Molecular modelling of protein adsorption on the surface of titanium dioxide polymorphs

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This paper reports a molecular modelling study of the adsorption of protein subdomains with unlike secondary structures on different surfaces of ceramic titanium dioxide (TiO₂), forming a passivating film on titanium biomaterials that provides the interface between the bulk metal and the physiological environment, affecting its biocompatibility and performance. Using molecular dynamics methods, we study the effect of the nanoscale structure of the common TiO₂ polymorphs (rutile, anatase and brookite) on the adsorption of an albumin subdomain and on two connected fibronectin modules, respectively containing α-helices and β-sheets. We find that the larger protein subdomain shows a stronger adsorption, as expected because of its size, but also that the three surfaces behave differently. In particular, brookite shows the weakest adsorption, whereas anatase leads to the strongest intrinsic adsorption, in particular for the fibronectin modules. Moreover, the simulations indicate a significant conformational change of the adsorbed protein subdomains with extensive surface nanopatterning. These results show that classical molecular dynamics methods can provide useful information about the influence of nanostructure and topology on protein physisorption at a fixed surface chemistry.

Keywords: molecular simulations; molecular dynamics; biomaterials; titanium dioxide; protein adsorption

1. Introduction

By definition, biomaterials interface with biological systems and come into contact with physiological fluids, which provide in general a polar environment. In this case, the first event taking place at the interface is the surface hydration, followed by the adsorption of small molecules and then proteins. The kinetics of this process is affected, among various factors, by the proteins’ concentration in the biological fluid and their diffusivity, related in turn to hydrodynamic volume, hence to size [1–3]. On the other hand, at thermodynamic equilibrium, the protein adsorption strength is controlled by the non-bonded interactions at the interface (for pure physisorption), affected by the nanoscale surface structure and topology.

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On a lengthier time scale, the biomaterial surface eventually shows cell adhesion, driven by the adsorbed proteins, and then cell proliferation and (possibly) differentiation. Owing to the importance of protein adsorption for the subsequent events, both the surface chemistry and its nanoscale structure and possible nanopatterning are particularly relevant.

From a theoretical viewpoint, the surface peculiarities and the hierarchical structure of proteins cannot be handled analytically or through coarse-grained simulations, except for very broad and general features, but require a fully atomistic description [1,4]. Moreover, owing to the large system size typically involving tens of thousands of atoms, quantum methods cannot be used. Hence, in recent years, an atomistic picture of protein adsorption on specific surfaces has been obtained through classical force fields mainly using molecular mechanics (MM) and molecular dynamics (MD) methods adopting a suitable simulation strategy, thus following the interactions, the fluctuations and the overall dynamics of the individual atoms. Because proteins are biological macromolecules formed by tens of residues, at least, their rearrangements, for instance close to a surface, involve motion at different length scales, ranging from the local ones within a single residue with very short time scales, to the collective ones involving long adjacent strands at much longer time scales, whereas macromolecular connectivity gives rise to cooperative processes. Such events pose a noticeable challenge to current simulations.

Classical MD methods were recently used to model protein adsorption on surfaces of various nature. For instance, in our group, we considered the adsorption of unlike protein subdomains and of lysozyme on hydrophobic graphite and on the amorphous surface of a hydrophilic polymer, together with sequential adsorption on graphite [4–10]. With similar methods, other groups considered different ceramic surfaces, such as titanium dioxide (\(\text{TiO}_2\)) (rutile) [11], MgO [12], SiO\(_2\) (assumed to mimic atomically flat mica) [13,14], hydroxyapatite, the mineral component of bones [15], or a silicate surface of chrysotile [16], but self-assembled monolayers were also modelled [17]. Some of these papers were already reviewed in earlier studies [10,18], and shall be briefly commented upon later. An excellent and a comprehensive review of protein adsorption on a surface recently summarized the current state of the art, focusing on both experimental and theoretical aspects of the phenomenon, mentioning also atomistic and coarse-grained simulation studies [1]. In the present study, we report new simulation results about protein adsorption on \(\text{TiO}_2\). An extensive review of the structure, morphology, and electronic and catalytic properties of \(\text{TiO}_2\) surfaces, and of their adsorption behaviour \textit{in vacuo} and in the air, but not as a biomaterial, can be found in Diebold [19].

Ceramic materials are currently used as bulk biomaterials, such as alumina, bioactive glasses, calcium phosphates or pyrolytic carbon [20], but they are also present in other cases as films of passivating oxides on metallic biomaterials. In particular, titanium is a metal widely used in medical implants and prostheses owing to its mechanical properties and relatively low density, which interacts with biological fluids through a passivating \(\text{TiO}_2\) film that ensures its inertness and biocompatibility [21]. Much effort has been devoted to control the thickness and structure of this film and to modulate its properties by incorporating appropriate ions (typically calcium and phosphate) to enhance its osseointegration. In particular, suitable chemical and electrochemical treatments can affect the

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thickness of the TiO$_2$ film and its submicrometre morphology, as well as the surface structure in terms of the exposed polymorph [22]. Three polymorphs are known at ambient pressure: rutile (the most common one), anatase (that can be favoured through specific surface treatments) and brookite (less common, and mostly absent in passivating films). These polymorphs expose unlike surfaces to physiological fluids, which may differently affect protein adsorption, hence the final biocompatibility and performance of the biomaterial. Therefore, here we report a simulation study of protein adsorption (physisorption) on ideal surfaces of the TiO$_2$ polymorphs. For simplicity, we assume that no surface reconstruction takes place compared with the bulk structure: such events may be important in vacuo for certain crystalline surfaces, but are generally minor in water [23]. Accordingly, only bridging oxygens will be present with no hydroxyls in all cases to have a surface chemistry as similar as possible for the three polymorphs, so as to investigate the effect of the (sub)nanoscale topology only. A fundamental issue addressed in the study is whether indeed the surface topology affects protein adsorption at a fixed surface chemistry, and to what extent, and whether current simulation methods based on MD can predict significant differences.

The plan of present study is the following. First, we give a brief, non-technical overview of the simulation methods focusing on MM and MD methods (concepts and machinery) in terms of a simple physico-mathematical picture most familiar to chemists. Then, we describe protein adsorption on TiO$_2$, considering both the initial and the final adsorption stage on bare surfaces. In the final section, after a summary of the new results, we briefly comment upon the results obtained by the same techniques for proteins on unlike ceramic surfaces, and provide an outlook to some open issues for future studies in this area.

2. Background on molecular mechanics and molecular dynamics methods

(a) The mathematical model: the force field

The physico-mathematical description of a molecular system is embodied in the force field, which yields the potential energy $V(R)$ as a function of the atomic coordinates, collectively indicated by $R$. Force fields account for intra- and intermolecular terms, but they are more conveniently described as formed by a valence term and a non-bonded term. The valence term accounts for the interactions involving chemically bonded atoms separated by at most four or five bonds. Within this term, force fields account for bond stretchings and angle bendings, for the torsional potentials and often for the out-of-plane deformation in planar moieties, corresponding to motions experimentally observed by vibrational spectroscopies [24–27]. Current force fields are expressed through slightly different mathematical expressions, including harmonic potentials for bond stretching and angle bending (often with optional anharmonic corrections); but in many cases, coupling terms enhance the simulation accuracy for isolated molecules. However, a most important point consists of the numerical parametrization of the force field in terms of the unlike atom types describing the specific local geometry (or hybridization) and environment (through the connected atoms, the bond order, the possible conjugation and so forth), hence the possible functional groups.
On the other hand, the non-bonded terms account for the interactions among atoms that either belong to different molecules, or to topologically distant strands of the same molecule—for instance, in synthetic and biological polymers or in proteins, that happen to be close in space because of the molecular flexibility. These terms include the coulombic terms with integer charges for ionic groups, and with fractional charges to account for the local dipoles in the point-charge approximation. Additionally, the dielectric constant of the medium is usually included for simulations with implicit solvent, thus avoiding the burden of modelling thousands of solvent molecules. Further non-bonded terms include the repulsive covolume interactions between atoms that come in contact, and the attractive dispersive interactions. These interactions are usually much weaker than the electrostatic ones on a per atom basis, but because they are always additive, their overall contribution may become very large, in particular with macromolecules. Covolume and dispersive interactions are usually comprised within the familiar 6–12 Lennard-Jones potential, or some of its variants with different exponents to account for softer potentials (as sometimes used for condensed matter) or for sharper potentials (used in some cases for hydrogen bonds).

Potentially relevant issues about some approximations implicit in these non-bonded potentials should be noted [28]. The first one is the above-mentioned point charge approximation to account for the local dipoles. The second issue is the neglect of molecular polarizability, which could be important in conjugated systems with mobile electrons, even though this effect can be somehow embodied in the parametrization of the covolume and dispersive interactions. Another issue involves the parameters of the Lennard-Jones potential for a given pair of unlike atoms. For practical reasons, and faute de mieux, these parameters are usually calculated as some appropriate (numerical or geometrical) average of those between identical atoms, ignoring their hybridization and chemical environment. This feature could be in principle relevant for physisorption on hydrophobic surfaces, for instance, and in molecular recognition phenomena with apolar moieties. In this case, however, we have recently shown [29] that molecular recognition in host–guest complexes can be predicted very accurately by current force fields through an appropriate simulation methodology, in full agreement with nuclear magnetic resonance experiments in solution and with X-ray data in the solid state. Furthermore, with macromolecules, the situation is actually quite different and more favourable, because specific interactions of individual residues are statistically less important, whereas cooperative effects have a paramount importance.

(b) The mathematical model: the simulation methods

A connectivity table specifying the molecular topology, i.e. the bonded atoms, accompanies the physico-mathematical description embodied in the force field. For a given set of atomic positions, the potential energy of the system is then obtained, and the properties of interest can be investigated by different simulation techniques. A number of books fully describe them in detail (e.g. [30–32]), and therefore here we give only a very brief overview of the most popular techniques used to model surface adsorption phenomena, namely MM and MD methods.

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MM methods are based on the minimization of the potential energy with respect to all the variables, i.e. the atomic coordinates. The minimization is carried out with standard numerical procedures, which guarantee convergence to some (possibly local) minimum. The problem of the huge number of coordinates, often tens of thousands, may eventually pose practical limits to current algorithms on today’s computers, but the main conceptual issue is the large number of local minima that they almost invariably produce. Appropriate strategies must be adopted to look for the most stable state with the absolute energy minimum, or at least a reasonable one. One strategy is to adopt different starting points: for protein physisorption, one possibility is to try different starting orientations, corresponding to the random approach from solution. Usually, each orientation yields a different adsorption geometry and interaction energy, while the protein is trapped in a (local) energy minimum. Other techniques can involve the simulated annealing procedure, consisting of giving an energy input to the system that allows overcoming the energy barriers separating the local minima. Such energy input can also be provided through the kinetic energy in MD simulations at a constant temperature. These simulations allow the system to better explore the conformational space and to probe different arrangements in an unbiased way. In turn, the independent conformations sampled at equilibrium in a dynamic trajectory may be used to calculate statistical averages of interest at the chosen temperature, or to minimize the system energy in search of the most stable state.

Concerning the MD simulations, we briefly recall that they are based on the numerical integration of Newton’s equations of motion were the force acting on each atom is obtained from the gradient of the potential energy given by the chosen force field. According to statistical thermodynamics, temperature is obtained by the average kinetic energy. Because there may be drifts in temperature, a thermostat is introduced in the simulations, fictitiously coupling an oscillator with appropriate mass and characteristic relaxation time to keep constant the average temperature with random fluctuations. Similar procedures can also be applied to control the pressure or the stress, and accordingly, one can perform simulations at a constant volume or pressure (hydrostatic pressure), or at a constant stress, with a change in the system volume. It is implicit in the last cases that these simulations must be carried out with periodic boundary conditions (PBCs) for the atomic positions and velocities. In this case, obviously, care must be taken to choose a large enough cell with PBCs; otherwise, the system will show artefacts due to its finiteness, involving for instance artificial self-interactions of molecules with their periodic images.

\( (c) \) The simulation procedure

The simulation strategies can be somewhat different, and here we report our simulation protocol \([4–6,8,10]\), followed also here. However, other groups have independently chosen a somewhat similar strategy \([12]\), or have explicitly adopted our procedure \([16]\). Our simulation protocol basically involves two steps: (i) first, we minimize the energy of the system using different initial orientations of the protein close to the surface, using an implicit solvent through an effective distance-dependent dielectric constant and (ii) then we perform MD runs in the implicit solvent at 300K for selected arrangements, considering both the lowest and the highest energy minimum obtained in the initial minimizations to follow.
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the surface rearrangements on a bare surface. In these runs, care must be taken to achieve equilibration of the system, at least within the available timeframe. Therefore, it is important to monitor the time variation of different quantities that may relax with unlike characteristic times, such as the total and potential energy and their components, the distance between the molecular centre of mass and the surface, or some typical molecular size such as the radius of gyration. In fact, the contact interactions with the surface can be accompanied by a lengthier intramolecular optimization of the contacts among distant strands of the adsorbed protein [4,8].

Note that this strategy is also physically meaningful because the first step corresponds to the initial adsorption stage, but it may also yield the final adsorption geometry on bare patches of the surface at a large coverage, provided that there are non-specific intermolecular interactions (such as the formation of intermolecular disulphide bridges). Conversely, the second stage yields the stable state with full molecular spreading on a bare surface under thermodynamic control. As anticipated in the first stage, many local energy minima are typically found, indicating the presence of a rugged energy landscape where the protein is easily trapped in some local minimum. On the other hand, the initial energy minima provide also a first estimate of the intrinsic interaction strength in terms of the number of amino acids in contact with the surface [4,10].

As a concluding remark of this section, we should comment on the use of an implicit solvent instead of explicit water. Modelling the solvent requires a huge computational effort owing to the very large number of water molecules (of the order of $10^4$, at least) and to the huge increase of the characteristic times (by some orders of magnitude). Therefore, an implicit solvent allows modelling much lengthier processes, both for the smaller size of the simulated system, and of its much faster relaxation. On the other hand, proteins are well solvated at their hydrophilic envelope through water molecules that, on average, show lower mobility than in bulk water, but are still mobile. Thus, using an implicit solvent, the changes in the solvation energy and in the entropy of the interfacial water molecules are neglected. Two arguments based on the comparison between simulations adopting both an implicit and an explicit solvent do lend support to this approximation. These arguments rely on the relatively modest change in solvation energy (about 10%) for surface-adsorbed and fully spread protein subdomains compared with that for the native state [7,10] and on the predicted molecular recognition by non-covalent inclusion complex, where the same most stable arrangement was determined with either simulation, in full agreement in all details with the experimental results in solution and in the solid state [29]. More details can be found in the cited papers and in Raffaini & Ganazzoli [18]. Let us only add that in an implicit solvent, the kinetics of the process could still be essentially unaffected, apart for some time-scale factor, if protein spreading is mostly controlled by the intramolecular interactions.

3. Protein adsorption on simple surfaces of the titanium dioxide polymorphs

In this section, we report new results about the adsorption of two protein subdomains with an unlike secondary structure on ideal simple surfaces of the three TiO$_2$ polymorphs: rutile, anatase and brookite (listed in appendix A).
Figure 1. The native state of (a) the albumin subdomain and of (b) the fibronectin type I modules highlighting their secondary structure: the α-helices are shown as red cylinders, and the antiparallel β-sheets as orange arrows, whereas the backbone is shown in green. The carbon atoms are displayed in green, the oxygen atoms in red, the nitrogen atoms in blue and the sulphur atoms in yellow (hydrogen atoms are not shown for clarity). (Online version in colour.)

The selected protein subdomains comprise an albumin subdomain containing only α-helices, and two connected fibronectin type I modules with antiparallel β-sheets, shown in figure 1, already modelled by us on hydrophobic crystalline graphite [5,6,8] and on hydrophilic amorphous poly(vinyl alcohol) [7]. More details on the protein subdomains can be found in Raffaini & Ganazzoli [5,6], whereas a brief description of the chosen surfaces and the simulation procedure are in appendix A (see also De Nardo et al. [22]).

(a) The initial adsorption stage

According to the simulation protocol described in §2, this adsorption stage was obtained for each protein subdomain on the surfaces of the three polymorphs by direct energy minimization assuming different starting orientations. These initial arrangements closely matched those reported for the albumin subdomain [5] and the fibronectin modules [6] on graphite. In all cases, adsorption took place on the surfaces with some intramolecular rearrangements to enhance the interaction strength. Interestingly, these rearrangements did either affect only the protein strands in contact with the surface, or led to major changes within the whole
proteins, preserving however part of the secondary structure (figure 2). This feature is most evident in the least stable initial geometries (second and fourth row of figure 2), and is clearly related to the relative weakness of the interaction taking place through a few residues only. However, it is also apparent in the most stable initial geometries (first and third row of figure 2), where the molecular rearrangements are somewhat larger, and a greater number of residues interact with the surface. Indeed, in keeping with previous results [5–8], we found that in all cases a linear correlation exists between the interaction energy $E_{\text{int}}$ and the number of residues that are in contact with the surface, $n_{6\AA}$, for the different starting orientations. Here, $E_{\text{int}}$ is defined as $E_{\text{int}} = (E_{\text{free}} + E_{\text{biomat}}) - E_{\text{tot}}$, where $E_{\text{free}}$ is the energy of the free native subdomain, $E_{\text{biomat}}$ is the energy of the biomaterial (which is a constant, conveniently set to zero because it is kept fixed) and $E_{\text{tot}}$ is the total energy of the whole system. According to this definition, $E_{\text{int}} > 0$ is the energy required to desorb the protein subdomain and bring it back to the free native state. Moreover, the number of amino acids in contact with the surface, $n_{6\AA}$, is determined by conveniently taking 6 Å as the upper distance for a contact interaction. The plots of $E_{\text{int}}$ versus $n_{6\AA}$ for the albumin subdomain and the fibronectin modules are reported in figure 3, where the simulation data are
shown together with the best-fitting lines through the origin, given by

\[ E_{\text{int}} = (39.4 \pm 1.5) \cdot n_{6\text{Å}} \text{ kJ mol}^{-1} \]  
for the albumin subdomain on rutile (3.1)

\[ E_{\text{int}} = (32.4 \pm 1.2) \cdot n_{6\text{Å}} \text{ kJ mol}^{-1} \]  
for the fibronectin modules on rutile (3.2)

\[ E_{\text{int}} = (41.5 \pm 1.0) \cdot n_{6\text{Å}} \text{ kJ mol}^{-1} \]  
for the albumin subdomain on anatase (3.3)

\[ E_{\text{int}} = (37.1 \pm 1.3) \cdot n_{6\text{Å}} \text{ kJ mol}^{-1} \]  
for the fibronectin modules on anatase

\[ E_{\text{int}} = (35.3 \pm 1.1) \cdot n_{6\text{Å}} \text{ kJ mol}^{-1} \]  
for the albumin subdomain on brookite

\[ E_{\text{int}} = (27.6 \pm 1.0) \cdot n_{6\text{Å}} \text{ kJ mol}^{-1} \]  
for the fibronectin modules on brookite

where the ‘±’ sign refers to the standard error of the fit. The numerical coefficients reported earlier (the slopes of the fitting lines in figure 3) yield the average interaction energy per amino acid in contact with the surface, henceforth denoted as the intrinsic interaction strength. It should, however, be remembered that the overall interaction energy depends on the total number of such amino acids, hence somehow on the molecular weight of the protein. Keeping in mind this caveat, a few conclusions about the intrinsic adsorption strength in the initial adsorption stage are apparent. First of all, anatase provides the stronger adsorption for both protein subdomains, even though for albumin the difference with rutile is only significant at the 1σ level, whereas brookite yields by far the weakest one. In the second place, in this stage, the albumin subdomain shows a stronger adsorption than the fibronectin modules on all surfaces, a result that can be related to the ‘soft’ nature of albumin [33], which may locally best adjust its conformation to optimize the surface interaction. For both subdomains, adsorption is mostly due to dipolar and dispersive interactions with few intermolecular hydrogen bonds, thus largely preserving the secondary structure.
Figure 4. The potential energy $E_{\text{pot}}$ (left scale) and the distance $D$ between the backbone centre of mass and the surface (right scale) plotted as a function of the simulation time in the MD runs. The runs, yielding the most stable states in all cases, are for the albumin subdomain on (a) rutile and on (b) anatase and for the fibronectin modules on (c) rutile and on (d) anatase.

The local surface deformations imply also a strain energy that is much smaller than the favourable interaction energy, in view of the observed adsorption. The strain energy does also increase with increasing $n_6\text{Å}$ (data not shown for brevity), but much more slowly than $E_{\text{int}}$, as previously found with other surfaces and proteins [5–8]. Accordingly, also in this case, we can expect that long MD runs at room temperature can allow the subdomains to fully optimize the interaction through further surface spreading, as described in §3b.

(b) The surface spreading of proteins and the final adsorption stage

The surface spreading of the subdomains on the TiO$_2$ surfaces of rutile and anatase was investigated through the MD runs at 300K starting from the geometries shown in figure 2, having both the lowest and the highest initial energy minima. In this stage, the brookite surface was no longer considered in view of the much less favourable initial adsorption. In the MD runs, the system energy showed a fast decrease in the initial part of the trajectory, and then a slower decrease, sometimes with more successive steps, while the protein approached the surface with a concomitant larger spreading. The kinetics of the process was followed through the time change of the potential energy $E_{\text{pot}}$ and of the distance $D$ between the backbone centre of mass and the surface, reported in figure 4 for the most stable arrangements found for each protein subdomain on rutile and anatase. Note incidentally that $D$ provides an indirect measure of the thickness of the adsorbed layer that may be qualitatively...
compared with experimental data (see also Raffaini & Ganazzoli [8]). It may be noted in the figure that $E_{\text{pot}}$ and $D$ show significant variations in the initial part of the run, but afterwards while $E_{\text{pot}}$ achieves an almost constant value, in some cases $D$ may show a second relaxation step (and possibly additional ones) that however do not significantly affect the overall system energy. A single exponential, which sometimes does also describe the whole time behaviour, can fit the initial relaxation, and interestingly in all runs the initial decrease of $E_{\text{pot}}$ was found to show a longer characteristic time than the change in $D$, as is also evident from simple visual inspection. Such behaviour indicates that the proteins flatten on the surface to maximize the surface interaction with a fast decrease of $D$, and then optimize more slowly the intramolecular interactions among topologically distant strands through lengthier surface rearrangements with a minor change of $E_{\text{pot}}$ [4,8]. The kinetics is somewhat more complicated when multiple relaxation steps are present: in such cases, after the initial spreading an induction time sets in, owing to an activation entropy arising from the collective motions required for the long-range rearrangements. Only when the correct path is eventually found can additional protein strands cooperatively enhance the surface interactions with a concomitant closer approach.

The final most favourable adsorption geometries eventually achieved by the two protein subdomains on rutile and anatase after the MD runs are shown in figure 5. These geometries correspond to the final snapshots after energy minimization and show a very large, and usually an almost complete spreading to a monolayer of amino acids. Interestingly, the ‘soft’ albumin subdomain does show such behaviour both on anatase and on rutile (figure 5a(i) and b(i)), whereas the fibronectin modules do so only on the rutile surface (figure 5a(ii)). On the other hand, on anatase, the fibronectin modules somewhat retain the globular shape, so that a smaller number of residues is in contact with the surface than in the other cases (figure 5b(ii)). As for the interaction strength, given by the interaction energy $E_{\text{int}}$ reported in the caption of figure 5, we found again that it is affected by the overall protein size; so in general the interaction energy $E_{\text{int}}$ is larger for the fibronectin modules than for the albumin subdomain on both surfaces. A more significant comparison can be carried out between the interaction energies of a given subdomain on rutile and on anatase. Overall, the adsorption of the fibronectin modules is stronger on rutile than on anatase by about 230 kJ mol$^{-1}$ ($=\Delta E_{\text{int}}$), whereas the opposite is true for the albumin subdomain, where the adsorption on anatase is favoured by 180 kJ mol$^{-1}$ compared with rutile. However, the intrinsic interaction strength, given by the (average) interaction energy $E_{\text{int}}$ per amino acid in contact with the surface, as said before, is shown by the fibronectin modules on anatase, with a value of 52.0 kJ mol$^{-1}$ of residues, whereas on rutile such value decreases to 43.9 kJ mol$^{-1}$. This result is clearly owing to the larger spreading on the latter surface, where the vast majority of residues are in contact with it, unlike what happens on anatase (compare the snapshots of figure 5). On the other hand, the albumin subdomain shows an intrinsic interaction strength of 48.4 kJ mol$^{-1}$ of residue on anatase, and of 46.9 kJ mol$^{-1}$ on rutile. These values indicate a stronger adsorption on anatase purely as an effect of the surface topology, with larger intrinsic interaction strength for the fibronectin modules. In turn, the topology effect is mainly related to the width of the surface grooves, clearly...
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Figure 5. The optimized geometries of the two protein subdomains at the end of the MD runs in a side and top view (in the latter case, the surface is not shown but simply outlined manually for visual clarity): the albumin subdomain on rutile, with $E_{\text{int}} = 2.66 \text{ MJ mol}^{-1}$ (a(i)), and on anatase, with $E_{\text{int}} = 2.84 \text{ MJ mol}^{-1}$ (b(i)); the fibronectin modules on rutile, with $E_{\text{int}} = 3.20 \text{ MJ mol}^{-1}$ (a(ii)), and on anatase, with $E_{\text{int}} = 2.97 \text{ MJ mol}^{-1}$ (b(ii)). The colour codes are the same as in figure 2. (Online version in colour.)

seen in the side views of figure 5, which show bridging oxygens at the upper edges that optimize the dipolar interactions and the hydrogen bonds with the adsorbate.

The loss of the secondary structure is quite evident from simple inspection of the backbone trajectory in figure 5. In the final adsorption stage, the protein strands optimize both the interaction with the surface and the interaction among themselves through a relatively ordered pattern. This pattern may be enhanced by an ordered surface structure, as already noted for graphite [34] and also pointed out before, but it is also driven by the interaction among the side groups of the facing strands (hydrogen bonds, charged and dipolar interactions). Therefore, these strands assume a roughly parallel arrangement mostly on the surface plane,
Figure 6. The Ramachandran plots showing the values of $\varphi$, $\psi$ torsion angles of the backbones for the albumin subdomain and the fibronectin modules in the native state (a(i) and a(ii), respectively) and in the final adsorption state on anatase (b(i) and b(ii), respectively). The plots for the adsorption on rutile are not shown for brevity, because they are qualitatively very similar to those on anatase.

but also on an orthogonal plane (see for instance figure 5b(ii)). Such optimization also entails a significant change of the distribution of the ($\varphi$, $\psi$) torsional angles along the backbone, usually analysed in the Ramachandran plot [35,36]. In fact, when adsorbed on rutile and anatase, we found a significant shift of the torsion angles of both subdomains towards the $\beta$-sheet region of the map, with large $\psi$ values, as shown in figure 6 by comparison between the native and the adsorbed state on anatase. This feature is most evident for the albumin subdomain, which contains only $\alpha$-helices in the native state: here, the $\psi$ values, initially around $-45^\circ$ (figure 6a(i)), become clustered around $+90^\circ$ on anatase (figure 6b(i)) and on rutile (data not shown). However, it is also evident in the fibronectin modules (formed by $\beta$-sheets) owing to a change in the random strands and in the regular turns (compare figure 6a(ii) and b(ii)). Because the main changes are found in these torsion angles, a histogram reporting the distribution of $\psi$ is shown in figure 7 for the two protein subdomains in the native and in the most stable adsorption state found on rutile and on anatase. In the native albumin subdomain, the $\alpha$-helices give rise to a large number of slightly negative $\psi$ values centred at about $-30^\circ$ (figure 7a(i)), whereas in the native fibronectin modules, the $\beta$-sheets yield large, positive values of $\psi$ centred at about $120^\circ$ (figure 7a(ii)). Upon adsorption, in both subdomains the $\psi$ values assume in all cases large positive

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values, mostly in the range of 60–90°, but also with a significant contribution for ψ around 120°. These values are highly suggestive of a new refolding of the subdomains to form β-sheet-like structures, which however lack the hydrogen bonds among the backbone atoms, being solely due to the interactions among the side groups.

4. Concluding remarks

In this study, we considered simple surfaces of the three TiO$_2$ polymorphs to model the adsorption of two protein subdomains with an unlike secondary structure with MD methods. We first show that adsorption on brookite is much weaker than on rutile and anatase. On the surfaces of the latter polymorphs, the albumin subdomain (a ‘soft’ protein) displays a very large spreading that maximizes the surface interaction, which is strongest on anatase as an effect of the surface topology, whereas the fibronectin modules show a larger spreading on rutile, but keep a roughly globular shape on anatase, showing a stronger interaction than albumin on both surfaces, thanks to the larger size. The intrinsic interaction strength, i.e. the interaction strength per residue in contact with the surface, is however largest for the fibronectin modules on anatase. Interestingly, competitive adsorption measurements [37] indicate that a significantly larger amount of fibronectin is adsorbed on variously prepared (but crystallographically not characterized) TiO$_2$ samples than human serum albumin when the two proteins are present in a 1:1 ratio. On the other hand, larger amounts of adsorbed albumin are observed at the physiological ratio owing to its much larger abundance. However, this amount is not as large as from a single protein solution, being effectively limited by fibronectin. These findings suggest a stronger
binding to the surface by fibronectin, in qualitative agreement with the calculated behaviour previously reported.

Other groups recently carried out other molecular modelling studies of protein adsorption on ceramic materials. We first mention a simulation study that modelled the adsorption on rutile (1 1 0) of a fibronectin type III module \textit{in vacuo} \cite{11}. This module did comprise antiparallel $\beta$-sheets arranged in two sets of seven strands that form a sandwich. The chosen surface is the most stable one among the TiO$_2$ polymorphs \cite{19}, but the study did also consider the influence of surface point defects (oxygen vacancies), of step defects and of grooves. Starting with a single arrangement, having the $\beta$-sheets roughly orthogonal to the surface, the point defects led to small deformations of the adsorbed fibronectin module, and the perfect surface produced larger deformations similar to those shown in figure 2, whereas major rearrangements were observed with larger defects. The surface adsorption was mainly driven by the interaction of the carboxylate anions with the exposed surface titanium atoms assisted by hydrogen bonds of the side groups with the bridging oxygens. This coordination geometry is in good agreement with that previously obtained through classical and quantum methods for mono- and dipeptides on the same surface \cite{38,39}. The presence of large defects with a high surface energy was suggested to promote a stable adsorption, while a stronger interaction can lead to a major loss of the protein secondary structure.

Further work involved MgO, SiO$_2$ (taken as a model for mica), hydroxyapatite and a silicate. MgO is a purely ionic ceramic, and therefore it exposes a charged surface to the environment. The adsorption of bovine pancreatic trypsin inhibitor (a small protein with 57 amino acids) on this surface was investigated by considering three different starting orientations \cite{12}. The whole system was fully hydrated by assuming a vacuum boundary, whereas the simulation strategy involved a hybrid procedure, with a local energy minimization every 8ps of the MD run, lasting for a total of 240ps only. Therefore, full equilibration was not achieved, and the protein dynamics mainly consisted of small rigid-body translations or partial rotations, corresponding to a weak initial adsorption. Some puzzling results were obtained \cite{12}, because, for instance, the arrangement with the most favourable interaction energy seemed to have more hydrophobic than charged amino acids close to the charged surface. This feature was partly attributed to the fact that adsorption was mediated by a thin water layer only two molecules thick, but anyway the lack of equilibration suggests that the results are not conclusive for the long-term adsorption. A covalent ceramic surface with polar bonds was used to model lysozyme adsorption on mica \cite{13,14}, effectively described as a SiO$_2$ surface with a slightly negative surface charge density equal to that observed in mica. However, the surface was described as a single flat plane of silicon and oxygen atoms arranged in a square array, unlike the tetrahedral coordination of silicon in SiO$_2$, neglecting any lower polar plane. Nevertheless, and even though the final adsorption stage was probably not achieved, the MD runs in solution indicated that electrostatic interactions were the main factor leading to adsorption, while interaction with the inner edge of two orthogonal Si–O planes led to partial lysozyme unfolding with a significant loss of the tertiary and secondary structure. Another study dealt with the adsorption of a fibronectin type III module on hydroxyapatite (0 0 1) \cite{15}, using a simulation strategy similar to that proposed by us. In this case, the simulations were carried.
out in explicit water, but owing to the relatively short MD runs (1 ns), only pseudo-equilibrium metastable states were likely achieved. Therefore, the final states described in the study may provide the initial adsorption geometry with only local rearrangements near the surface. After that, a steered MD simulation was carried out assuming a pulling force acting on the fibronectin module to mimic the desorption experimentally induced by an atomic force microscope tip. Finally, another paper reported MD simulations of the adsorption of the same albumin subdomain considered by us on a silicate surface of chrysotile, $\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$ \cite{16}. Adopting an implicit solvent, the albumin subdomain was found to better interact with this polar, hydrophilic surface through its polar sides, but eventually to show a very large spreading to almost a single monolayer of amino acids. As previously mentioned, such behaviour is also related to the ‘soft’ nature of albumin, which strongly interacts with significant spreading on almost all surfaces, irrespective of their nature or net charge \cite{33}.

In conclusion, as an outlook to future work, we point out that classical atomistic simulations are increasingly used to model protein adsorption on biomaterial surfaces, offering a new theoretical tool for understanding such important phenomena. We expect that in the near future, these methods will focus on a systematic study of the effect of surface chemistry and/or nanoscale topology considering either defective or regularly patterned surfaces. Such a research programme obviously includes the study of the protein interactions with nanoparticles having a size in the range of $1–10$ nm, i.e. the same as most proteins. Another issue involves modelling protein adsorption at different ionic strengths, in particular as a function of pH, because it modifies the charge distribution (and the electrostatic potential) at the protein envelope, but also possibly on the material surface. We can confidently expect that this ongoing theoretical study of the performance of biomaterials in a physiological environment will provide increasing insights about the bio–non-bio interactions and the performance of biomaterials.

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Appendix A

The simulations were performed with MATERIALS STUDIO \cite{40} using the COMPASS force field \cite{41}. The albumin subdomain, its structure and hydropathy are described in detail in previous work (the A subdomain in Raffaini & Ganazzoli \cite{5}), while the fibronectin type I modules are similarly described in Raffaini & Ganazzoli \cite{6}, where we incorrectly mentioned it as formed by a single module instead of two. In both cases, the appropriate charges for the ionizable residues were considered at the physiological pH = 7.4. The surfaces of the TiO$_2$ polymorphs were generated as a relatively thin slab from the known crystallographic coordinates already available in the MATERIALS STUDIO database by creating a supercell through periodically repeating the crystal cell along the three axes. We then hugely increased the axis perpendicular to the chosen surfaces, namely rutile (0 0 1), anatase (1 0 0) and brookite (1 0 0), so as to generate a vast empty space above it, but kept otherwise fixed the atom positions. This choice of the surface was made for simplicity in order to have surfaces with common features from the chemical viewpoint (in particular, the lack of any hydroxyl group) to better probe to effects of the (sub)nanoscale
topology. The thermodynamic stability of these surfaces is quite different, and to avoid reconstruction effects we kept fixed throughout the atomic position of the TiO$_2$ slab. The surface sizes and thickness of the TiO$_2$ samples were respectively $91.9 \times 68.9\,\text{Å}^2$ and $8.9\,\text{Å}$ for rutile, $85.6 \times 56.8\,\text{Å}^2$ and $11.4\,\text{Å}$ for anatase, and $81.8 \times 77.1\,\text{Å}^2$ and $9.2\,\text{Å}$ for brookite. The subdomains were placed close to the surfaces in various trial orientations so that all the main sides of each subdomain could interact with the surface. These starting arrangements, not shown for brevity being equal to those reported in Raffaini & Ganazzoli [5,6], were then optimized in an implicit solvent using a distance-dependent dielectric constant by keeping fixed the TiO$_2$ slabs. The MD simulations were performed with implicit solvent at a constant temperature (300 K), controlled through the Berendsen thermostat. Integration of the dynamical equations was carried out with the Verlet algorithm with a time step of 1 fs, and the instantaneous coordinates were periodically saved for further analysis or geometry optimization. Equilibration of the systems was monitored through the time changes of the total and potential energy, together with its components, and of the distance between the centre of mass of the backbone of the subdomains and the surface. These quantities showed an initial decrease possibly with a few separate kinetic stages, but eventually fluctuated around a constant value, indicating eventual achievement of an equilibrium state.

References

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