Study of bovine serum albumin interaction with zinc oxide surfaces by force field simulations

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Abstract. We studied the interactions of bovine serum albumin (BSA) molecule with ZnO surfaces by force field simulations. The different orientations of BSA over the two main nonpolar wurtzite ZnO surfaces (10\overline{1}0) and (11\overline{2}0) have been investigated. These surfaces contain both Zn and O atoms. We compare the results also with polar Zn-face (0001) surface. The results demonstrate tendency of BSA molecule for being adsorbed on the ZnO surface in specific orientations. This happens either by bonding between a hydrogen atom of BSA molecule to an oxygen atom of the ZnO surface or by physical attraction inducing ZnO atoms displacement in some cases.

1. Introduction
Serum albumin (SA) is the most plentiful protein in blood plasma and has been investigated intensively for its numerous vital functions such as maintaining the oncotic pressure, transporting drugs as well as contribution in functionality of the immune system. The interactions of SA with a wide range of nanomaterials have been the subject of many investigations [1,2]. The adsorption of SA on diamond as one of the most popular materials in biomedicine and electronics has been already investigated [3–6]. Besides diamond, zinc oxide (ZnO) as a wide and direct band-gap semiconductor with well-known optical properties, low level of toxicity and great biocompatibility possesses luminescence property that can be extremely useful in various biomedical and pharmaceutical applications [7]. The interactions of ZnO with a range of biomolecules and proteins have been reported by multiple experimental studies [8,9]. In [8], an in vitro toxicological investigation supported by theoretical modelling has shown multilayer adsorption of lactate dehydrogenase (LDH) on ZnO nanomaterials. Strong interaction of adenosine triphosphate (ATP) with ZnO nanostructures through chelate formations has also been demonstrated by Raman spectroscopy, XRD, and electron microscopy in [9]. In addition, using UV–vis absorption, fluorescence, synchronous fluorescence, and Raman spectroscopy to study of ZnO nanoparticles covalently and non-covalently coated by bovine serum albumin (BSA) has shown that the binding between ZnO nanoparticles and BSA decreases the cytotoxicity of ZnO nanoparticles [10]. Despite availability of large amount of experimental data conducive to perceiving the essence of ZnO-proteins interactions, fine aspects behind the interactions still need to be studied theoretically to understand the details of the phenomena on atomic scale.

However, the main challenge in computational study of the systems constituting nanomaterials and complex molecules such as albumin is the huge number of the atoms in the system which can make the calculations extremely costly if a proper approach is not adopted. One possible approach to cope
with large systems is selecting more computationally efficient methods, which are still sufficiently accurate, over more costly ones with higher levels of accuracy (such as DFT and \textit{ab initio} methods). Another complementary way can be considering a reasonable simplification in the system under study, such as fixing or excluding some non-interacting atoms in computations.

In this study, we aimed to elucidate the interaction of a BSA molecule with ZnO surfaces on atomic scale. Force field simulations have been carried out under some simplifying assumptions to overcome the computational limitations imposed by the huge size of the system. The interactions between BSA and ZnO for nonpolar and polar surfaces of ZnO have been compared.

2. \textbf{Method}

The adsorption of BSA on ZnO nonpolar surfaces which contain both Zn and O atoms has been investigated by implementing force field method in QuantumATK software which enables the possibility of dealing with the huge number of atoms in our system. The results have also been compared to polar Zn-face (0001) surface. HP system with a Core i5 8th generation processor and 8 GB RAM was used to perform the computations.

The system under the study consists of a BSA molecule and ZnO slab that are available from the RCSB Protein Data Bank website and QuantumATK software database respectively. Since hydrogen atoms are generally not observed in X-ray crystal structures, BSA structure taken from the Protein Data Bank lacks hydrogen atoms. Mol Probity has been employed to add hydrogen atoms to the molecule. Some approximations were also considered to provide the feasibility of using predefined force field potential sets as there are no sets of force fields defined for all types of atoms in BSA+ZnO system. Sulphur atoms have been excluded from the structure of BSA molecule to enable ReaxFF potential for force field simulations. This seems a reasonable simplification as sulphur atoms in BSA are not situated at or near the locations where the interaction of the molecule with ZnO occurs. To deduct the volume and duration of the calculations, different orientations of BSA molecule have been manually adjusted over ZnO surfaces in the distance range of 3-20 Å. The optimization trajectory and energy of the system then have been observed to discuss the interactions of the BSA molecule with ZnO planes.

3. \textbf{Results and discussion}

The simulations have been carried out for different orientations of BSA over nonpolar and polar ZnO planes. Figure 1 depicts five non-equivalent configurations used for forcefield simulations which are named based on the orientation of BSA molecule versus ZnO surface: Head, Side, Flat, Legs, and Tip. The distance between the nearest atom of BSA molecule and the surface of ZnO slab is set manually at around 3 Å for all configurations. The simulations have also been performed for the distance of 20 Å where there was no interaction between BSA molecule and ZnO surface detected regardless of the orientation of BSA.

![Figure 1. Different orientations of BSA molecule versus ZnO.](image-url)
Figure 2. Magnified images of BSA-ZnO interaction sites for different orientations of BSA over (10\bar{1}0), (1\bar{1}20), and (0001) ZnO planes. The structures are results of force field simulations. Yellow frames denote the configurations in which chemical bonding happens in the calculations.
Figure 2 summarizes the results of forcefield simulations for BSA molecule over ZnO non-polar and polar planes ((10\overline{1}0), (11\overline{2}0), and (0001)). Magnified images of the interaction sites between BSA molecule and ZnO planes are illustrated for different orientations of BSA. In all cases, the proximity of BSA molecule to ZnO surface has culminated in mutual attraction, followed by re-arrangement of surface atoms on ZnO slab. In addition to these displacements, chemical bonding of atoms from BSA molecule with atoms on ZnO surface can also be seen at interaction sites in Side and Tip orientations over (10\overline{1}0) plane, as well as Side and Flat orientations over (11\overline{2}0) plane. In these cases, which are highlighted by yellow frames in figure 2, chemical bonding happens between hydrogen atoms of BSA molecule and surface oxygen atoms of ZnO. For (0001) surface, no chemical bonding has been observed since it contains only Zn atoms compared to nonpolar surfaces which include both Zn and O atoms. It must be mentioned here that more accurate electron-based calculations such as DFT have to be employed to investigate the chemical bonding phenomena and to validate the observed chemical bonds. Nevertheless, the results obtained from the current force field simulations will be helpful for selection of relevant parts of the system for more expensive calculations. Validating the chemical bonding phenomena observed in force field simulations by employing DFT method is going to be done as the next phase of the current study.

To compare different orientations of BSA and determine the most likely one over each ZnO plane, total energy of the system is displayed via bar-graphs in figure 3. Since orientation of BSA molecule is the only varying aspect and other characteristics of the systems remain completely unaffected, total energy of the systems can be observed to deduce the favourable position. According to the total energy graphs in figure 3, tendency of BSA molecule for being adsorbed on ZnO surfaces is noticeably different for different orientations. The head orientation has the lowest energy in the BSA+ZnO(0001) system and thus it is the most likely orientation over ZnO (0001) surface. The side orientation has...
been observed as the most favourable for BSA+ZnO(10\{1\}0) and BSA+ZnO(11\{2\}0) systems. It is also worth mentioning that mechanism of interactions is not identical in the most favourable positions over different planes and it depends on the different groups of atoms from the BSA molecule and type of the ZnO plane. Atoms are differently re-arranged, and the adsorption might occur through either physisorption or chemisorption.

4. Conclusion
We employed forcefield simulations to investigate the interactions of BSA molecule with ZnO nonpolar and polar surfaces. The total energy indicates that BSA molecule is likely to adsorb in different orientation on different types of ZnO surfaces. The way how BSA molecule interacts with ZnO surfaces depends on the type of ZnO surface as well as the orientation of the BSA molecule. The computational results demonstrate that the vicinity of BSA molecule with ZnO surfaces results in rearrangement of atoms on ZnO surfaces that lowers the total energy and gives rise to physical binding. Furthermore, in several configurations, chemical bonding between BSA atoms and ZnO surface has also been observed which needs to be validated by more accurate (but more costly) electron-based simulation methods in further studies. This may have important implications for interaction of ZnO with biological environment as well as impact its optical and electronic properties.

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