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Research paper

# Insulin adsorption on crystalline $SiO_2$: Comparison between polar and nonpolar surfaces using accelerated molecular-dynamics simulations

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ABSTRACT

Adsorption of insulin on polar and nonpolar surfaces of crystalline $SiO_2$ (cristobalite and $\alpha$-quartz) is studied using molecular dynamics simulation. Acceleration techniques are used in order to sample adsorption phase space efficiently and to identify realistic adsorption conformations. We find major differences between the polar and nonpolar surfaces. Electrostatic interactions govern the adsorption on polar surfaces and can be described by the alignment of the protein dipole with the surface dipole; hence spreading of the protein on the surface is irrelevant. On nonpolar surfaces, on the other hand, van-der-Waals interaction dominates, inducing surface spreading of the protein.

## 1. Introduction

The interaction of proteins with inorganic surfaces is vital for describing their biocompatibility. Upon adsorption on the surface, the protein conformation may be strongly influenced, and depending on the surface hydrophilicity, the protein may be denatured [1]. In particular proteins tend to unfold on hydrophobic surfaces, while they keep their structure on hydrophilic surfaces [2]. In the past, molecular dynamics (MD) simulations have proven to be a useful tool for studying such conformation changes upon adsorption at an atomistic scale [3].

Insulin is an important protein of considerable medical relevance, in particular for the treatment of diabetes. For drug delivery, the protein is often kept in glass containers, since it is often believed to interact only weakly with it. The question of how strongly insulin binds to the container wall and how much it denatures thereby is therefore of interest when regulating the rate of drug delivery and the quality of the delivered drug [4]. Pertinent studies have been undertaken for instance by Mollmann et al. [5] who study insulin adsorption on Teflon using total internal reflection fluorescence.

Previous experimental studies mainly turned their attention to amorphous silica. They investigated the conformational stability of insulin on silica surfaces and showed that insulin is more strongly adsorbed to a (methylated) hydrophobic silica surface than to a hydrophilic silica surface [6]. Jorgensen et al. [7] studied insulin adsorption on a Teflon surface and Ademovic et al. [8] on a hydrophobic poly(vinylidene) fluoride surface, and showed that major conformational changes are induced.

Simulation studies of insulin adsorption have been carried out using MD by Liang et al. [9] on graphene. They find major conformational changes caused by the adsorption, which is in line with other studies of protein adsorption on this hydrophobic surface [10–13]. While we are not aware of previous MD studies of insulin adsorption to silica surfaces, the adsorption of other molecules on such surfaces has been investigated. Thus, for example, Tosaka et al. [14] study the adsorption of the ribosomal protein L2 (RPL2) on silica and find that adsorption becomes stronger with the pH value of the solvent.

In a series of papers, Kubiak-Ossowska et al. [15–17] study the adsorption of proteins on silica surfaces, considering both lysozyme and fibronectin proteins. The role of the protein dipole moment in establishing the electrostatic interaction and guiding the molecule into a favorable orientation during adsorption is discussed. These studies, however, focus on polar and charged surfaces. In addition, due to their relatively short simulation times of $\leqslant$100 ns, they may not have explored phase space sufficiently to identify the realistic adsorption conformations.

In the present paper, we use MD simulation to study the adsorption of insulin on crystalline $SiO_2$ surfaces. By considering both polar and nonpolar surfaces, we identify the influence of electrostatic interaction on the adsorption process. This allows us to go

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beyond previous work that focused on polar and charged surfaces. The use of accelerated MD (aMD) [18,19] allows us to sample the protein phase space more efficiently and to obtain final protein conformations that represent the real adsorbed conformation more closely.

## 2. Methods

We study two crystalline modifications of $SiO_2$, cristobalite and $\alpha$-quartz. Cristobalite has a tetragonal crystal structure, while $\alpha$-quartz is trigonal, see Fig. 1. Depending on the orientation and the atomic termination, the surfaces of these crystals have strongly different properties. We study Si-terminated surfaces of both $\alpha$-quartz and cristobalite; since Si is positively charged, these surfaces are polar. In addition we study a nonpolar cristobalite surface, in which the surface atom concentration is stoichiometric; this surface is hence neutral. ‘Negative’ – i.e., O-terminated – surfaces do not show insulin adsorption; the protein moves within the first 2 ns far away from the surface and will not approach it again. Positive and negative surfaces are polar in that they are characterized by a strong dipole moment; the neutral surface is nonpolar. In this study, we choose the (001) surfaces of cristobalite and $\alpha$-quartz as polar surfaces, and the (010) surface of cristobalite as the nonpolar surface.

In our simulations, the surfaces are realized by thin $SiO_2$ slabs with thicknesses of around 4–6 Å. They have an area of around 55–80 $nm^2$ and contain roughly 2700 atoms. We note that all slabs used in the simulation are stoichiometric and hence electrically neutral. The atoms of the slab are held fixed during the simulation, such that our study concentrates on the motion of the protein. We note that – similar to previous work [15–17] – no surface hydroxylation [20] was considered in our simulations.

The charges of Si and O in the silica model were chosen as $+1|e|$ and $-0.5|e|$, respectively, as proposed by Cruz-Chu et al. [21] in their study of silica-water interaction. From similar considerations, the well depth of the vdW attraction of Si was fixed to 0.3 kcal/mol to obtain the experimentally measured water contact angle of 42° for a silanol-free surface.

The structure of the 2Zn pig insulin was obtained from the protein data base (ID: 4INS [22]). We use the chain A of insulin; it has 313 atoms and includes 21 residues of which 13 are polar, 6 are hydrophobic and 2 are negatively charged (glutamate), see Fig. 2. We study two orientations of the insulin, which are obtained by a rotation by an angle of 90°. Note that the residues situated close to the surface are hydrophobic while the charged residues are roughly in the center of the protein, see Fig. 2.

The entire system extends around 104 Å parallel to the surface and 67 Å normal to it. It contains 11,811 water molecules which are described by the TIP3P [23] water model. 2 $Na^+$ ions are added in order to neutralize the protein charge. We use periodic boundary conditions in order to be able to study a bulk system. The protein is put initially at a distance of 6 Å above the surface. The system is subjected to an energy minimization and is then equilibrated for 10 ps at a temperature of 300 K and constant pressure of 1 bar. Then the adsorption simulations are performed for a total time of 20 ns.

We note that besides the counterions introduced in our system, the water contains no additional ion concentration. It is known that an increase of the ionic strength of the solvent leads to increased screening of the electrostatic interactions, and hence may induce changes in the adsorption process, at least by delaying the time scale of adsorption. This feature has been studied both experimentally [24] and by MD simulation [17].

We use accelerated molecular dynamics (aMD) simulations to study the adsorption of insulin on crystalline $SiO_2$ surfaces. In Sec-

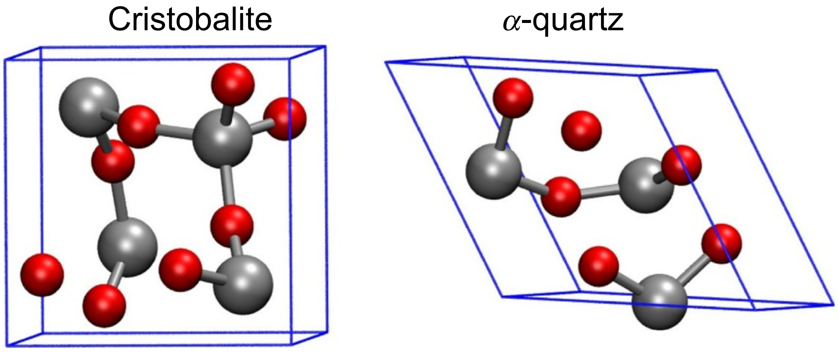

**Fig. 1.** Unit cell of cristobalite and $\alpha$-quartz. Gray: Si; red: O. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

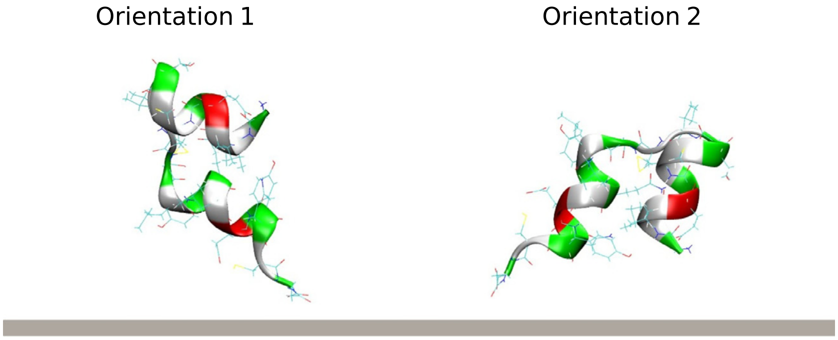

**Fig. 2.** First (left) and second (right) orientation of insulin. Charged residues are plotted in red and hydrophobic residues in white. The surface is indicated schematically. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)