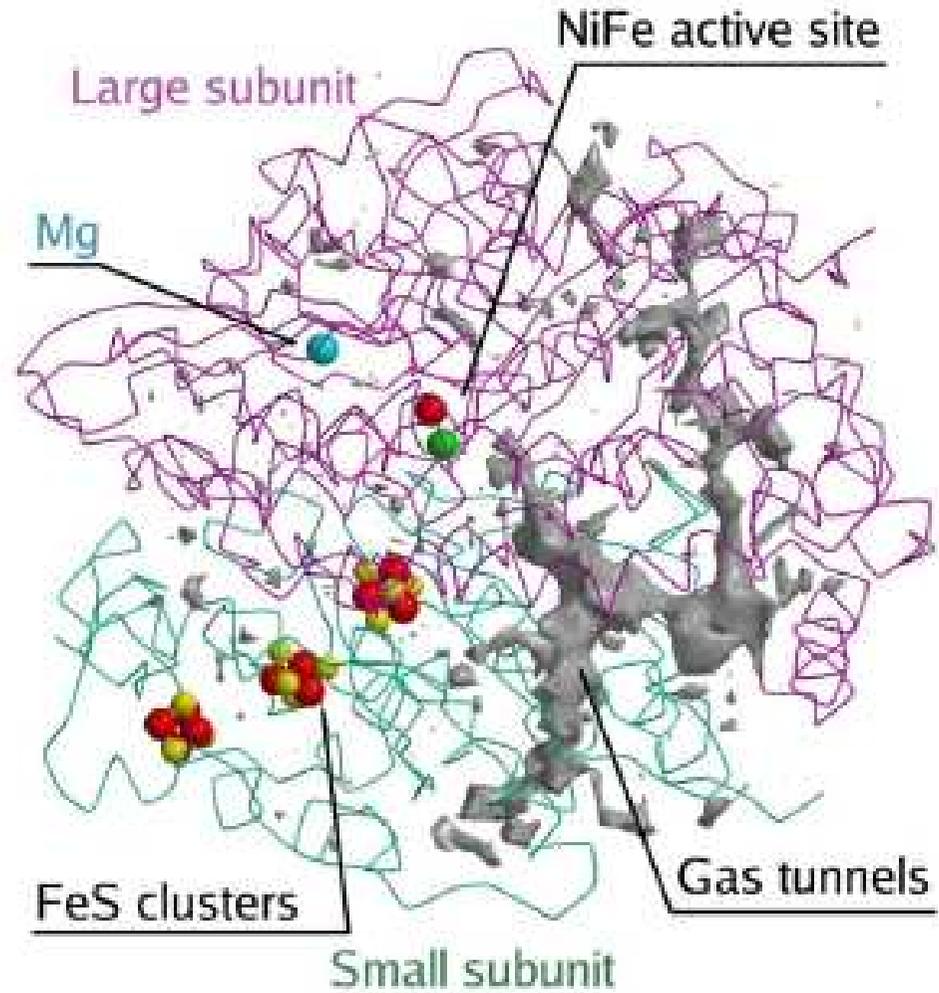
500-2000 μmol H₂ min⁻¹mg⁻¹



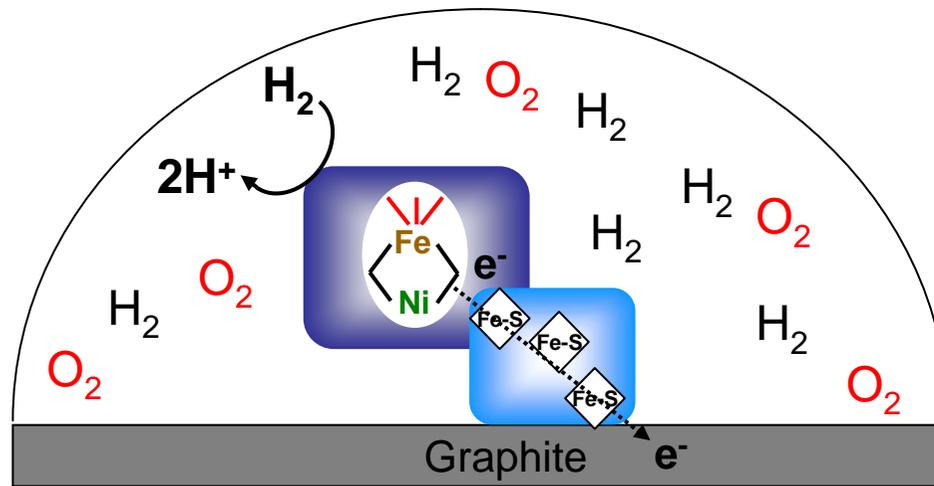
Inactivation des [NiFe] hydrogénases par l'oxygène



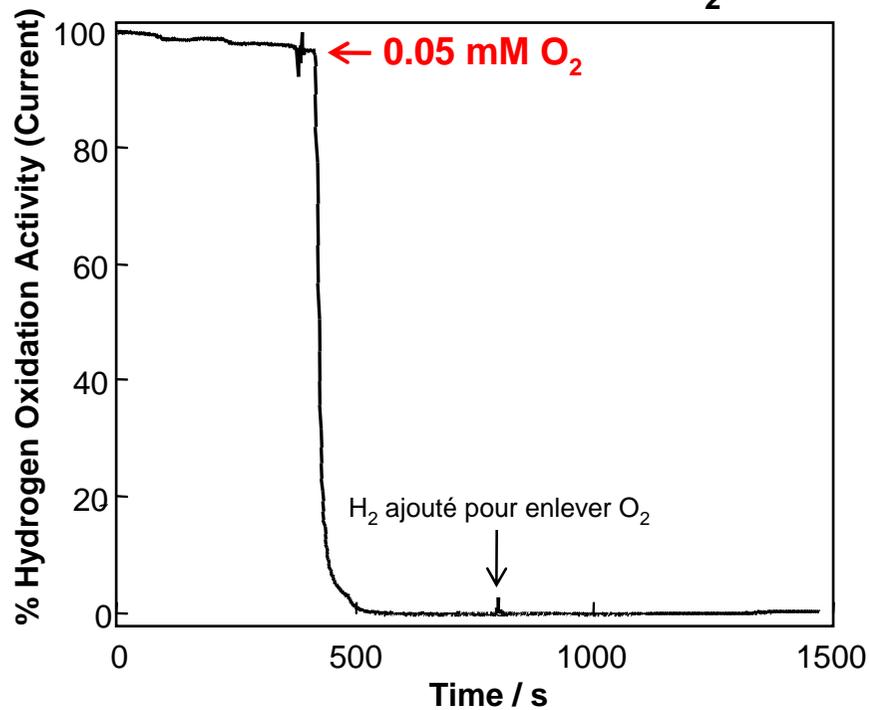
Compétition H₂-O₂: sensibilité à l'air



Les [NiFe]-hydrogénases sont sensibles à O₂



Allochromatium vinosum H₂ase

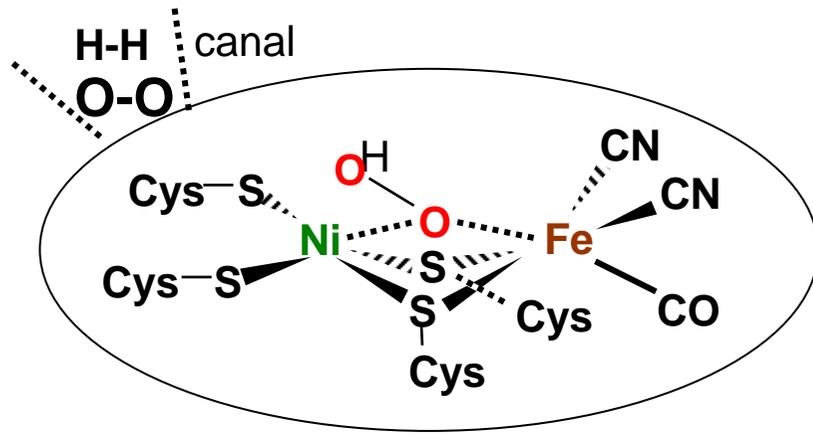


Vincent, K.A., Cracknell, J.A., Lenz, O., Zebger, I., Friedrich, B., Armstrong, F.A. (2005) *Proc. Natl. Acad. Sci. USA* 102:16951-16954.

Ludwig, M., Cracknell, J.A., Vincent, K.A., Armstrong, F.A., Lenz, O. (2009) *J. Biol. Chem.* 284:465-77

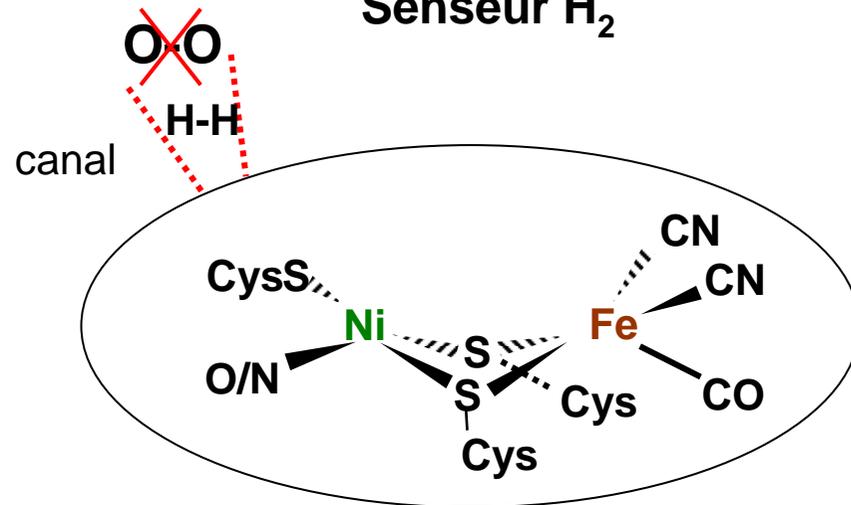
Conclusion: la taille du canal contrôle l'accès d' O₂ au site actif

Hydrogénase sensible



Volbeda *et al.* (2005) JBIC 10:239-49
Ogata *et al.* (2005) Structure 13:1635-42

Senseur H₂

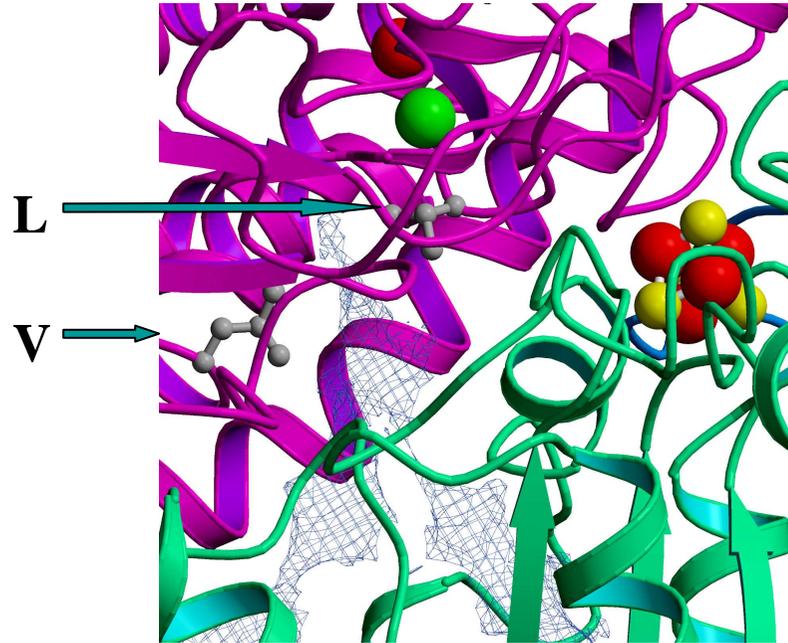


Haumann *et al.* (2003) Biochemistry 42:11004-15
Buhrke *et al.* (2005) JBC 280:23791-6

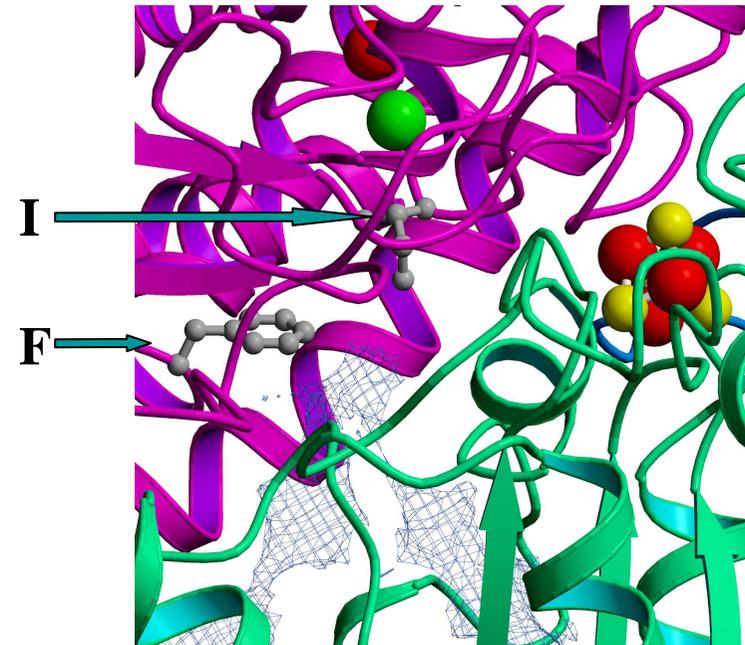


Résistance à O₂

Hydrogénases sensibles



H₂ senseurs (résistants mais peu actifs)



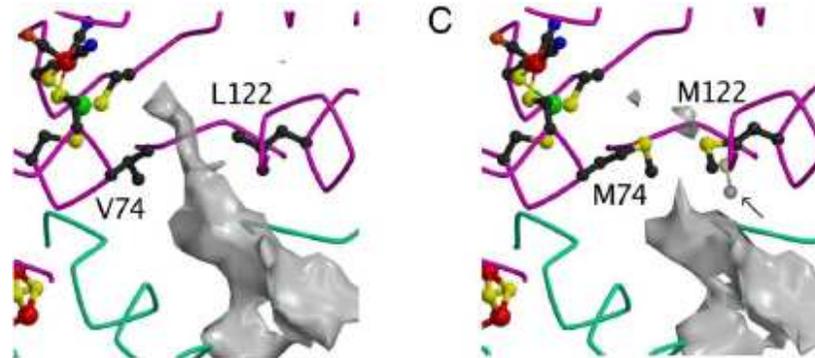
Volbeda et al., *Int. J. Hydr. Energy* (2002), **27**, 1449-1461

Senseurs H₂ : Mutation Ile + Phe en Leu + Val => **augmentation de la sensibilité à O₂**

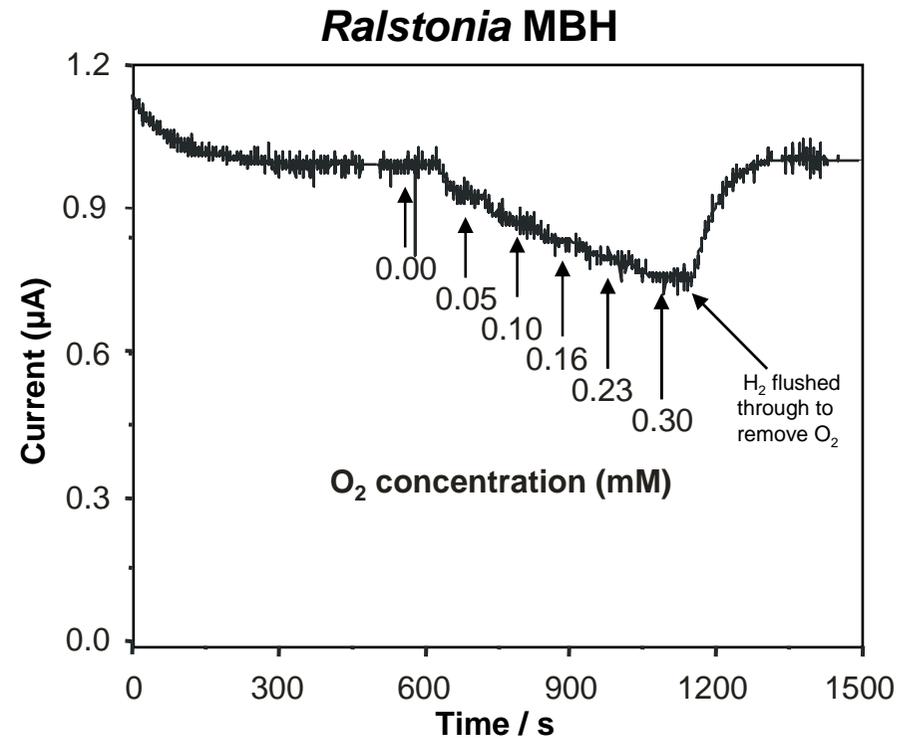
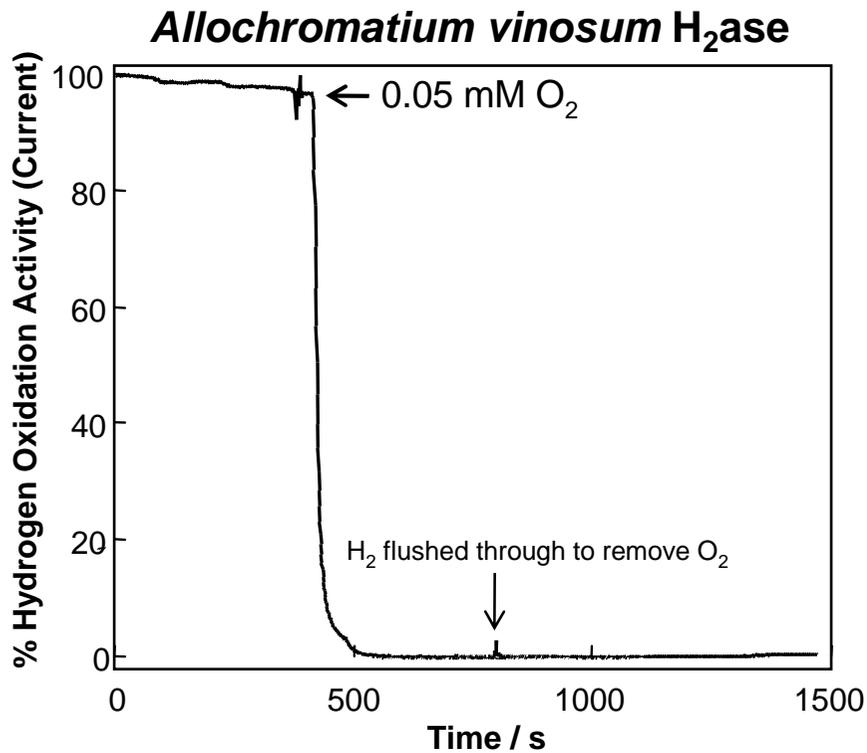
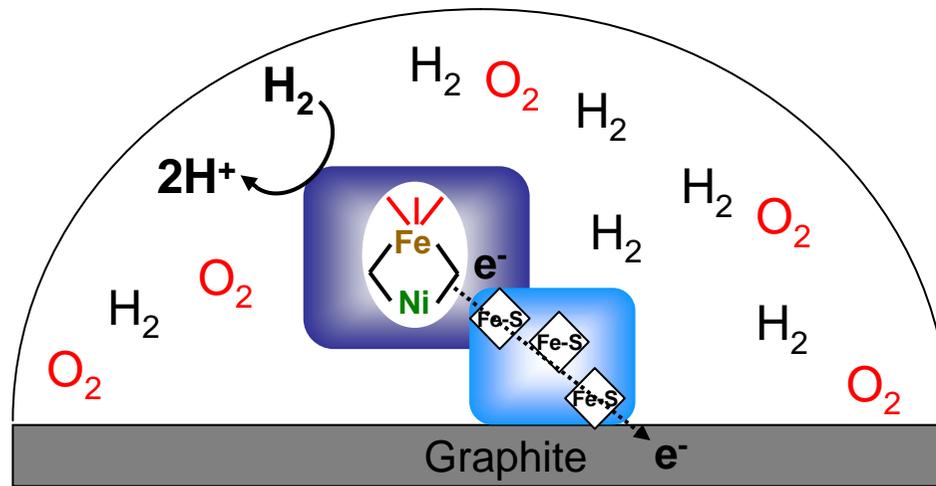
Duche, O. et coll *FEBS J.* **2005**, 272, 3899; Buhrke, T. et coll. *J. Biol. Chem.* **2005**, 280, 23791

H₂ase *D. fructosovorans* Mutation V74M-L122M => **resistance à O₂ accrue, bonne activité**

Dementin, S. et coll *J. am. Chem. Soc.* **2009**, 131, 10156; Leroux F. et coll *PNAS* **2009**, 105, 11188

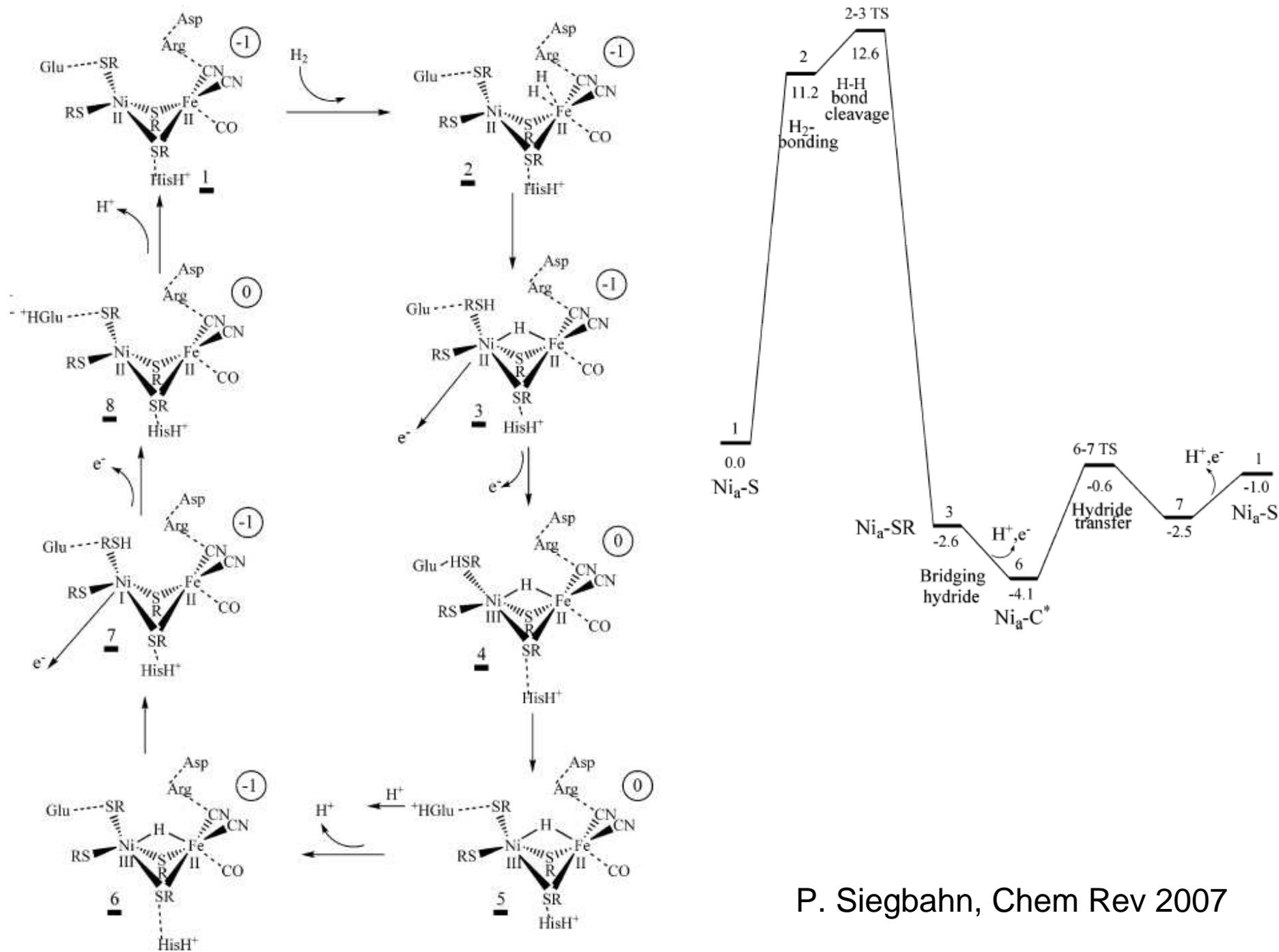


L'Hydrogénase de *R. eutropha* est résistante à O₂

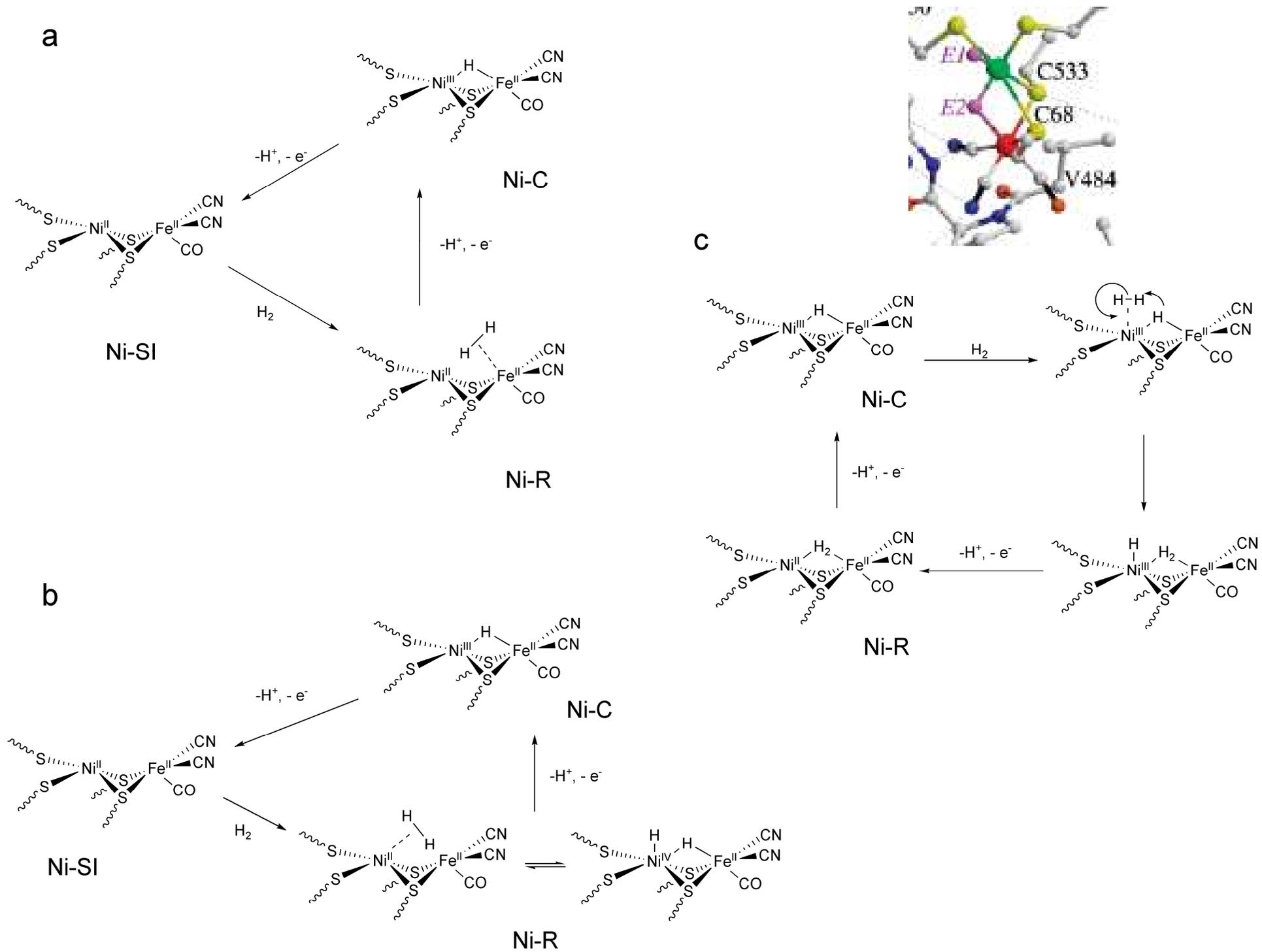


Vincent, K.A., Cracknell, J.A., Lenz, O., Zebger, I., Friedrich, B., Armstrong, F.A. (2005) *Proc. Natl. Acad. Sci. USA* 102:16951-16954.

Ludwig, M., Cracknell, J.A., Vincent, K.A., Armstrong, F.A., Lenz, O. (2009) *J. Biol. Chem.* 284:465-77



P. Siegbahn, Chem Rev 2007



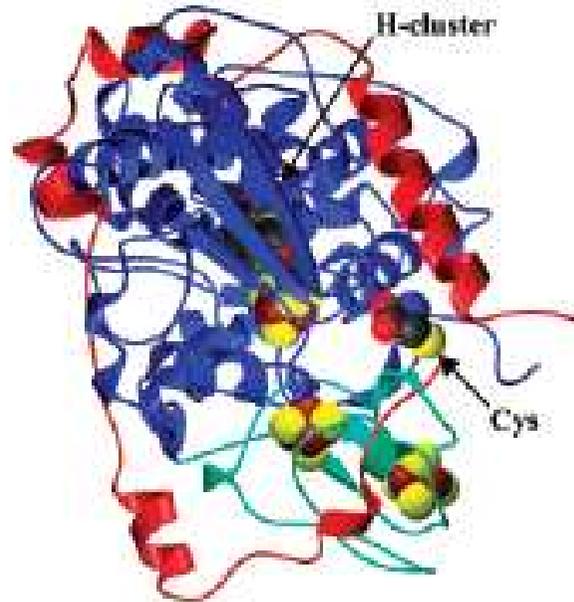
Deux classes d'hydrogénases:

2. [Fe-Fe]-H₂ase

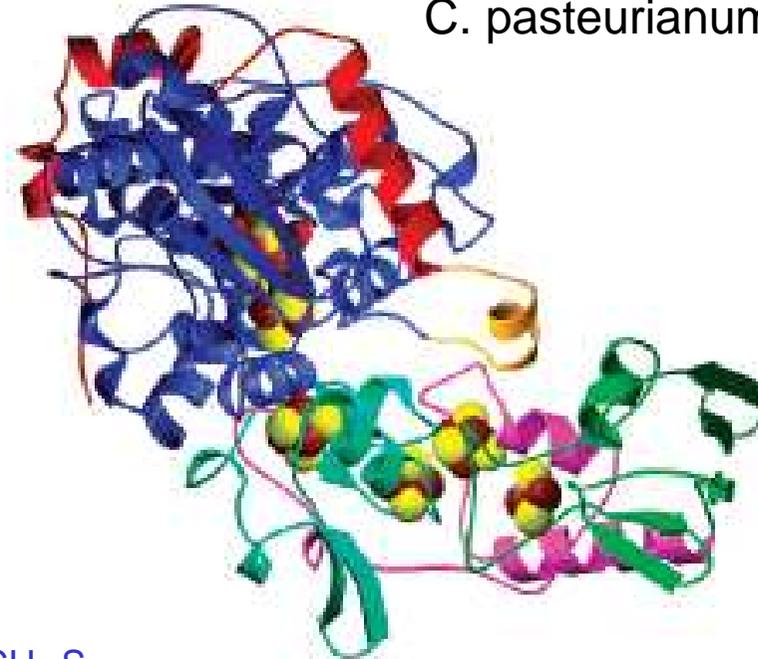
Production H₂

1000-8000 $\mu\text{mol H}_2 \text{ min}^{-1} \text{ mg}^{-1}$

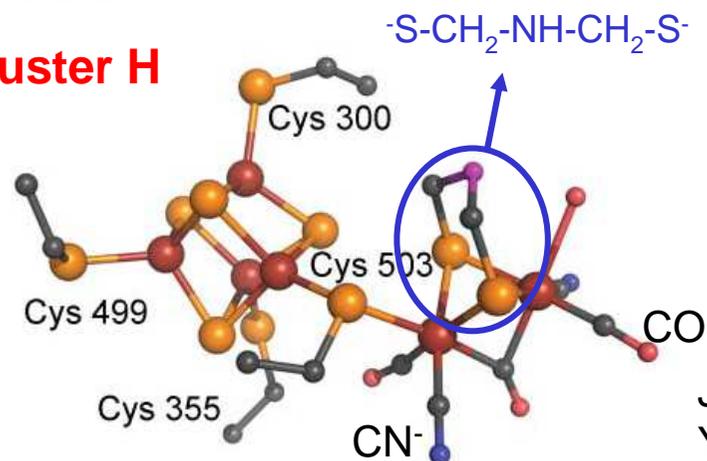
D. desulfuricans



C. pasteurianum



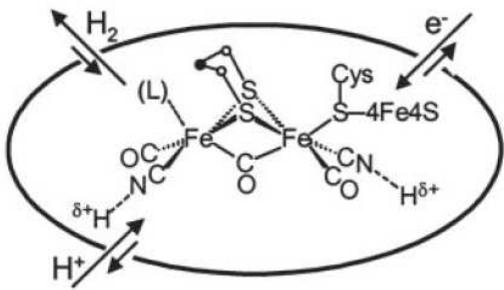
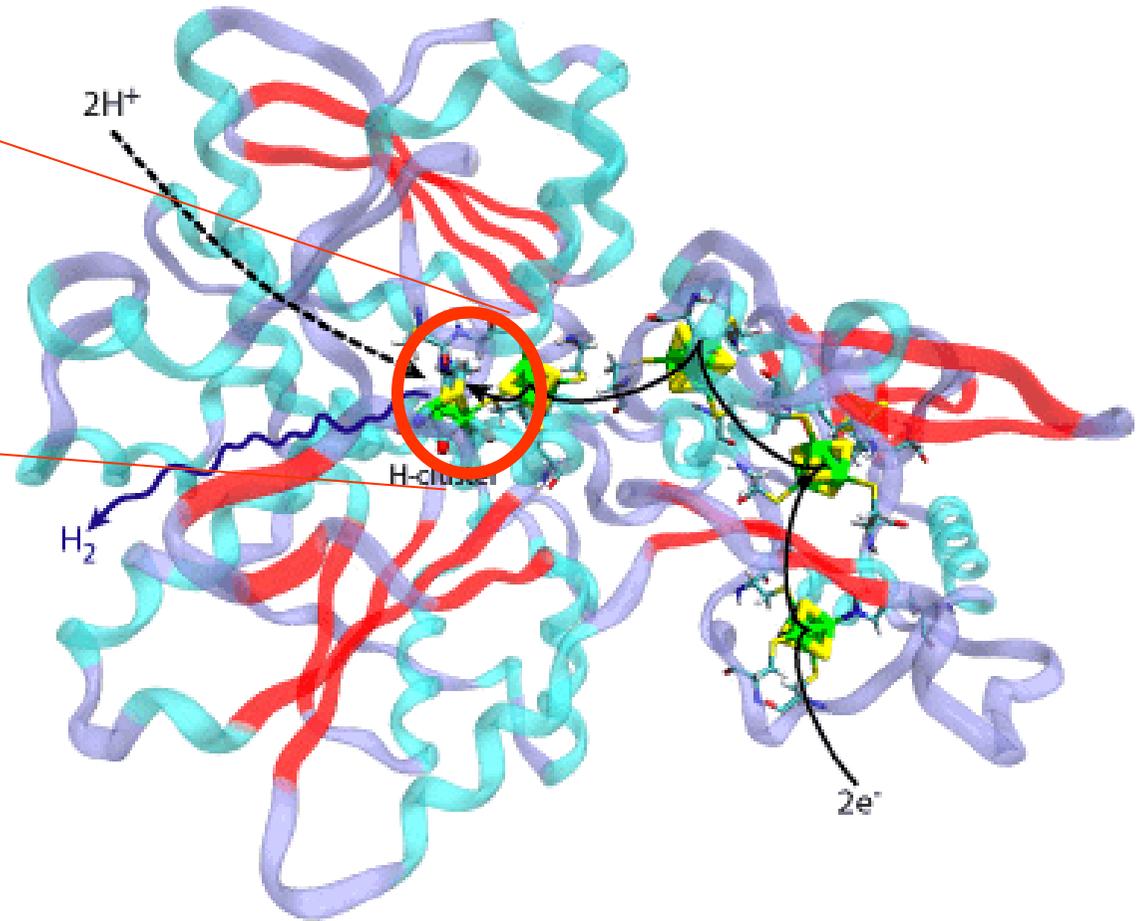
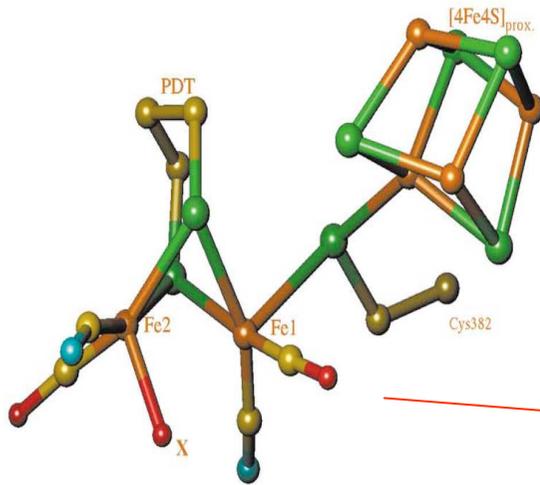
Cluster H



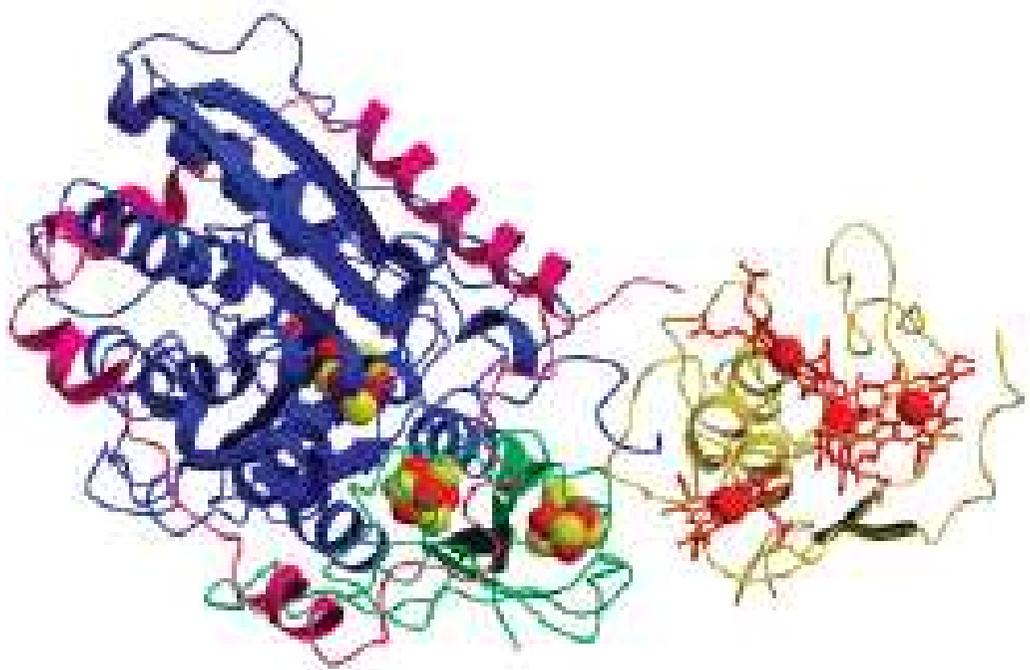
JW Peters et coll *Science* (1998) 282, 1853

Y Nicolet et col *Structure* (1999) 7, 13

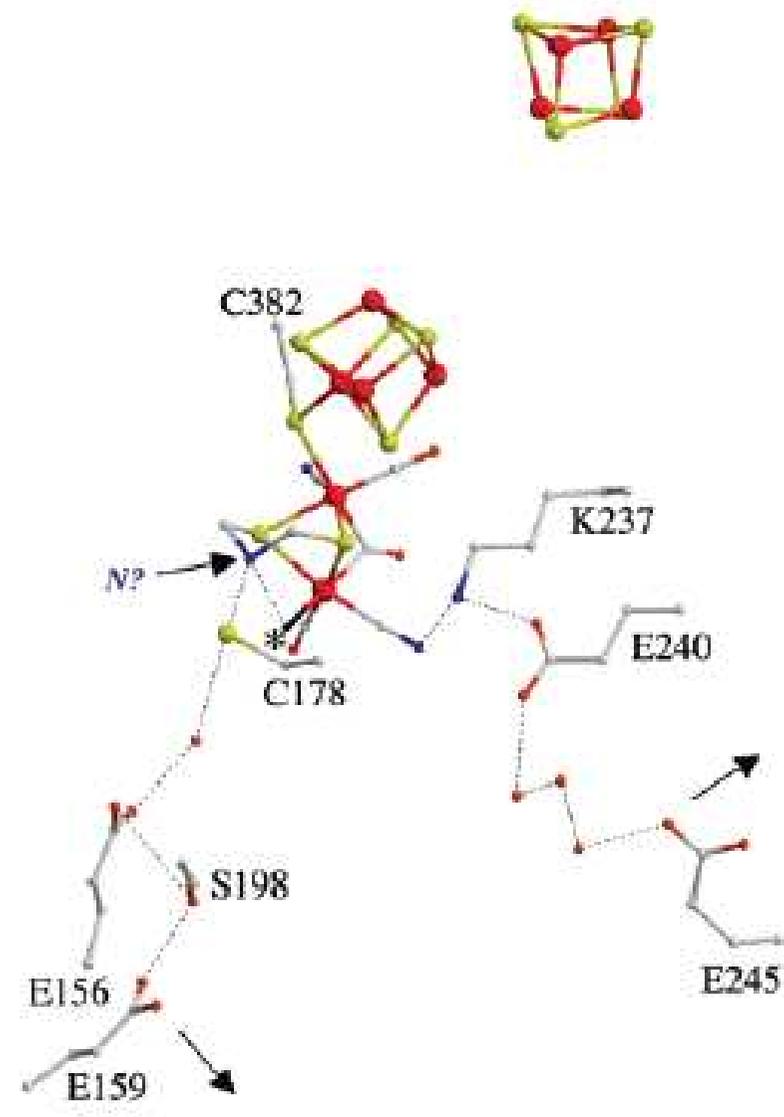
Site actif [Fe₂]H₂ase



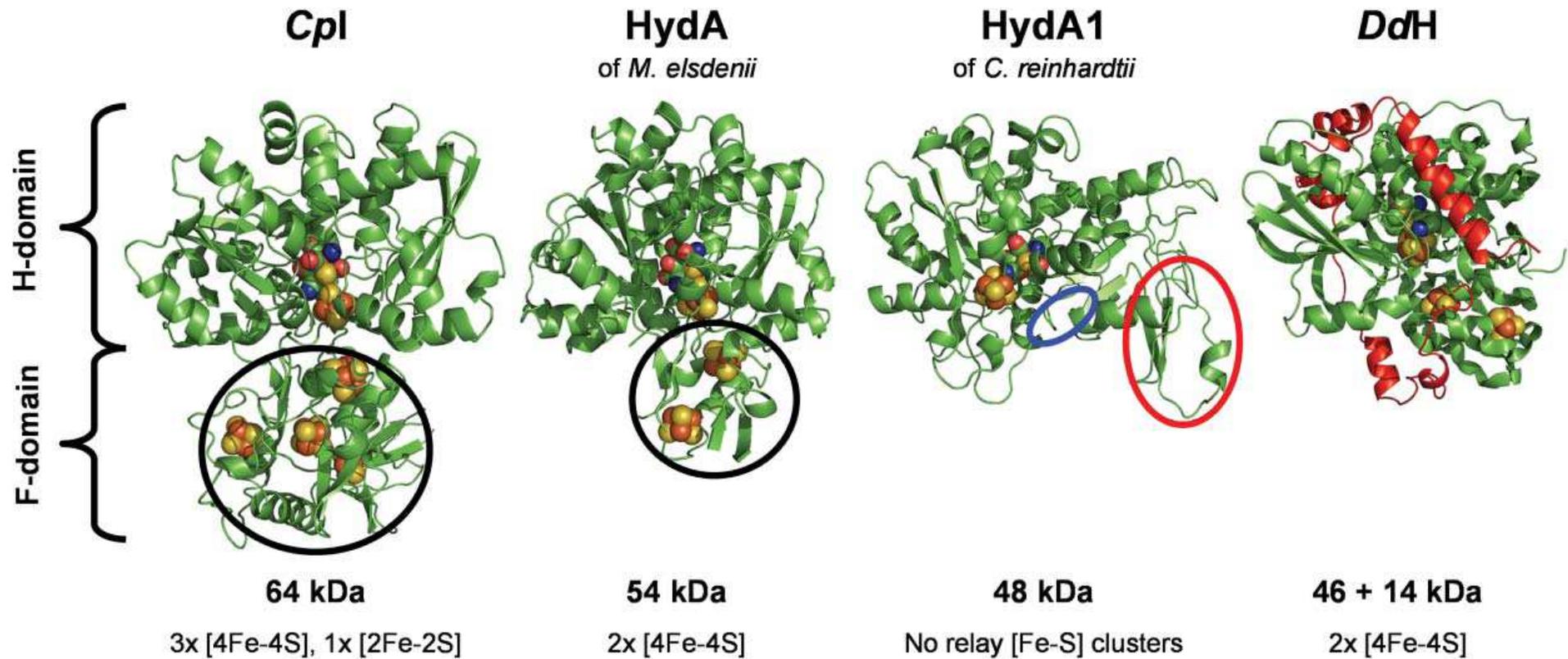
Active site of Fe-H₂ase



Des chaines de transferts de:
 -Électrons
 -Protons

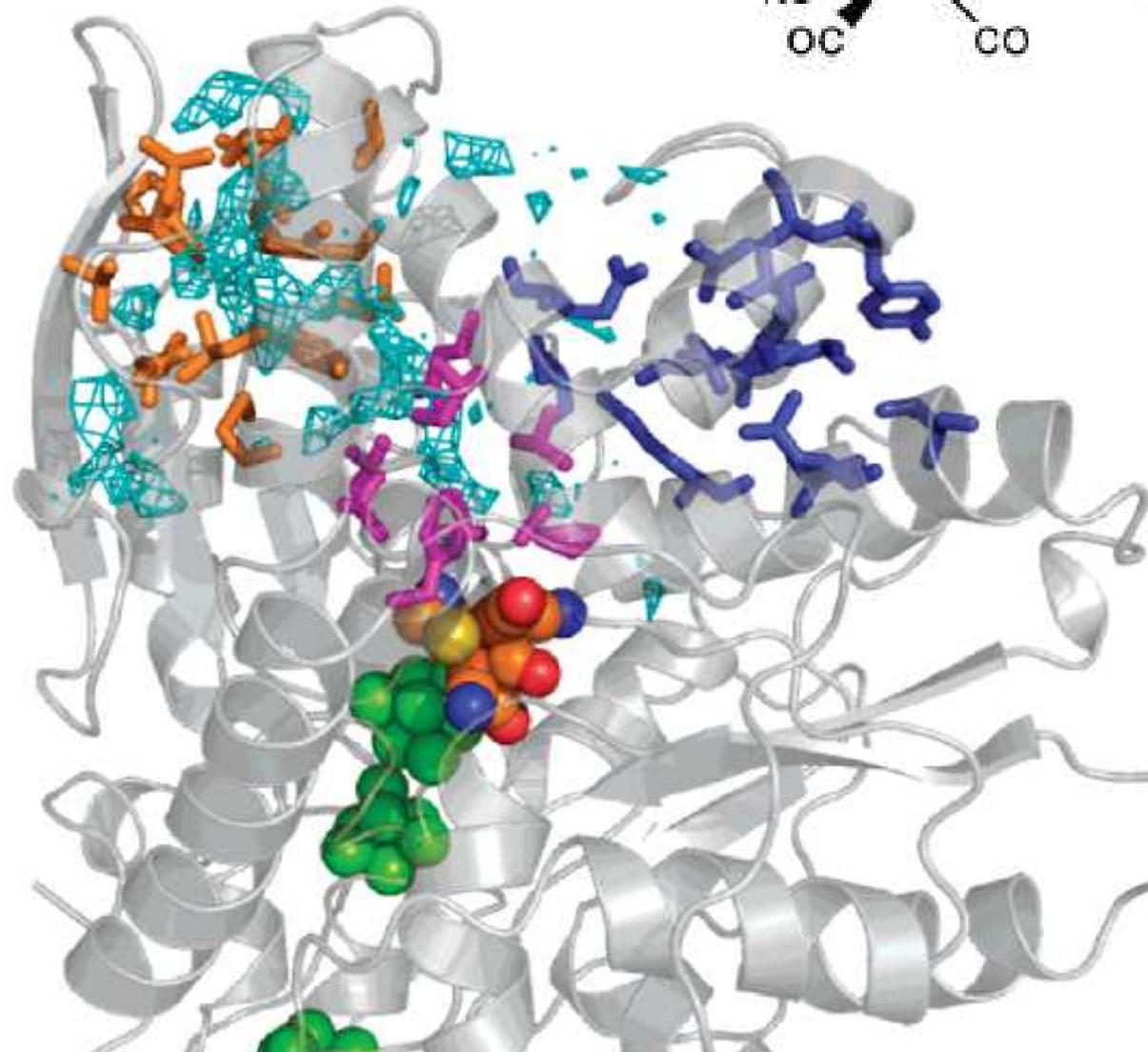
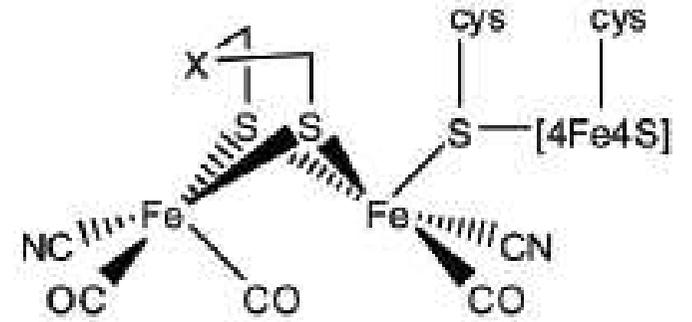


Fe₂-Hydrogénases:
Des chaînes de clusters Fe-S variables

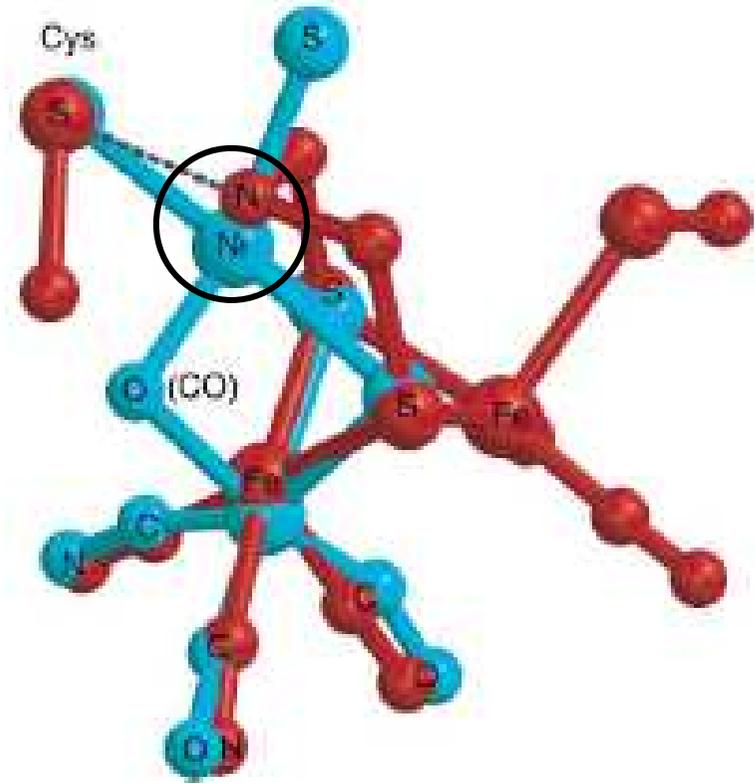
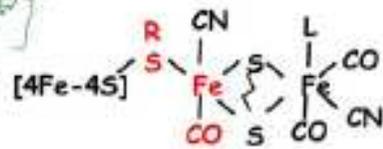
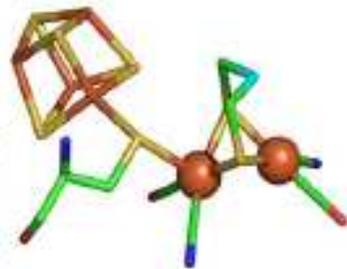
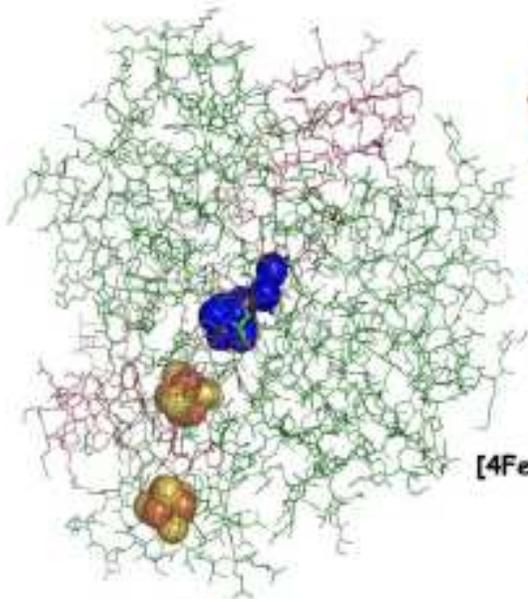
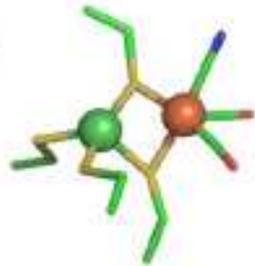
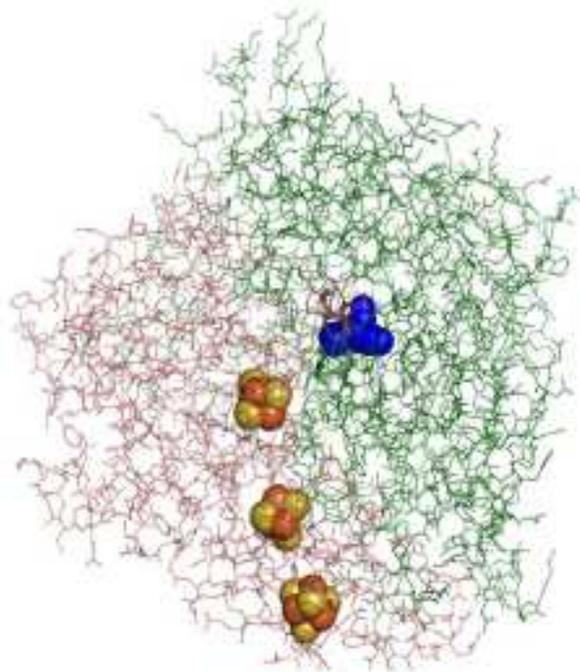


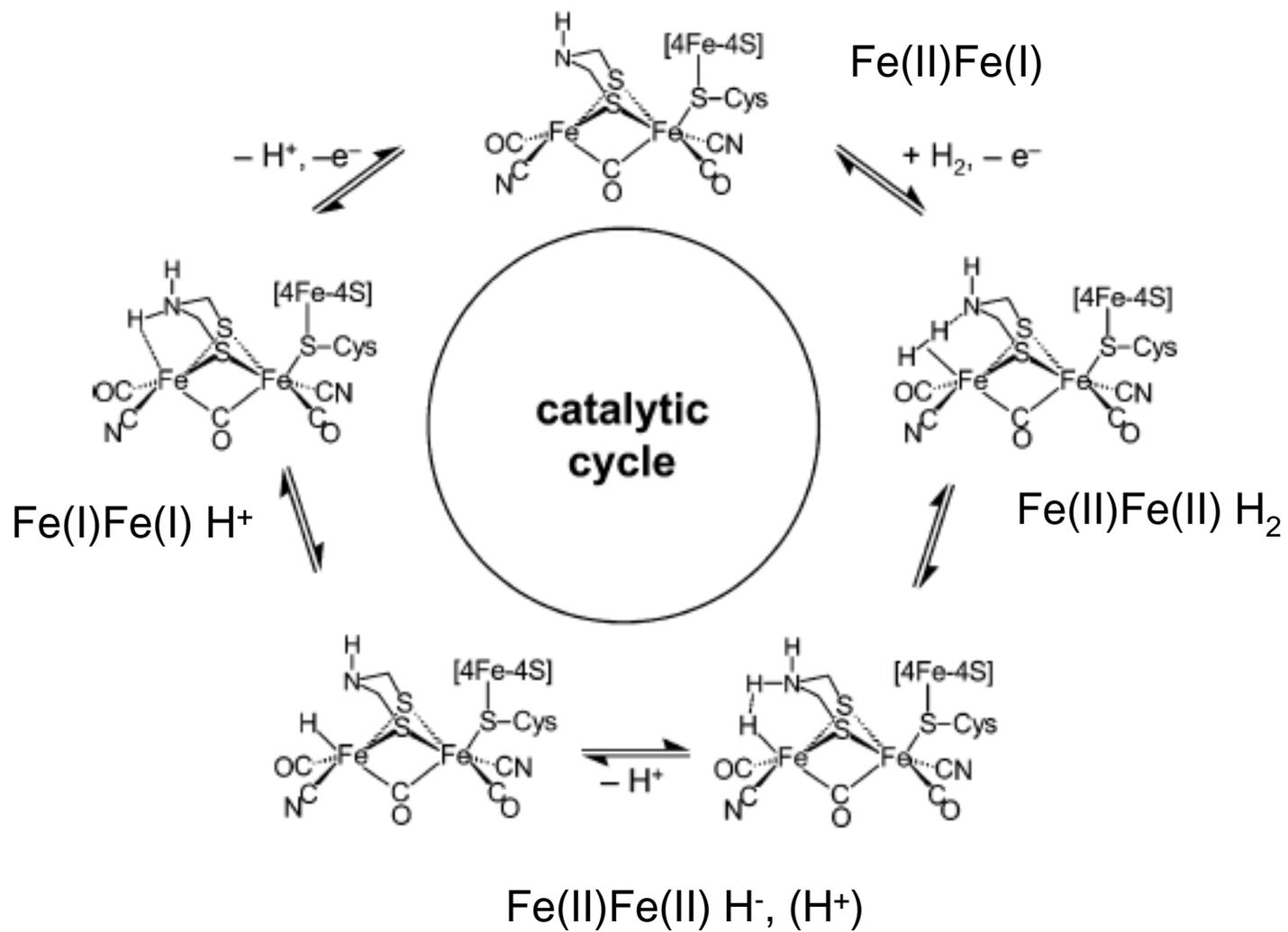
FeFe-H₂ase

Des canaux de transport de gazs

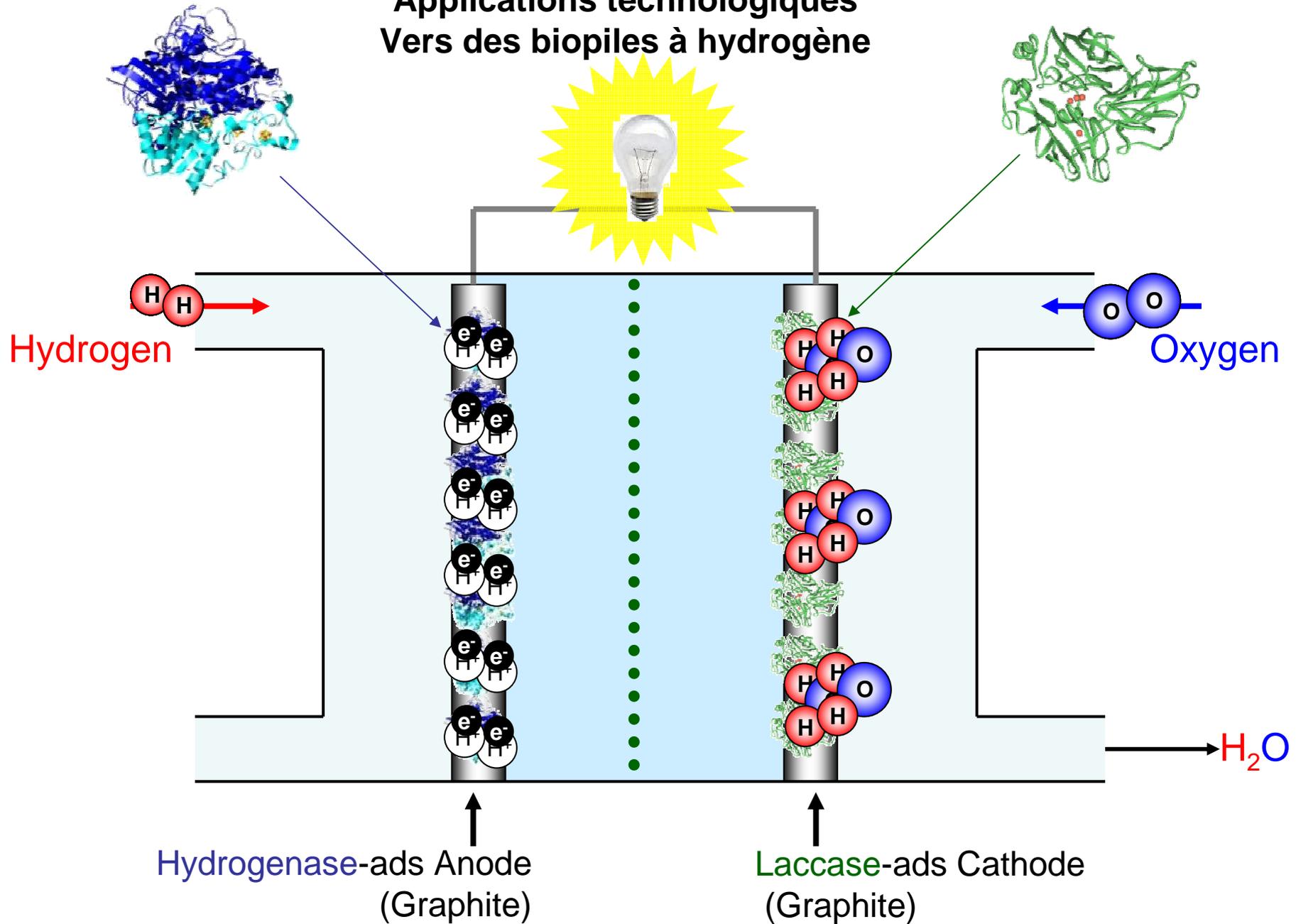


Des sites actifs superposables
N ~ Ni

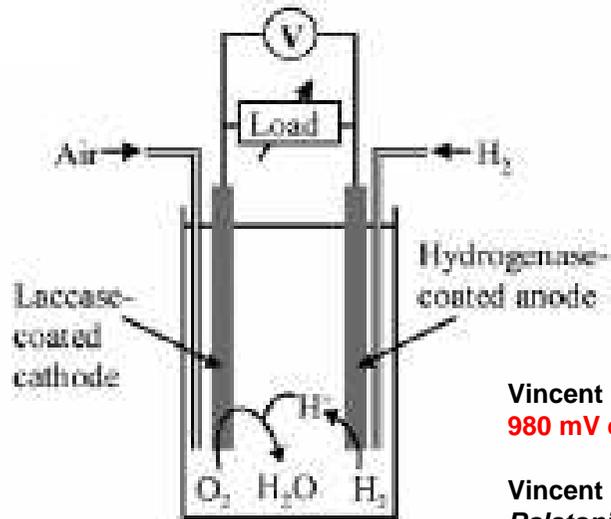




Applications technologiques Vers des biopiles à hydrogène

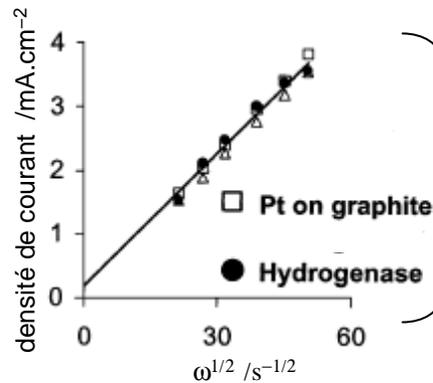


Applications technologiques Vers des biopiles à hydrogène



Vincent *et al.* (2005) PNAS 102:16951-4
980 mV en circuit ouvert; 7 $\mu\text{W}\cdot\text{cm}^{-2}$; CO n'inhibe pas

Vincent *et al.* (2006) Chem. Commun. (Camb). 48:5033-5.
Ralstonia metallidurans, *R. eutropha*
950 mV en circuit ouvert; 5 $\mu\text{W}\cdot\text{cm}^{-2}$ tension 500mV

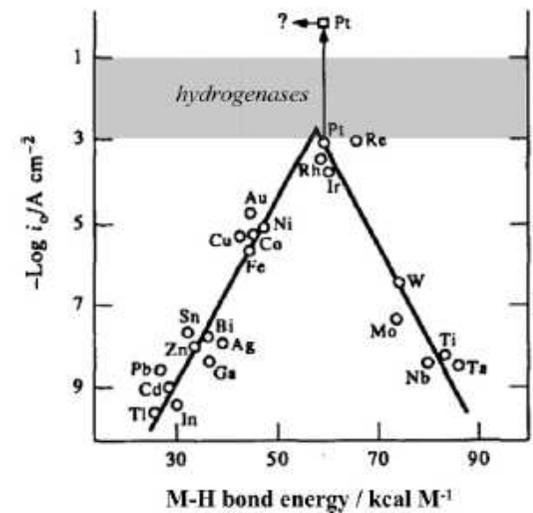
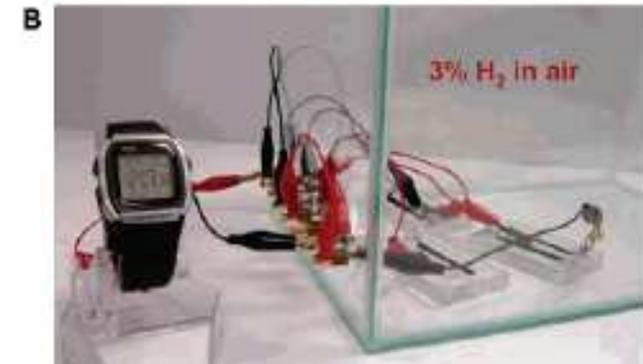
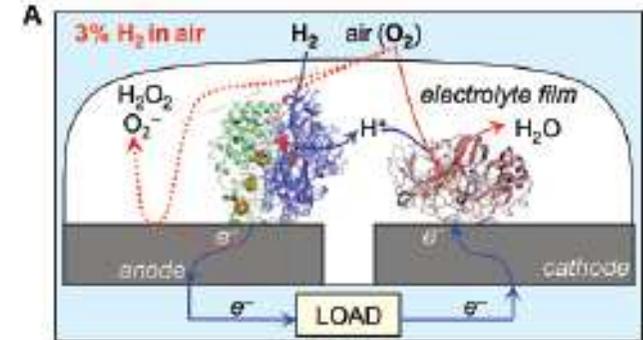


- Mêmes densités de courant
- Même activité par site actif
- Surtension $\text{H}_2\text{ase} > \text{Pt}$

Armstrong *et coll.*, *ChemComm* 2002, 866

[NiFe]-[H_2]ase de *Allochromatium vinosum* adsorbé sur graphite forme un système aussi réversible que le platine colloïdal

$$E_{\text{isos}} = -400 \text{ mV/ENH} \\ (30^\circ\text{C}; \text{pH } 7; 0,1 \text{ bar } \text{H}_2)$$





COLLÈGE
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Les hydrogénases: des biocatalyseurs pour la production et l'oxydation de l'hydrogène

Marc Fontecave

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F. Armstrong Chem Rev 2007 107, 4366,
Chem Rev 2008, 108, 2439
J. Fontecilla-Camps Chem Rev 2007 107, 4273