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
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 CO$_2$ and production of sustainable fuels

1. **Carbon monOxide DeHydrogenase (CODH)**

   \[ \text{CO}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2 \text{O} + \text{CO} \]

   Sequestration of CO$_2$ by its conversion to liquid fuels

2. **Hydrogenase (H$_2$ase)**

   \[ 2\text{H}^+ + 2e^- \rightarrow \text{H}_2 \]

   Viable convertible form of energy for the future

3. **Lytic Polysaccharide MonoOxygenases (LPMO)**

   oxidative polysaccharidae chain breakage

   Second-generation biofuels from non-edible biomass

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University of Milano-Bicocca

23/11/2018
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oxidative polysaccharide chain breakage
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Sequestration of CO$_2$ by its conversion to liquid fuels

1. [NiFe]-CODH

2. [CuMo]-CODH

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3. **Lytic Polysaccharide MonoOxygenases (LPMO)**

![Diagram of LPMO with arrows indicating oxidative polysaccharide chain breakage](image)

- C1 to C4
- Oxidative polysaccharide chain breakage
- Second-generation biofuels from non-edible biomass

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Prof. Maurizio Bruschi  
University of Milano-Bicocca  
23/11/2018
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.
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Quantum mechanical investigation; in the Density Functional Theory (DFT) framework, of the CODH, H₂ase and LPMO active sites.

2. Hydrogenase (H₂ase)

- **[FeFe]-H₂ase**
  - [Fe₄S₄]²⁺ binding site
  - [FeH]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 synthetized so far in order to rationalize the stereoelectronic properties required for the design of efficient biomimetic catalysts**

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

DFT study of the stereoelectronic properties of the active site of [NiFe]-hydrogenases with the final aim of developing O₂-tolerant biomimetic catalysts.
### Quantum mechanical investigation; in the Density Functional Theory (DFT) framework, of the CODH, H$_2$ase and LPMO active sites.

#### Lytic Polysaccharide MonoOxygenases (LPMO)

The main unsolved issues on the LPMO catalytic mechanism concern:

1. **To what extent the presence of the sugar might promote oxidative addition of dioxygen to Cu**
2. **The identity of the intermediate that performs the hydrogen atom abstraction step**
3. **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

Prof. Maurizio Bruschi
Laboratorio di Chimica Computazionale Inorganica per l’Ambiente
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 O₂ and H₂O₂.

- 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.
The aim of the project is to investigate the conformational effects due to replacing Zn with Cd in the active site of the protein and to study the catalytic mechanism with Zn replaced by Cd. This will be done through molecular dynamics and quantum mechanical investigations. The main questions to be addressed are:

1. Does replacement of Zn with Cd induce conformational modification in the protein that enhances its aggregation properties?
2. Are there synergic effects due to the concomitant replacement of Zn with Cd and mutations in the protein?
3. How does the replacement of Zn with Cd affect the catalytic mechanism?

The project is carried out in collaboration with experimental biologists and doctors from UNIMIB, UNIMI, UNIPO, and Ospedale Maggiore Novara.