Modelling Interactions between Titanium Surfaces and Biomolecules Using Molecular Dynamics Methods

Summary of the PhD theses

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1. Introduction

The development of computational capacity has facilitated the molecular dynamics (MD) simulations of various biomolecules such as proteins and peptides [1]. In the recent two decades the MD methods were successfully applied to study and simulate the interactions between surfaces and biomolecules [2]. In my work I have studied the interactions between titanium dioxide (TiO_2) surfaces and various biomolecules using MD simulations. Titanium plays a significant role from both medical and dental perspectives, often serving as a fundamental material for implants [3]. During the process of osseointegration, i.e. fixation of implants into the bone, various biomolecules from the bloodstream adhere to the thin TiO_2 layer that covers titanium surfaces. These molecules form a bio-layer, which facilitates the attachment of bone cells [4]. In my study, I focused on the adhesion of short tri-, and tetrapeptides to various TiO_2 surfaces.

2. Aims

In my thesis, I aimed to investigate the adhesion of peptides potentially affecting the process of osseointegration. I studied the peptide-TiO_2 interactions using MD methods. For this purpose, I constructed computational models of TiO_2 with various polymorphic states and amorphous structures. My objective was to identify physical quantities that can characterize, and permit a comparison of distinct peptide-TiO_2 interactions. Such quantities include the adsorption time ratio (e.g. how much time the molecule spends interacting with the surface compared to the total simulation time), the binding energy or the binding free energy.

3. Methods

First, I studied the KRSR molecule (Lys-Arg-Ser-Arg), which plays an important role during the first steps of osseointegration, on TiO_2 surface. This molecule has not been studied yet with simulation methods so far. My

simulations were carried out with the GROMACS software package [5]. The anatase TiO_2 structure was prepared from its unit cell, and subsequently, I investigated the adhesion of the KRSR tetrapeptide in aqueous environment. Following a brief equilibration procedure, a 200 ns MD simulation was carried out at a room temperature of 310 K. For potential parametrization, the widely used CHARMM36 force field was applied to the peptide and water molecules, while the potential parameters for the titanium and its oxygen atoms were defined according to the methodology described by Luan *et al*. [6].

To investigate the conformational states of the adhered KRSR tetrapeptide, I conducted additional REMD (Replica Exchange Molecular Dynamics) simulations, encompassing a total of 96 parallel simulation runs (replicas) at different temperatures, each lasting 50 ns. The REMD simulations were configured to have a 30% exchange probability among the individual replicas, corresponding to a temperature difference of 1,5 K. A time step of 2 fs was utilized throughout the MD simulations,

and each simulation was preceded by an equilibration phase (under NVT and NPT ensembles).

I conducted a pulling simulation, in which I used a harmonic potential to pull the adhered KRSR tetrapeptide from the anatase TiO_2 surface at a rate of 5 nm/ns. To further analyze the binding energy, I performed umbrella sampling at 24 different points along unit distance from the surface for a duration of 10 ns (240 ns total sampling time). Using the WHAM algorithm, I computed the potential of mean force (PMF) curve based on the average force exerted on the molecule, and from these results, I determined the binding free energy [7].

In addition to the anatase TiO_2 structure, I constructed a rutile model using the rutile unit cell and an additional amorphous TiO_2 model by elevating the temperature of the anatase model to 3000 K followed by a rapid quenching it to 310 K. On these three surfaces, I conducted additional 500 ns simulations involving six different peptides: KGD, KRSR, LGD, LRSR, RGD, and RSR. To compare the adhesion tendencies, I calculated adsorption time ratios, which represent the proportion of time intervals that

molecules spent within a proximity of 0.5 nm to the TiO_2 surface compared to the total simulation time.

I performed additional pulling simulations with the six peptides on the three different surface models. In these simulations, I employed a harmonic potential to pull the molecular centers of mass (COM) of the peptides. By analyzing the extracted force and distance curves, I determined the binding energies of the tri- and tetrapeptides and compared them.

4. Theses, Novel Scientific Findings

Thesis 1. I performed molecular dynamics simulations in an aqueous environment to investigate the interaction between the KRSR (Lys-Arg-Ser-Arg) peptide and the anatase TiO_2 surface. The KRSR peptide starting from the water phase have adsorbed to the TiO_2 surface during a 200 ns MD simulation. I observed that the binding of the KRSR molecule to the TiO_2 surface can be divided into three phases. During the first phase, within 1.5 ns, the KRSR peptide is diffusing in the water phase and approaches the surface by 0.3 nm (migration), and then in the second phase it maintains this distance for the following 6 ns. In the third phase, a tighter adhesion was established, with the distance between the KRSR peptide and the nearest surface atom settling at 0.148±0.008 nm during the last 193 ns of the simulation. Furthermore, these simulation results highlighted the significance of the charged amino acid residue K (Lys, located at the N-terminus of the peptide) in the adhesion of the KRSR

molecule, as it adheres to the anatase surface through its charged amino group [T1].

Thesis 2. I determined the molecular conformations of the KRSR tetrapeptide on the anatase TiO_2 surface using REMD (Replica Exchange Molecular Dynamics) method in an aqueous environment. The REMD simulation performed for this purpose consisted of 96 parallel replicas, ranging from 310 K to 454 K temperatures, each lasting 50 ns. This approach allowed me to identify the molecular conformation states of the KRSR molecule on the TiO_2 surfaces on the given temperature. Various molecular conformations were explored through a 0.1 nm RMSD LINCS clustering. Among these clusters, I selected the one with the greatest population, which assuming Boltzmann distribution, also has the lowest energy, the cluster size of this conformation was 4751. The KRSR molecule was present in this state approximately 40% of the simulation time at the lowest temperature (310 K) [T1].

Thesis 3. Using the dominant conformation of the KRSR peptide obtained through the REMD method on the anatase TiO_2 surface, I conducted a pull simulation to determine the binding free energy in the aqueous environment. During the pull simulation the KRSR peptide center of mass was pulled along the axis perpendicular to the surface. To further evaluate the binding energy, I performed umbrella sampling at 24 points over a 10 ns period (240 ns total simulation time), where I selected KRSR peptide states from the pull process at uniform distances from the TiO_2 surface, the smallest distance was 0.156 nm, the largest was 2.77 nm. Using the curves obtained during the sampling, I determined the free binding energy based on the potential of mean force (PMF) curve using weighted histogram analysis (the WHAM algorithm). The resulting value for the binding free energy was $\Delta G = 8.817 \frac{kcal}{mol}$ (equivalent to 0.3823 eV) [T1].

Thesis 4. I determined the adsorption time ratios of six small-sized peptides on various TiO_2 surfaces in an

aqueous environment. The comparison was conducted for the peptides KGD, KRSR, LGD, LRSR, RGD, and RSR on the anatase, rutile, and amorphous TiO_2 surfaces. The adsorption time ratio was calculated as the proportion of simulation time spent interacting with the TiO_2 surface (distance < 0.5 nm) compared to the total simulation time. Based on the results, I identified peptides that exhibited outstanding binding characteristics on different surface types. In terms of the adsorption time ratio, KRSR and RGD demonstrated high values across all the three surface types, averaging 82.93±1.13% and 87.26±10.11%, respectively. LGD and RSR values lagged slightly behind, with values of 77.99±14.7% and 69.79±18.2% respectively. KGD also showed a high average value of 80.31±14.43%, with exceptionally elevated adsorption residence time ratio of 91.26% on the amorphous TiO_2 surface. Conversely, LRSR exhibited a substantially lower adsorption time ratio compared to the other peptides: 31.72±16.34%. It was observed that peptides carrying positively charged amino acids, Lys (K) and Arg (R) at their N-termini demonstrated prolonged adhesion. Conversely, peptides with a non-charged N-terminal

amino acid, Leu (L) resulted a decrease in their adsorption time ratio [T2].

Thesis 5. I determined the binding energies of the selected peptides (KGD, KRSR, LGD, LRSR, RGD, and RSR) adhering to different TiO_2 surfaces (anatase, rutile, and amorphous) using pulling simulations. The calculated binding energies are in the order of a few eV. The lowest binding energies were obtained for peptides starting with N-terminal Leu (L); 2.72±0.77 eV in case of LGD and 2.37±1.51 eV in case of LRSR averaged for the three surface types. The highest average value was observed for KRSR at 4.98±1.94 eV. Furthermore, a linear relationship was found between the maximum pulling force and the calculated binding energies [T2].

Publications related to the theses

[T1] Tarjányi, T.; Bogár, F.; Minarovits, J.; Gajdács, M.; Tóth, Z. Interaction of KRSR Peptide with Titanium Dioxide Anatase (100) Surface: A Molecular Dynamics Simulation Study. *Int. J. Mol. Sci* **2021**, *22*, 13251.

[T2] Tarjányi, T.; Bogár, F.; Minárovits, J.; Gajdács, M.; Tóth, Z.; Interaction of biomolecules with anatase, rutile and amorphous TiO_2 surfaces: A molecular dynamics study. *PLOS ONE* **2023,** *18*, e0289467.

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