Multiscale approaches for modeling proteins on solid surfaces

All-atom and coarse-grain simulations of bilirubin oxidase adsorbed on SAM functionalized surfaces

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In Short

- Predicting protein orientation on functionalized surfaces
- Impact of adsorption on the protein conformational stability and internal dynamics.

The interaction of proteins with solid surface is of crucial importance in the field of biomaterials [1], since it plays a key role in numerous applications, such as tissue engineering and regenerative medicine, the optimization of surfaces for biosensors, the development of bioactive nanoparticles, biocatalysist or bioanalytical systems. One of the main issues that are addressed when investigating protein/surface interaction, is the matter of protein orientation. Controlled adsorption with ordered proteins is essential for devices such as biosensors, where antibodies should be immobilized with a specific orientation favoring the following antibody-antigen binding, or biofuel cells, where a correct enzyme orientation is crucial for direct electron transfer between the adsorbed protein and the electrode [2]. The adsorbed proteins orientation depends on many factors, such as their charge, size or shape, their surfaces properties, or external conditions like the temperature and pH. Hence theses devices require a specific functionalization of the surface, in order to fine tune its charge and physico-chemical properties, that can be achieved with self assembled monolayers (SAMs) for example. For both cases, the conservation of the adsorbed proteins native conformation (and hence its biological function) is another a key aspect that has to be taken in consideration.

All these phenomenons have now been under scrutiny for several decades, but reaching a detailed understanding of the molecular mechanisms associated with biomolecular adsorption on functionalized surfaces is far from being achieved. In particular, considerable experimental works have been conducted until now, such as atomic force microscopy, mass spectrometry and various spectroscopies. However, the resolution of current experimental techniques is still insufficient to quantitatively determine the orientation and conformation of adsorbed proteins on the atomistic level. In that perspective, molecular simulations play an increasingly important role in revealing the mechanisms of chemical and biological processes taking place on the bio-nano interface, and designing new products [3,4].

This work on protein-surfaces interactions is part of a collaboration with the electrochemistry group of Dr. Lojou at the Bioénergetique et Ingénierie des Protéines Laboratory in Marseille, France. These project aims to develop biofuel cells were redox enzymes are grafted on each electrode in order to serve as biocatalysts (see **Figure 1**). The resulting biofuel cell is inspired from the pathway microorganisms use for the production of ATP, with the advantages of biocatalysts over classical chemical catalysts (like Pt): bioavailability, biodegradability, and substrate specificity. The H2/O2 biofuell cells can be regarded as a promising system for small power devices such as environmental sensors [5].

As theoreticians, our part in these project is to develop molecular simulation tools that will help to understand the protein adsorption process on the electrodes on the atomic level. During the first step of the project, we used the ProPhet program, which was developed in my research group in Paris, to calculate protein mechanical properties on the residue

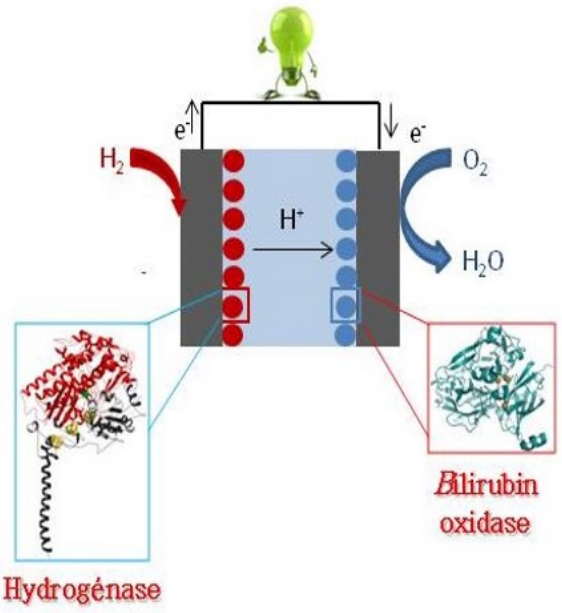

Figure 1: *A schematic view of the biofuel cell developed in the BIP Laboratory in Marseille. [NiFe] hydrogenases are adsorbed on the anode, while multicopper bilirubin oxidases are used at the cathode.*

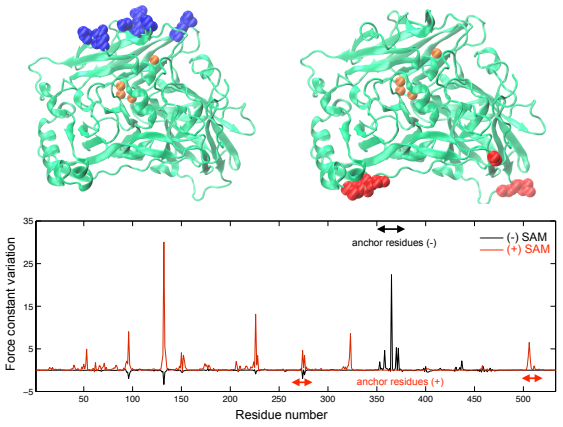

Figure 2: *Upper panel: Bilirubin oxidase with the residues in direct contact with the solid surface shown in blue (for negatively charged SAMs) or in red (for positively charged SAMs). Lower panel: Force constant variations for the BOD adsorbed on negatively (black line) or positively charged SAMs (red line)*

level [6]. Proteins are highly flexible objects, and a proper understanding of their flexibility is essential to understand how they function [7]. ProPHet was modified to investigate how the adsorption on a solid surface can impact a protein mechanics, internal dynamics, and therefore its biological activity. We showed how the adsorption on a surface functionalized with positively charged self-assembled monolayers (SAMs) undergoes larger mechanical perturbations than when adsorbed on negatively charged SAMS (see **Figure 2** [8]). As a consequence BOD adsorbed on positively charged SAMs are more likely to see their function perturbed. These results concur with the experimental results produced by the electrochemistry group of Dr. Lojou, who obtained much lower catalytic currents for BODs adsorbed on positively charged SAMs than for negatively charged SAMs [9]. This is of particular interest since it shows that protein function also depends on their internal dynamics, and that seemingly negligible structural changes can nevertheless result in modifications of the protein mechanics that will considerably alter its activity [10]. In the case of enzyme grafted on electrodes, the conservation of the protein native structure is no guarantee that the enzyme will remain functional once it has been immobilized.

A limitation of this work is that this simulation technique does not explicitly model the interaction between the protein and the surface it is adsorbed on. The implicit modeling of the impact of adsorption on protein mechanics relies on structural information regarding the protein orientation on the surface. Therefore, this project represents the next step in our study of protein/surface interactions. Combined with the coarse-grain calculation tools, this will enable us to set up a complete, multiscale, approach for investigating the mechanical behavior of redox enzymes grafted on functionalized surfaces. With a first MD step on the classical all-atom level, to predict the enzyme orientation on the surface, and potential conformational changes resulting from the adsorption process. Followed by a coarse-grain step that will give us information regarding the impact of adsorption on the enzyme mechanical properties as a function of the residues in contact with the surface. The MD simulations will be done using the NAMD software, which is available on the HLRN architecture.

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More Information

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Project Partners

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