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REGULAR ARTICLE

Interaction of biomolecular systems with titanium-based materials: computational investigations

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Abstract Ab initio calculations and molecular dynamics simulations were performed to investigate the adsorption mode of various oligopeptides on titanium dioxide surfaces and to characterize their conformational behavior upon adsorption. The models were progressively improved obtaining a description compatible with the experimental observations.

Keywords Peptides · Titanium dioxide · Adsorption

1 Introduction

In recent years much work has been devoted to the design, development and study of novel biocompatible materials with appropriate biological and mechanical functionalities that could be used effectively in medical implants as tissue regenerators. Titanium has been extensively employed for its advantageous mechanical, and chemical properties such as tensile strength, fracture toughness, corrosion resistance, and biocompatibility, which derives mainly from the presence of the strongly adherent surface oxide film, 0.5–10.0 nm thick, that passivates the metal when it is in contact with aqueous solutions or water vapors [1]. These characteristics make titanium one of the best candidates for the creation of artificial biomimetic surfaces, which can be obtained through the incorporation of bioadhesive motifs from the extracellular matrix proteins. Bioactive molecules such as oligopeptides and proteins, are able to adsorb on medical implants through complex and poorly understood mechanisms, regulating the biological response to the new material in contact with biological fluids and directly influencing implant biocompatibility. The nature of the interaction of the biomolecule with the thin titanium oxide surface at the molecular level will ultimately determine its conformation. An alteration of the conformation with respect to its naturally occurring state could cause an autoimmune response and as a consequence rejection of the implant. The intrinsic complexity of biological systems and the numerous mechanisms influencing the evolving cellular environment render the developing of new biocompatible materials a highly dynamic process where it is not always possible to separate the various mechanisms experimentally. However, to improve functional properties and control their evolution, every step of the design strategy remains the subject of intensive multidisciplinary research integrating biology, biochemistry, clinical medicine, material science, physics, engineering, and last but not least computational modeling.

Theoretical methods are essential to interpret experimental results, to identify the dominating mechanisms, and can have a huge potential as predictive tools both in the optimization of culture conditions and in the design of experimental protocols. However, in comparison to the extensive experimental studies regarding the reactions of various molecular species on TiO_2 surfaces only a few computational investigations on peptide/surface interactions using different simplified approaches have been

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performed. Molecular self-assembly has attracted much attention as a powerful breakthrough technology that enables the bottom–up fabrication of three-dimensional (3D) structures on a nanometer scale and peptides have demonstrated to be versatile building blocks which are able to self-assemble in nanoarchitectures of specific shape such as micelles, vesicles, fibers, networks, and a variety of other morphologies. Self-organized peptide molecules are expected to have potential as novel functional biomaterials with a wide range of applications including drug delivery, biological surface engineering, and scaffolding for tissue regeneration. Peptide scaffolds not only represent a powerful nanobiomedical technology for tissue repair and restoration but also raise the possibility of effective treatment of different tissue or organ trauma. The study of their complexes with medical implants made of titanium-based materials and the comprehension of the mechanisms which regulate the association of these two organic/inorganic bioregenerators is of fundamental interest and of practical importance. Controlling peptide assembling mechanisms on the nanoscale, tuning the molecular structure precisely, would allow the accomplishment of more easy and accurate nanofabrications of designed 3D objects with respect to the conventional top–down approaches.

Ionic self-complementary oligopeptides are of special interest due to the formation of complementary ionic pairs which affect single chain properties and stabilize aggregates through electrostatic interactions. They share common features of uncharged peptides such as hydrophobicity, hydrogen bonding, etc. and possess unique charge properties that can sensitively control their aggregation behavior. The simultaneous presence of different types of interactions leads to a rich and subtle molecular self-assembling behavior. Many important questions about the formation of stable adsorbates remain unanswered, for example what are the main conformational characteristics of oligopeptide molecules when they adsorb on inorganic surfaces from water solutions and what are the mechanisms which regulate peptide surface interactions? What is the role played by the amino acid side-chains and what are the most favorable side-chain arrangements? How sensitive is the formation of a stable surface coverage to changes in environmental conditions such as pH, temperature, and salt concentration? As already stated the study of peptide–surface interactions is fundamental in many areas of biotechnology and extremely important in the field of biomaterials. However, due to the complex nature of the surrounding environment, adsorption mechanisms and structural rearrangements have not been characterized in detail and accurately predicted.

To address the aforementioned questions we have performed a series of ab initio periodic optimizations and quantum molecular dynamics (MD) simulations on different peptide systems. Moreover, force field-based MD simulations which have succeeded, in many cases, in combination with experimental techniques, in interpreting and explaining experimental data, have been subsequently employed to study the adsorption of peptide clusters on titanium dioxide non-hydroxylated and hydroxylated surfaces and have provided valuable insight into factors responsible for peptide surface adsorption. Classical MD simulations are able to describe realistically complex systems, where different interaction mechanisms cannot be practically treated at a more sophisticated ab initio level, at a reasonable computational cost. The interaction potential parameters necessary to carry out classical molecular dynamics simulations of the TiO_2-oligopeptide-aqueous solution systems were taken from published data [2, 3], existing force fields [4, 5] and derived, when missing, using procedures similar to that reported in [2]. In order to derive the force field parameters, for the representation of the peptide structural and dynamic properties in proximity of the TiO_2 surface, ab initio calculations were performed using representative model systems.

The computational procedures employed consisted of several steps:

- ab initio determination of force field parameters needed for classical MD simulations and not available in the literature;
- periodic DFT calculations to estimate the strength of different adsorption modes of single amino acids;
- classical MD simulations of the binding of amino acids to a specific TiO_2 surface [rutile (110)] in water solution;
- quantum MD simulations to validate the classical MD approach.

In Sect. 2 the main characteristics of the systems studied are briefly described. The computational procedure is accounted for in Sect. 3 and the results of the various simulations for the adsorption of different types of peptides and peptide supramolecular structures are reported in Sect. 4.

2 Molecular models

2.1 Surface structure and water adsorption

The rutile (110) surface was chosen for these studies because it is one of the most important and extensively investigated TiO_2 surfaces and it is highly stable. It is formed by a complete Ti–O layer, or surface plane, including rows of exposed, fivefold Ti sites and rows of doubly coordinated bridging O atoms that protrude above the surface and are bound to two sixfold Ti atoms located

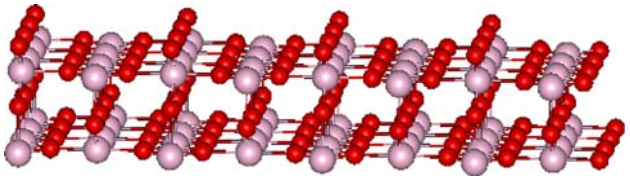

Fig. 1 Ball and stick model of two planes of a portion of the perfect TiO_2 rutile (110) surface used in DFT calculations and MD simulations. *Pink balls* represent Ti atoms whereas *red balls* represent oxygen atoms

in the surface plane. Rows of exposed threefold oxygens are also included in the surface plane. The rutile (110) surface (Fig. 1) derives from the relaxation of the rutile bulk structure cleaved by the (110) plane, and when in contact with a water solution its relaxation, at room temperature, is minimal and perpendicular to the surface itself [6]. The surface unit mesh has dimensions $a = 6.497$ Å and $b = 2.959$ Å along the rutile [$\bar{1}$10] and [001] crystallographic directions.

The adsorption of water on the rutile (110) surface has been investigated extensively both from an experimental and theoretical point of view. However, the extent to which water dissociates to produce surface hydroxyl species aroused a lot of controversy between theory and experiment, between various experimental procedures used to prepare the TiO_2 surface and between different theoretical investigations.

From an experimental point of view water is adsorbed, on the surface, molecularly at low temperatures, whereas dissociation may be caused by the presence of surface defects due to oxygen vacancies [7, 8]. On the other hand, at room temperature, molecular and dissociative adsorption may occur simultaneously [9, 10].

Form a theoretical point of view dissociative adsorption [10], molecular absorption [11–14], or both molecular and dissociative absorption are all possible [15–19].

In order to characterize water/rutile (110) adsorption we performed accurate quantum MD simulations using a model system sensibly larger than the ones reported in the literature (the details regarding the model and procedures employed are described in a recent paper of ours [20]). The results suggested, in agreement with previous experimental and theoretical findings [21, 28], that a mixed molecular-dissociative adsorption could take place. Indeed, a fraction ($\sim$20%) of water molecules dissociated on the perfect TiO_2 (110) surface at 300 K through a mechanism assisted by hydrogen bond interactions among neighbor water molecules. This dissociative adsorption, which could occur even when a few waters were randomly placed far away from the surface, was realized on the same time scale of the molecular adsorption. The calculations also confirmed that the building of a second solvation layer could start before the first layer was complete. The average Ti–O bond length of the first layer of chemisorbed water was estimated as 2.21 Å. The electronic localization function (ELF), which is an indicator of bond type and bond strength [22], is shown in (Fig. 2) for waters on rutile TiO_2 (110) surface. This picture is aimed to show the electronic interaction between water and water or rutile surface. Such interaction is not as strong as a normal covalent bond, so a relative low iso-surface value (0.648) is here adopted. We can observe a

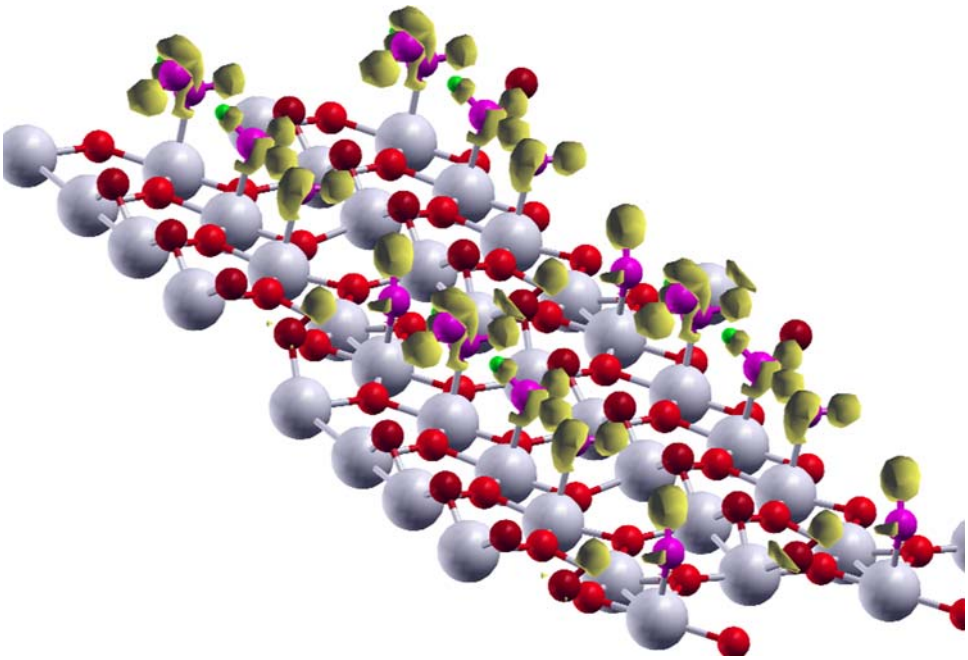

Fig. 2 Electronic localization function (ELF) for water molecules adsorbed on rutile TiO_2 (110) surface. The representative structure was extracted from an ab initio MD simulation

relatively small function distribution between Ti and oxygen atoms of chemisorbed waters, which indicates a moderate bond strength for the adsorbate. The presence of some strong hydrogen bonds is also pointed out by this figure.

Taking into account the fact that both types of surface-water attachments, that is molecular and dissociative adsorption, can coexist on the perfect TiO_2 layer, only the molecular one has been considered in our classical simulations where the water model used (TIP3P [23]) does not allow for dissociation.

2.2 Biomolecular systems: single peptides, multiple peptides and peptide supramolecular stuctures

Some of the most promising new synthetic biomaterial scaffolds are composed of self-assembling oligopeptides [24–32] such as the EAK16-II and RAD16-II sequences [33–35] which consists of regular repeating units of positively charged residues, such as lysine (Lys = K) and arginine (Arg = R), and negatively charged residues, such as aspartic (Asp = D) and glutamic acid (Glu = E), separated by hydrophobic alanine amino acids (Ala = A). They display singular secondary structure plasticity and multifaceted behavior, and spontaneously assemble to form macroscopic structures that can be manufactured into various geometric shapes. They can be organized as strong β-sheets in aqueous solutions, but they can also be able to undergo secondary structure transition from β-sheet to α-helix in response to changes in temperature, pH and salt concentration. They are soluble at low millimolar concentrations in salt-free aqueous solutions and when exposed to physiological media or salt solutions they can form hydrogel-like matrices with a high water content. EAK16-II and RAD16-II self-assembling peptides robustly support cell attachment, proliferation and differentiation.

Notwithstanding that extensive studies of EAK16-II and RAD16-II filaments have elucidated many aspects of their

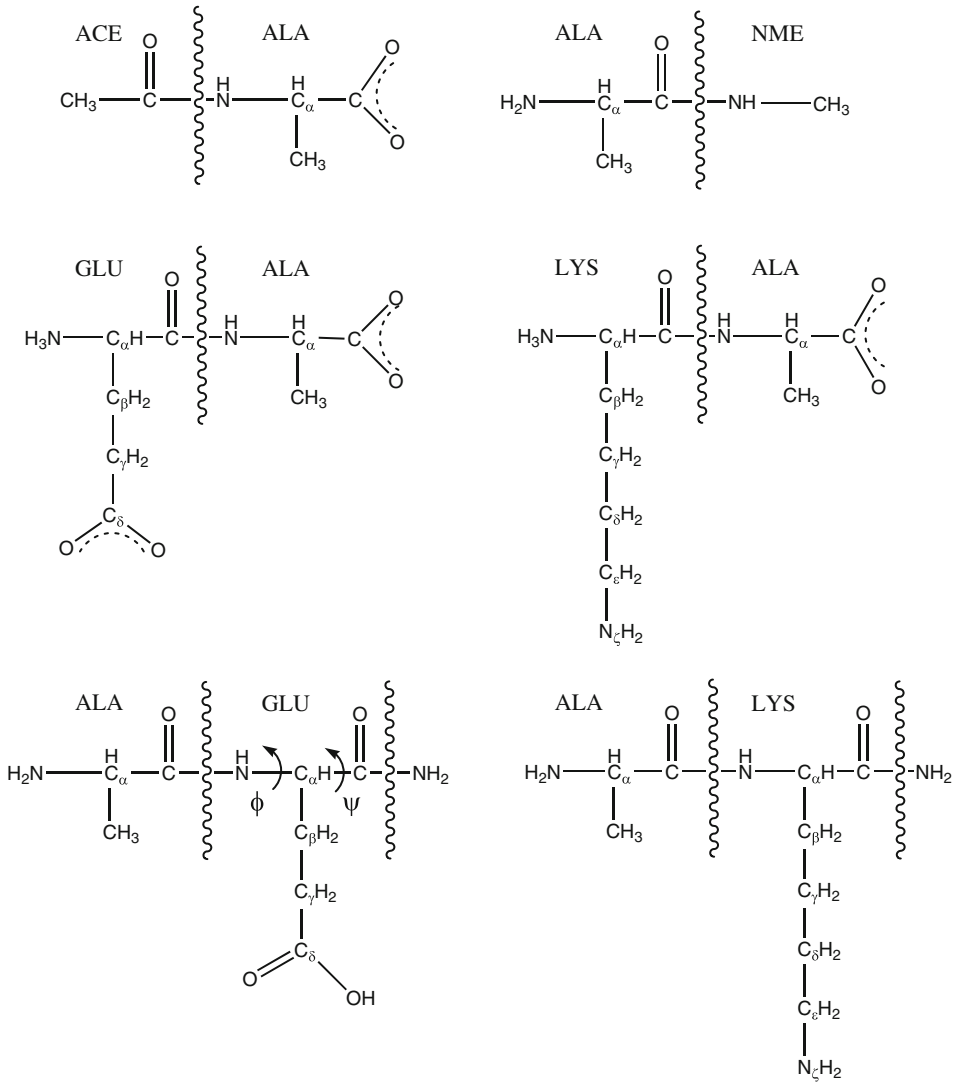

Fig. 3 Structure of the small peptide molecules whose adsorption onto the TiO_2 (110) surface was simulated

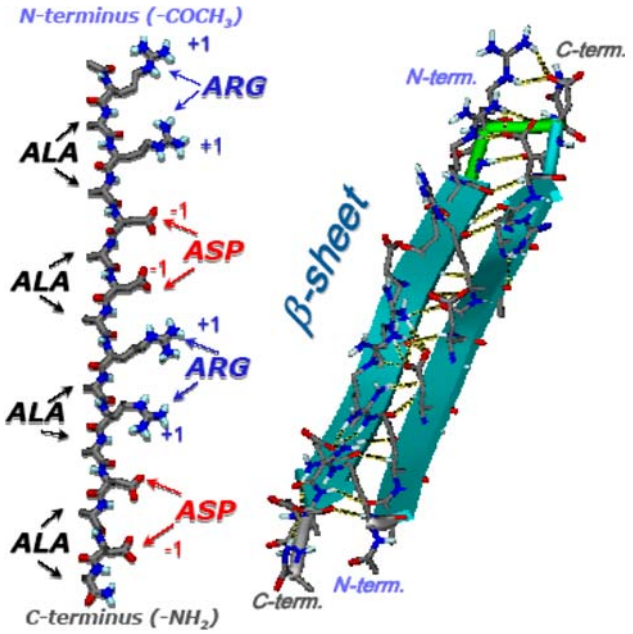

Fig. 4 RADA16II peptide investigated using classical MD simulations

structure, elasticity and in vivo behavior, important issues concerning their adsorption onto titanium-based materials and thus their possible use as surface coating bioadhesive motifs, remain to be resolved. In order to understand the mechanisms which determine the formation of stable peptide/TiO_2 complexes, a series of calculations (ab initio optimizations and MD simulations) regarding the adsorption properties of simplified model systems were carried out: ACE-ALA, that is an ALA residue N-terminated with a $–COCH_3$ group to have a peptide bond on account of the continuation of the peptide sequence and with a carboxyl group (total charge = −1); ALA-NME, that is an ALA residue N-terminated with an NH_2 amine group and C-terminated with an $–NHCH_3$ group, to mimic, also in this case, a peptide bond (total charge = 0); LYS-ALA, GLU-ALA, ALA-LYS and ALA-GLU dipeptides with different charges determined by the protonation state of the terminus groups and the charge of the sidechains (Fig. 3). Then a small filament made of 16 RARADADARARADA peptide chains with N and C termini blocked by acetylation and amidation (to prevent rapid degradation) (Fig. 4), organized as a β-sheet bilayer [36], was placed near the TiO_2 surface in two specific orientations with its β-sheet planes parallel to the titanium dioxide surface and with the ribbon β-sheet planes almost perpendicular to the TiO_2 layer (the major side in contact with the surface) (Fig. 5). Thereafter, long molecular dynamic simulations were run to identify the preferential arrangement of the filament and evaluate the strength of its adsorption.

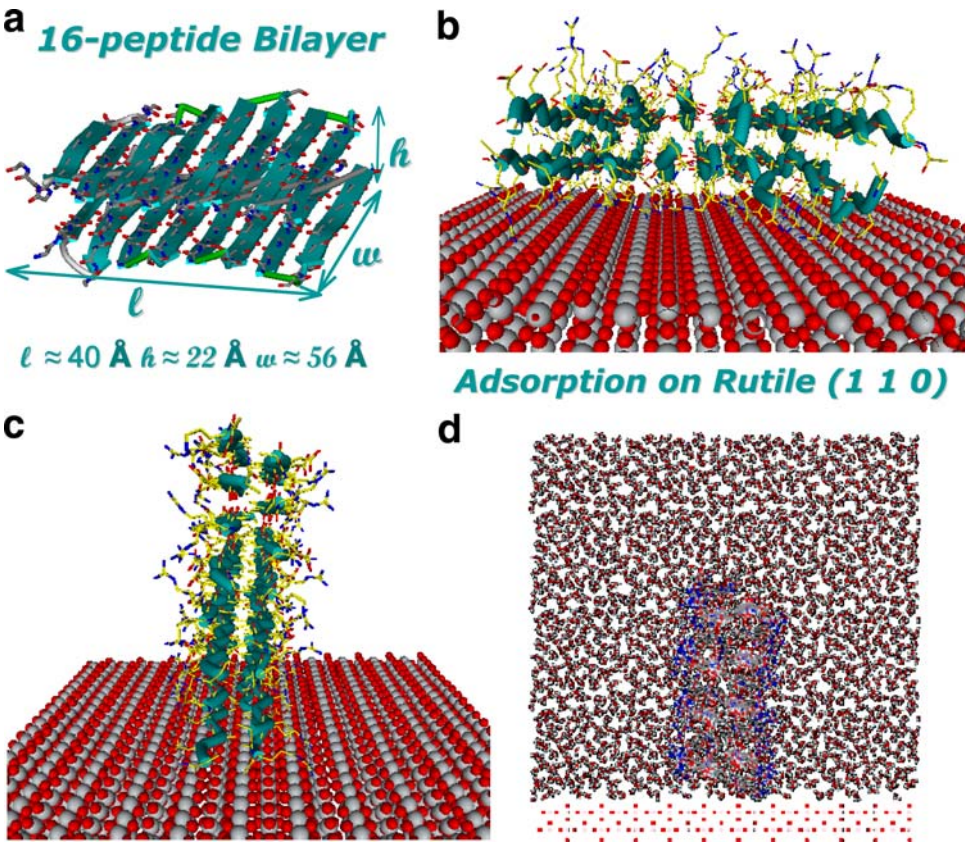

Fig. 5 Short filament made of RADA16II peptides employed in MD simulations. β-strands are highlighted by *solid arrows* (**a**). Perspective view of the filament model on rutile (110) with its β-sheet planes (β-strands are highlighted by *solid dark green ribbons*) in parallel (**b**) and perpendicular (**c**, **d**) orientation with the respect to TiO_2 layer [displayed as *grey* (Titanium) and *red* (Oxygen) balls]. The sidechain carbons are *yellow* and hydrogen atoms are undisplayed. **d** Orthographic view of the simulation box containing the TiO_2 surface, the filament in perpendicular orientation and water molecules. Color codes: carbon, *yellow*; oxygen, *red*; nitrogen, *blue* and titanium, *grey*

3 Computational methods

3.1 Ab initio calculations

Second order Møller–Plesset (MP2) and periodic density functional theory calculations (DFT) were used to complement the surface adsorption studies in two different ways.

First, MP2 calculations with a mixed basis set, namely 6-311+G** for Ti atoms and aug-cc-pVDZ for O, C, N, and H atoms as implemented in Gaussian03 [37], were performed on reduced model systems consisting of small molecules such as H_2CO and NH_3 complexed with TiO_5H_6 clusters, whose geometry was frozen at the rutile crystal surface, in order to derive the force field parameters necessary for a realistic representation of the interactions between the peptides and the TiO_2 layer. To derive the nonbonded interaction parameters the binding energy of the model molecule was defined as the energy of the complex minus the energies of the isolated partners and was corrected for the basis set superposition error (BSSE) using the counterpoise (CP) method (for more details about the computational approach and the chosen basis sets see [38]).

Second, periodic DFT optimizations were carried out to investigate the adsorption of single amino acids and dipeptides on TiO_2 to test if the hypothesized modes of attachment would have produced stable configurations of the system and to validate if a classical approach could have been applied successfully to larger structures. The plane wave total energy code DA CAPO [39, 40] was employed to optimize the complex systems consisting of a periodic two-layer slab of TiO_2 rutile (110) and an alanine molecule. The two most probable adsorption mechanisms, that is monodentate and bidentate coordination of the carboxyl oxygens with Ti atoms of the surface, were considered and analyzed in detail. All of the surface atoms were fixed at the crystal geometry, a vacuum of 30 Å, sufficient to prevent any substantial interaction between the first and the second surface, was used to separate the slabs and the core electrons of all of the atoms were treated via the ultrasoft pseudopotentials with an energy cutoff of 340 eV for both the electronic wave function and the density. The PW91 gradient-corrected exchange-correlation functional was employed.

Most stable dipeptide–surface complexes (of both AE and AK molecules) extracted from the classical MD simulation trajectories were instead checked through ab initio optimizations performed with the Vienna ab initio simulation package (VASP) [41–43]. Also in this case the generalized gradient approximation (GGA) with the spin-polarized Perdew–Wang functional (PW91), ultrasoft pseudo potentials for inner electrons and a plane wave basis set with a cut off at 270.2 eV for the valence states were used. A three-layer slab of TiO_2 rutile (110) with a vacuum gap of about 20 Å, a (2 × 5) supercell (12.994 Å × 14.795 Å) with Monkhorst–Pack [44] 2 × 2 × 1 *k*-point sampling were employed. The positions of the atoms of the first layer were allowed to relax whereas the central layer was fixed at the bulk crystal geometry. A few selected water molecules were included in the calculations and the whole configurations were relaxed until self-consistent forces were lower than 0.04 eV/Å. More details can be found in [45].

3.2 Classical molecular dynamics simulations

The biomolecular systems, already described in Sect. 2.2, were inserted in rectangular parallelepiped boxes and solvated with TIP3P water molecules removing those waters falling within 2 Å from the surface/peptide complex. All simulations were performed by using the DL_POLY [46] or AMBER9 [47] suite of programs with the amber force field to describe the peptides and developed parameters for the TiO_2 atoms. Before starting the MD simulations the cell height was progressively reduced and the energy of the system was minimized after each reduction in order to achieve the correct water density. Then the systems, consisting of the TiO_2 surface, oligopeptides and water molecules, were subjected to constant volume MD at high temperature ($T = 600$ K) with solute and surface frozen, in order to randomize water positions. Then pre-equilibration in the NVT ensemble ($T = 310$ K) was performed. Bond lengths were constrained using the SHAKE algorithm [48] and the time step was set to 2 fs. Periodic boundary conditions were applied in x, y, and z directions and the particle mesh Ewald method was used to deal with electrostatic forces. As motivated in [38], the surface was kept fixed at the crystal geometry during all the simulations. All the details regarding the methodology employed are described in our previous papers [38, 45, 49].

3.3 Quantum molecular dynamics simulations

The adsorption of the two neutral dipeptides ALA-LYS and ALA-GLU on a rutile (110) three-layer slab in the presence of water molecules was studied [45] by fixed volume first principles MD carried out with the plane-wave-based VASP package using the GGA approximation in the PW91 parametrization and ultrasoft pseudo-potentials. The Brillouin zone sampling was restricted to the Γ point because the computational supercell, whose size was about (13 × 15 × 20) $Å^3$, was sufficiently large. All of the simulations were performed using a plane wave cutoff energy of 270.2 eV. Optimizations were performed before MD simulations, then production was run in the canonical

ensemble coupling the system to a thermal bath at $T = 300$ K with the Nosé thermostat procedure. The Verlet velocity algorithm with a time step of 1 fs was used and the total simulation time was about 2.8 ps.

4 Results and discussion

4.1 Short oligopeptides

All of the short oligopeptides under study, namely ACE-ALA, ALA-NME, LYS-ALA, GLU-ALA, ALA-LYS, and ALA-GLU adsorbed on the TiO_2 (110) rutile surface in a quite stable arrangement and with relatively limited hinge-bending movements. Once attached to the surface the peptide structures had a reasonable propensity to remain there with their polar groups providing an anchor to the surface. Substantial changes only involved side chains dihedral angles, whereas backbone atoms were less flexible due to the fact that most of the time they were in contact with the surface. In order to characterize the location of the molecules with respect to the surface the distance of selected atoms from the TiO_2 layer was analyzed in detail and statistical parameters are reported in Table 1. As it can be seen clearly, carboxyl oxygens in close contact with Ti surface atoms were restricted in their motion and substantially remained at a distance of about 2.1 Å from the interface, during the time scale sampled, oscillating between 1.8 and 2.7 Å (maximum range) with standard deviations σ, from the average distance, of about 0.1 Å (Fig. 6).

Distance fluctuations of the other groups increased with the growth of their separation from the attached portion of the molecule. The distance of the side chains from the interface had more marked fluctuations due to the decreased influence of the layer atoms and to the significantly increased interaction with the surrounding water molecules.

The conformational flexibility of GLU and LYS side chains was highly variable and together with their spatial extent allowed the carboxyl and amino groups to stretch toward the surface at interacting distance. Such a behavior was apparent during all the MD runs where the side chains were observed approaching the surface and then shifting to longer distances, reaching, sometimes, the bulk region of the solvent (Fig. 6).

Water molecules hydrogen bonded to the dipeptide hydrophilic groups made a portion of a well-defined first solvation shell which, from one side prevented a strong peptide–surface interaction, whereas on the other side acted as an intermediary between the peptides and the surface. The adsorbed water layer formed hydrogen bonding interactions with the hydrophilic groups of the peptides and as a consequence an indirect interaction with the surface (Fig. 6).

The most probable contact points between the peptide molecules and the TiO_2 layer were represented by carbonyl oxygens as well as nitrogen atoms and at low concentration the peptides had the tendency to lay flat upon the surface,

Table 1 Atom-surface distances in Å (statistical analysis)

Atom	Max	Min	Mean	σ
ACE-ALA (total charge = −1)				
O_{COO^-}	2.29	1.91	2.12	0.09
O_{COO^-}	2.28	1.87	2.10	0.09
O_{CO}	7.05	5.77	6.52	0.35
NNH	6.12	3.63	4.35	0.62
ALA-NME (total charge = 0)				
O_{CO}	2.40	1.99	2.10	0.08
N_{NH_2}	3.38	2.89	3.11	0.08
GLU-ALA (total charge = −1)				
O_{COO^-ALA}	2.13	1.86	1.98	0.05
O_{COO^-ALA}	2.14	1.86	1.98	0.05
O_{COO^-GLU}	11.45	5.21	8.43	1.44
O_{COO^-GLU}	11.55	5.78	8.79	1.15
O_{CO}	7.06	5.85	6.68	0.18
N_{NH}	5.43	4.01	5.08	0.14
$N_{NH_3^+-terminus}$	8.50	5.59	6.94	0.78
LYS-ALA (total charge = +1)				
O_{COO^-}	2.09	1.84	1.97	0.05
O_{COO^-}	2.15	1.86	2.00	0.05
O_{CO}	6.94	5.98	6.57	0.17
N_{NH}	5.76	4.12	4.33	0.16
$N_{NH_3^+-terminus}$	7.63	5.72	6.34	0.32
$N_{NH_3^+-sidechain}$	11.33	3.58	6.40	1.31
ALA-GLU (total charge = 0)				
O_{COO^-GLU}	8.75	5.22	7.39	0.64
	7.06	3.59	4.45	0.75
O_{COO^-GLU}	9.11	5.59	7.62	0.70
	7.73	4.00	5.45	0.62
O_{COGLU}	2.26	1.89	2.06	0.05
	6.33	1.90	5.26	0.19
O_{COALA}	6.32	3.75	5.08	0.32
	2.26	1.91	2.07	0.06
N_{NH_2ALA}	8.07	3.38	4.84	0.45
	2.51	2.18	2.21	0.06
ALA-LYS (total charge = 0)				
O_{COALA}	6.94	1.86	4.24	0.28
O_{COLYS}	2.66	1.83	2.10	0.08
N_{C-term}	3.65	2.63	3.32	0.10
N_{NH_2ALA}	8.95	2.54	4.44	0.30
N_{NHLYS}	7.48	3.59	4.49	0.11
$N_{NH_2LYSside-chain}$	9.49	2.67	5.07	0.45

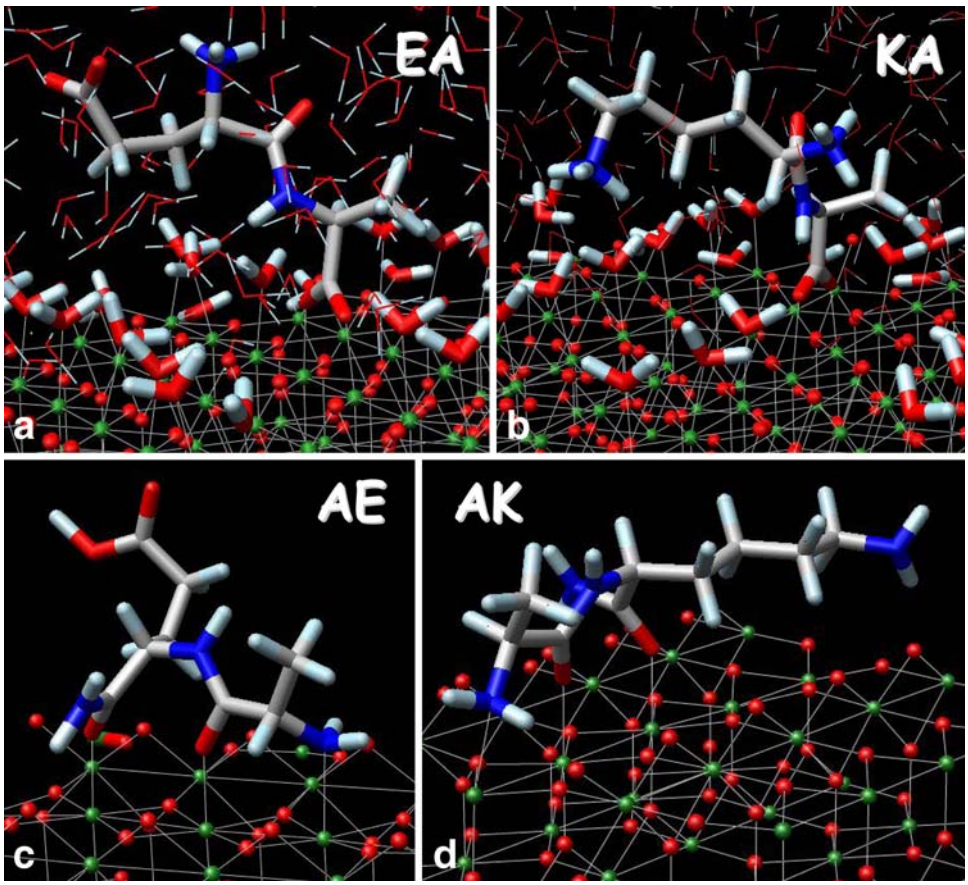

Fig. 6 Representative structures of adsorbed EA, AE, KA and AK oligopeptides; structures extracted from classical MD simulations

in agreement with experimental data, hydrogen bonding their side chain groups to surface atoms to adsorbed waters and to other side chain or backbone atoms. The balance between optimization of the local surface–adsorbate interaction and adsorbate structural variations did not play a significant role because the deposited molecules were flexible and had just a few contacts with the surface.

Many different arrangements of the various peptides on the TiO_2 surface could be found, especially when different conformers were simultaneously adsorbed upon the interface; however, preferential interactions with the backbone atoms were observed, namely coordination of the oxygen atoms to Ti sites and hydrogen bonds of the amino groups to surface oxygens. Depending on local effects, interpeptide interactions could promote or hinder peptide attachment to the inorganic layer, whereas self-interaction effects were able to induce molecular reorientations weakening the adsorption.

In order to validate the classical MD approach and to check if the final most stable arrangements identified could represent possible stable binding modes, selected molecules were subjected to ab initio optimization and MD in the presence of a reduced number of solvent molecules extracted from the classical simulations (Fig. 7). The conformations, obtained after energy optimization were very similar to the classical ones having the same backbone arrangement and only a slightly different orientation of the solvated side chain, essentially due to the different description of the surrounding solvent. As far as ab initio MD simulations are concerned they were performed to identify some probable mechanisms which took place near the surface during the very early steps of the peptide adsorption process. Indeed, the total simulation time (2.8 ps) was not sufficient to verify the stability of the attachment and explore the motion of the various portions of the peptide when in contact with the surface, but it was long enough to obtain some interesting results concerning the influence of the peptide on the mechanism of water dissociation and adsorption onto the surface.

As can be observed in Fig. 7a, b the primary interaction between the peptide and the surface is a coordination of the carboxylate oxygens of GLU with two titanium atoms. This interaction is supported by an additional, but weaker interaction (hydrogen bond) of the protonated amino terminus group with a surface out of plane oxygen. Even though the bidentate adsorption of the molecule can be considered of moderate strength, the peptide did not show the tendency to desorb easily at $T = 300$ K within the simulation time. Interestingly, a proton of the N-terminus moiety was dynamically shuttling back and forth between

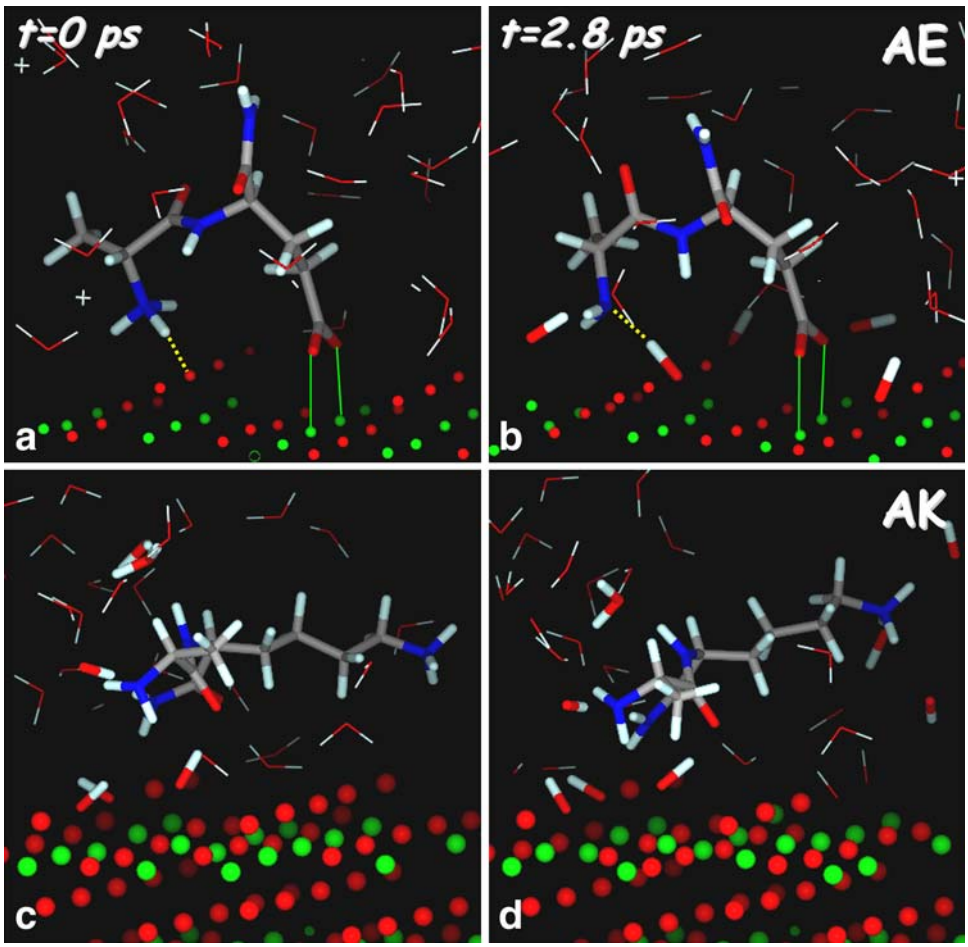

Fig. 7 Representative structures of charged AE and AK oligopeptides adsorbed on rutile (110). Structures extracted from ab initio MD simulations

the nitrogen and the oxygen atom of the surface; the hydrogen bond was maintained and the process did not affect the motion and location of the carboxylate group of the molecule which remained at a distance of about 2.2 Å from the titanium sites. The results suggest that the water activity does not cause desorption; in fact after 2.8 ps the molecule was still stably adsorbed on the TiO_2 layer.

4.2 Oligopeptide bilayer

Classical MD simulations of small systems and their validation through quantum methodologies provided us with reliable means to study the adsorption of peptide supramolecular structures, with dimension compatible with experimental observations, on the TiO_2 layer. Indeed, the study was focused on the conformational dynamics of a model of RAD16II self assembled β-sheet filament when in contact with the rutile (110) surface (Fig. 5).

A bilayer made of two eight-strand β-sheets was sufficient to provide a stable template for further elongation of the fibrillar ordered peptide aggregates and it was proven in a previous work [50] that addition of new strands was able to stabilize highly ordered configurations that could strongly adsorb onto titanium-based materials, engaging direct and indirect interactions with the surface atoms through their amino acid side chains.

The comparison between two different possible arrangements of the peptide bilayer on the surface, namely parallel and perpendicular alignments, indicated clearly that the strength of adsorption was strictly connected with the number and type of peptide–surface contacts, which was maximum when the filament laid flat on the TiO_2 layer, that is its β-sheet planes were parallel to the interface. The tendency to adopt such an arrangement was evidenced analyzing the filament's mobility and its propensity, when placed perpendicular to the interface, to rotate and orientate the hydrophilic faces towards the TiO_2 layer.

The main collective degrees of freedom necessary to describe the dynamics of the filaments on the surface were derived from the eigenvectors of the diagonalized positional covariance matrix of the backbone atoms [principal component analysis (PCA)]. The resulting eigenvectors described the nature of deformation movements, whereas eigenvalues represented the amount of variance explained by each movement, that is its relative importance.

Eigenvectors with sufficiently high eigenvalues were mainly anharmonic fluctuations [51, 52], slower correlated modes of motion relevant to biological functions, and the first two eigenvectors were sufficient to describe the most relevant movements of the filaments with respect to the surface [52]. Translational and rotational motions of the two bilayers (parallel and perpendicular to the surface) were quite different and spanned different time scales (from a few nanoseconds to more than 10 ns). Indeed, the motion of parallel segment was a combination of three different rotations, namely about the backbone of a border chain, around an axis laying in the β-sheet plane perpendicular to the strands' orientation, and about an axis almost perpendicular to the TiO_2 surface. The rotation of the bilayer about its edge was the prevailing movement and was responsible for the detachment of part of the system from the surface. However, the amplitude of the identified displacements was restricted by the presence of many contacts between the amino acid side chains and the surface atoms.

Instead, as expected, the perpendicular arrangement was more flexible and broader rotations of the bilayer with respect to the surface were observed. The filament turned about the basal strand adsorbed on the surface and about an in-plane axis perpendicular to the strands' orientation and showed the tendency to bend down toward the inorganic interface.

Despite the dissimilar behavior of the two systems the filament geometry in the central region, the β-sheet secondary structure and the bilayer packing were well preserved. However, once laid flat on the oxide interface the filament was not rigidly adsorbed but moved moderately on the surface, in spite of the great number of contacts between the oligopeptides side chains and the atoms of the TiO_2 layer.

5 Conclusions

Classical MD simulations have provided some insights into the conformational dynamics of small peptides and a short filament of RAD16II, self assembled as a β-sheet bilayer, when located near the rutile (110) surface. The atomistic description of the rutile surface and its interactions with water and peptide molecules were based on ab initio calculations. In some cases, static and MD ab initio calculations have been performed for the smallest adsorbates in order to validate the adopted procedure, but also to get detailed information, as in the case of water on rutile surface, about dissociation mechanisms that are not described by classical MD simulations. Such simulations have allowed, anyway, to capture the essence of peptide/TiO_2 surface interactions in solution at least on a short time scale. The small peptides studied, adsorbed onto the (110) TiO_2 rutile layer, are quite stable and undergo relatively limited hinge-bending motions. Once bonded to the surface the peptides have a reasonable propensity to remain there with the C-terminal polar group providing an anchor to the surface. The inherent complexity of the solvated biomolecules has been recognized in terms of multiple coordination, and the individuation of most probable contact points between the molecule and the surface has allowed to build a coherent structural model of the adsorbed species. A comparison, not reported here but fully discussed in our previous papers [20, 49, 53], of computed K-edge spectra with the available experimental spectra, gave further support to the adsorption structures and mechanisms suggested by the present studies. The stability of the computed configurations for a large number of similar adsorbates has provided clear evidence that small peptide molecules, their aggregates, and peptide matrices containing alternating positively and negatively charged residues can strongly adsorb on titanium-based materials, engaging direct and indirect interactions with the surface atoms through their amino acid side chains.

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