Redox State-Dependent Structural Changes in [NiFeSe] Hydrogenase from *Desulfovibrio vulgaris* Hildenborough

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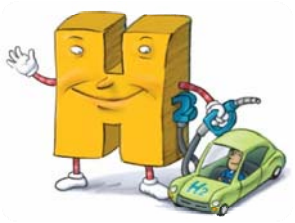
[NiFeSe] Hases

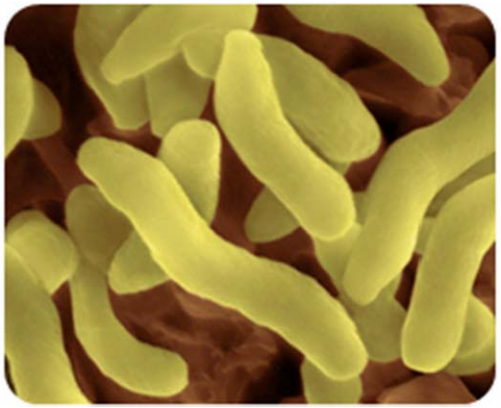


- ✓ Included in the [NiFe] group
- ✓ Higher activities for H_2 production
- ✓ Less H_2 inhibition
- ✓ Fast reactivation at a low redox potential
- ✓ Display some level of protection to O_2 exposure

Attractive candidates for:

- Biological H_2 production from renewable sources
- Use in bioelectrical devices

Desulfovibrio vulgaris Hildenborough

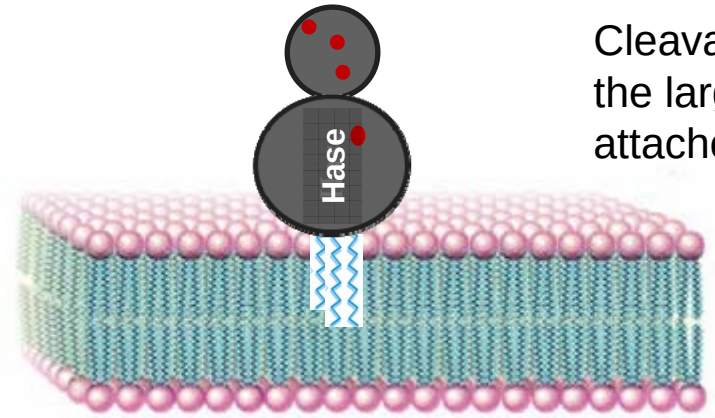


- ✓ 7 Hases in genome, 4 are periplasmic
- ✓ [NiFeSe] Hase uses Type I cyrochrome c_3 as electron acceptor
- ✓ Expression levels of different Hases depend on metal availability and H_2 concentration
- ✓ [NiFeSe] Hase is preferentially expressed in the presence of Se

Pereira, IAC et al. 2011, Frontiers in Microbiology 2:69

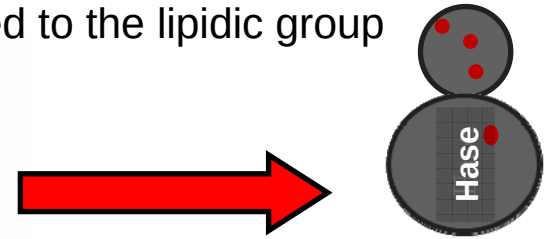
D. vulgaris Hildenborough [NiFeSe] Hase

- Periplasmic bacterial lipoprotein (lipobox)
- Two subunits
- Three [4Fe-4S] clusters
- The large subunit binds the NiFe active site
- One of the terminal Ni-bound Cys is a SeCys
- During purification a soluble protein is also obtained



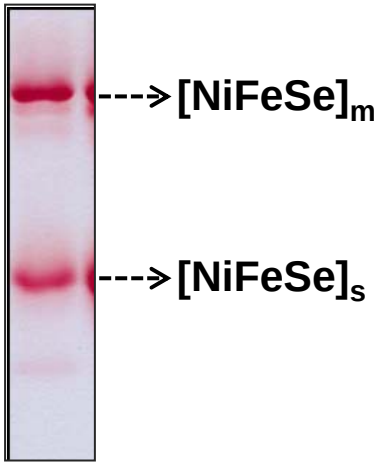
$[NiFeSe]_m$ Hase

Cleavage of the first 11 residues of the large subunit containing the Cys attached to the lipidic group



$[NiFeSe]_s$ Hase

Activity-stained native PAGE



D. vulgaris Hildenborough [NiFeSe] Hase

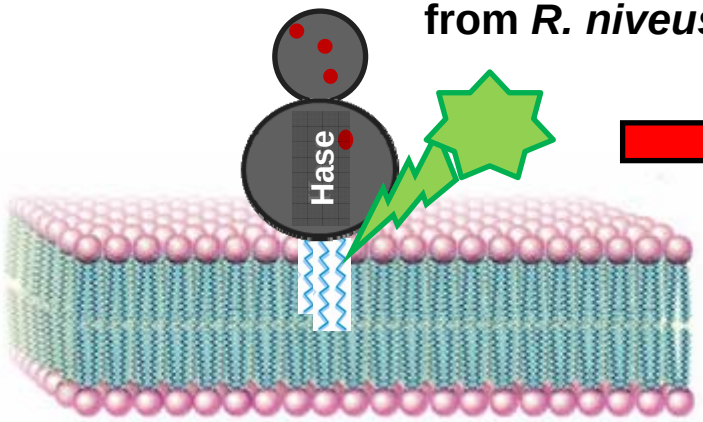
Specific activity (U mg^{-1})

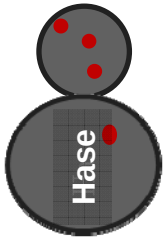
Hase	Phospholipids	Tris-HCl buffer
$[NiFeSe]_m$	6908	2755
$[NiFeSe]_s$	-	460
$[NiFe]_1$	495	366

Valente, FMA et al., 2005, J. Biol. Inorg. Chem, 10:667-682.

Production of $[NiFeSe]_s$ Hase from $[NiFeSe]_m$

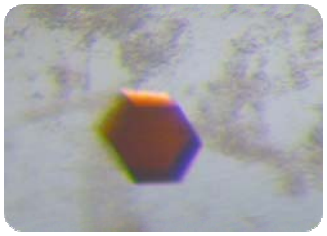
Commercial Lipase from *R. niveus*

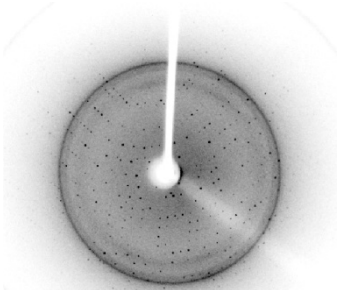




soluble [NiFeSe] Hase

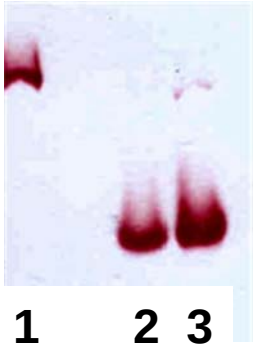
Crystals





X-ray diffraction

Activity-stained native PAGE after 12h incubation with lipase



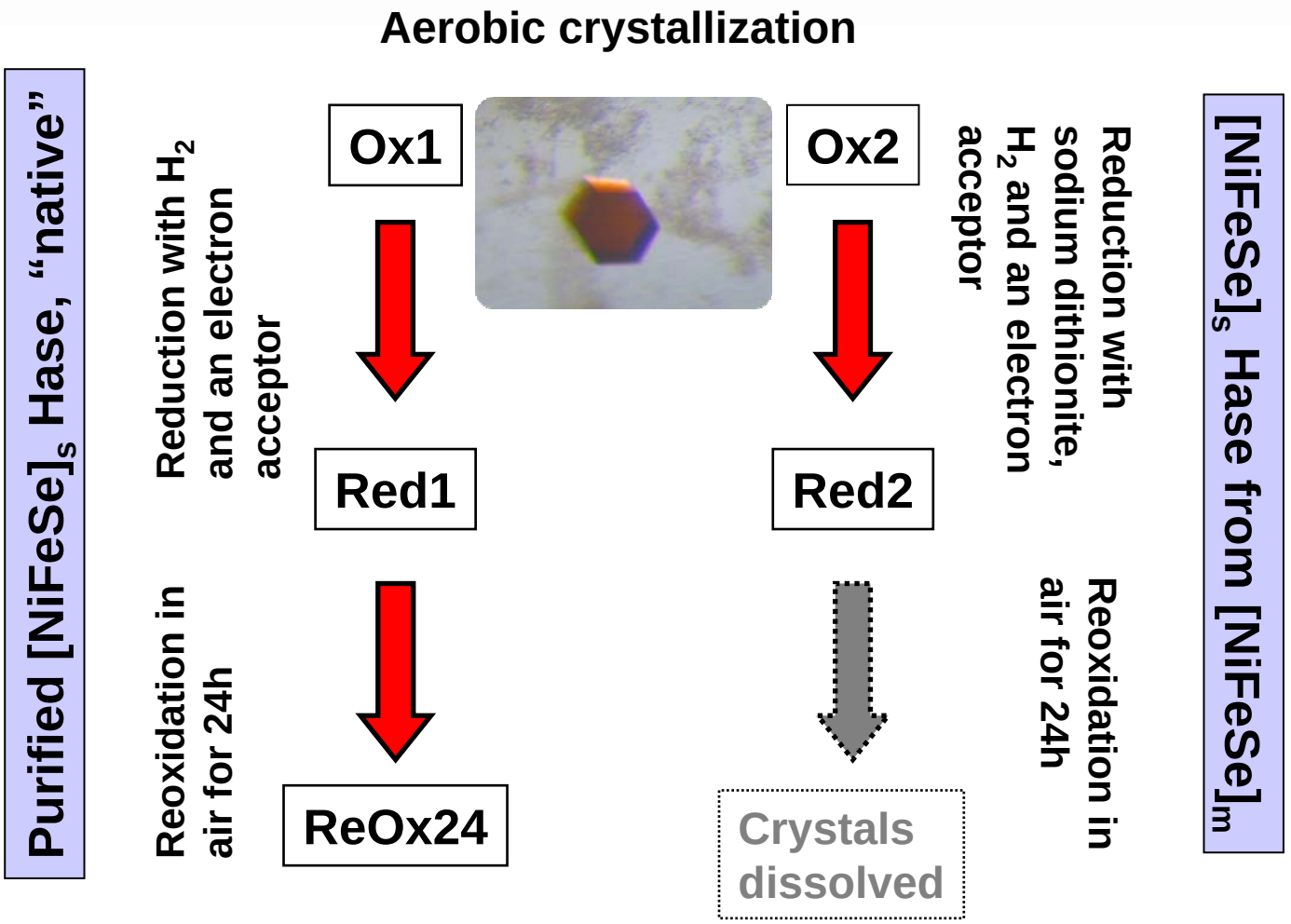
1 2 3

1 – $[NiFeSe]_m$ Hase from purification

2 – $[NiFeSe]_s$ Hase from purification

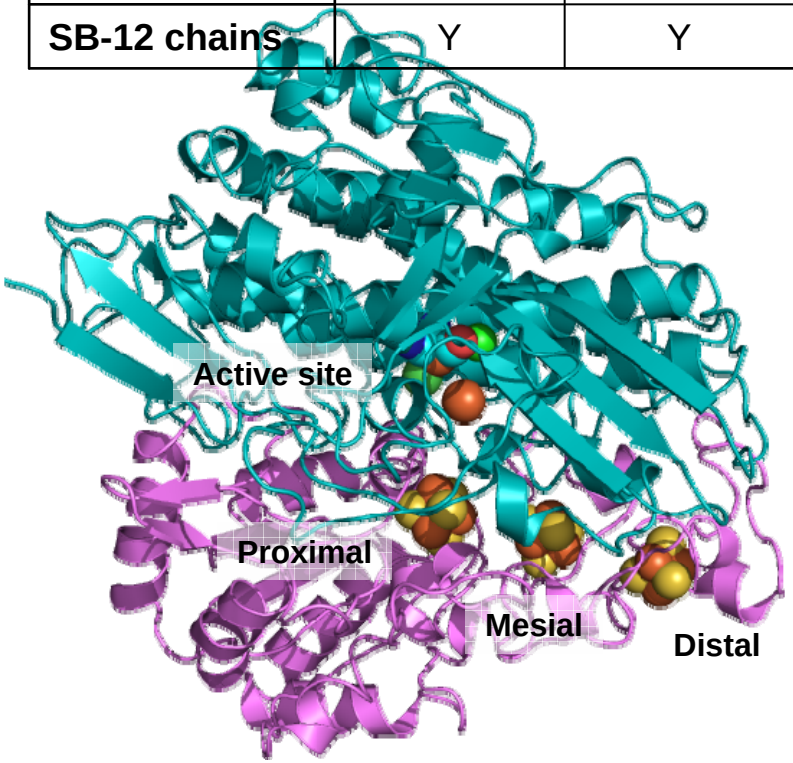
3 – $[NiFeSe]_s$ Hase from lipase

Crystals of $[NiFeSe]_s$ in different redox forms



X-ray data collection & 3D structure

Dataset	Ox	Ox1	Ox2	Red1	Red2	ReOx24
Beamline	DLS I04	SLS PXIII	ESRF ID29	SLS PXIII	ESRF ID29	SLS PXIII
Resolution (Å)	2.05	1.50	1.33	1.95	1.82	1.80
R / R_{free} (%)	14.4 / 20.1	13.5 / 15.4	13.1 / 14.8	15.3 / 19.0	12.4 / 14.7	13.5 / 16.6
Space Group	*P* 2_1	*P* $2_12_12_1$	*C* 2	*P* $2_12_12_1$	*P* 3_121	*P* $2_12_12_1$
SB-12 chains	Y	Y	N	N	N	Y



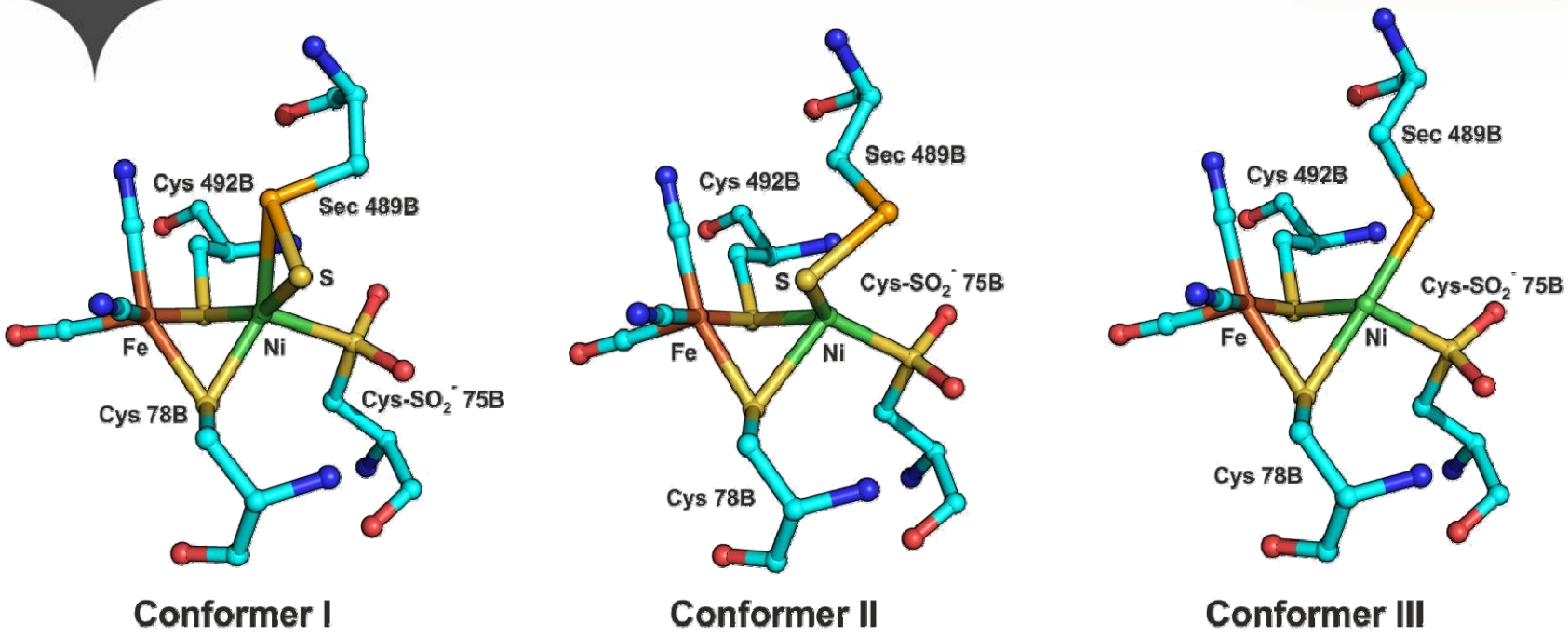
Large subunit (B)

Typical fold of a [NiFe] Hase

Small subunit (A)

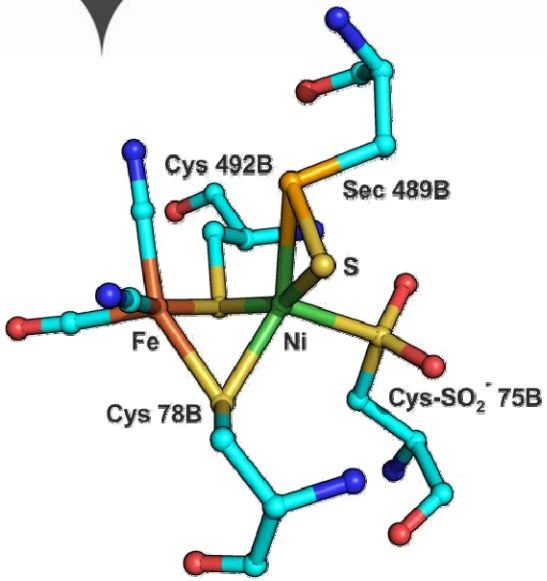
Marques *et al*. 2010, *J Mol Biol*, 396:893-907
Marques *et al.* 2013, *Int J Hydrogen Energy*, 38:8664–8682

The active site

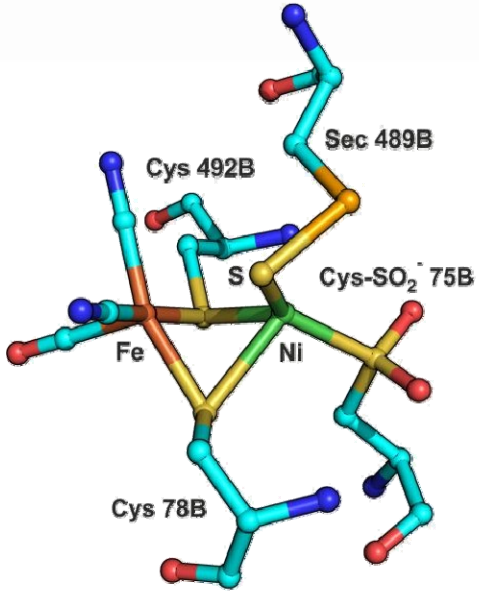


- Side chain of SeCys 489B in **three different conformers**
- Terminal Cys 75B **irreversibly** oxidized to **sulfinate**

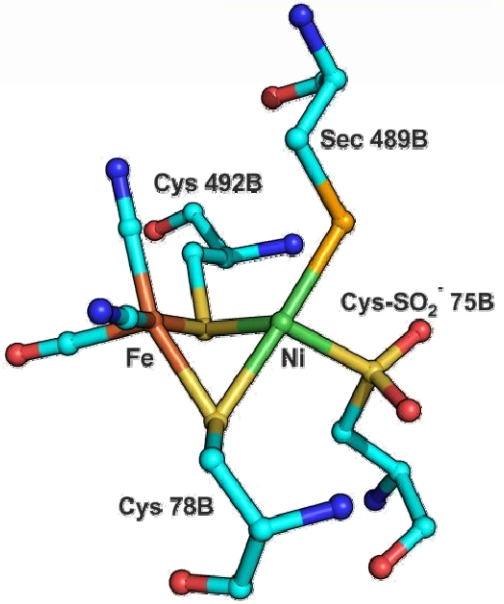
The active site



Conformer I



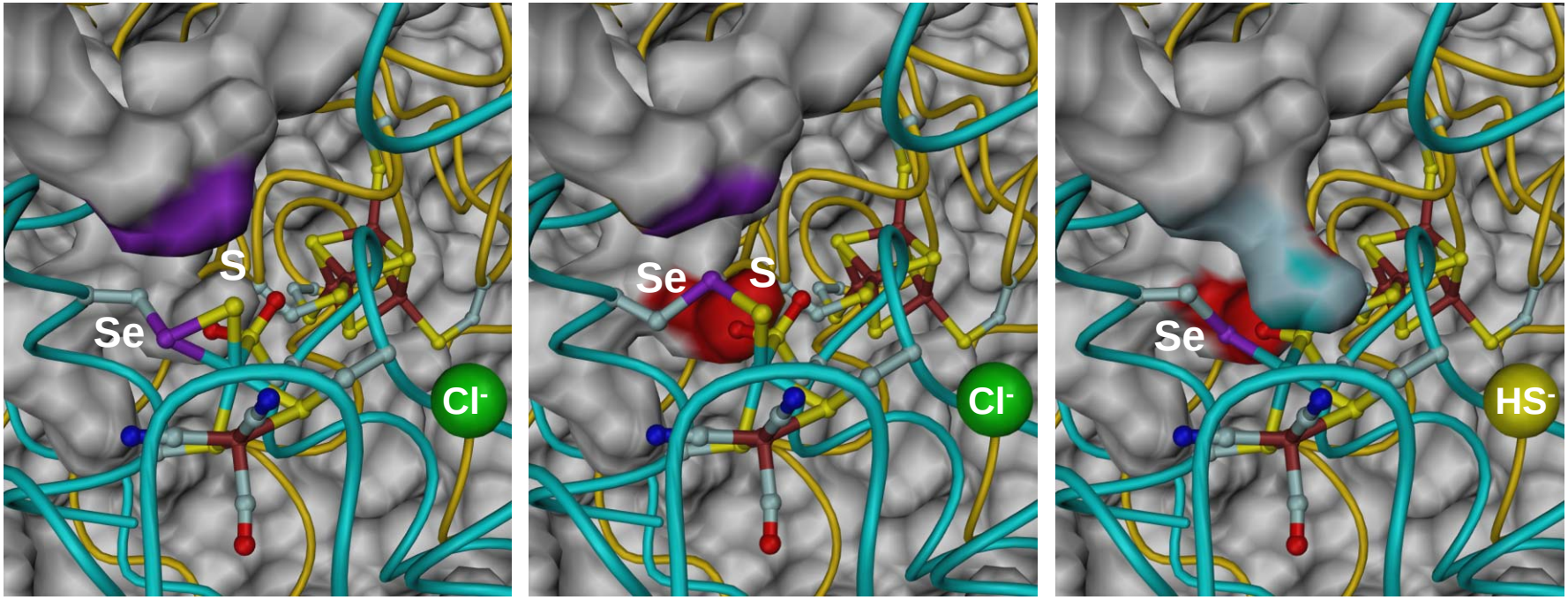
Conformer II



Conformer III

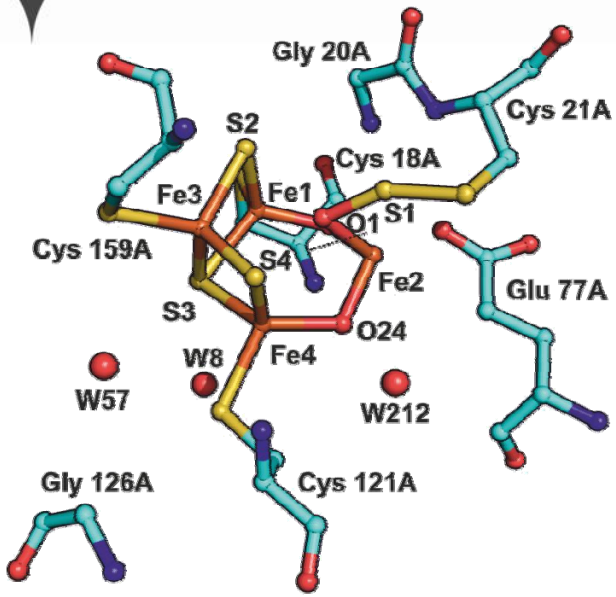
Ox	70 %	15 %	15 %
Ox1	74 %	16 %	10 %
Ox2	73 %	13 %	14 %
Red1	-	-	100 %
Red2	-	12 %	88 %
ReOx24	-	38 %	62 %

The active site

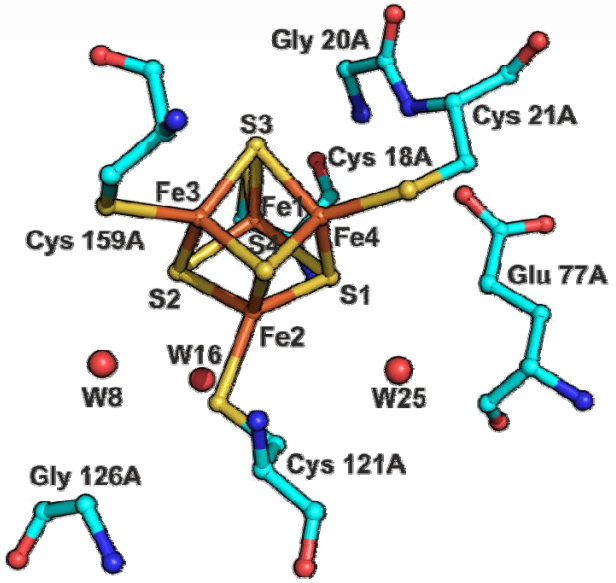


- Se atom in conformers I and II **blocks access to bridging position**
- No oxy/hydroxy bridging species
- **No Ni-A/Ni-B EPR signal**

The proximal [4Fe-4S] cluster



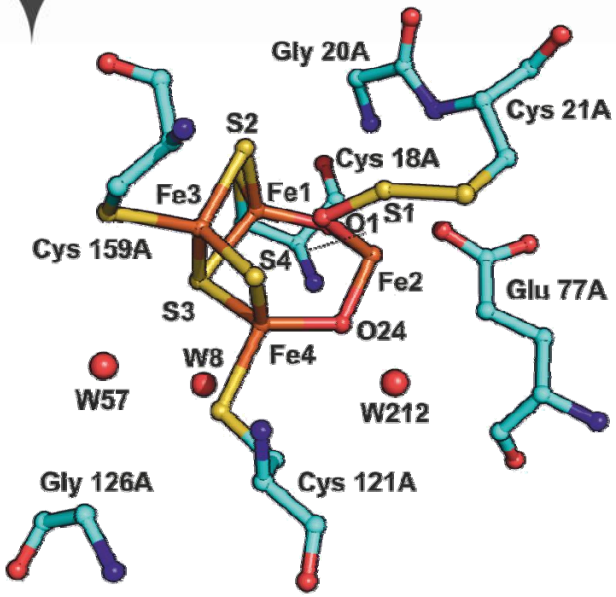
Ox, Ox1, Ox2

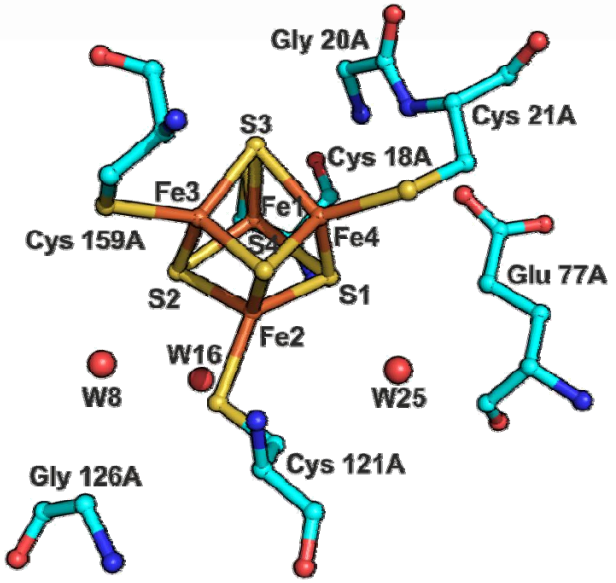


Red1, Red2, ReOx24

- [4Fe-4S] **reversibly** oxidized to [4Fe-4S-O3]
- oxidation occurs during aerobic purification and crystallization

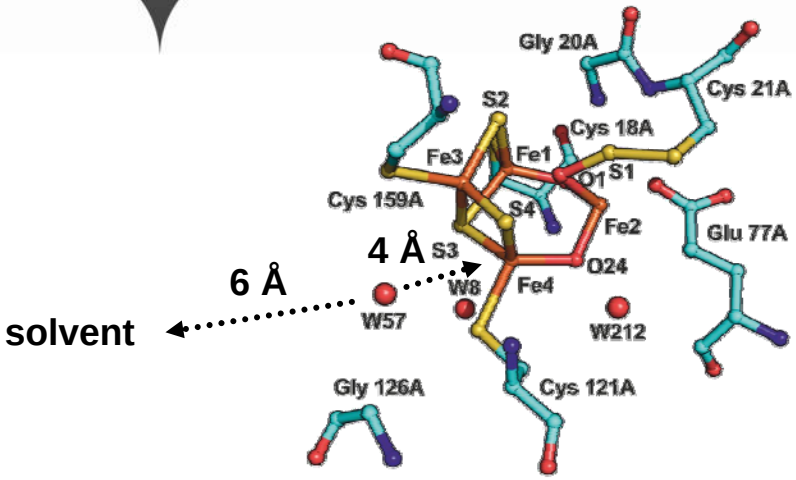
The proximal [4Fe-4S] cluster



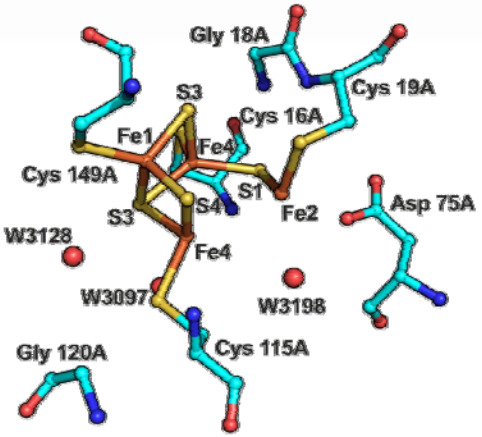


	$[Fe_4S_4O_3]$	$[Fe_4S_4]$
Ox	40 %	60 %
Ox1	80 %	20 %
Ox2	~100 %	-
Red1	-	100 %
Red2	-	100 %
ReOx24	-	100 %

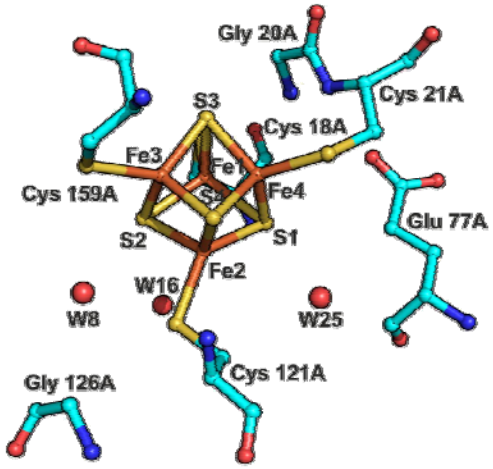
The proximal [4Fe-4S] cluster



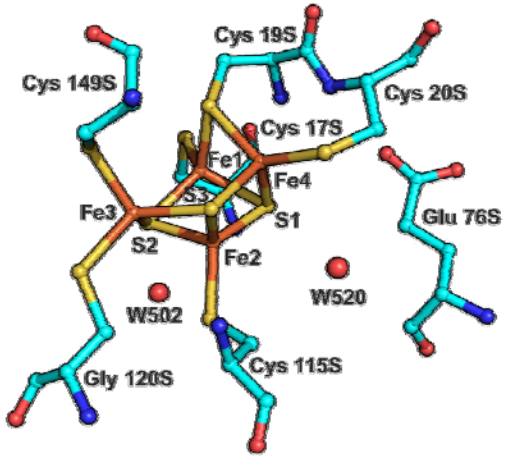
D. Vulgaris Hildenborough Ox



A. vinosum [NiFe] Ni-A – 3myr
(Ogata et al, 2010)



D. Vulgaris Hildenborough Red



E. coli Red – 3uqy
(Volbeda et al., (2012)

The inactivation of [NiFeSe] Hase from *Dv*H

Inactive states of [NiFeSe] Hases different from [NiFe] Hases?

In *D. vulgaris* Hildenborough:

- **No access** to bridging site by oxy/hydroxy bridging species
- Proximal [4Fe-4S] cluster **reversibly** oxidized to [4Fe-4S-3O]
- Terminal Cys 75B **irreversibly** oxidized to sulfinate
  - Does this modification completely inactivate the enzyme ?

New activity measurements of $[NiFeSe]_s$ Hase :

5707 U mg^{-1} after purification

782 U mg^{-1} after 16 days (from redissolved crystals with ~100% sulfinate)

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