Interaction of biologically relevant ions and organic molecules with titanium oxide (rutile) surfaces: A review on molecular dynamics studies

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Abstract

The surface of a biomaterial can play a major role in its biological fate since the surface is the primary pathway for its interaction with the body. As the natural response of the body to a foreign material is to encapsulate it with a fibrous material, the interactions between the body and the biomaterial are mediated by this fibrous layer. Initial interactions occur between the biomaterial surface, water, ionic species and organic molecules, which then mediate further interactions with body tissues. Surface engineering can influence these interactions and hence, improve the biocompatibility of the biomaterial. Therefore, both experimental and computational studies have been interested in phenomena happening at the solid-solution interface as their mechanisms and driving forces can point to new directions for biomaterial design and evaluation. In this review, we summarize the computational work on the interaction of titanium oxide surfaces (mainly rutile) with solvated ions and organic molecules by means of molecular dynamics, with a certain relevance to bioactivity testing protocols. The

primary goal of this review is to present the current state of the art and draw attention to points where further investigations are required.

Keywords: Titanium oxide; Rutile; Simulated Body Fluid (SBF); Solid-solution interface; Molecular dynamics

Contents

1. Introduction

Titanium alloys are nowadays extensively used for biomedical applications since they have proven to be biocompatible (the ability to exist in contact with human body tissue without causing an unacceptable degree of harm to the body[1]) for many biomedical applications (e.g., artificial bones, joints and dental implants[2]). It is argued that they owe their biocompatibility

for such applications to the oxide layer that forms when the metal is in contact with either oxygen or water.[2] The oxide layer on Ti implants that interacts with the surrounding environment is a mixture of amorphous and crystalline forms of titanium oxide (hereafter, titanium oxide will refer to both crystalline and amorphous states).[3] The three major crystalline polymorphs of titanium dioxide are rutile, anatase and brookite, rutile being the most abundant naturally occurring phase at ambient pressure, while anatase is stable in nanomaterials.[2,4–8] At high temperatures, anatase and brookite can irreversibly transform to rutile.[9,10] Chemical treatments are often used to render inert surfaces bioactive. For example, Kokubo *et al.* showed that a sodium titanate hydrogel layer forms on a titanium metal surface after its immersion in sodium hydroxide.[11] Heat treatment of this sodium titanate hydrogel layer-covered titanium showed that it transforms into an amorphous sodium titanate at 400-500 °C and crystalline sodium titanate and rutile at temperatures above 700 °C.[12] These chemically modified surfaces show an excellent biocompatibility for specific applications and are under clinical trials for artificial hip joints and spinal fusion devices.[13] This highlights the importance of rutile, which is the titanium oxide phase studied in most publications on this topic.

The biocompatibility of an implant with a given surface preparation can most reliably be evaluated by *in vivo* testing where the assessment is done with the implant inserted in a living body. However, for economic as well as ethical reasons it is desirable to perform reliable *in vitro* tests, in which samples are tested in laboratories and outside any living bodies. In the latter case, researchers try to achieve experimental conditions close to those found *in vivo*. While in some cases *in vitro* results agree with *in vivo* results, other studies have shown that there are unidentified factors during *in vitro* tests that cause discrepancies between the results obtained by these two methodologies.[14,15] In a recent study, eight different European universities carried out *in vivo* and *in vitro* studies on 93 different biomaterials, showing a weak correlation between *in vivo* and *in vitro* results.[16]

One of the sources of this discrepancy is the solution used for *in vitro* testing.[17,18] Depending on the purpose of the study, different aqueous *in vitro* solutions have been proposed, Simulated Body Fluid solutions (SBFs) being a popular category.[19,20] The ionic concentrations in different SBFs (table 1) are very close to those in blood plasma. The variations say between the Kokubo and Bohner solutions have a minor effect on supersaturation[18] and it is the possible specific adsorption of ions onto different surfaces that should be of importance in the hydroxyapatite formation on implants. There are certainly

differences between blood plasma and SBF solutions; for example, the buffer used to maintain the solution pH near the 7.4 found in human blood. The use of a carbonate buffer (i.e., a P_{CO_2} of 5%) instead of an organic molecule such as Tris should render the *in vitro* test more representative. This can change the amount of carbonate or bicarbonate species in solution,[18] which may lead to modifications in the inorganic species adsorbed on implant surfaces. This, in turn, may influence the nucleation and growth of calcium phosphates on implant surfaces *in vitro*. Also, one of the most commonly used SBF solutions, proposed by Kokubo *et al.* and used in the ISO standard,[21] lacks the proteins present in the blood plasma.[17,22] In the human body proteins adsorb onto an implant surface in a variety of orientations and configurations shortly after implantation. Further interactions between the cells and the implant surface will occur through this organic layer.[2,23,24] Since cells recognize only a few specific proteins in well-defined orientations and configurations, the composition and structure of the adsorbed organic layer will influence the biocompatibility of the biomaterial.[25–28] Therefore, the absence of proteins during *in vitro* testing could be another reason for the discrepancies found between *in vivo* and *in vitro* testing.

Table 1. Ionic concentration [mM] of the human blood plasma and some Simulated Body Fluid solutions (SBFs).[18,21,22,29]

	Human blood plasma	**ISO 23317 (pH 7.4)**	**Kokubo *et al.***	**Bohner *et al.***
Na^+	142.00	142.00	142.00	142.00
K^+	5.00	5.00	5.00	-
Mg^{2+}	1.50	1.50	1.50	-
Ca^{2+}	2.50	2.50	2.50	2.31
Cl^-	103.00	147.80	148.80	109.90
HCO_3^-	27.00	4.20	4.20	34.88
HPO_4^{2-}	1.00	1.00	1.00	1.39
SO_4^{2-}	0.50	0.50	0.50	-

Understanding protein adsorption on biomaterial surfaces is therefore of great importance since, alongside water-surface interactions, it can significantly affect the performance of a biomaterial.[19,24,30] With this knowledge, it would be possible to design implants with surfaces

that trigger or boost biocompatibility and bioactivity when in contact with blood-plasma proteins,[14,25] since "*there is a causal connection between the detailed properties of a native implant surface and the ultimate tissue response*".[31] It should be borne in mind that the extent and the manner of protein adsorption on surfaces are significantly influenced by certain properties of the surface[31,32] and the local environment such as wettability, hydrophobicity, surface charge, pH, the concentration of ions and temperature.[23,24,26,33,34] Also, the presence of organic molecules can control some surface features[33] such as step and edge formation and crystal growth.[35]

Despite steady advances in experimental methods and techniques, computational methods can be more suitable for answering certain questions.[27,36,37] Depending on the property under study, different computational methods can be used. Even though surface science aspects of titanium oxide have been extensively studied using density functional theory (DFT), investigation of surface-protein interactions is currently beyond the reach of this method. Due to the large number of atoms (simulation of even a single organic molecule via DFT can be impractical) and the extended time scales, classical molecular dynamics (MD) is better suited to these tasks. Reactive force fields can describe changes in bonding and charge transfer, but there are only a few MD studies using this type of force field at the moment[38–41] and hence, in this review we focus on studies done with non-reactive force fields.

To have a proper time average, as many configurations as possible must be sampled, which is hindered by high energy barriers, the crossing of which often occurs only on millisecond timescales (such as protein folding) as well as the finite sampling time.[42] Enhanced sampling methods, such as metadynamics, make it possible to overcome these barriers and to sample configurations inaccessible within the nanosecond time limit inherent to classic molecular dynamics (it is worth mentioning that affording even a few hundred picoseconds via DFT is impractical).[37] During metadynamics, the free energy of a system is biased to the point that the system can cross an energy barrier and explore neighboring energy wells. Currently, only a few groups have used enhanced sampling methods and we discuss their results in this review.

While some review papers have summarized the experimental work on the interaction of organic molecules with titanium oxide (alongside other materials),[2,24,43] to the knowledge of the authors, hardly any reviews exist for computational studies on bio-related titanium oxide systems.[44] Here, we assess what is known from classical and enhanced molecular dynamics about the interactions of ions and organic molecules with titanium oxide. The limited number

of studies presented here on anatase, brookite and amorphous titanium oxide stems from the fact that most researchers have been conducting their studies on rutile (the most thermodynamically stable phase of titanium dioxide) and not because we have narrowed the scope of this review.

While we focus mainly on studies in aqueous solution, we do present computational studies in vacuum (in four instances) since they provide the fundamental knowledge required to investigate more complex systems. As it will be discussed later in this review, water plays a crucial role in the adsorption process of ions and organic molecules on the surface. We would like the reader to be cautious about studies carried out in vacuum and keep in mind that they ignore the irreplaceable role of the solvent in an adsorption event.

A concise discussion of the available force fields is presented in section 2. In section 3, we discuss the interaction of rutile surfaces with ions in aqueous solution. Section 4 covers different surface features which affect the organic-inorganic interactions. The effect of the initial orientation of the organic on its adsorption on the surface is also addressed in this section. The paper concludes with a summary section. The temperature of the simulations is in the range of 25 - 37 ºC unless otherwise stated. We do not provide a description of the atomistic simulation methods, which are mentioned throughout the review but refer the reader to other sources for a detailed discussion.[27,37,45] A list of the abbreviations and the chemical representation of most of the organic molecules investigated in this review are presented in the supplementary information.

2. Force fields

The accuracy of any atomistic simulation depends highly on its underlying force field. Great care should, therefore, be taken in choosing the force field (range of validity) as well setting it up in a specific code. Moreover, determining undefined parameters should be done judiciously - for example, in case one has force field parameters for the interaction of atom type *i* with itself and for atom type *j* with itself, there are several ways to obtain parameters for the interactions between types *i* and *j*.

In molecular dynamics simulations, the force applied on atoms, which is described by the force field, is used in Newton's law of motion equation to propagate velocities and atomic coordinates using a timestep shorter than the fastest atomic vibrations in the system. The types of interaction between atoms can be divided into bonded and non-bonded or

intramolecular and intermolecular interactions. The bonded interactions are defined for the atoms of the same molecule that are covalently bonded to each other. The non-bonded interactions include Coulombic and van der Waals interactions between the atoms of different molecules. Both bonded and non-bonded interactions are described by a variety of parametric functions of the atom coordinates and types, which we will not discuss in details here.

The system of an inorganic surface with organic molecules in an ionic solution includes the inorganic surface, the organic molecule, ions and water. Atomistic simulations of such systems require five primary groups of interaction parameters; the first four groups will be the potential sets of the inorganic, organic, ions and water; the last group contains the cross-term interactions between the components: inorganic-organic, inorganic-ions, inorganic-water, organic-ions, organic-water and ions-water.

Given that accurate force fields for the first four groups are known, deriving the cross-term interactions is the most challenging task. Where feasible, for example when the organic molecule is relatively small, *ab initio* calculations can be carried out to extract cross-term interaction parameters.[42,46] However, this approach becomes impractical for more complex organic molecules.[47] Freeman *et al*.[48] proposed a methodology which uses the existing potential sets and generates only the cross-term interactions between different components of a system such as water-mineral, mineral-organic and mineral-ions. In this method, expensive fitting steps can be avoided. If we assume that atom A belongs to a different component than atom B, the fitting can be carried out on a mineral that contains both atoms A and B. For example, A can be the calcium in calcite ($CaCO_3$) and B can be the oxygen atom of the organic molecule and one can use Ca-O interaction parameters of calcite to obtain the new cross-term interactions of $Ca_{mineral}$-$O_{organic}$.

Another method to obtain the cross-term interactions is via the Lorentz-Berthelot mixing rules. This method requires the Lennard-Jones parameters for atom *i* and atom *j* to generate the Lennard-Jones parameters for the interaction between atoms *i* and *j* (Eq. 3).[49,50] Several studies presented in this review have used Lorentz-Berthelot mixing rules to obtain the organic-inorganic, organic-water and ion-water interactions.[51–53]

In the following, we will mention some of the force fields used for the components of the under-study system. Since caution should be taken when trying to use an existing force field for a particular system, we do not present the force field parameters in this review and refer the reader to the original manuscript for parameters and validation.

2.1. Titanium oxide

A broad range of titanium oxidation states for stoichiometries varying from Ti_2O to TiO_2 can be present in the surface oxide layer.[2] Several force fields have been suggested for modelling titanium oxide systems.[54–58] One of the most well-established force fields for titanium dioxide polymorphs was first developed by Matsui and Akaogi.[54] While this force field is not very successful at reproducing the anisotropic static relative permittivity of rutile, its simplicity and capability in reproducing the structures of titanium dioxide polymorphs have led to its extensive usage in molecular dynamics simulations.[59–61]

Kim *et al*. developed an alternative force field for rutile and tested the transferability of this force field to anatase and brookite.[55] Several properties such as the lattice constants, bulk modulus and heat capacity for titanium dioxide polymorphs were well reproduced.

The majority of studies on oxidized titanium surfaces assume a perfect crystalline structure. However, the force field developed by Schneider *et al*. is capable of modelling an amorphous oxidized titanium surface.[62,63] In this parameterization, the interface between titanium and TiO_x (surface oxide layer) was described using a Finnis-Sinclair form of a many-body potential. The parameters were chosen such that they reproduce the same atomic charges as the Matsui-Akaogi set when the TiO_x structure is bulk TiO_2 rutile. The amorphous oxidized titanium layer was modeled using electrostatic Coulomb interactions and short-range repulsive terms. Despite its simplicity, this force field is successful in describing the amorphous oxide layer.

2.2. Water and its interaction with titanium dioxide

At least 46 water models were developed between 1933 and 2002.[64] However, for titanium oxide in biological systems mainly simple three-site SPC/E and TIP3P (three-site transferable intermolecular potential) water models have been used.[65–67] Despite their simplicity, these models have been able to reproduce many properties of water accurately.[64,68–71] Although more evolved water models such as TIP4P (four-site transferable intermolecular potential)[72,73] might be able to present a better model of water and its interactions, adding just one more interaction site to the water model can significantly increase the computational cost in biologically-relevant studies.[70]

In both *in vivo* and *in vitro* conditions, titanium oxide is in contact with an aqueous environment which will lead to the hydroxylation of the surface via dissociative adsorption of water molecules.[74,75] The hydroxyl group on the rutile surface which forms as a consequence of the protonation of a surface oxygen atom is called the bridging hydroxyl while the hydroxyl which forms as a result of the attachment of an OH group to a surface Ti atom is called the terminal hydroxyl. The degree of hydroxylation, conventionally, refers to the fraction of the available surface O and Ti sites that carry bridging and terminal hydroxyl groups, respectively. Non-neutral pH, however, will lead to a selective protonation or deprotonation of surface sites, which affects both the degree of hydroxylation and the balance between the number of bridging and terminal hydroxyl groups and thus induces a surface charge. We will, in the following, use the term 'partial hydroxylation' to refer to the presence of unequal numbers of either type of hydroxyl groups. The experimentally observed negative surface charge at biologically relevant pHs above the isoelectric point of rutile can be achieved using two approaches. One possibility is to have a partial coverage of terminal hydroxyl groups with no bridging hydroxyl groups. Another possibility is to have a full coverage of terminal hydroxyl groups with a partial coverage of bridging hydroxyl groups.[66] Figure 1 is a schematic of the rutile (110) surface in the non-hydroxylated, fully hydroxylated and partially hydroxylated states; in the latter case, shown in Fig. 1-c, the surface charge is provided through partial coverage with only terminal hydroxyls and in Fig. 1-d, the surface charge is the result of partial coverage of bridging hydroxyls on a surface with a full coverage of terminal hydroxyls. This figure shows the surface in the unrelaxed state for the sake of clarity. Upon relaxation, the hydroxyl groups are tilted and hydrogen bonds form between adjacent groups.

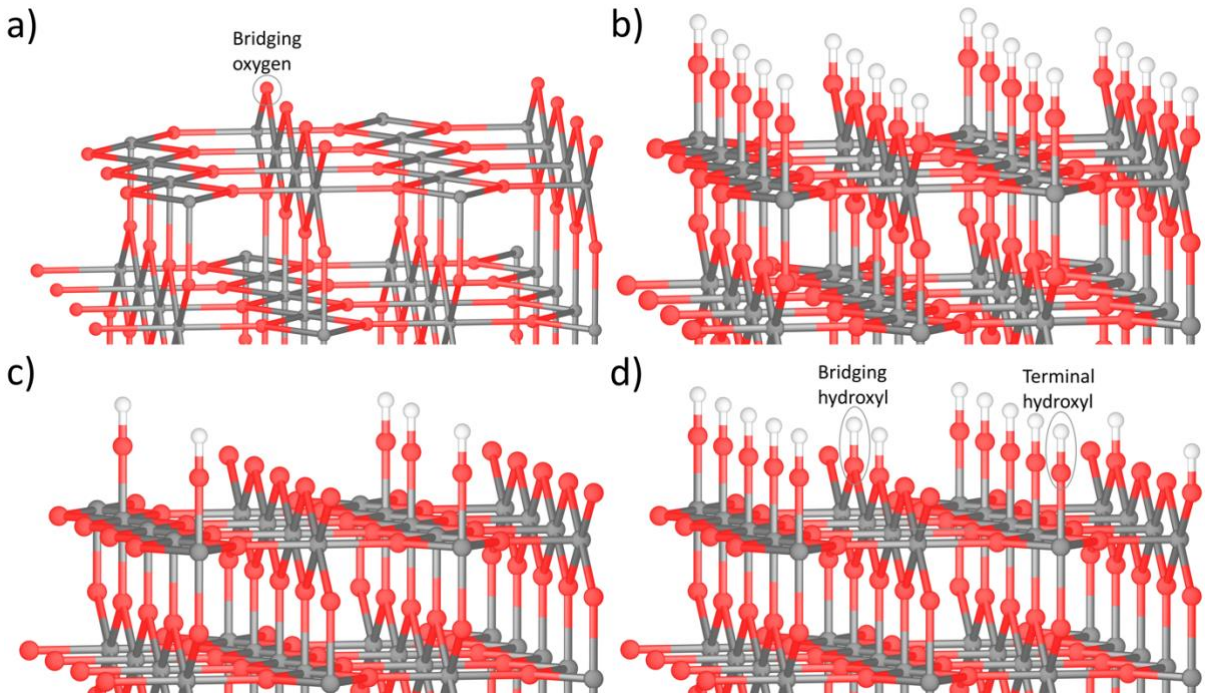

Figure 1. Unrelaxed rutile (110) surface: a) non-hydroxylated surface, b) fully hydroxylated surface highlighting the bridging and terminal hydroxyl groups, c) partial coverage of terminal hydroxyl groups on the surface and d) full coverage of terminal hydroxyl groups and partial coverage of bridging hydroxyl groups. Color code: Ti: gray, O: red and H: white. Adapted from Ref. 66. © 2004 American Chemical Society.

From *ab initio* calculations, Predota *et al*. found that surface Ti atoms and hydroxyl groups are variable-charge atoms.[66] Thus, Predota *et al*. developed slightly different charge schemes for the rutile surface in the neutral (non-hydroxylated or fully hydroxylated) and negatively charged (partially hydroxylated) states.[76]

The Matsui and Akaogi force field was amended by Bandura *et al*.[60] to include TiO_2-H_2O interactions. A*b initio* calculations were carried out to validate this force field for the interaction of the SPC/E (extended simple point charge) water model with the rutile (110) surface. In comparison to another titanium dioxide parameterization (developed by Kim *et al*.[55]), they observed that the Matsui and Akaogi force field yields better agreement with *ab initio* results. Predota *et al*. further adapted the force field derived by Bandura *et al*. to introduce different surface charges on the rutile (110) surface.[66]

Alimohammadi *et al*.[58] also modified the force field developed by Bandura *et al*.[60] to refine the interaction of water with rutile and anatase surfaces. This refined force field contains new cross-term interactions between titanium and oxygen atoms of titanium dioxide with oxygen of water. The binding energies and conformations obtained by using this refined force field yielded results which were in good agreement with first-principle DFT calculations and experiments.

Several computational studies have used implicit water instead of explicit water molecules,[6,77] in which no actual water molecule is present in the system and the solvent is modelled as a dielectric continuum.[78] While this can significantly reduce the computational cost, details of the interfacial structure might be ignored and the results should be interpreted cautiously.[47] The main problem with using an implicit water model is ignoring the competition of the water molecules and the organic residue for the surface and the incapacity of the implicit model to represent hydrophobic effects.[42]

Water is known to adopt a layered structure close to different rutile planes, as well as the amorphous titanium oxide.[65,67,79,80] By studying the water structure close to these two rutile surfaces, strongly structured water layers were observed close to both of them. The density distribution of water in the surface normal direction revealed that water is more strongly orientated on the (110) surface compared to the (001) surface.[79] An organic molecule often binds to the surface through the first layer of water molecules, which is known as indirect bonding.[52] Many studies have investigated the water structure close to the titanium oxide surfaces,[67,74,75,80–86] but we will not discuss them further in this review.

2.3. Ions and their interaction with rutile and water

Some of the proposed force fields for ions which are present in the human blood plasma and SBF solutions (table 1) can be found in following references.[66,87–93]

Predota *et al*. defined the short-range van der Waals interaction between ions and rutile oxygen as being similar to the interaction between ions and the oxygen atom of water. Due to the lack of available force fields for the short-range interaction between the titanium atoms of rutile and ions, they ignored the short-range interactions and considered the Ti-ion interactions to be purely electrostatic.[66]

2.4. Organic molecules and their interaction with rutile and water

The force field for an organic residue can be established based on different force fields such as AMBER, CHARMM, GROMOS, etc.[42,94–96]

For investigating the interaction of a dipeptide with the rutile surface, Carravetta *et al*. used *ab initio* calculations on small sections of the organic residue and the inorganic surface to have a better approach to predicting the surface-dipeptide interactions.[46] Other groups have mainly used the Lorentz-Berthelot mixing rules to obtain the organic-inorganic or organic-water interactions. Since this requires having the force field of rutile in the Lennard-Jones scheme, several groups have fitted Lennard-Jones parameterizations to the original Matsui-Akaogi Buckingham set.[50,51,97,98]

3. Interactions of ions with rutile surfaces

Interaction of ionic species with surfaces can provide insight into the affinity of the ion for the surface, preferred adsorption sites, adsorption energy, etc. Interaction of some of the ions that are present in SBF solutions or human blood plasma (table 1) with rutile surfaces has been studied.

In this section, the interaction of three monovalent (Na^+, Rb^+, K^+) and four divalent (Ca^{2+}, Sr^{2+}, Mg^{2+} and Zn^{2+}) cations with two rutile surfaces ((110) and (100)) is discussed. Different temperatures, surface charge densities and pH levels have been tested and it was shown that all these parameters, along with the ionic size, affect the adsorption energy as well as the adsorption site (adsorption geometry) on the surface.

Predota *et al*. studied the adsorption geometry and binding strength of several monovalent and divalent cations (Na^+, Rb^+, Ca^{2+}, Sr^{2+} and Zn^{2+}) solvated in water (SPC/E model) on neutral and negatively charged (partially hydroxylated) rutile (110) surfaces.[99] X-ray structure determinations were also carried out to compare the results of simulation and experiment.[100] The simulation box was electrically neutralized by adding a sufficient number of chlorine anions. It was seen by means of molecular dynamics simulations that smaller cations (e.g., Na^+, Ca^{2+} and Zn^{2+}) adsorb closer to the surface compared to larger ones (e.g., Rb^+ and Sr^{2+}). Zn^{2+}, the smallest cation in this study, adsorbed the closest to both neutral and negatively charged rutile surfaces. Its small size also makes it the only cation, which adsorbs in a bidentate site (between two terminal oxygens or between one terminal and one bridging oxygen), DFT calculations confirming this to be the energetically most favorable adsorption mode.[101] However, we want to note that in *ab initio* calculations[101] water hydrolysis was

observed in the first hydration shell, which adds complexity to Zn^{2+} adsorption that cannot easily be captured by classical MD. Based on the X-ray results, all other cations adsorbed at tetradentate sites (between two terminal and two bridging oxygens).[100] During the molecular dynamics simulations, however, adsorption in both tetradentate and bidentate sites was observed for all cations with different occupation probabilities.[99] Different adsorption sites for Rb^{+}, Sr^{2+} and Zn^{2+} are shown in Fig. S1.

The binding between divalent cations and their hydration shell is stronger than for monovalent cations.[102] Therefore, divalent cations tend to retain their hydration shell and remain solvated, especially in the case of a neutral surface. For all of the studied cations, inner-sphere adsorption on the rutile surface was observed. Inner-sphere adsorption implies that the cation adsorbs directly on the surface. However, water molecules are involved in the indirect binding of the ion on the surface in the case of an outer-sphere adsorption. Outer-sphere adsorption was also observed for Ca^{2+} and Sr^{2+} but much less frequently than the inner-sphere adsorption. By developing a method to predict the adsorption geometry of the cations, Predota *et al.* found that the adsorption geometry depends on the cation size.[99] While using this method one can predict all the possible adsorption geometries, molecular dynamics simulations are still required to find the preferred adsorption site based on the probability of the occupation of the adsorption site.

In agreement with the work of Predota *et al.*,[99] Wu *et al.* also observed that the ionic size notably affects the adsorption geometry and adsorption mechanism on the surface.[102] By studying the adsorption of a group of monovalent (Na^{+}, K^{+} and Rb^{+}) and divalent (Mg^{2+}, Ca^{2+} and Sr^{2+}) cations solvated in water (SPC/E model) on the negatively charged (partially hydroxylated) rutile (110) surface, they observed that the preferred adsorption mechanism for all cations except Mg^{2+} and Ca^{2+} is inner-sphere adsorption. Magnesium, due to its small size, adsorbs in an outer-sphere configuration. It was however observed that there is no significant preference between inner-sphere and outer-sphere adsorption on the surface for the calcium cation. The residence time of water in the hydration shell of cations is significantly shorter for monovalent cations (5-25 ps) compared to divalent cations (150 ps-∞; which is limited by simulation time of 6 ns). This shows that the binding between the monovalent cations and their surrounding water molecules is not permanent; occasionally, the cation is free to bind to the surface or a peptide, if present in the system. Among the three divalent cations, magnesium has the largest residence time of water within its hydration shell, which is due to its small ionic size. This suggests strong binding energetics for Mg ions, implying that SBF

solutions for *in vitro* studies should contain Mg ions despite the slow kinetics of their binding.[15,17,18]

The effect of temperature and surface charge density on the adsorption frequency and adsorption site on the rutile (110) surface at 25, 150 and 250 ℃ were studied for Na^+, Rb^+ and Sr^{2+}.[76] Water was modelled using the SPC/E model. Using experimental titration tests, the surface charge density was calculated for the three temperatures mentioned above at different pH values. Five surface charge densities of -0.416, -0.208, -0.104, 0.0 and +0.104 $C \cdot m^{-2}$ were studied. The negative surface charge densities were produced by a partial coverage of bridging hydroxyl groups while the positive surface charge density was achieved by replacing some terminal hydroxyl groups with water molecules. Four different adsorption sites were observed, which included three inner-sphere adsorption sites and one outer-sphere adsorption site. The inner-sphere adsorption sites are closer to the rutile surface and consist of i) the TD tetradentate site in which the ion interacts with two bridging and two terminal hydroxyl groups; ii) the BOTO bidentate site in which the ion interacts with one bridging and one terminal hydroxyl group and iii) the TOTO bidentate site in which two terminal hydroxyl groups interact with the ion. The proximity of the adsorption site to the rutile surface varies in the order of TD, BOTO and TOTO; from the closest site to the farthest one.[76] The frequency of outer-sphere adsorption for different ions is lower than inner-sphere adsorption for all temperatures and all surface charge densities.

Increasing the temperature enables the ions to overcome energy barriers and to adsorb on sites closer to the surface. The adsorption frequency decreases significantly on the non-charged and positively charged rutile surfaces (almost zero) at higher temperatures. For the surface charge densities of -0.208 and -0.104 $C \cdot m^{-2}$, the TOTO adsorption sites are favored by Na^+ and Sr^{2+} while Rb^+ adsorbs most frequently in the TD adsorption sites. This can be explained by the weaker binding of water molecules in the Rb^+ hydration shell compared to the ones around the smaller Na^+ ion. The water molecules around Rb^+ can be removed more easily, which facilitates its adsorption to the tetradentate site.[76]

Koppen *et al*. studied the interaction of sodium and chlorine ions in solution (using TIP3P water model) with the rutile (100) surface at three pH values of 4.0, 7.4 and 9.0.[103] Considering the isoelectric point of rutile (~ 5.3 at 35 ℃ [104] and between 5 - 6.7,[105] in general), the rutile surface carried a positive charge at the first pH and negative charge at the latter two pH values. The pH was adjusted by adding protons or hydroxyl groups to the stoichiometric rutile surface. The density distribution of the structured water layers close to

the surface was affected by the ionic solution. While ions with a charge opposite to that of the surface (counter-ions) like to approach the surface, the ions with the same charge as the surface prefer to diffuse into the aqueous solution.

From the above studies, it can be seen that the interaction of ions present in a simulated body fluid with the titanium surface remains incomplete. In most cases, chlorine is used as the counter-ion, whereas other anions such as sulphate, bicarbonate and hydrogen phosphate are also present in SBFs (table 1). Despite the fact that rutile and anatase surfaces are negatively charged at the temperature and pH of *in vivo* condition, the interaction of other anions present in the simulated body fluid can also be interesting.

4. Interaction of organic molecules with titanium oxide surfaces

A summary of studies on protein adsorption on different substrates, using experimental and computational methods, can be found elsewhere.[24] Here we will discuss those that have applied computational methods to study the interaction of organic molecules with titanium oxide surfaces. The sub-sections are divided according to the type of the organic molecule.

Due to their simplicity, single amino acids or oligomers have been the first residues to be computationally studied. Among the many possible organic molecules that can be studied, the RGD (Arg-Gly-Asp) and RKLPDA sequences of amino acids are of great interest. After the placement of a Ti implant inside the body, integrin receptors at the cell membrane will search for specific ligands on the surface to bind to. If the ligand is present and its conformation on the surface is suitable, further interaction between the cell and the implant can occur. Protein ligands such as fibronection, vitronectin and collagen are present in the extracellular matrix (ECM). The cellular response induced by these extracellular matrix proteins, however, is mainly through the Arg-Gly-Asp (RGD) sequence.[9,106] The RGD sequence is a polypeptide; in the zwitterion state, the Arg residue is positively charged and the Asp residue is negatively charged. It has been reported that RGD can mediate cell attachment onto several ECM proteins such as type I collagen and has a high specificity for integrin receptors.[107] Consequently, coating Ti implants with RGD can enhance its bioactivity and biocompatibility.[108]

The RKLPDA hexapeptide is also known as a titanium binding peptide (TBP) since it has shown high affinity towards surfaces such as Ti but little affinity towards other surfaces such as Au, Cr, Pt, Zn etc.[109] In 2005, Sano *et al*. showed that TBP-1, a sequence containing 12

amino acids, where the very first six amino acids are RKLPDA (TBP), recognizes Ti, Si and Ag surfaces. Since the electronic and crystallographic structures of these three are not similar, they hypothesized that there is an unknown parameter which controls the surface recognition by TBP-1.[109] With the continuous increase of computational resources, it has been possible to study more complex organic units.

4.1. Surface crystal structure and phase

i. The RGD polypeptide: Zhang *et al*. compared the binding energy of the RGD sequence to non-hydroxylated rutile (110) and anatase (101) surfaces in three different initial configurations, both in vacuum and in water (TIP3P model).[110] It was shown that the effect of the crystal structure is more important than the initial configuration of RGD. The higher binding energy of RGD to the anatase surface compared to the rutile surface was attributed to the fact that the anatase (101) surface consists of O and Ti atoms (the vertical distance between them is about 1 Å), but the rutile (110) surface is oxygen terminated. It was concluded that the presence of Ti atoms influences the adsorption process of RGD on the surface.

The binding energy of RGD to the surface was significantly smaller in water compared to vacuum for both rutile and anatase. In fact, water molecules can affect the adsorption process via different mechanisms. Before the adsorption of RGD to the surface, structured water layers form close to the surface. Hydrogen bonds between the surface and the water molecules should be broken before RGD can bind to the surface. Also, the binding between RGD and water molecules could be stronger than that of RGD and the surface. In this case, RGD will not be able to interact with the surface strongly.[110]

ii. An albumin subdomain and two fibronectin modules: In another study, the adsorption of an albumin subdomain and two connected fibronectin type I modules onto the non-hydroxylated rutile (001), anatase (100) and brookite (100) surfaces were compared using an implicit water model.[6] The interaction energy for both organic segments was the highest on anatase and the lowest for brookite. The binding strength between the surface and the organic molecules was not directly compared. During energy minimization, albumin showed a strong interaction with all three polymorph surfaces, while during the following molecular dynamics run, fibronectin modules had stronger interactions with the surfaces. Both organic segments showed structural changes to increase their interaction with the surface.

4.2. Surface hydrophobicity

i. Human lactoferrin and human bone morphogenetic protein-2: Surface characteristics like its chemical composition can affect the interaction between the surface and the protein. Sun *et al*. studied the effect of the hydrophobicity of the fully hydroxylated rutile (110) surface on the strength and the nature of the interaction of this surface with two proteins.[111] Human lactoferrin (LF), which has antibacterial activity and is a part of the immune system of the body, and human bone morphogenetic protein-2 (BMP2), which is important in the development of bones and cartilage (Protein data bank ID codes: 1CB6 and 3BMP, respectively) were chosen as the organic residues. The TIP3P model was used to describe water molecules. The charges of rutile Ti and O atoms were scaled by a factor of 0.5 and 1.4 to create a more hydrophobic and a more hydrophilic surface, respectively, when compared to the original surface. Results revealed that both proteins have a stronger interaction with the more hydrophobic surface. On approaching this surface, water molecules are displaced by the protein residues and the protein binds directly to the surface. On the more hydrophilic surface, water competes with the protein more strongly. This leads to indirect adsorption of the protein on the surface; instead of interacting directly with the surface, the protein mainly interacts with the water layer on the surface.

4.3. Surface charge

The isoelectric point of rutile and anatase is generally below 7.[105] In physiological conditions (pH ~ 7.4), these surfaces are hence negatively charged, which is why the cases presented in this section are either on neutral or negatively charged surfaces (positively charged surfaces not being relevant in physiological conditions). A neutral rutile surface can either be non-hydroxylated or fully hydroxylated while exchanging some of the surface hydroxyl groups with surface atoms leads to a net negative charge on the surface.

4.3.1. Charge neutral surfaces

i. Simple organic residues: Nada *et al*. studied the adsorption of the glycolate anion ($CH_2(OH)COO^-$) in water to two non-hydroxylated rutile surface directions: (110) and (001).[79] The TIP3P model was used for water. Density distributions were studied for the two

carbon atoms of the glycolate anion close to the rutile surfaces. The adsorption on the (110) surface was through the carboxylate carbon, while for the (001) surface, it was through the hydroxyl carbon. Bonding of the glycolate ion was shown to be more stable to the (110) surface compared to the (001) surface. Since strong bonding between an organic and the surface can hinder the crystal growth, it can be expected that the (001) surface should have a higher growth rate compared to the (110) surface in the presence of the glycolate ion, which was in agreement with experimental results.[79]

Metadynamics was used to assess the binding/unbinding process of the formate anion ($HCOO^-$) to the rutile (110) surface in water (TIP3P model).[112] The rutile surface was non-hydroxylated and charge neutral. The free energy landscape was measured in the two cases where the ion is closer to the surface than 4 Å and where it is farther than 4 Å. In the first instance, two collective variables were chosen: the distance of the ion from the surface in the surface normal direction and the coordination number of the binding site on the rutile surface. In the latter case where the ion is not close to the surface, only one collective variable (the ion-surface distance in the normal direction) was considered.

Three energy basins were detected when the formate ion is close to the surface. The basins include the doubly bound, singly bound and unbound states; in the singly bound state, the formate ion is bonded to the surface by one of its oxygens while in the doubly bound state both oxygens of the ion are involved in bonding. Doubly and singly bound states were in more favorable energy states compared to the unbound state, but it was also shown that the ion has to cross energy barriers to be able to leave the unbound state and undergo the transition to first, the singly bound state and then the doubly bound state.

In the case where the formate ion is farther than 4Å from the surface (in the unbound state), there is a local minimum around 4.5Å which corresponds to the point when the ion is moving into bulk water.

ii. Oligopeptides: It has been shown previously that amino acids bind to surfaces through their side-chains.[113] Brandt *et al*. used molecular dynamics (unbiased sampling), umbrella sampling and adaptive well-tempered metadynamics (biased sampling) to study the adsorption of amino acid side-chain analogues (SCA) and a titanium-binding peptide (TBP; the RKLPDA hexapeptide) on a charge neutral (non-hydroxylated) rutile (100) surface.[53] The C_α of the amino acid (the backbone carbon to which the carbonyl carbon is attached) was replaced by a hydrogen in different amino acids to obtain 19 SCAs (Fig. S2). These SCAs can be divided

into four groups of polar, charged, aromatic and hydrophobic residues. The organic residues were solvated in water (TIP3P model).[53] Umbrella sampling and adaptive well-tempered metadynamics are both known as enhanced sampling molecular dynamics methods. Comparison of the results obtained from these two methods in this study showed that they are in good agreement with each other.

Among the SCAs, polar and aromatic residues showed stronger adsorption to the surface while hydrophobic groups showed less affinity for the titanium dioxide surface. A general statement could not be made for charged residues. In general, residues with oxygen or nitrogen in their terminal groups can bind to the surface through hydrogen bonding while residues with carbon or sulfur terminations have less favorable interaction with the surface. Serine and tyrosine have the strongest binding to the surface; they can displace water molecules and bind directly to the surface.

Histidine was considered in its two forms (HID and HIE) with protonation on two different nitrogen atoms on the side group. Still, this minor difference was found to affect the free energy of adsorption.

The free energy of adsorption for the side-chain analogues can be used to predict the adsorption behavior of proteins. For example, a protein is expected to bind to the surface in a way that a higher number of SCAs with more favorable interactions is exposed to the surface. The binding energy of TBP was significantly larger than the accumulative free energy of adsorption of its SCAs, meaning its adsorption is more favorable than that of its residues, pointing out that this hexapeptide must have a strong affinity for titanium dioxide surfaces.[65] Two binding modes for TBP to TiO_2 surface were observed. The first mode is a worm-like mode which has more mobility compared to the second binding mode (compact c-like mode). The second binding mode was energetically more favorable than the first binding mode. Figure 2 shows the two-dimensional free energy landscape of TBP binding to the rutile (100) surface with respect to the peptide end-to-end distance (EED) and its surface separation distance (SSD). The two adsorption modes are marked with crosses in this figure.

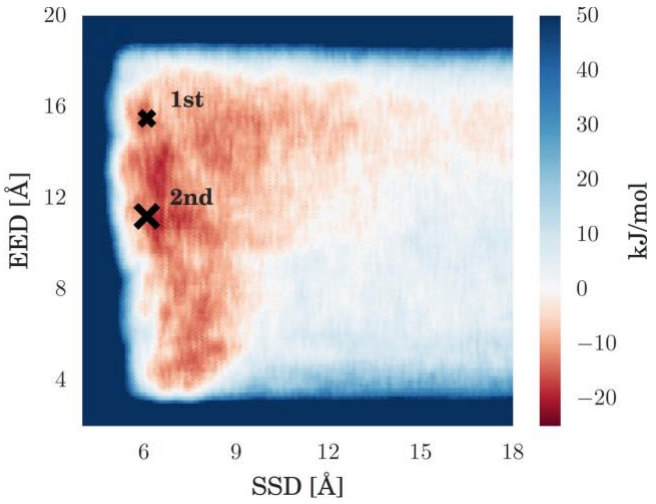

Figure 2. Two-dimensional free energy landscape of TBP based on its separation distance from the rutile (100) surface (SSD) and its end-to-end distance (EED). Crosses mark the two binding modes of the peptide on the surface. Reprinted with permission from Ref. 53. © 2015 American Chemical Society.

The interaction of C-terminated and N-terminated Ala amino acid (Ala-Ace and Ala-Nme, respectively) and Ala-Glu and Ala-Lys dipeptides (with -1 and +1 charges, respectively) with the non-hydroxylated rutile (110) surface was studied by Carravetta *et al*. .[46] The Ace blocking group which is added to the N-terminus, and the Nme blocking group which is added to the C-terminus, create peptide bonds for the Ala amino acid as it would appear in a protein. Water was described by the TIP3P model.

The radial distribution function revealed strong interactions between the carbonyl, carboxyl and amide groups of the Ala-Ace molecule and the water molecules (Fig. S3-a). The amine group of the Ala-Nme molecule also showed a sharp peak in the radial distribution function with water molecules (Fig. S3-b). The absence of a prominent peak between the Ala-Nme carbonyl oxygen and water can be explained by the direct interaction of this side group with the TiO_2 surface.

The trend of the interaction strength of different atom pairs in Ala-Glu and Ala-Lys with water molecules was similar. The interaction of Ala-Lys dipeptide with the surface is slightly more favorable than Ala-Glu dipeptide, which is also supported by the lower flexibility of the Ala-Lys dipeptide.

RAD (Arg-Ala-Asp) is similar to RGD and it can also be involved in cell attachment. Monti used molecular dynamics to study the interaction of a bilayer with the non-hydroxylated rutile (110) surface.[114] Each layer consisted of eight peptide chains. The bilayer was investigated in a parallel orientation and a perpendicular orientation towards the surface. Even though the water molecules (described by TIP3P model) between the bilayer and the surface were initially removed, some water molecules were found in this region at the end of the simulation. Thus, the water-surface interactions are in general more favorable than the bilayer-surface interaction and there is competitive adsorption between the organic and water on the surface. Nevertheless, the bilayer had direct and indirect interactions with the surface in both orientations; through direct bonds with the surface and hydrogen bonds with the adsorbed water molecules on the surface, respectively. The bilayer in the parallel orientation formed more bonds (95% through the Arg residue) with the surface compared to its perpendicular orientation. It was observed that in the perpendicular orientation, the bilayer is capable of significant conformational rearrangements to increase favorable interaction points with the surface. The reason for its higher mobility and flexibility was attributed to the smaller number of bonds with the surface compared to the parallel orientation.

iii. An albumin subdomain: In order to study the effect of surface hydroxylation on protein adsorption, Kang *et al*. compared the adsorption of an albumin subdomain (HSA-IIIb made of 85 amino acids; Protein data bank ID code: 1AO6) on the non-hydroxylated and fully hydroxylated charge neutral rutile (110) surface in contact with water (SPC/E model).[51] The electrostatic interaction between albumin and the hydroxylated surface was found to be more favorable than with the non-hydroxylated surface. While on the hydroxylated surface some of the residues were able to displace water molecules and form hydrogen bonds with surface hydroxyls, the adsorbed residues could not perturb the first two water layers on the non-hydroxylated surface (Fig. 3). This was the case even for the same amino acids in the albumin subdomain which were adsorbed onto the surface in both hydroxylated and non-hydroxylated states.

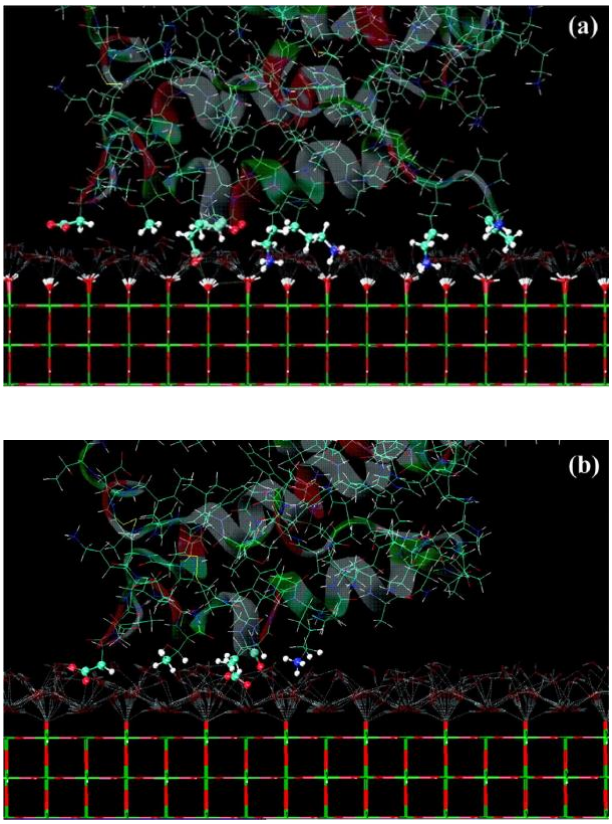

Figure 3. Adsorbed residues on a) fully hydroxylated and b) non-hydroxylated rutile (110) surfaces at 5 ns. Albumin atoms closer than 7 Å to the surface are shown using the ball-and-stick model. Water molecules except the interfacial water molecules have been removed for clarity. Hydrogen bonds are shown in white dashed lines. Color code: Ti: green, C: turquoise, O: red, N: blue and H: white. Reprinted with permission from Ref. 51. © 2010 American Chemical Society.

The first two water layers are bonded to each other via more hydrogen bonds on the non-hydroxylated surface compared to the number of hydrogen bonds between the surface hydroxyls and the first water layer on the hydroxylated surface, before and after adsorption of the protein. As a result, the movement of the albumin subdomain towards the non-hydroxylated rutile surface can be hindered by the stronger bonding between the water layers close to the surface.

iv. The RGD polypeptide: Schneider *et al*. performed umbrella sampling to measure the desorption energy of RGD from an amorphous oxidized titanium surface.[63] The free energy of desorption of the RGD polypeptide solvated in water on a neutral amorphous titanium oxide surface was calculated to be -0.32 eV.[63] In the presence of external surfaces (substrates),

adsorption of organic molecules on surface can be in competition with binding of integrin receptors to the surface, emphasizing the importance of surface modification and surface engineering. The interaction of RGD (Arg-Gly-Asp) with the oxidized titanium surface was through direct binding of the R residue (Arg) to the surface and indirect binding of the D residue (Asp).

4.3.2. Negatively charged surfaces

i. Simple organic residues: Sultan *et al*. considered six amino acid analogues rather than the complete amino acid.[115] There are numerous studies on the interaction of single amino acids with surfaces. Nevertheless, such results cannot be generalized to cases where the amino acid is part of a polypeptide or a protein because the exposed amine and carboxylate terminal groups of the non-bonded amino acids are absent in the polypeptide/protein.

The amino acid analogues covered nonpolar, uncharged polar and charged polar molecules. From the alanine, phenylalanine, serine, arginine, lysine and aspartic acid amino acids, the chosen analogues were methane, benzene, methanol, guanidinium cation, ammonium cation and methanoate anion, respectively. The first three analogues are neutral. The adsorption of these analogues was investigated in an aqueous environment (using a modified TIP3P water model), on a neutral non-hydroxylated rutile (110) surface using molecular dynamics and on a negatively charged (partially hydroxylated) rutile (110) surface using metadynamics.[115,116]

Among the neutral analogues, the two hydrophobic analogues, methane and benzene, revealed no attraction towards neither the neutral nor the charged surface. This suggests that the hydrophobic parts of peptides are expected to be found as far as possible from the hydrophilic titanium dioxide surface.[31,116] Methanol showed a weak binding to the negatively charged rutile surface.

Benzene and the guanidinium cation have relatively planar and rigid geometries. While benzene did not adsorb on either of the neutral or charged surfaces, the guanidinium cation showed the strongest binding to the charged rutile surface among all studied analogues (Fig. 4-a). The adsorption of the ammonium cation on the negatively charged rutile surface was similar to that of the guanidinium cation. Nevertheless, the methanoate anion also showed a favorable binding to the charged rutile surface. It was suggested that the binding of the anion to the negatively charged surface is due to the nanometer spatial variation of charges (missing bridging hydrogen) on the surface.[115,117]

In general, binding was always stronger and energetically more favorable on the charged surface compared to the neutral surface (Fig. 4-b). The free energy of adsorption was defined as the difference between the free energy of the system when the organic analogue is free in water and when it was adsorbed onto the surface. Since the adsorption of the organic analogue was possible on both faces of the solid slab, the free energy of the system in the adsorbed state was the average value of the adsorption on each face. The weak adsorption of the uncharged residues to the charged surface can even turn repulsive on the neutral surface.[115,116]

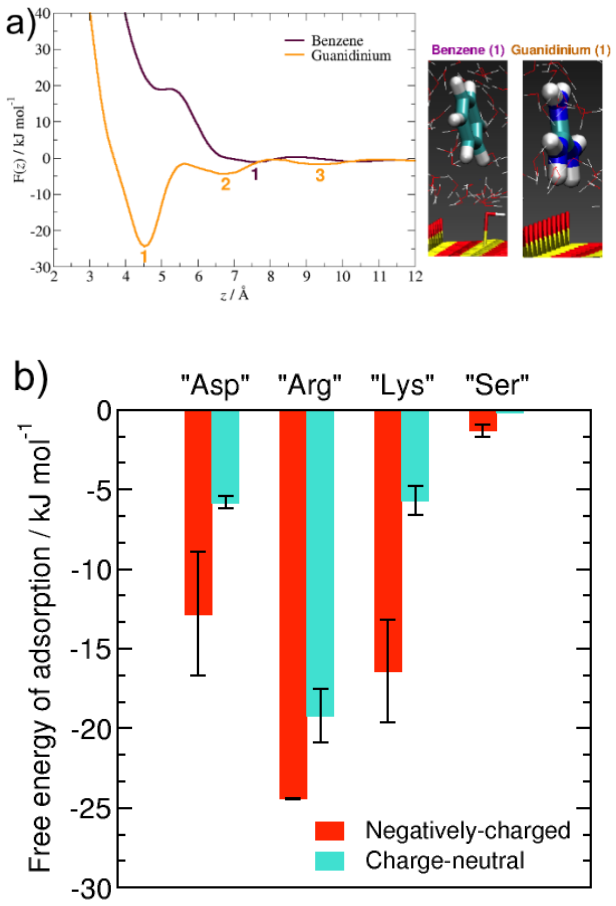

Figure 4. a) Variation of free energy of adsorption as a function of distance from the negatively charged rutile (110) surface for benzene and the guanidinium cation. The local (2 and 3) and global (1) energy minima are numbered. The lowest-energy configurations (1) are shown on the right. Color code: Ti: yellow, C: turquoise, N: blue, O: red and H: white. b) Free energy of adsorption for the adsorbed analogues on the charge neutral[116] and negatively charged rutile (110) surfaces. Reprinted with permission from Ref. 115. © 2014 American Chemical Society.

ii. The RGD polypeptide: Wu *et al*. studied the effect of the presence of Na^+ cations on the adsorption of the RGD polypeptide on the negatively charged (partially hydroxylated) rutile (110) surface in contact with water (SPC/E model).[118] In the absence of Na^+ cations, bonding between the negatively charged rutile surface and the positively charged Arg residue is expected. However, the presence of Na^+ cations in the solution forces RGD to change its conformation. Consequently, the Arg residue in RGD detaches from the rutile surface in the presence of Na^+ ions to reduce the repulsion between its amine group and the sodium cations. On the other hand, the Na^+ cations bridge the COO^- group to the rutile surface; making it possible for the negatively charged Asp residue to bind to the negatively charged rutile surface.

In order to see if monovalent and divalent cations mediate surface-organic bonding differently, Wu *et al*. studied the effect of different cations on the adsorption strength and conformation of RGD (the negatively charged Asp residue, in specific), onto the negatively charged (partially hydroxylated) rutile (110) surface.[102] Water molecules were described using the SPC/E water model. Monovalent cations, similar to sodium cations, help the COO^- group to form hydrogen bonds with the negatively charged rutile surface (Fig. 5-a).[118] If the number of these hydrogen bonds is sufficient to keep the RGD sequence attached to the surface, the monovalent cations are free to leave the surface. However, the adsorption of the peptide to the surface is quite different in the presence of divalent cations. In this case, the adsorption is through indirect binding between the surface and the divalent cation. This binding is robust enough to keep the peptide attached to the surface without the need to have direct hydrogen bonding between the rutile surface hydroxyl groups and the COO^- group of RGD (Fig. 5-b).

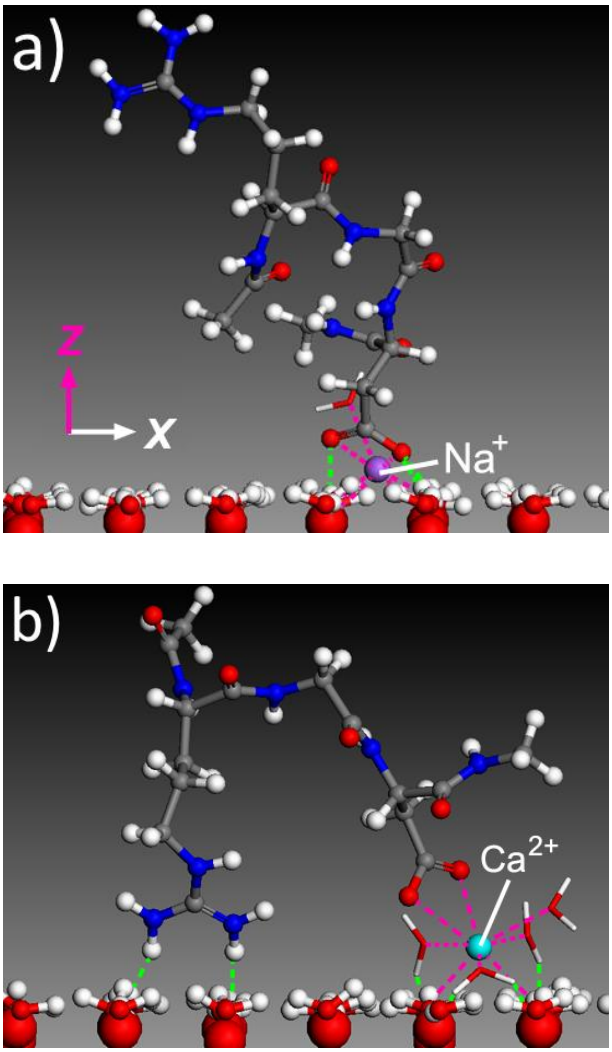

Figure 5. Adsorption configuration of RGD on the negatively charged (partially hydroxylated) rutile (110) surface in the presence of a) sodium and b) calcium cations. Color code: C: gray, N: blue, O: red, H: white, Na: purple and Ca: turquoise. Reprinted with permission from Ref. 102. © 2012 American Chemical Society.

iii. Nucleotide bases: Monti *et al*. studied the interaction of four nucleotide bases (adenine, thymine, guanine and cytosine) with the partially hydroxylated rutile (110) surface with a negative charge density of -0.104 $C \cdot m^{-2}$.[119] The total charge of the system was neutralized by adding Ca^{2+} and Cl^- ions to the solution. Ca^{2+} ions did not move freely in the solution and strongly adsorbed to surface terminal oxygens.

Since the surface carries a net negative charge, chlorine ions are expected to be far from the surface. However, a permanent interaction between calcium ions and chlorine ions was found

(Fig. S4). It had been speculated that in the presence of multivalent cations, the chlorine ion could approach the surface up to small distances but not closer than the first water layer on the surface.[100,119] Although the surface was designed to mimic the charged titanium dioxide surface in the physiological pH, the presence of the calcium and chlorine ions close to the surface modifies the acid-base nature of the surface by compensating the surface charge.

The density distribution of the center of mass of the four nucleotide bases versus their distance from the surface showed that their preferred distance from the surface is between the structured water layers and the bulk water. This can be seen in Fig. S4 in which the density peak for the center of mass of the nucleotide bases is located farther than water peaks with respect to the rutile surface. The study of the variation of the free energy as a function of distance during the adsorption of these four nucleotide bases shows that the structured water layers close to the surface can postpone or significantly weaken the direct surface-nucleotide base interaction. In fact, none of the studied nucleotide bases showed a strong binding with the surface and their migration away from the surface was frequently seen.[119]

iv. Lipids: The adsorption strength of three lipids solvated in water (TIP3P model) on the rutile (110) surface with different levels of hydroxylation was studied by Fortunelli *et al*. [120] The hydroxylation percentage of the partially hydroxylated surface was designed such that it will resemble the state of the surface in physiological conditions. The mobility and flexibility of the lipids decreased in the order of hydroxylated, partially hydroxylated and non-hydroxylated surface. Hence, the adsorption became more favorable in this order. Since pH affects the balance between the bridging and terminal hydroxyl groups on the surface, it will affect the attachment of organics on the surfaces.

v. Polypeptides: Sultan *et al*. used the Replica Exchange Solute Tempering (REST) method coupled with metadynamics to look at the adsorption mechanism and behavior of two polypeptides on a negatively charged rutile (110) surface.[117] The TIPS3P model was used to describe water. The surface charge density was set to -0.104 $C \cdot m^{-2}$. The two polypeptides were different in their total charge and the number of hydrophobic residues (Ti-1: QPYLFATDSLIK and Ti-2: GHTHYHAVRTQT). Despite their different building blocks, both residues showed a strong affinity for the rutile (110) surface. The free energy of adsorption was found to be -12.7 $\pm$ 0.4 $kJ \cdot mol^{-1}$ for Ti-1 and -16.34 $\pm$ 3.7 $kJ \cdot mol^{-1}$ for Ti-2. The same trend for the adsorption energy of two peptides was found using experimental methods. The absolute energy of the adsorption, however, was different from the experimental results which can be attributed to various parameters including the use of a non-

reactive force field. Different characteristics of Ti-1 and Ti-2 and at the same time their similar adsorption energy on the titania surface indicate that the adsorption of these two residues should be via different mechanisms. The contribution of the entropy in the adsorption was estimated for the two residues. This parameter had a positive value for Ti-1 while it had a negative value for Ti-2. This indicates that the adsorption of Ti-1 on the rutile surface is driven by the entropy while Ti-2 adsorption is mainly driven by enthalpy.

vi. The RKLPDA hexapeptide: The force field which was developed by Schneider *et al*. for amorphous oxidized titanium[63] was used to study the adsorption of the RKLPDA hexapeptide on the non-crystalline titanium oxidized surface in contact with water (TIP3P model) using the metadynamics method.[65] The surface carried a surface charge density of -0.123 $C \cdot m^{-2}$, which corresponds to the surface charge at physiological pH. The binding of the hexapeptide was through direct binding of the Arg residue to the surface.

4.4. Surface defects

Compared to defect-free surfaces, surfaces containing structural defects have a higher surface energy, which may enhance protein adsorption and cell attachment.[9,121] The strong interaction of the protein with surfaces containing structural defects might restrain the movement of the protein and affect further cell recognition and adhesion. Water density distribution close to the surface can also be affected by surface defects, which can provide more active interaction sites on the surface for the organic.[122]

i. Fibronectin module: Wu *et al*. compared the interaction of a fibronectin module ($FN\text{-}III_{10}$) with perfect and various defects on non-hydroxylated rutile (110) surfaces in vacuum. The surface defects included oxygen vacancies, steps and grooves.[123] While adsorption occurred on all surfaces, the binding strength differed for different surface topographies. Both side-chain and backbone atoms were involved in the adsorption process. The carbonyl and carboxylate groups showed dominant interaction with the surface while the interaction of the amine and amide groups were relatively weak. The surface with step defects showed the highest binding energy with the fibronectin module. The fibronectin module contains the RGD sequence. The adsorption of RGD, especially on the surface with steps, significantly reduced its mobility. While this can be beneficial in having stable protein adsorption on the surfaces, it should not hinder further cell recognition by the surface. Although these trends

seem generally interesting, further work in the presence of water needs to be made to verify such findings in vacuum.

ii. Collagen triple helix: Ebrahimi *et al*. studied the effect of the degree of surface roughness of the non-hydroxylated rutile (100) surface on the adhesive energy of type I collagen, consisting of a triple-helix, in vacuum.[124] Compared to the defect-free (100) surface, collagen experienced significant conformational changes while adsorbing on the surface defects and the surface-collagen equilibration distance was also relatively smaller. The interaction of the collagen segment to the rutile surface defects was much more favorable and the collagen bonded through more contact points to this surface.

iii. The RGD polypeptide: Song *et al*. investigated the effect of surface defects, in the form of pits, on the adsorption of RGD solvated in water (TIP3P model) onto a non-hydroxylated rutile (110) surface.[9] Adsorption of RGD polypeptides onto a surface containing defects happened much faster compared to the defect-free surface and was more stable due to its stronger binding. The same trend for adsorption kinetics and adsorption strength was observed when the adsorption of RGD onto the non-hydroxylated (110) rutile surface in vacuum was compared between the defect-free surface and surfaces with grooves of different dimensions.[125]

Chen *et al*. compared the adsorption energy of RGD solvated in water (TIP3P model) on non-hydroxylated, defect-free and rutile (110) surfaces with defects represented by three different depths of grooves (3.25, 6.50 and 9.75 Å).[108] The binding state of RGD was initially through the carboxylate group. On the defect-free surface, RGD maintained this binding mode and the amine group was far from the rutile surface. On the grooved surfaces, however, RGD underwent significant conformational changes until the RGD long axis was parallel to the surface. RGD adsorption onto grooved surfaces was much more favorable than onto defect-free surfaces (almost 1.6 times, similar to Liang *et al*.[126]) which can be attributed to a higher number of active sites on the grooved surfaces.

iv. Collagen segment: In another study, the effect of the width and the depth of surface grooves on the adsorption of a collagen segment (2KLW), solvated in water (SPC/E model), on the non-hydroxylated rutile (110) surface was investigated.[127] Adsorption was favored when the groove width matched well with the dimension of the collagen segment. This is in agreement with Kasemo's suggestion that topographical surface features with dimensions

similar to those of the adsorbing protein can significantly affect its configuration, binding strength and activity.[31]

4.5. Surface contamination

i. Two peptides and the RKLPDA hexapeptide: Air-exposed titanium oxide surface can be contaminated with hydrocarbons and small alcohols, which are present in the ambient atmosphere. The adsorption of two peptides (TiOBP1: RPRGFGMSRERQ sequence and TiOBP2: WFCLLGCDAGCW sequence) and a hexapeptide (RKLPDA) on rutile (100) surfaces, with two different levels of contamination by pentanol, were compared to the partially hydroxylated clean surface in water (TIP3P model).[50]

Hydrophobicity of the peptide and the ratio of the hydrophobic to hydrophilic residues can affect its adsorption onto the surface. TiOBP1 has nine hydrophilic and three hydrophobic residues. As long as one of the faces of the slab was a clean surface, the adsorption of TiOBP1 on this surface was more favorable. When both surfaces of the slab were contaminated and no clean surface was present in the system, the adsorption occurred on the contaminated surface. Adsorption on both clean and contaminated surfaces was stable. In the case of the contaminated surface, the peptide underwent structural changes, during which it tried to expose more hydrophobic residues to the surface. Two of the residues with charged end groups penetrated the pentanol layer and bound directly to the titanium dioxide surface.

TiOBP2 has an equal number of hydrophobic and hydrophilic residues (6:6). In contrast to TiBP1, TiOBP2 did not adsorb on the clean surface while it quickly adsorbed on the contaminated surface. The hexapeptide (RKLPDA) is similar to TiOBP2 in terms of having the same ratio of the number of hydrophobic to hydrophilic residues (3:3). However, unlike TiOBP2, RKLPDA adsorbed on both clean and contaminated surfaces. It was concluded that the adsorption on different surfaces is driven by the ability of the organic molecule to undergo structural changes to rearrange its residues in a manner that allows more favorable interactions with the surrounding environment.

4.6. Initial orientation of the organic molecule

i. Ala dipeptides: Adsorption of two uncharged peptides (Ala-Lys (AK) and Ala-Glu (AE)) on the non-hydroxylated rutile (110) surface in the presence of water (TIP3P model) was

studied.[128] Each peptide was studied in a separate system. In each system, nine different initial orientations of the peptide were simultaneously placed on top of the surface. In other words, each peptide was studied in the presence of eight other orientations of its own. This was done to investigate the perturbation effect resulting from the presence of neighboring peptides. During the equilibration step, one out of nine orientations of each peptide led to its detachment from the surface. Both peptides (AK and AE) interacted with the surface mainly through the oxygen atoms of their carboxylate groups and the nitrogen atoms of their amine groups. Further investigation revealed that the titanium dioxide surface constrains the movement of the peptides due to the surface-peptide binding.[129] It was shown that having several contact points between the backbone and the surface or even a single such contact point in addition to hydrogen bonding with other peptides that are strongly bonded to the surface can keep the peptide bonded to the surface throughout the simulation time of 6 ns.

ii. The RGD polypeptide: The effect of the initial orientation of the RGD polypeptide on its adsorption to the non-hydroxylated rutile (110) surface was studied using two different water models (SPC/E and TIP3P).[106] In agreement with other studies, the initial orientation proved to be important since, for some orientations, the peptide moved away from the surface as its interaction with water was more favorable. The interaction of amine groups was dominant compared to the carboxylate groups and it was mainly through the Arg residue; this is in contrast with DFT results of adsorption of RGD on rutile (110) surface, albeit in vacuum, which occurs through the aspartic acid carboxyl groups and not the arginine side group.[130] The presence of the RGD polypeptide did not affect the water structure close to the hydrophilic rutile surface.

The results of surface-organic-water interactions using the two three-point rigid water models (SPC/E and TIP3P) showed that the SPC/E water model leads to slightly stronger interactions between the peptide and the surface oxygen atoms. Also, the peptide shows more flexibility while solvated in the SPC/E water model which helps it attain its equilibration state in a shorter time.

iii. Collagen segment: While one of the most important segments of collagen is the RGD sequence, Monti studied the possibility of the adsorption of a collagen segment in the absence of any RGD sequence or charged amino acid to the non-hydroxylated rutile (110) surface.[131] The collagen segment was chosen to be a triple helical segment (THS) of collagen, consisting of 21 amino acids. Water molecules were modelled using the TIP3P model. The long axis of THS was orientated parallel to the rutile surface and its interactions with the surface were

studied in six different rotations of THS around this axis. The attachment of the THS segment to the rutile surface was observed through hydrogen bonds.

The stability of the THS can be attributed to the stability of the hydrogen bonds between its helices. In proximity to the rutile surface, several residues in the helix will engage in bonding with the surface and will not be available for hydrogen bonding with the other two helices anymore. The other two helices, as a result, now have the possibility to interact with their surrounding solvent. The stronger the interaction of the helices far from the surface with their surrounding water molecules, the more likely the unfolding of the THS segment. In fact, in two out of six different initial orientations of THS, the helices started to expand over time to a point in which the initial THS orientation was completely lost and disrupted.

Even though the THS segment studied in this work lacks the carboxylate groups, it was seen that peptides rich in Hyp residues could also adsorb to the rutile surface but the binding stability depends on their initial orientation.

The adsorption of a collagen segment (2KLW) in three different orientations, solvated in water (SPC/E model), to the non-hydroxylated rutile (110) surface with defects has also been studied.[122] The initial orientation significantly affects the interaction frequency of the functional groups as well as the binding strength. No adsorption was observed in one out of three different orientations but in the other two orientations, the collagen segment adsorbed to the surface at the beginning of the simulation. Adsorption in these two cases occurred both indirectly through water molecules and directly through direct bonds between the collagen segment and the surface. The indirect bonding between the carboxylate group of the Asp residue was insufficient to keep the collagen segment bound to the surface and after some time it detached (Fig. 6). On the other hand, direct binding between the amino group of Lys and the rutile surface was stable over the simulation time of 6 ns.

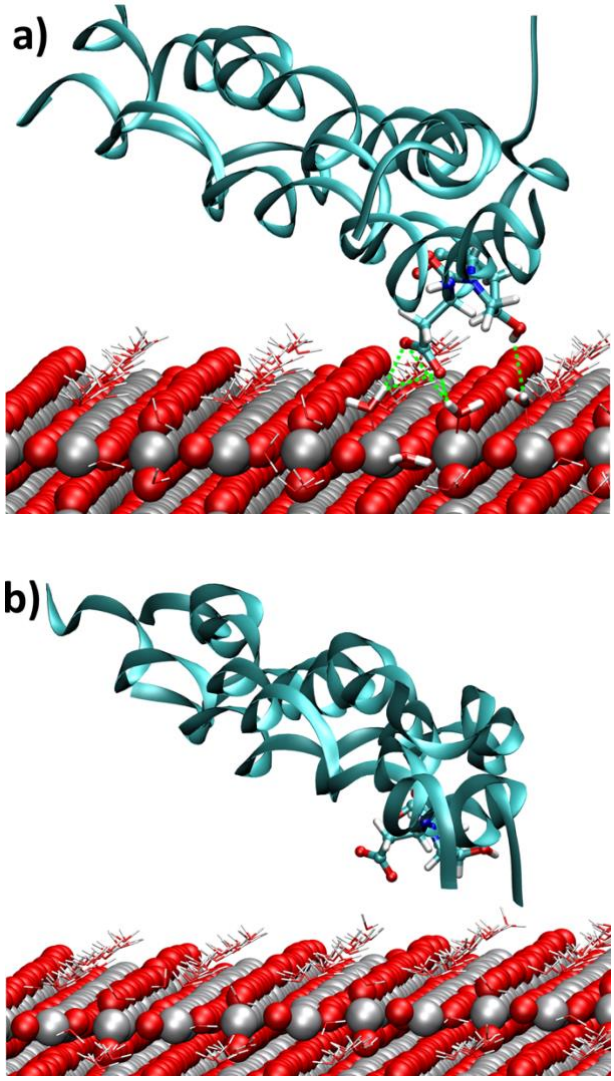

Figure 6. a) Collagen interaction with the non-hydroxylated rutile (110) surface at t = 3 ns and b) its detachment from the rutile surface at t = 6 ns. Color code: Ti: gray, C: turquoise, N: blue, O: red and H: white. Reprinted with permission from Ref. 122. ©2013 Elsevier.

iv. Bone morphogenetic protein-2: The interaction of bone morphogenetic protein-2 (BMP-2) with the non-hydroxylated (001) rutile surface, solvated in water (TIP3P model), was investigated by Utesch *et al*. in six different initial orientations with respect to the surface.[132] The BMP-2 protein was placed on the surface in two end-on and four side-on orientations. Molecular dynamics and steered molecular dynamics methods were used. During steered molecular dynamics, an external force was applied on the biomolecule to accelerate its conformational changes and to observe its possible adsorption/desorption from the surface. Although the surface-biomolecule interaction was favorable, the adsorption of the BMP-2

molecule to the surface was loose and strongly hindered by the two structured water layers close to the hydrophilic TiO_2 surface. The force exerted by water molecules on BMP-2 was measured at different distances from the surface. At distances where the first water layer is located, this force was repulsive. At a distance close to the second water layer, this force was attractive and when far from the surface, the force was negligible. It was concluded that direct binding between BMP-2 and the surface is hindered by the repulsive force exerted on the biomolecule from the first water layer. Nevertheless, the attractive force between BMP-2 and the molecules of the second water layer was enough to keep the biomolecule loosely bonded to the surface. By applying an external force on BMP-2 to pull it towards the surface, BMP-2 penetrated the second water layer but not the first water layer close to the surface. Application of larger forces led to unrealistic conformational changes in BMP-2.

5. Summary

Here we present a summary of points which were made in different studies. Adsorption of ions and biologically relevant organic molecules readily take place on rutile surfaces at pHs near to physiological conditions and molecular dynamics studies have been able to shed significant light on the mechanisms involved. From these simulations, it can be concluded that surface characteristics (crystal structure, hydrophobicity, surface charge, surface defects and contamination) and organic molecule characteristics (its functional groups and orientation with respect to the surface) affect the organic-inorganic interactions as well as the conformational and structural changes that the organic molecule might experience during adsorption on the surface. Some other points include:

- There is competitive adsorption between water and the organic on the surface. Since water molecules cover the titanium oxide surface before organic molecules (on a non-coated surface), the organic residues have to displace the water molecules to bind directly to the surface.
- On a neutral surface, the interaction of functional groups containing oxygen or nitrogen with the titanium dioxide surface is more favorable than that of functional groups containing sulfur or carbon atoms. In general, adsorption onto a charged surface is more favorable than onto a neutral surface.
- Defect sites can provide additional binding sites for organic molecules. Their higher activity can lead to stronger binding. It should be noted that, in some cases, this strong

binding can significantly restrict protein mobility and negatively affect cell recognition and attachment since cells recognize certain proteins in specific orientations and configurations.

- Surface contamination, like other surface characteristics, can control the arrangement of hydrophobic and hydrophilic residues of the organic unit before and during its adsorption on the surface.
- The initial orientation of the organic molecule affects its adsorption behavior. Several studies observed that for some orientations the molecule preferred to stay solvated while the same molecule bonded to the surface in other orientations. While this is not always problematic, caution should be taken when studying an organic molecule with several parts that are bonded to each other through hydrogen bonds. If some parts of the organic engage in surface binding, other sections might start compensating their missing hydrogen bonds through hydrogen bonding with surrounding water molecules. The unfolding of the initial conformation of the organic molecule, in this case, is probable.

There are still fundamental questions concerning the interaction of organic molecules with inorganic surfaces and research is still required on *in vitro* conditions. Computational methods are certainly useful in answering some of these questions. Some possible research points in this area are:

- The interaction of other SBF anions with titanium oxide surfaces, such as hydrogen phosphate, hydrogen carbonate and sulphate.
- The competitive adsorption of SBF ions and organics on titanium oxide surfaces.
- Enhanced sampling methods in line with molecular dynamics simulations to confirm adsorption energetics and kinetics at different sites both for SBF ions and organic molecules.

Ideally one should investigate the interaction of Ti surfaces with organic components solvated in a solution close to SBF - this could be computationally very expensive but it is an important next step in the realm of computational studies. Within the limitations of molecular dynamics modelling (limited timescale and no chemical reactions), some interesting and pertinent insights have already been gained. As outlined above, further work with well-defined systems (mimicking experimental conditions derived from thermodynamic modelling) will lead to a better understanding of the interaction of these complex solutions with solid surfaces. Further work on specific problems, where “reactive” situations are of high relevance, could then be carried out using first-principles molecular dynamics potentially

combined with classical molecular dynamics for the non-reactive part (QM/MM scheme) or enhanced sampling methods to overcome timescale limitations.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Supplementary Information

The supplementary information contains the list of abbreviations and chemical formula for most of the organic molecules mentioned through this review.

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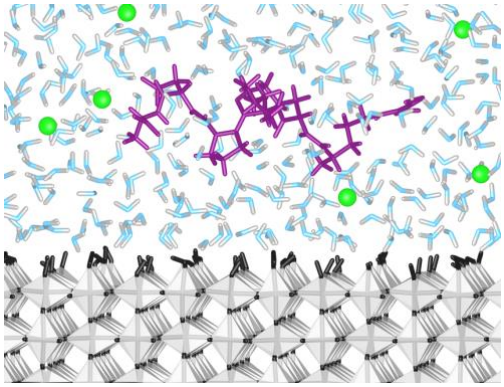
Graphical abstract



Atomistic representation of a system containing an inorganic surface (grey), an organic molecule (purple), ions (green) and water.

Highlights

- There is a vast number of computational work regarding titanium oxide surfaces.
- Surface properties control its interactions with ions and organic molecules.
- Water can delay or prevent adsorption of species on the surface.