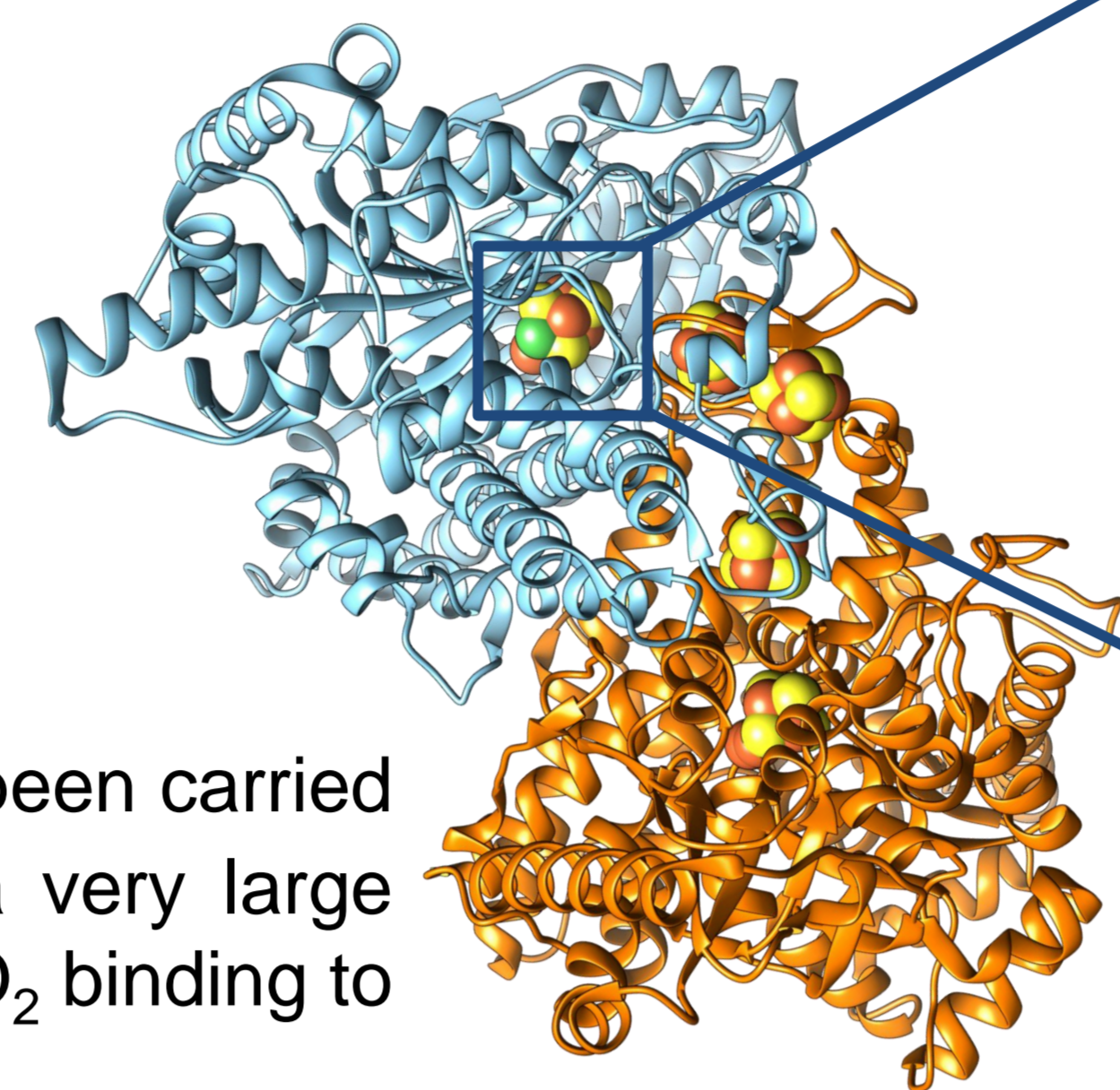
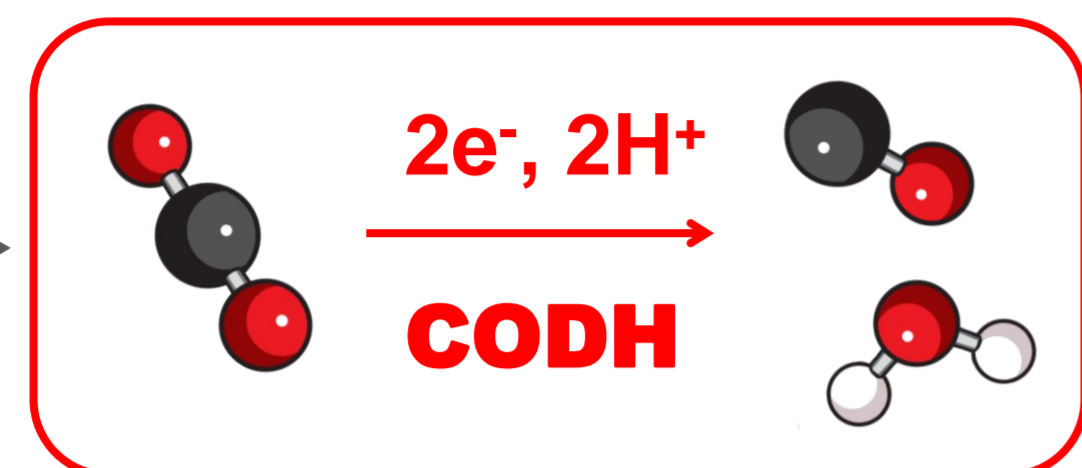
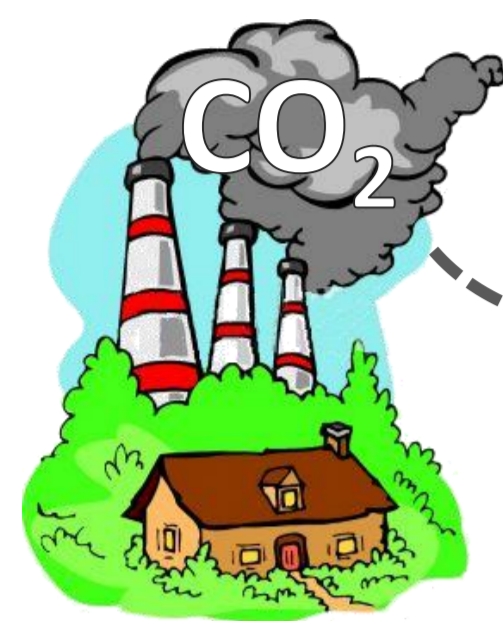


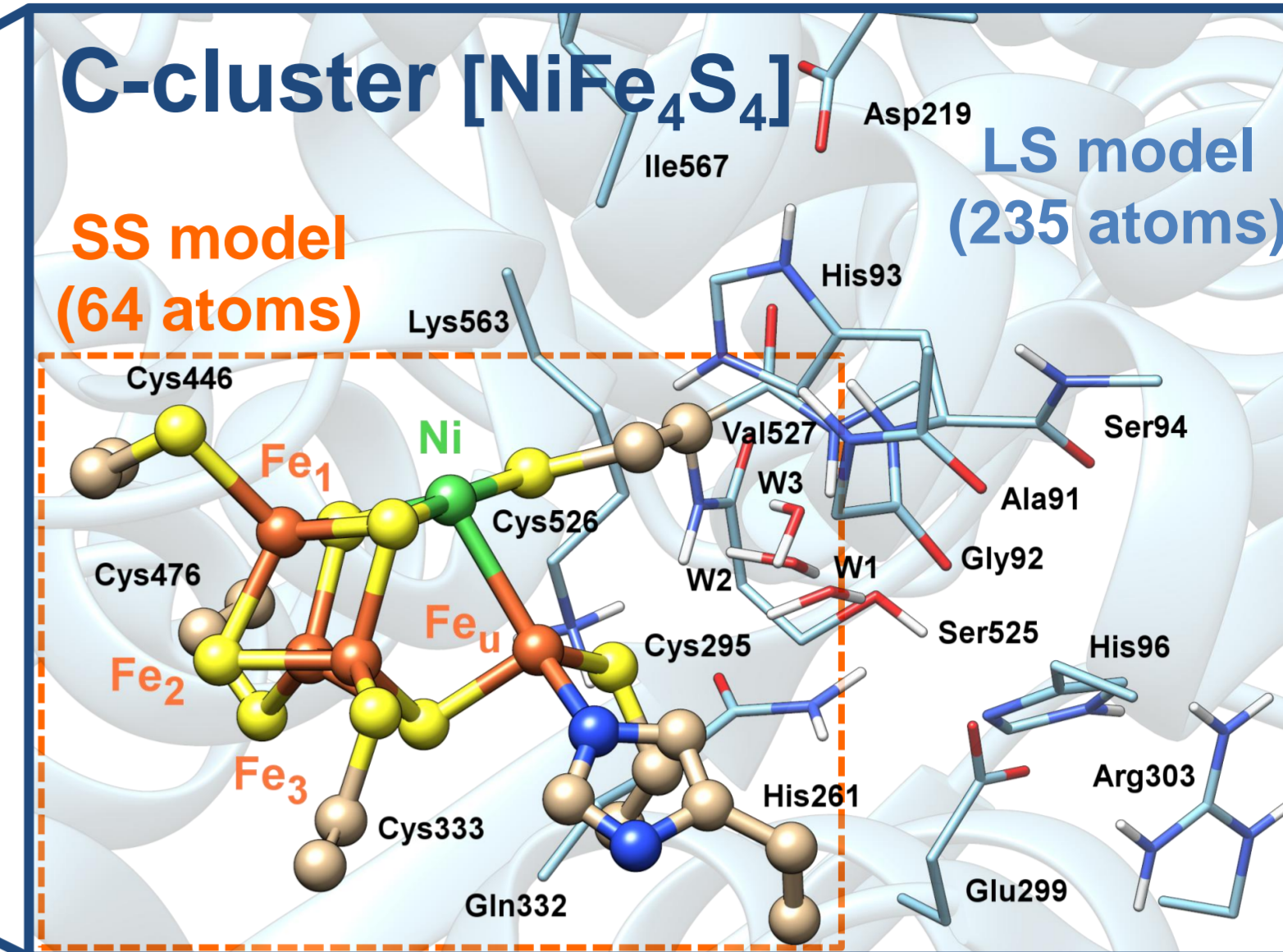
**Introduction:** The sustainable production of chemical fuels from non-fossil sources and reduction of greenhouse gas emissions are two of the biggest societal challenges. Sequestration of CO<sub>2</sub> by its conversion to liquid fuels could play a role to solve these problems. In this context, carbon monoxide dehydrogenases are of fundamental interest for their capability to catalyze the reversible reduction of CO<sub>2</sub> to CO. The high efficiency and the absence of expensive metals in their active site make the CODHs a very promising target for reverse engineering studies aimed at the development of bioinspired catalysts.



**Aim of the work:** Quantum mechanics calculations have been carried out in the framework of the Density Functional Theory (DFT) on a very large model of the active site to better understand the enzyme reactivity. CO<sub>2</sub> binding to different redox states of the active site have been investigated.

**Methods:** Two DFT models obtained from the active site of the 3B52 X-ray structure. [1]

BP86/def-TZVP-SVP



Terminal atoms restrained to the X-ray positions. Protein environment modelled by continuum dielectric with  $\epsilon=4$  (COSMO).

**Spectroscopic studies:** EPR, ENDOR, Mössbauer, and IR spectroscopies allowed to characterize three redox states of the C-cluster: [2]

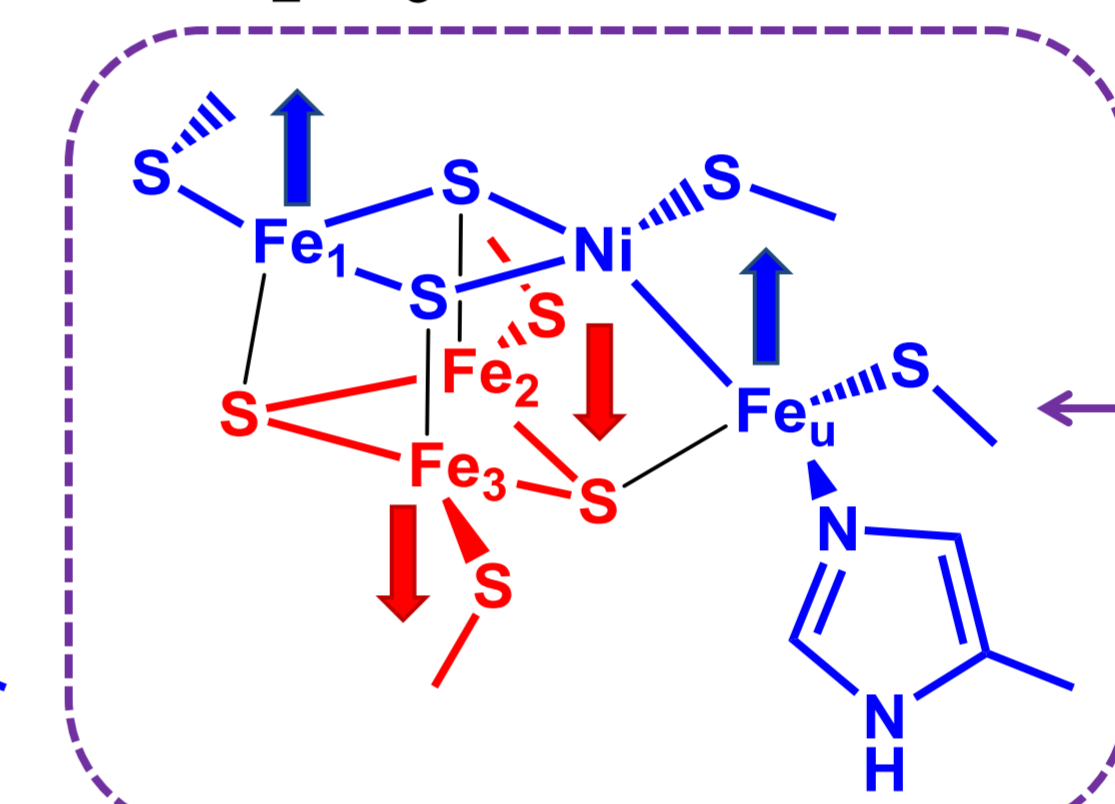
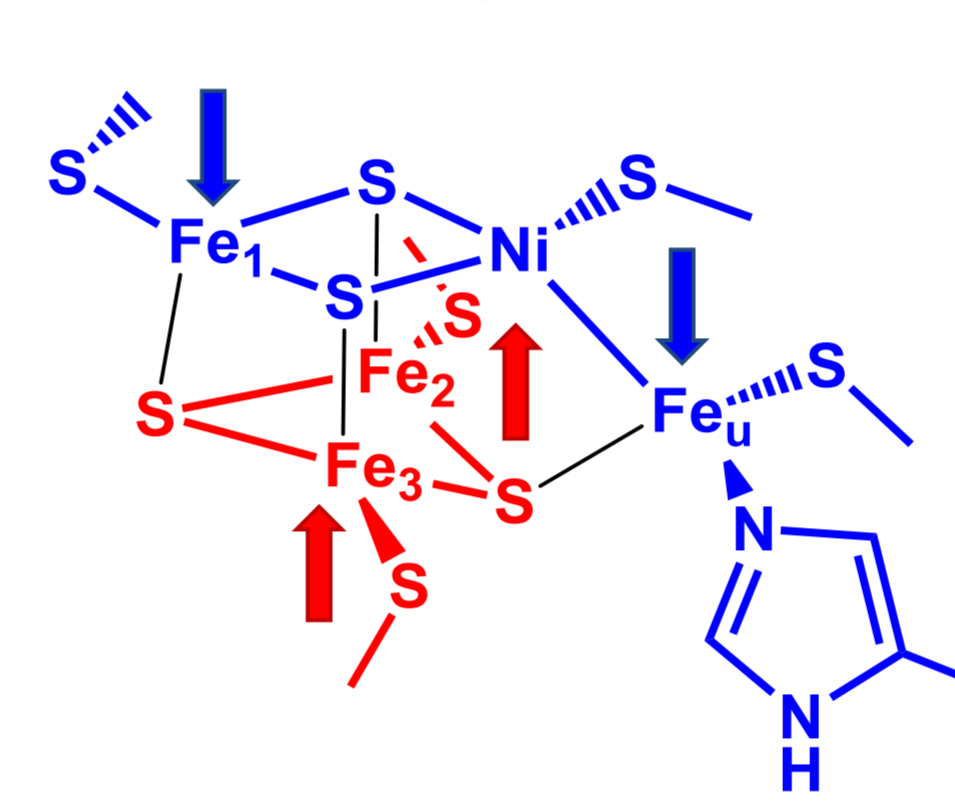
- **C<sub>ox</sub>**: diamagnetic inactive state occurring in oxidizing conditions, S=0
- **C<sub>red1</sub>**: active state obtained from the mono-electron reduction of C<sub>ox</sub>, S=1/2
- **C<sub>red2</sub>**: two electrons more reduced than C<sub>red1</sub>, S=1/2

Another undetected diamagnetic state, called **C<sub>int</sub>**, is postulated to arise from the one-electron reduction of C<sub>red1</sub> or from the one-electron oxidation of C<sub>red2</sub>.

In all of the characterized redox state the **Ni atom is diamagnetic** and may switch between Ni<sup>2+</sup> and Ni<sup>0</sup> during the redox process. [3][4]

For C<sub>red1</sub> Mössbauer measurements suggest high spin Fe<sup>2+</sup>, Fe<sup>2+</sup>, Fe<sup>3+</sup> formal oxidation states for the [Fe<sub>3</sub>S<sub>4</sub>] subsite and high spin Fe<sup>2+</sup> state for Fe<sub>u</sub>. [2][5]

**Electronic structure of the C-cluster:** The antiferromagnetic coupling of the Fe atoms have been treated within the broken symmetry approach. Of the six possible non equivalent spin coupling schemes, the broken symmetry states in which the Fe<sub>1</sub>Fe<sub>u</sub> pair is coupled antiferromagnetically with the Fe<sub>2</sub>Fe<sub>3</sub> pair have been found of lower energy.

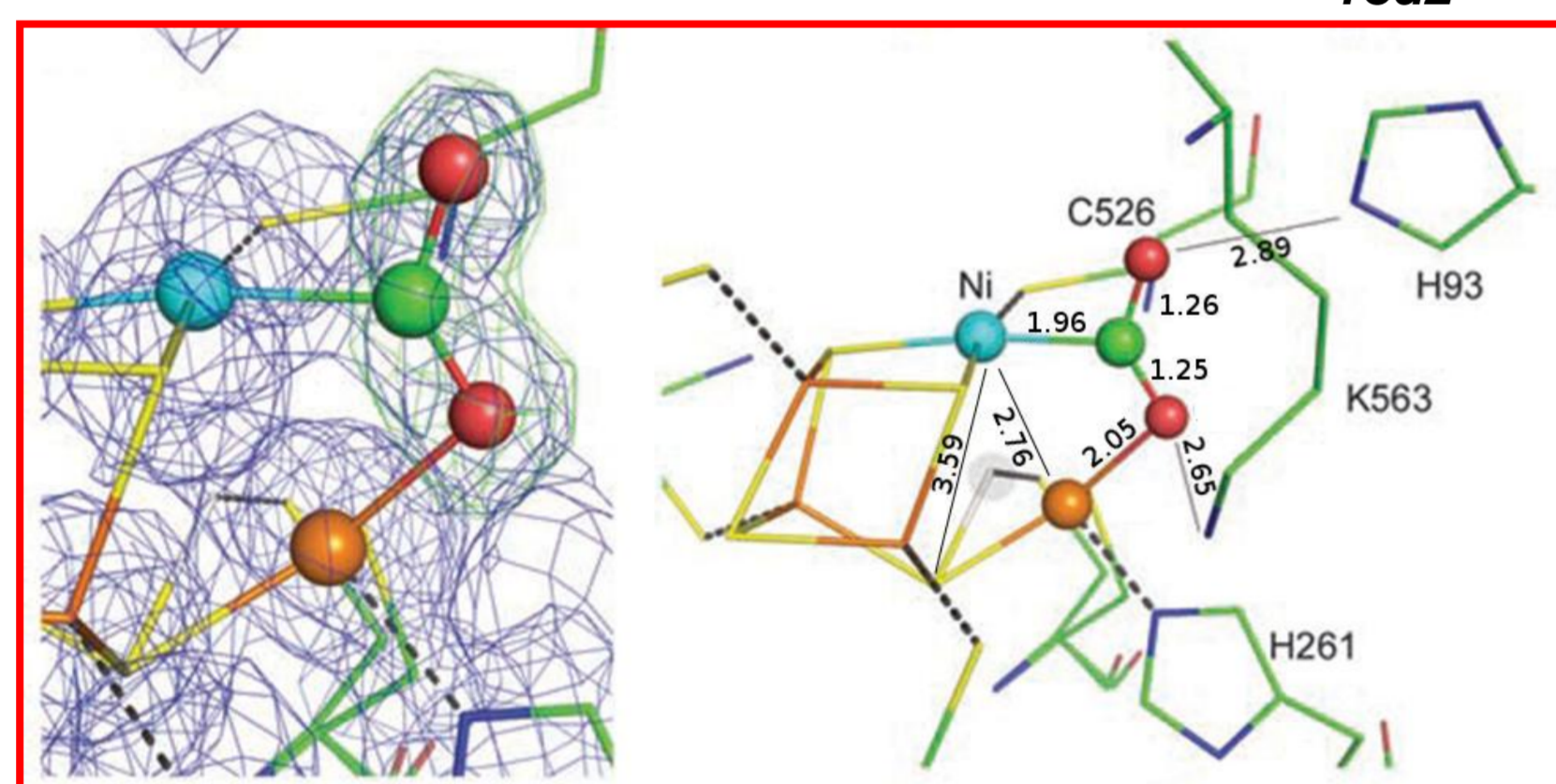


all results reported in this work refer to structures optimized according to this spin coupling scheme

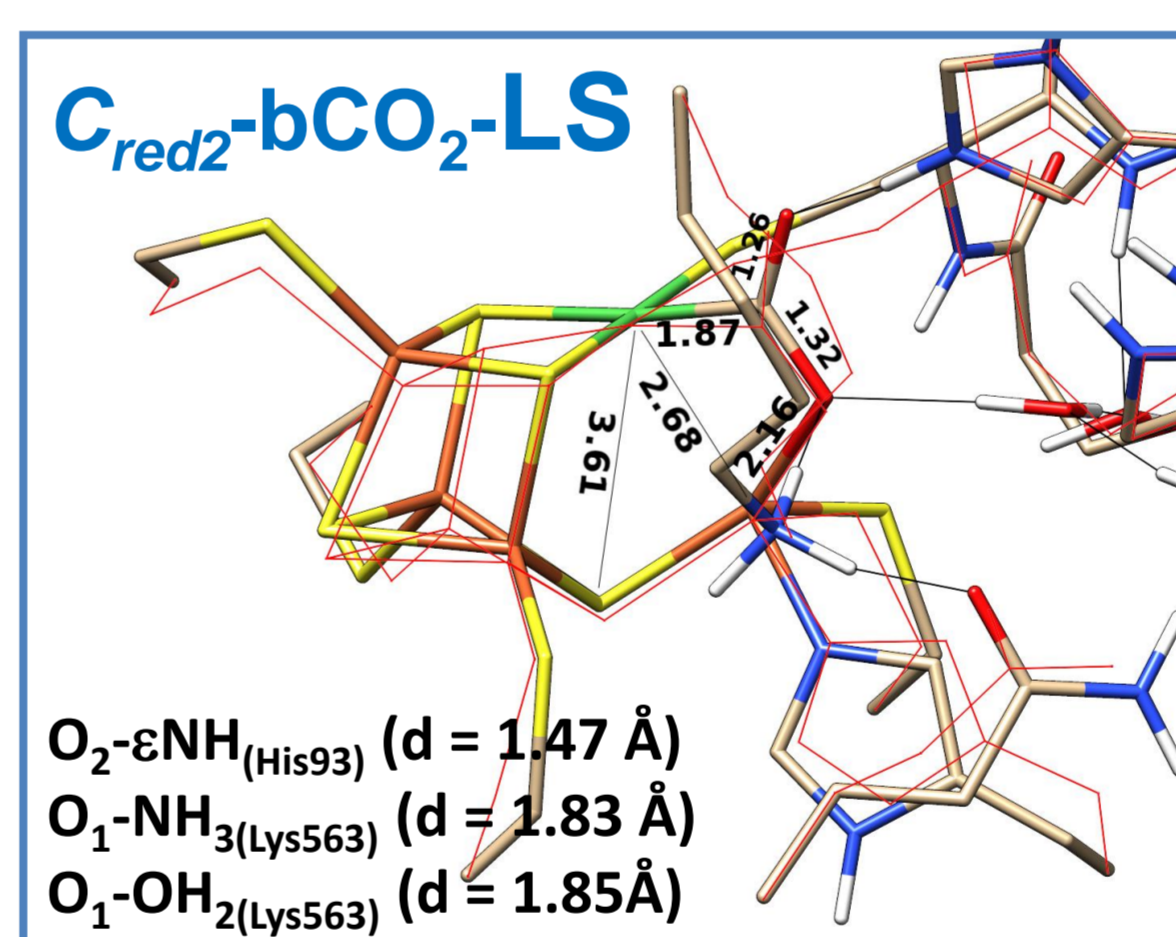
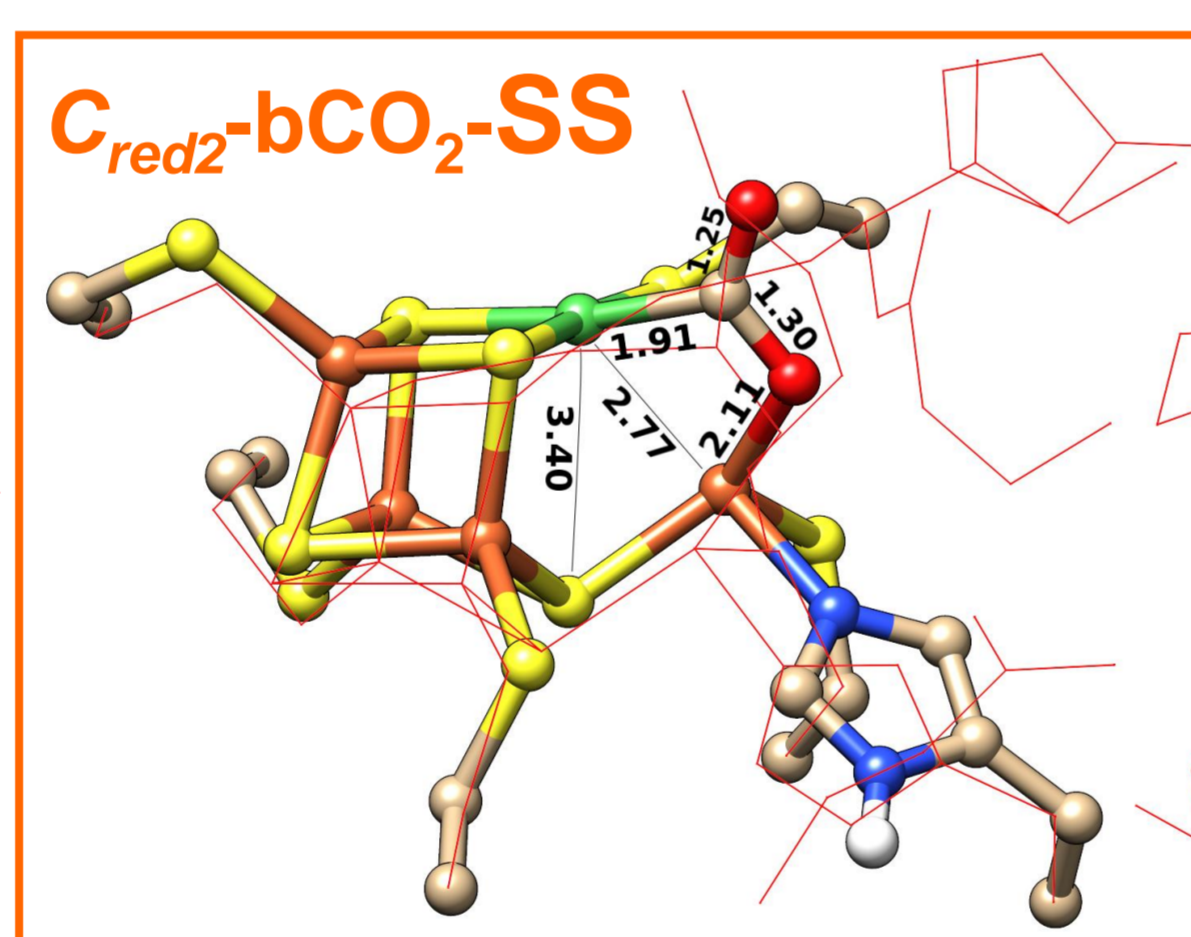
Two different binding modes of CO<sub>2</sub> to the C-cluster, using the SS and the LS models of the active site, have been investigated:

## $\mu_2\text{-}\eta^2\text{C,O}$ CO<sub>2</sub> binding mode:

3B52 x-ray structure ( $d_{\min}=1.5 \text{ \AA}$ ) of the CO<sub>2</sub> bounded form of the C-cluster in the C<sub>red2</sub> state



Both models adequately reproduce the 3B52 structure



In addition, the predicted bent CO<sub>2</sub> geometry is very similar to that found in the recent true atomic resolution 4UDX structure ( $d_{\min}=1.03 \text{ \AA}$ ). [6] Distortion from linearity of bound CO<sub>2</sub> suggests the **CO<sub>2</sub> reductive activation**.

Selected bond distances (Å) and angles (°)

	C-O <sub>1</sub>	C-O <sub>2</sub>	O <sub>1</sub> -C-O <sub>2</sub>
SS	1.30	1.25	124.1
LS	1.32	1.26	122.0
3B52	1.25	1.26	132.6
4UDX	1.32	1.30	117.2
free CO <sub>2</sub>	1.17	1.17	179.9
CO <sub>2</sub> <sup>-</sup>	1.25	1.25	135.2
CO <sub>2</sub> <sup>2-</sup>	1.32	1.32	117.9

NBO charge and Mulliken spin population of the bound CO<sub>2</sub>

	q CO <sub>2</sub>	S CO <sub>2</sub>
SS	-1.02	0.09
LS	-1.06	0.03

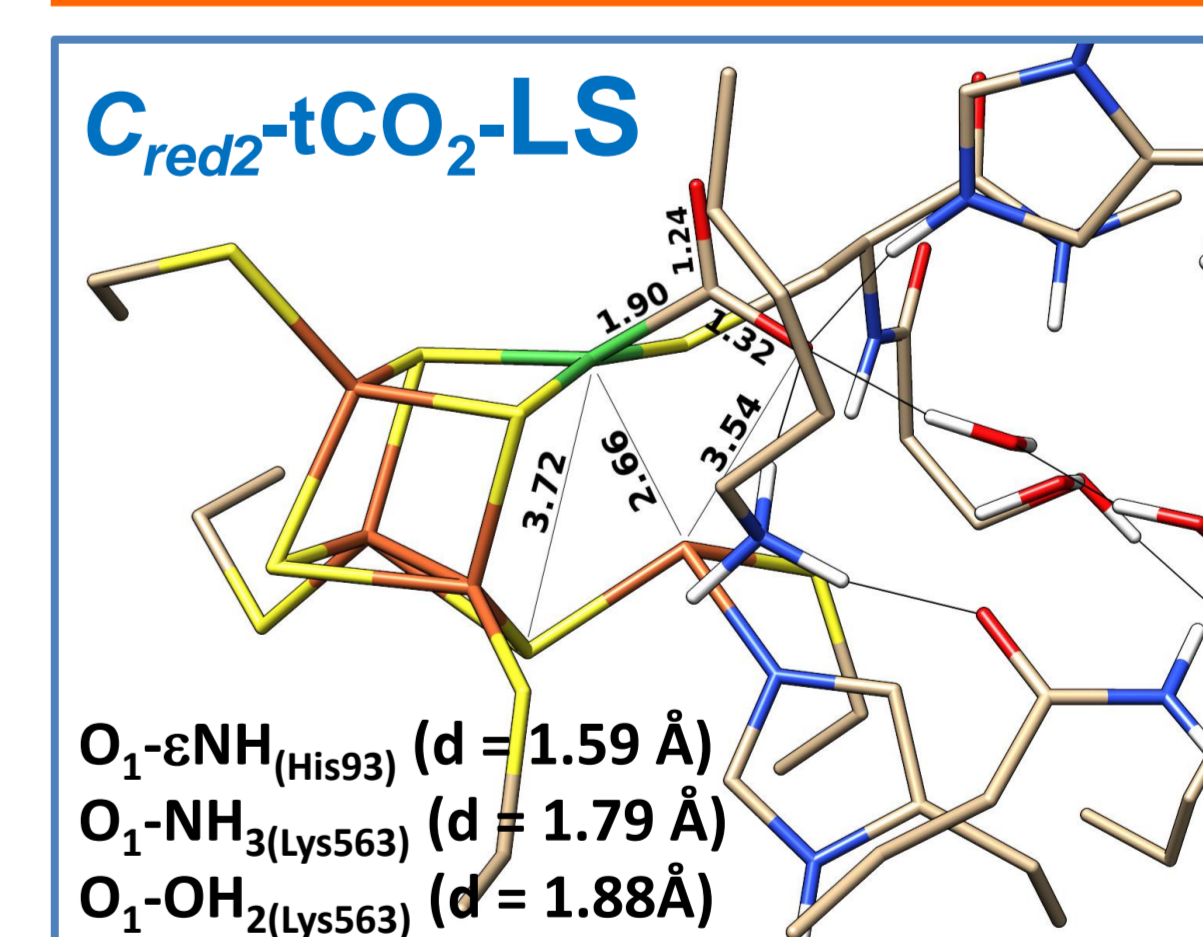
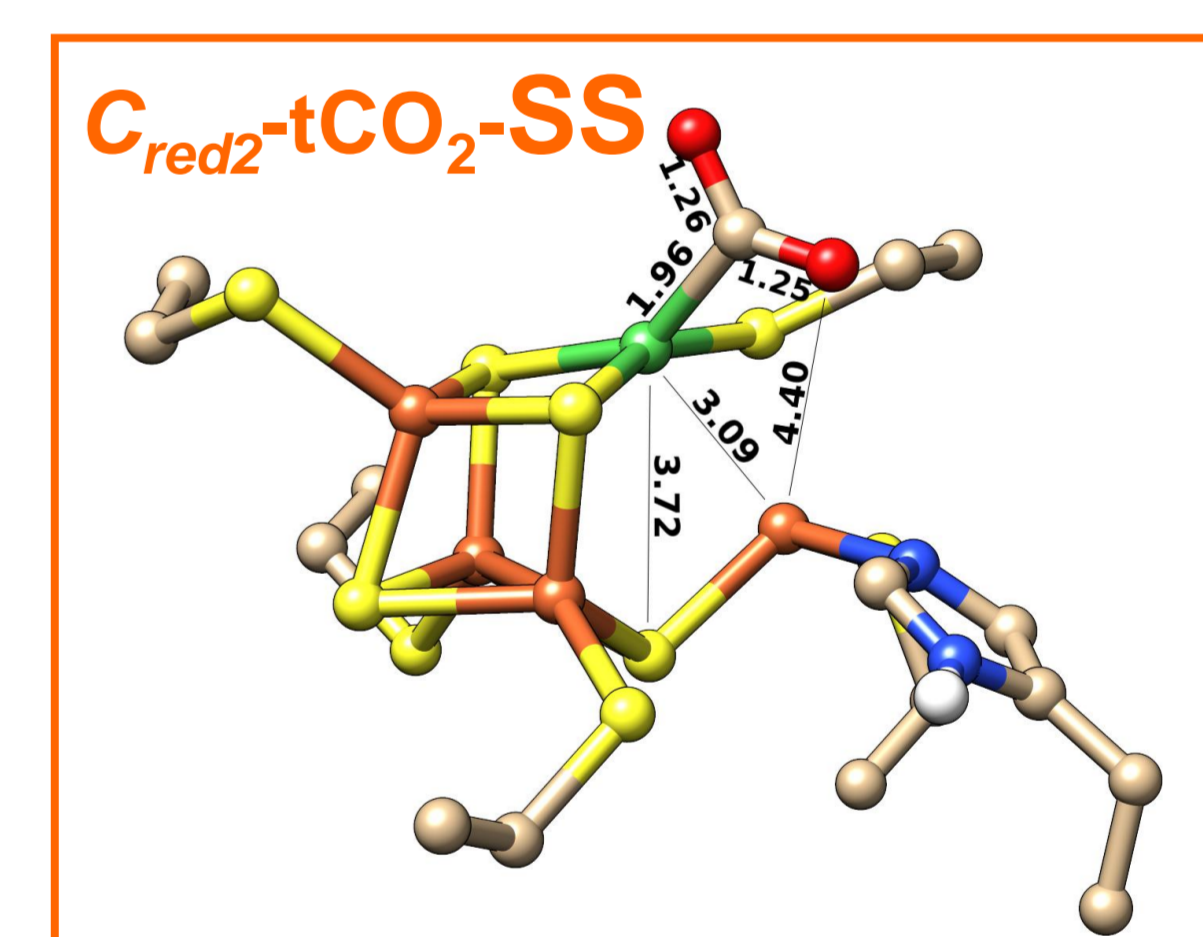
Partial charge of the bound CO<sub>2</sub> confirm a **net electron transfer from the C-cluster to CO<sub>2</sub>**.

The spin population and the geometry comparison of the bound CO<sub>2</sub> with those computed for the free CO<sub>2</sub> and its mono- and di-reduced species suggest the **two-electron reduction to the formal CO<sub>2</sub><sup>2-</sup> species**:

**C<sub>red2</sub>-CO<sub>2</sub> adduct better described as C<sub>red1</sub>-CO<sub>2</sub><sup>2-</sup>**

## $\eta^1\text{C}$ CO<sub>2</sub> binding mode:

No experimental structures corresponding to the terminal binding of CO<sub>2</sub> to Ni.



As found for the bCO<sub>2</sub> isomers, the partial charge of the terminally bound CO<sub>2</sub> is about -1.

$\Delta E$  (tCO<sub>2</sub> → bCO<sub>2</sub>) (Kcal/mol)

	SS	LS
C <sub>red2</sub>	+5.2	-16.5
C <sub>int</sub>	+3.2	/
C <sub>red1</sub>	+1.5	/

**SS model:** tCO<sub>2</sub> slightly more stable than bCO<sub>2</sub>  
**LS model:** only in the C<sub>red2</sub> state the CO<sub>2</sub> terminally binds the Ni atom. However, C<sub>red2</sub>-bCO<sub>2</sub> is largely more stable than C<sub>red2</sub>-tCO<sub>2</sub>. The C<sub>int</sub>-tCO<sub>2</sub> and the C<sub>red1</sub>-tCO<sub>2</sub> isomers are unstable as they converge to the bCO<sub>2</sub> species.

E bCO<sub>2</sub>-binding (Kcal/mol)

	SS	LS
C <sub>red2</sub> -bCO <sub>2</sub>	-12.1	-35.1
C <sub>int</sub> -bCO <sub>2</sub>	+1.4	-27.3
C <sub>red1</sub> -bCO <sub>2</sub>	+6.4	-12.9

E tCO<sub>2</sub>-binding (Kcal/mol)

	SS	LS
C <sub>red2</sub> -tCO <sub>2</sub>	-17.4	-18.7
C <sub>int</sub> -tCO <sub>2</sub>	-1.8	/
C <sub>red1</sub> -tCO <sub>2</sub>	+4.9	/

$$\Delta E_{LS} > \Delta E_{SS}$$

$$\Delta E_{C_{red2}} > \Delta E_{C_{int}} > \Delta E_{C_{red1}}$$

## Conclusions:

- The **crucial role of the protein environment** in tuning the CO<sub>2</sub>-binding to the C-cluster is highlighted by the comparison of results obtained using the different sized models.
- The  **$\mu_2\text{-}\eta^2\text{C,O}$  CO<sub>2</sub> binding is largely stabilized by the formation of a network of H-bond**.
- The conformation in which CO<sub>2</sub> is terminally coordinated to Ni can play a key role in the binding/dissociation of this molecule.
- The release of CO<sub>2</sub> should promote an electron transfer from the C-cluster to the other FeS clusters.
- According to experiments, CO<sub>2</sub> binds in the C<sub>red2</sub> state. [7]