



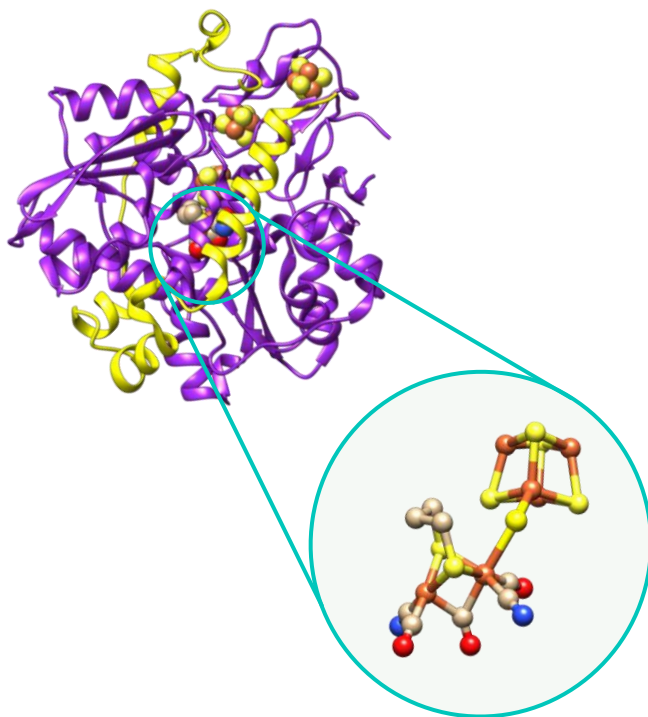
Quantum-mechanical study of the stereoelectronic and catalytic properties of metallo-enzymes involved in reactions of environmental and technological relevance

Prof. Maurizio Bruschi

Laboratorio di Chimica Computazionale Inorganica per l'Ambiente

Towards a Green Economy: Learning from Nature

The use of **enzymes** in the industrial catalysis is one of the most promising strategies to develop sustainable technologies and processes with minimal impact on the environment



Development of chemical and biotechnological solutions to two of the biggest challenges of our society:

- ❑ reduction of greenhouse gas emissions
- ❑ sustainable production of chemical fuels from non-petrochemical sources



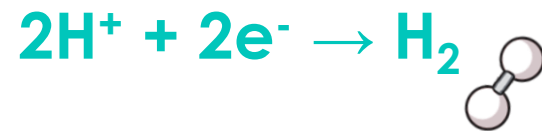
Reduction of CO₂ and production of sustainable fuels

1. Carbon monOxide DeHydrogenase (CODH)



Sequestration of CO₂
by its conversion to
liquid fuels

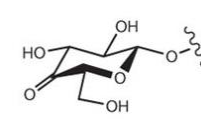
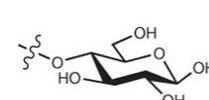
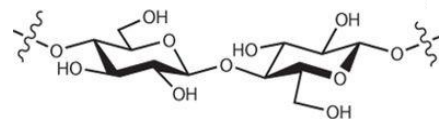
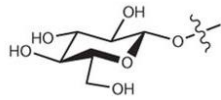
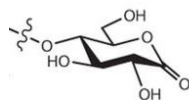
2. Hydrogenase (H₂ase)



Viable convertible form of energy
for the future



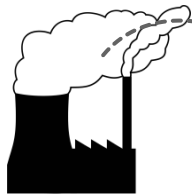
3. Lytic Polysaccharide MonoOxygenases (LPMO)



oxidative polysaccharide chain breakage

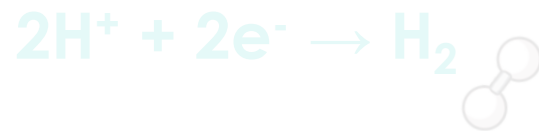
Second-generation
biofuels from non-edible
biomass

1. Carbon monOxide DeHydrogenase (CODH)



Sequestration of CO_2
by its conversion to
liquid fuels

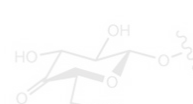
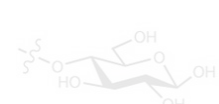
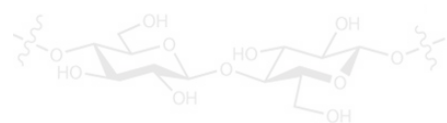
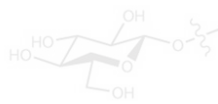
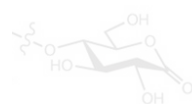
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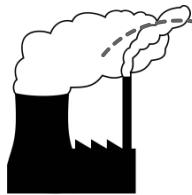


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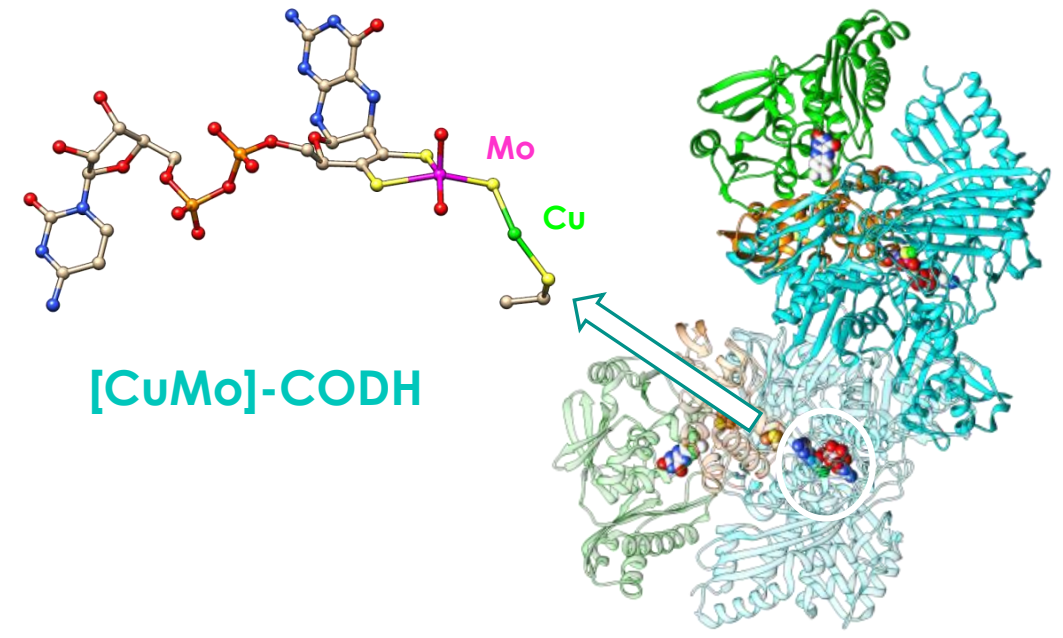
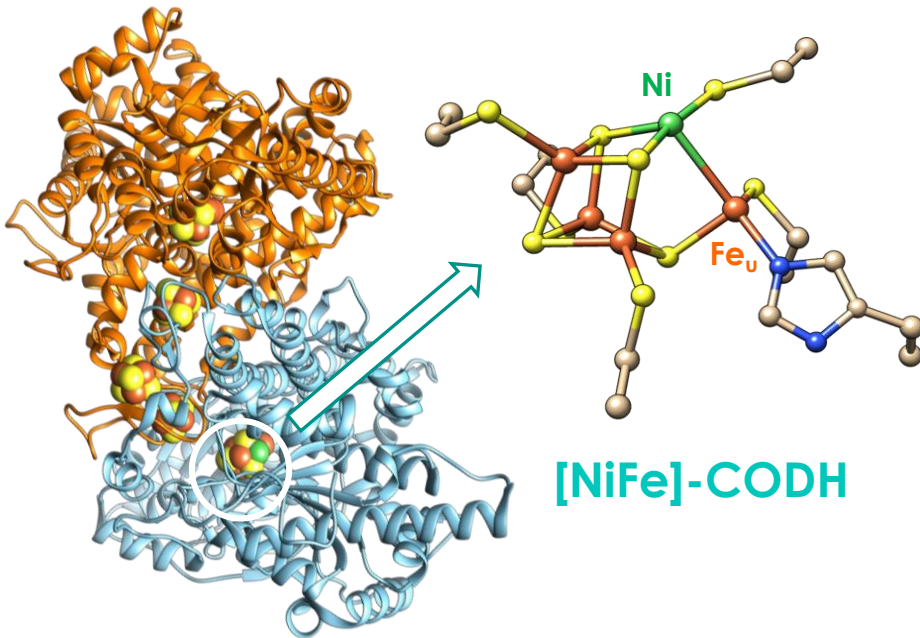
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CODH

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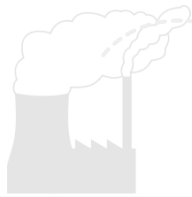


Sequestration of CO_2
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liquid fuels



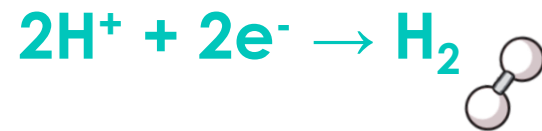
H₂ase

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Sequestration of CO₂
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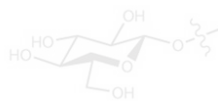
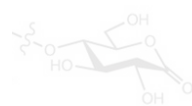
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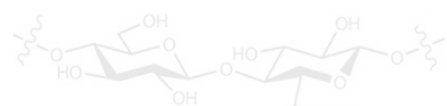
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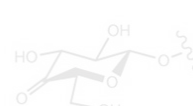
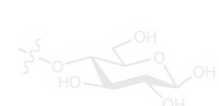
3. Lytic Polysaccharide MonoOxygenases (LPMO)



C1



C4

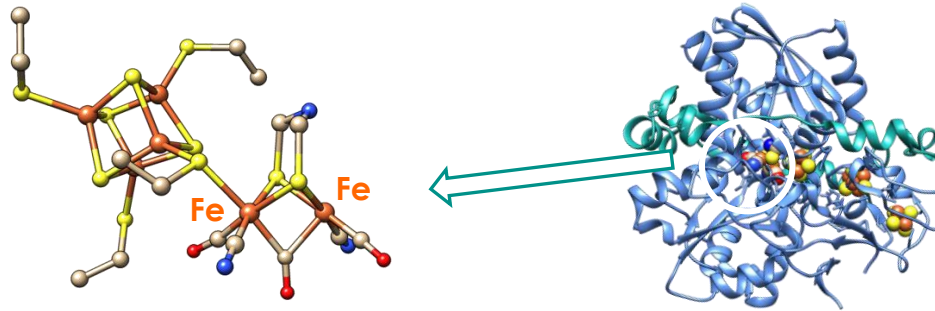


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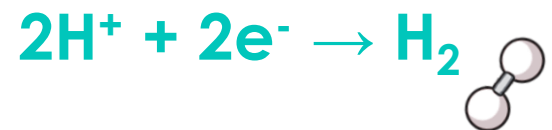
H₂ase

[FeFe]-H₂ase

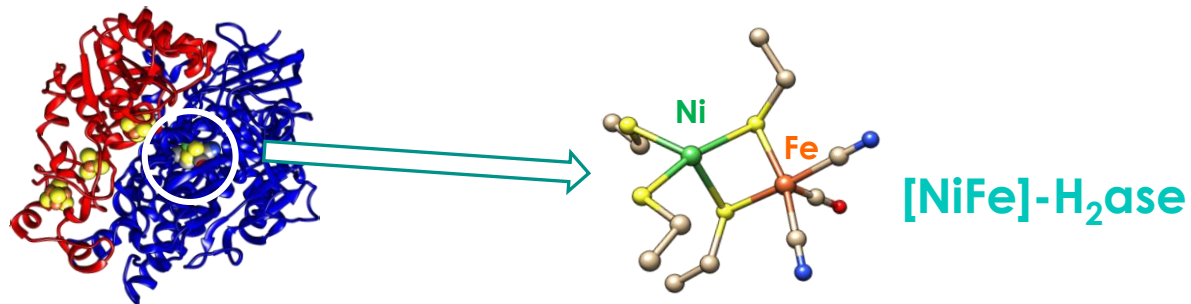


2.

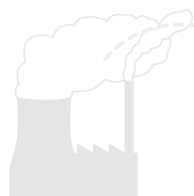
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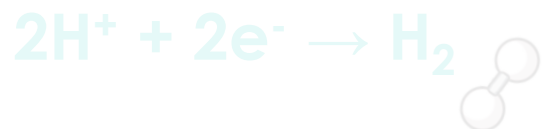


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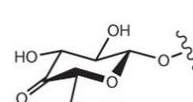
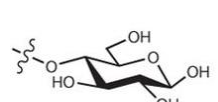
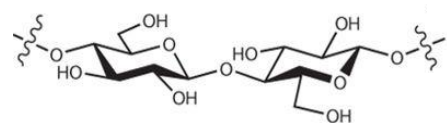
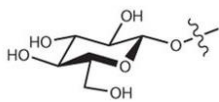
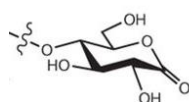
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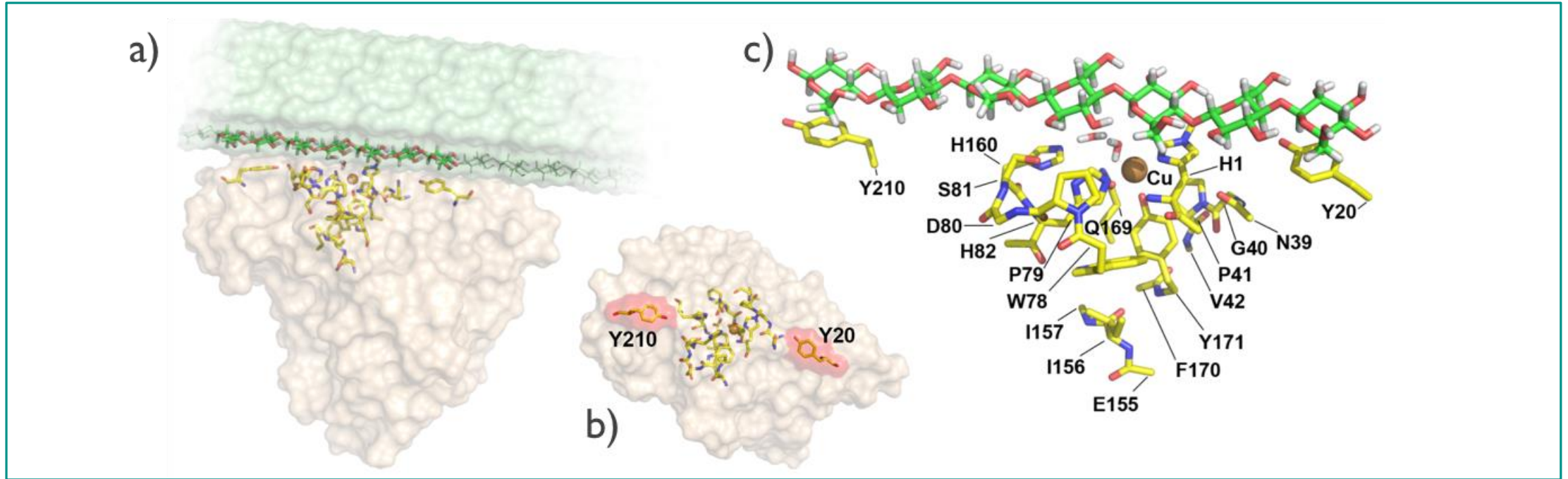


3. Lytic Polysaccharide MonoOxygenases (LPMO)

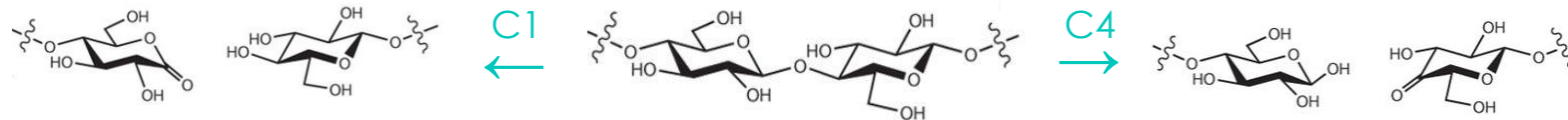


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3. Lytic Polysaccharide MonoOxygenases (LPMO)



oxidative polysaccharide chain breakage

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Aim of the project

Quantum mechanical investigation, in the Density Functional Theory (DFT) framework, of the CODH, H₂ase and LPMO **active sites**.

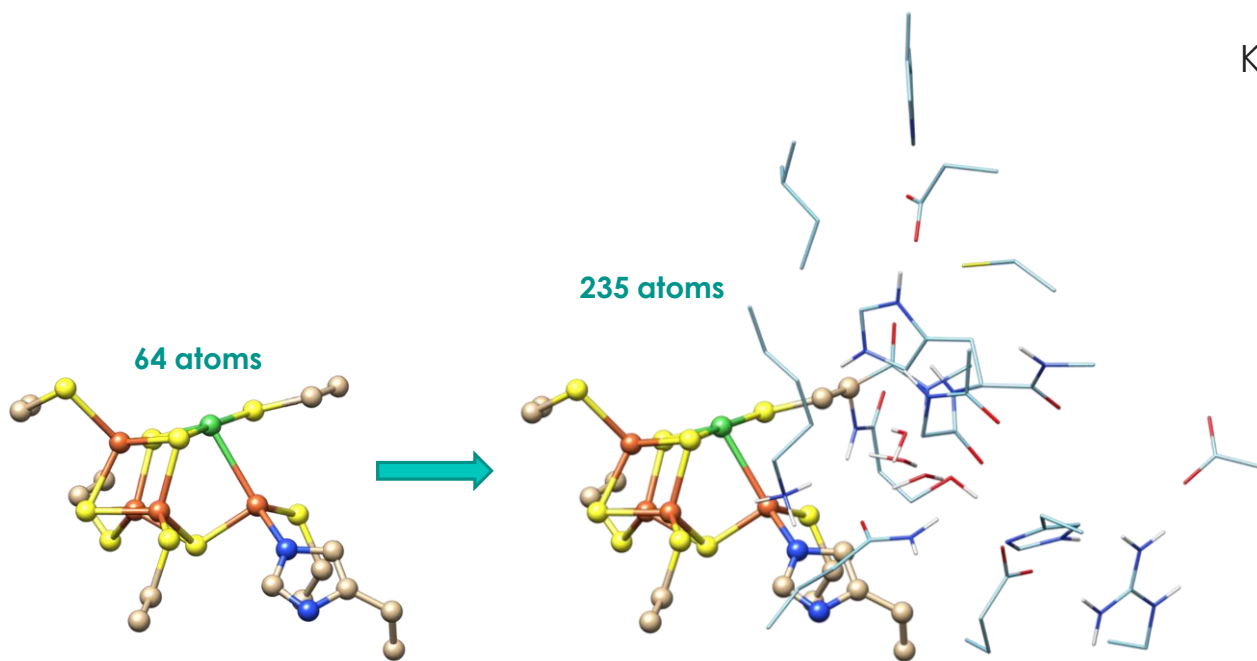
- ❑ Broken-symmetry formalism
- ❑ Cluster models approach
- ❑ Conductor-like screening model (COSMO)

- ❑ Analysis of electronic structure and geometry of potential intermediates
- ❑ Role of the protein environment
- ❑ Investigation of the catalytic mechanism
- ❑ Calculation of spectroscopic properties (such as IR, EPR and Mössbauer parameters)

Aim of the project

Quantum mechanical investigation, in the Density Functional Theory (**DFT**) framework, of the CODH, H₂ase and LPMO **active sites**.

1. Carbon monOxide DeHydrogenase (CODH)



Knowledge achieved on CODHs is still very limited

- ❑ Electronic structure of [NiFe₄S₄] ?
- ❑ Role of the protein environment ?
- ❑ Reactivity towards CO₂ and CO ?
- ❑ Catalytic mechanism ?

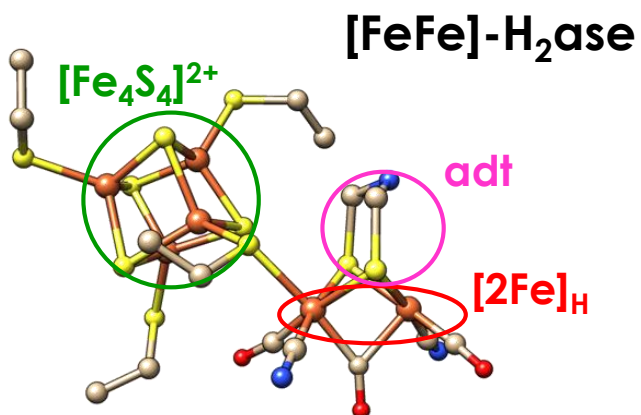
DFT investigation of the stereoelectronic and catalytic properties of the enzyme using two different sized model of the active site.

Aim of the project

Quantum mechanical investigation, in the Density Functional Theory (DFT) framework, of the CODH, H₂ase and LPMO **active sites**.

2.

Hydrogenase (H₂ase)



[2Fe]_H → binding site

[Fe₄S₄]²⁺ → electron transfer

-SCH₂XCH₂S- → proton transfer

High efficiency in H₂ production, **absence of expensive metals** in their active site and presence of an **easily assembled cofactor**



[FeFe]-H₂ases very promising target for reverse engineering studies aimed at the development of bioinspired catalysts.



Numerous biomimetic compounds were assembled and tested. However, the **catalytic efficiency of complexes synthesized so far is much lower than that of the enzyme**.

DFT calculation of spectroscopic properties of the active site and of complexes synthesized so far in order to rationalize the stereoelectronic properties required for the design of efficient biomimetic catalysts

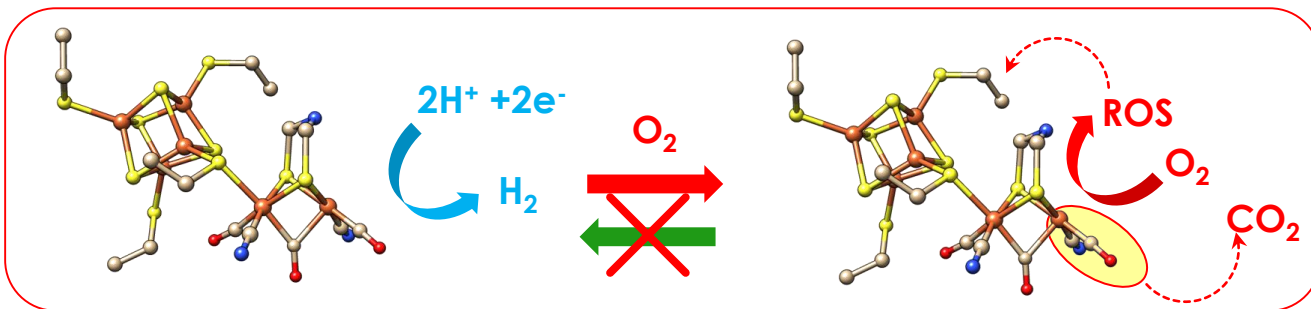
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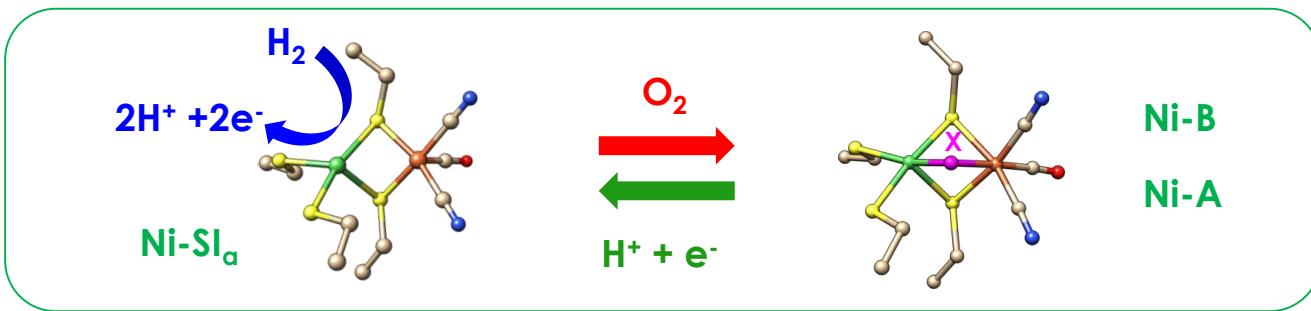
2.

Hydrogenase (H₂ase)

[FeFe]-H₂ases



[NiFe]-H₂ases

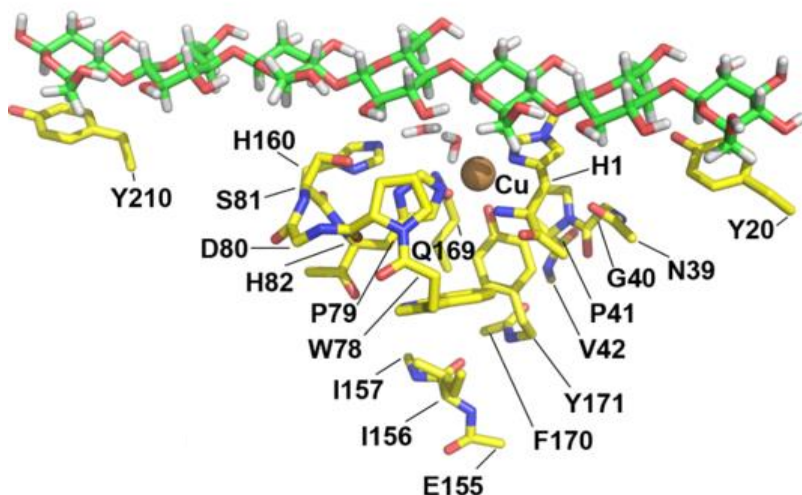


DFT study of the stereoelectronic properties of the active site of [NiFe]-hydrogenases with the final aim of developing O₂-tolerant biomimetic catalysts

Aim of the project

Quantum mechanical investigation, in the Density Functional Theory (DFT) framework, of the CODH, H₂ase and LPMO **active sites**.

3. Lytic Polysaccharide MonoOxygenases (LPMO)



The main unsolved issues on the LPMO catalytic mechanism concern:

- i) to what extent the presence of the sugar might promote oxidative addition of dioxygen to Cu^I;
- ii) the identity of the intermediate that performs the hydrogen atom abstraction step;
- iii) whether the necessary electrons and protons are transferred separately or in combined steps.

DFT study of the reaction pathways suggested to date, exploiting a very large active site model and using a celloheptaose unit as a substrate mimic, in order to provide new insights into the monooxygenase activity of LPMOs and disclose the role played by second sphere residues in assisting the catalytic mechanism.



Theoretical investigation of the effects due to the replacement of Zn with Cd in the active site of CuZn-Superoxide dismutase enzymes and their implication in the development of ALS

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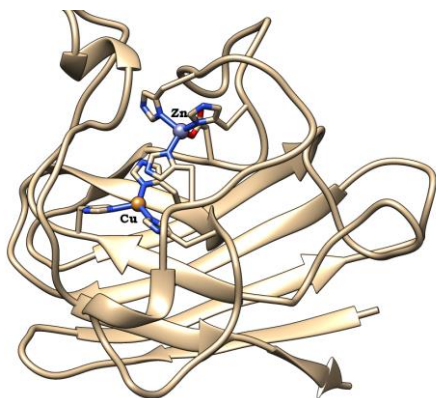
Aim of the project

- CuZn-SOD is a very important enzyme involved in the reduction of the oxidative stress in humans by catalyzing the dismutation of the superoxide anion, produced as a byproduct in the oxygen reduction, into O_2 and H_2O_2
- Dysfunction of CuZn-SOD may be implicated in the development of Amyotrophic Lateral Sclerosis (ALS), as it has been found that several patients affected by ALS have mutated aminoacids in this enzyme
- Recently it has been suggested that the replacement of the Zn ion contained in the SOD active site with Cd may be an important factor for the development of ALS

Aim of the project

- Molecular dynamics investigation of the conformational effects due to replacing of Zn with Cd in the active site of the protein
- Quantum mechanical investigation, in the Density Functional Theory (DFT) framework, of the catalytic mechanism with Zn replaced Cd.

CuZn-SOD



Main questions to be addressed

- Replacement of Zn with Cd induces conformational modification in the protein that enhances its aggregation properties?
- Are there synergic effects due to the concomitant replacement of Zn with Cd and mutations in the protein?
- How the replacement of Zn with Cd affects the catalytic mechanism?

This project is carried out in collaboration with experimental biologists and doctors of UNIMIB, UNIMI, UNIPO, and Ospedale Maggiore Novara