Minimized [NiFe]-hydrogenase as a biocatalyst

Molecular dynamics simulations of the large subunit using enhanced sampling techniques

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In Short

- Immobilization on SAM-coated Au surface
- Conformational dynamics

Hydrogenase enzymes catalyze reversible production and oxidation of H_2 in metabolism of microorganisms, which makes them primary candidates for bio-fuel cell engineering and generally in the field of energy production and storage.They contain metallic centers acting as active sites for oxidation/reduction, usually containing one Ni and one Fe atom or two Fe atoms coordinated to the protein via cysteine residues and CO and CN^- ligands in addition. Of critical interest for controlled biocatalysis is immobilization of hydrogenases on biocompatible gold electrode, *i.e.* Self-Assembled Monolayer (SAMs) coated Au surface. Immobilized proteins can be reversibly oxidized/reduced without additional agents and also can be used for coupled enzyme designs (with another enzyme or assembly of the original heterodimer) on top of an electrode and its subsequent control.

Immobilization of the entire heterodimeric membrane-bound [NiFe]-hydrogenase (MBH) from Ralstonia eutropha (ReH16) (**Fig. 1, up**) on various SAM surfaces [1,2] have been investigated in recent years: enzyme orientation relevant to the surface, SAM type and the effect of its protonation degree. Recently, hydrogenase research recently delved into applications involving only the catalytically irreplaceable large subunit (HoxG) of a membrane-bound [NiFe]-hydrogenase (MBH) containing only [NiFe] metallic center and acting as the minimal [NiFe]-hydrogenase [3]. Our unpublished results from the first phase of the HLRN project bec00218 confirm that the isolated large subunit of [NiFe]-hydrogenase is very stable as a monomer but also has tendencies for aggregation in solution. Investigation of the interaction of hydrogenase with bio-electrodes may be simplified by utilizing only the large subunit of MBH, HoxG, which is the focus of this project. It has been experimentally confirmed that immobilization of monomeric HoxG on certain SAM types under specific pH conditions as a monolayer is possible. The highest amount of protein was immobilized in case of amino-terminated ($-S(CH_2)_8NH_2$) SAM, followed by carboxyl-terminated ($-S(CH_2)_7COOH$) and hydrophobic ($-S(CH_2)_7CH_3$) SAMs.

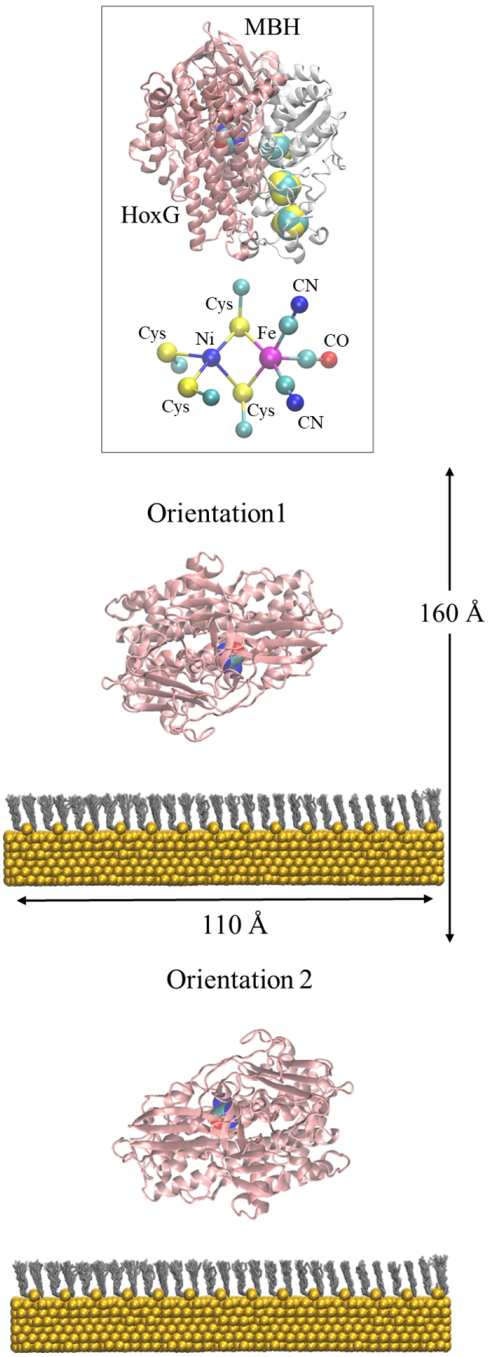

Figure 1: *[NiFe]-hydrogenase heterodimer (MBH) and two modeled orientations of thermally equilibrated HoxG subunit above the hydrophobic SAM-coated Au surface.* ***Up****: MBH heterodimer (up) and [NiFe]-active site (down).* ***Middle****: Orientation 1 of HoxG where an open HoxG interface and the active site are facing the surface.* ***Down****: Orientation 2 of HoxG where an open HoxG interface and the active site are facing away from the surface towards the solution.*

Here, several questions arise regarding the effect of the chain length and SAM type, the effect of the buffer pH and orientation of the protein on the surface. These details are needed in order to improve electrochemical control of HoxG and understand immobilization dynamics, which can be answered by looking into the immobilization at atomic level, *i.e.* performing long MD simulations. In summary, the extension of the project bec00218 attempts to shed light on how to improve immobilization of HoxG subunit on SAM-coated Au surface, by providing atomistic details regarding conformational dynamics of immobilization process by varying SAM type and chain length, buffer pH and investigating preferable protein orientation during the process (**Fig. 1**). This will be mostly done by performing Gaussian-accelerated molecular dynamics (GaMD) simulations. The prepared HoxG structures in project bec00218 can now be utilized in this project extension.

Molecular dynamics is a widely used method to virtually observe the time-dependent behavior of proteins and other systems. Set of function and parameters describing simulated molecules are contained in the force field, in this case CHARMM [4]. The dynamics of the system is obtained by numerically solving Newtons equation of motion for the present atoms. In order to thoroughly explore the conformational dynamics during protein immobilization, Gaussian-accelerated molecular dynamics (GaMD) will be utilized. This is a type of accelerated molecular dynamics, where potential energy surface gets smoothened by adding a boost potential which follows a Gaussian distribution [5]. Thereby, by raising the potential energy is raised and this allows sampling of the conformational space not accessible with classical MD simulations (cMD). Considering the system size and possible conformational change on larger time scales, this is the method of choice. NAMD software [4], used for molecular dynamics simulations is available on the HLRN architecture.

Following the sampling of the conformational space of the immobilization of HoxG monomer on SAM-Au surfaces, interaction energy with the surface as well as protonation and conformational changes in the enzyme itself will be evaluated (using locally available resources at TU Berlin).

WWW

https://www.biomodeling.tu-berlin.de

More Information

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Project Partners

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