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Interaction at the nanoscale of fundamental biological molecules with minerals

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Abstract. The availability of advanced nanotechnological methodologies (experimental and theoretical) has widened the investigation of biological/organic matter in interaction with substrates. Minerals are good candidates as substrates because they may present a wide variety of physico-chemical properties and surface nanostructures that can be used to actively condense and manipulate the biomolecules. Scanning Probe Microscopy (SPM) is one of the best suited techniques used to investigate at a single molecule level the surface interactions. In addition, the recent availability of high performance computing has increased the possibility to study quantum mechanically the interaction phenomena extending the number of atoms involved in the simulation. In the present paper, firstly we will briefly introduce new SPM technological developments and applications to investigate mineral surfaces and mineral-biomolecule interaction, then we will present results on the specific RNA-mineral interaction and recent basics and applicative achievements in the field of the interactions between other fundamental biological molecules and mineral surfaces from both an experimental and theoretical point of view.

Keywords: minerals; nucleic acids; amino acids; SPM; ab initio simulations; molecular dynamics

1. Introduction

Interest in the interaction between biomolecules and inorganic materials arises from the many possible applications in various and different scientific fields such as, for example, biomedicine, catalysis, chromatography and molecular electronics (Chen *et al.* 2007, Hanczyc *et al.* 2007, Kim *et al.* 2010, Lin *et al.* 2007, Tiselius *et al.* 1956, Bankston *et al.* 2010). The understanding and control of mineral-biomolecule interaction may find usefulness in specific applications as self-assembly, nanopatterning, biomolecular arrays and also in prebiotic chemistry and origin of life research (Hanczyc *et al.* 2007, Hazen and Sverjensky 2010, Valdrè *et al.* 2004). Recent researches have shown that some layered silicate minerals present a reliable biomolecular adsorption and nanopatterning of single DNA molecules (Valdrè *et al.* 2004).

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Natural silicates such as those pertaining to layer silicates can be successfully used in the applications cited above. In vitro and in vivo experiments with exfoliated nanoclay (mineral montmorillonite) showed there are no mutagenic effects, nor variations in cell morphology after adhesion of mineral particles on cell surface (Li *et al.* 2010). Mineral surface nanotopography and its physico-chemical properties have also revealed a fundamental role in the relationship with these complex bio-entities in a partnership of mutual reciprocity.

Valdrè and Fabbrizioli (2006) studied the bioadsorption of human red blood cells (RBCs) on various layer silicate minerals by Scanning Probe Microscopy (SPM). The experiments were conducted in air without the use of any fixative and binding chemical agent, comparing the fixing behaviour of the examined minerals to that of the commercial substrate made of muscovite (mica). Biotite, chlorite and vermiculite showed similar good adhesion properties, allowing for reliable imaging of RBCs morphology, in contrast with muscovite where RBCs were loosely bound and thus needed binding agents. Vermiculite revealed a higher surface adsorption density. Furthermore, mineral-cell adhesion was enough to allow the observation of membrane channels at the nanoscale.

Also other minerals have fundamental applications. Working on apatite microtopographies, Dangaria *et al.* (2011) have very recently demonstrated that natural tooth root topographies induce integrin-mediated extracellular matrix signalling cascades in tandem with cell elongation and polarization to generate physiological periodontium-like tissues. Furthermore, the natural extracellular surface topographies revealed the capacity to instruct progenitor cell populations to fully regenerate complex cellular and structural morphologies of tissues once lost to disease. They replanted surface topography instructed periodontal progenitors into rat alveolar bone sockets resulting in complete reattachment of tooth roots to the surrounding alveolar bone with a periodontal fibre apparatus closely matching physiological controls along the entire root surface.

SPM-based techniques have revealed to be one of the best suited methods to investigate experimentally at a single molecule level the surface interactions. At the same time, because of high performance computing, quantum mechanics (QM) can tackle the complex modelling of the interaction phenomena extending the number of atoms in the simulation (being closer to a real system) in a reasonable time.

We found very few interdisciplinary and combined SPM-QM researches and they refer only to organics (Gross *et al.* 2009, 2010, Swart *et al.* 2011). There is a lack of a comprehensive integrated review condensing the important results so far achieved by SPM and QM in the same research field of the interaction, at a single-molecule level, between minerals and biomolecules. In the present paper we try to fill the gap, giving the reader of one specific field what has been and can be done in the other.

First, after a brief section introducing innovative SPM-based methods, which can determine at high spatial resolution various physical properties related to mineral-biomolecule interaction, we report on recent SPM investigations of RNA in interaction with minerals. Then, we will review recent basics and applicative achievements in the field of the interactions between fundamental biological molecules and mineral surfaces from both an experimental (SPM) and theoretical (QM) point of view. QM *ab initio* and molecular dynamics computational methods will be introduced and their application to the study of mineral-biomolecule interaction from a quantum mechanics point of view presented and reviewed. The present approach is important to determine the main forces ruling adsorption and binding and to understand how they operate.

2. Scanning probe microscopy

A peculiar characteristic of scanning probe microscopy (SPM) is identified by its versatility to investigate at high spatial resolution materials surfaces, both carrying out measurements of a great variety of physico-chemical properties and also directly manipulating the sample surface. The information obtained from the surface can complement those usually obtained by the well-established bulk characterization techniques and electron microscopy and microprobe methods applied to biomaterials (Bocchi and Valdrè 1993, Borgia *et al.* 1992, Gatti *et al.* 1996). For this reason SPM is a well-suited technique to investigate surface-molecule interaction at a single-molecule level.

The higher spatial resolution has been achieved implementing a frequency modulation (FM-SPM) mode of operation in ultrahigh vacuum, resolving sub-atomic features of silicon surfaces (Giessibl 2003). Furthermore, thanks to the high sensitivity of FM-SPM the chemical identification of individual surface atoms was achieved in ultrahigh vacuum at room temperature by detecting the short-range chemical forces between the tip and the surface (Sugimoto *et al.* 2007). Working in liquid and air environment, the most used mode of operation is amplitude modulation (AM-SPM). However, recently, FM-SPM has also revealed great potentials for imaging of inorganic and organic systems both in air and liquid. For instance, the surface of a muscovite was atomically resolved in water, and high resolution was also obtained on biomolecules, visualizing the hydration layers on a lipid bilayer and β-strands constituting an amyloid fibril (Fukuma 2009). Generally speaking, dynamic SPM modes can also provide qualitative information about composition, adhesion, friction and viscoelasticity, by mapping the phase shift between the excitation signal and the response of the cantilever during the scan.

SPM can also provide quantitative information on the surface potential of minerals and biomolecules. This measurement can be performed by Kelvin probe force microscopy (KPFM) and static or dynamic electric force microscopy (EFM).

In Kelvin probe force microscopy the electrostatic surface potential can be quantitatively measured by electrically exciting the cantilever oscillation in a dual pass mode (Elings and Gurley 1994) or in a single pass mode (Ziegler *et al.* 2007). The spatial and voltage resolution can reach a few nanometres and millivolts.

In electrostatic force microscopy, the static mode works at the equilibrium when the elastic force of the cantilever is balanced by the electrostatic force acting between the probe and the specimen surface. The measured deflection is directly related to the surface potential of the mineral. The effective electrostatic force depends on the probe-to-specimen voltage difference and also on both cantilever and tip geometry. This dependence can be analysed by a numerical simulation to relate the probe's deflection to the real mineral surface potential (Valdrè and Moro 2008a, b). Furthermore, by means of a Force Volume experiment, a tri-dimensional data set can be acquired, quantifying the electrostatic force distribution above the sample surface. In dynamic mode EFM, a conductive cantilever is mechanically excited to vibrate above the sample surface, and frequency, amplitude and phase detections are employed to investigate the tip–surface interaction.

Fig. 1 shows an SPM observation of the surface of a freshly-cleaved chlorite, a mineral substrate suitable for bio-applications (Antognozzi *et al.* 2006, Valdrè 2007). This mineral is a layered silicate whose structure consists of tetrahedral-octahedral-tetrahedral (TOT) layers (with siloxane surface) about 1 nm thick, weakly bound to $Mg(OH)_2$ brucite-like octahedral sheets, about 0.5 nm thick. The layers are stacked in an alternating sequence. After cleavage, the surface

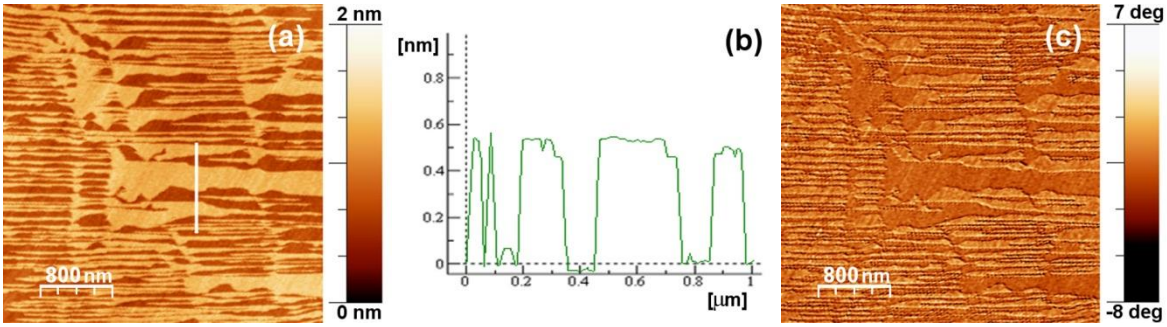

Fig. 1 (a) Typical nanomorphology of a chlorite crystal imaged by SPM. Micrometer long brucite-like stripes, about 0.5 nm thick, extended from few to hundreds of nanometres are bound to the underneath TOT layer (dark contrast). (b) Profile of the brucite-like stripes measured along the vertical white line in the image (a). (c) Phase signal from the same area shown in (a) revealing the presence of two different materials ($Mg(OH)_2$ and siloxane TOT surface)

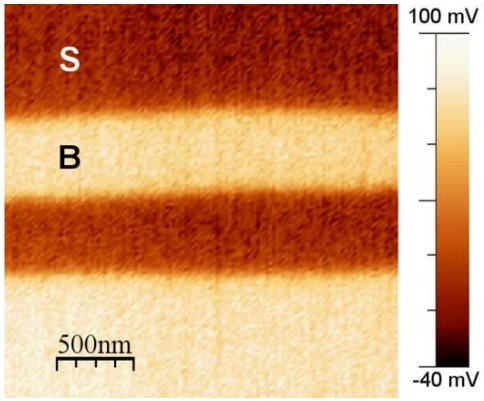

Fig. 2 Typical surface potential map of a cleaved chlorite with the potential of brucite-like B and siloxane surface S of the TOT measured by dual-pass KPFM

was imaged in air by AM-SPM showing micrometre long brucite-like stripes (bright contrast) over the continuous TOT background (dark contrast) (Fig. 1(a)). The stripes were measured by profile analysis (Fig. 1(b)) from hundreds to few tens of nm wide and about 0.5 nm thick, in agreement with single-crystal X-Ray Diffraction (XRD) data of the height of a single brucite-like sheet (Valdrè *et al.* 2009). The phase shift signal, that is material dependent (Fig. 1(c)) confirms the presence of the two different materials at the surface (i.e., brucite-like and TOT siloxane surfaces).

Fig. 2 shows, as an example, a surface potential nano-distribution of a cleaved chlorite. Two-pass KPFM was used for imaging surface bands of brucite, B, on TOT siloxane surface, S, by keeping the probe at 30 nm above the mineral surface. The alternating potential bands are associated to the different surface potentials of the two materials. By KPFM, catalytic Brønsted–Lowry sites were recently discovered at the surface of the siloxane in Al-rich chlorites (Valdrè *et al.* 2011). This technique and quantum mechanical modelling were used to correlate the nanoscale contrast due to the electrostatic surface potential (related to Brønsted–Lowry sites) with the surface morphology and crystal-chemistry of the mineral.

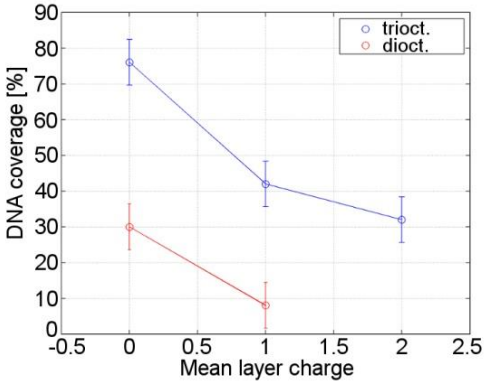

Fig. 3 Experimental trend of DNA coverage of the (001) surface of di- and trioctahedral layer silicates as a function of the mean layer charge

Recently, Valdrè *et al.* (2004) studied by SPM the surface affinity, self-assembly and nanopatterning of DNA molecules adsorbed onto several layered silicates and minerals. Talc, pyrophyllite, muscovite, biotite, brucite, chlorite and vermiculite surfaces were observed to present a considerable variety of DNA adsorption mechanisms, that were associated to the differences in surface structure and chemistry among the mineral surfaces. Investigations on dioctahedral (with trivalent cations, mainly Al^{3+}, in the octahedral sites) and trioctahedral structures (with divalent cations, mainly Mg^{2+}, Fe^{2+}, in the octahedral sites) revealed different deposition patterns in terms of configurations and amount of adsorbed molecules (e.g., low charge trioctahedral layer silicates presented about 80% DNA coverage in comparison to 30% of dioctahedral ones; see Fig. 3).

Because of the heterogeneous layered structure of chlorite, later works were focused on the study of the surface properties of chlorite at the interaction with simple biomolecules. Nucleotides and DNA interaction was investigated by SPM analysis. A high affinity with the positively charged brucite-like $Mg(OH)_2$ sheet was revealed for both molecules, together with an almost completely absence of adsorption on negatively charged siloxane zones (Antognozzi *et al.* 2006, Valdrè 2007). Furthermore, alignments of nucleotides and single DNA molecules were observed at the brucite-like layers edges.

In our laboratory we have also investigated the adsorption selectivity of chlorite towards another fundamental macromolecule, the ribonucleic acid (RNA). Despite the inherent difficulty of dealing with an intrinsically fragile molecule that can adopt a lot of nearly isoenergetic structures (Giro *et al.* 2004), preliminary studies carried out with RNA extracted from the bacteriophage MS2 show selective adsorption on the brucite-like sheet. The analysis were conducted at RT (25° C), atmospheric pressure and relative humidity of 40%.

The used RNA is single-stranded, with an approximate molecular weight of 1200 kD, 3569 nucleotides, and stored in a buffer solution with 10 mM Tris-HCl, 1 mM EDTA and a pH of 7.0 (Roche Diagnostics GmbH, Germany). The RNA was diluted with an ultrapure aqueous solution (Gibco, USA; Ultrapure water DNAse, RNAse free) to concentrations of 1nM and 0.1 nM. Drops of 20 μl were deposited onto freshly cleaved chlorite surfaces. Before the deposition all the surfaces were sterilized by means of a quartz sterilizer, then characterized morphologically, down to a nanoscale level, by SPM. After deposition, the RNA was incubated for 10 minutes, then rinsed with ultrapure water and finally dried in a flow of pure nitrogen. Fig. 4(a) reports the surface

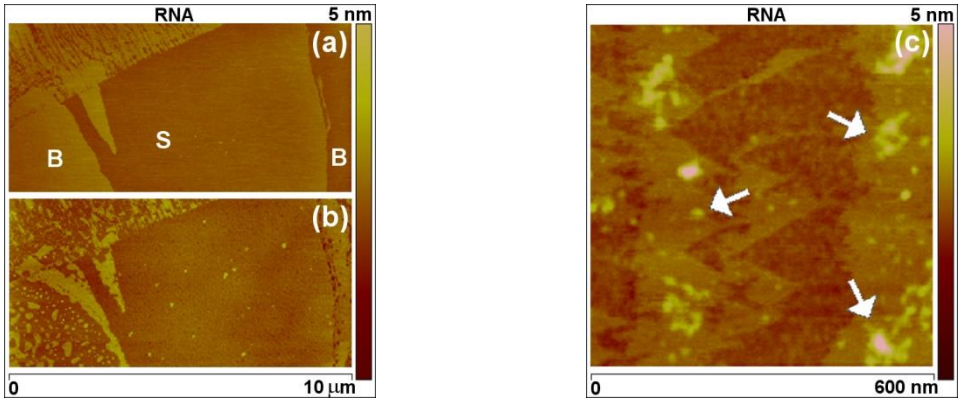

Fig. 4 SPM images of RNA molecules adsorbed on the surface of chlorite. (a) Chlorite surface before RNA deposition. Once cleaved, wide areas of brucite-like sheet, B, and siloxane S are present at the surface. (b) After deposition from a high concentrated solution (1 nM), huge amount of RNA molecules selectively adsorb on brucite-like sheet B forming continuous films or islands. (c) When RNA is deposited from a low concentrated solution (0.1 nM), as expected, only isolated single or multiple RNA agglomerates adsorbed on the brucite-like sheet are observed (see arrows)

nanomorphology of as-cleaved chlorite that presents continuous and fragmented brucite-like zones B bound to the siloxane surface S. Fig. 4(b) shows the same area after RNA deposition from the 1 nM solution. The comparison of the same area before and after RNA deposition shows a selective adsorption of the molecules on the brucite-like surface, which presents a surface potential more positive than the TOT one, suggesting an electrostatic binding of the RNA molecule. In fact, a higher RNA concentration was observed at the edge of brucite-like steps that are zones of high electrostatic force gradient. At these edges RNA was observed also as linearized filaments instead of a continuum film or islands.

Fig. 4(c) shows an SPM image of the surface of chlorite after RNA deposition from the 0.1 nM solution. Because of the reduced amount of RNA molecules, they are observed only as single or multiple agglomerates (globular domains). However, they are still selectively adsorbed on the positive brucite-like surface and no RNA molecules are adsorbed on the negatively charged siloxane surface of the TOT.

Chemical Force Microscopy (CFM) is another SPM-based method capable to map the surface forces probing specific molecular interactions (Noy *et al.* 1997). However, it implies the chemical functionalization of the SPM probe in order to selectively measure and image the probe-surface interaction with chemical sensitivity. Kirkham *et al.* (2000) used this technique to investigate the dental enamel crystals surface properties, central for the understanding of the matrix-mineral interactions. Negatively (carboxylated) and positively (amino-terminated) charged functionalized tips were used for imaging in liquid environment at various pH. Parallel alternating domains of surface charge in the direction of the crystallographic "c" axis were detected at the surface of individual maturation-stage crystals from developing enamel. The periodicity consisted of broad bands (positive) from 30 to 50 nm in width, interrupted by narrower domains (negative) about 15 nm in width. Amelogenin protein aggregates were observed to bind to the positively charged domains.

Other AFM studies in this scientific area concern the development of enamel crystals, because

they can be individually extracted with relative ease (Kirkham *et al.* 2002). During the enamel growth, two processes have been observed: (i) initially ribbon-like hydroxyapatite crystals are bound to a protein-rich matrix that acts as growth inhibitor and (ii) then the degradation and removal of the matrix permits secondary crystal growth in the a–b and b–c crystallographic directions. The nature of the interaction hydroxyapatite-protein is predominantly electrostatic and can occur via stereo-specific charged residues of the matrix or through the formation of self-assembled aggregate structures (nanospheres). In the first case, given the net positive charge of the hydroxyapatite surface in "in-vivo-like condition" of pH and ionic strength, the binding process involves principally acidic matrix proteins. It has been also pointed that the secondary structure of the protein in the matrix is fundamental in maintaining charged side chains in the correct spatial alignment for binding. However, it is known that amelogenin, the principle protein of the developing enamel matrix, usually forms the cited nanospheres, suggesting that it is the predominant mechanism of enamel biomineralization. In this case, the interaction is not related to specific sites, but to the overall charge density or to other macro properties. Other interactions may also take place, such as hydrogen bonding between protonated groups in the protein and electronegative sites domains at the crystal surface and van der Waals forces. In general, preferential binding of matrix proteins to specific crystal faces, related to interfacial hydrophobicity/hydrophobicity, was shown to control crystal habit.

Some years later Vandiver *et al.* (2005) used CFM to study a dense polycrystalline phase of pure synthetic hydroxyapatite (HA) in 0.001–1 m NaCl solution at about pH 6. They obtained an estimation of the surface charge per unit area of the samples by using the nonlinear Poisson-Boltzmann-based electrostatic double layer theory. The average surface charge per unit area of the HA was found to be ~ -0.02 C/m^2 and to vary from -0.0037 to -0.072 C/m^2. The surface linear gradient moving aside grain boundaries and crystallographic planes reaches figures up to -0.00019 $C/m^2/nm$. Surface migration of PO_4^{3-} groups has been suggested to cause all surfaces to have a negative charge and variance is most likely due to different arrangements on each crystal plane of the additional charged ions making up the HA lattice. These methodologies can give important contributions to the understanding of the electrostatic effects on the bioactivity of HA.

3. Integrated inter-disciplinary experimental and theoretical investigations of the interaction of fundamental biomolecules with mineral surfaces

A further understanding of the interactions between biomatter – mineral surfaces, at a single molecule level, can be obtained also from quantum mechanics (QM) computational simulations.

There are basically two QM approaches used for the simulations: *ab initio*, or static (Dovesi *et al.* 2005) and molecular dynamics, MD (Cygan *et al.* 2009) methods. These approaches use computer codes such as CRYSTAL (Dovesi *et al.* 2009), VASP (Kresse and Furthmuller 1996, Kresse and Hafner 1993, Kresse and Joubert 1999), NAMD (Phillips *et al.* 2005) and AMBER (Case *et al.* 2005). The choice of the specific computational method depends on the complexity of the system and the rate of accuracy required. Both *ab initio* and MD methods make use of quantum mechanics theories and can evaluate fundamental and excited states (reactions, adsorption, etc.). *Ab initio* methods are adaptable to many type of systems, are very accurate, however with high computational constraints. MD methods overcome this latter inconvenient, but introducing extensive approximations and the need of empirical parameters from experimental or *ab initio* data. However, they can provide 'good pictures' of what is happening in the system, as

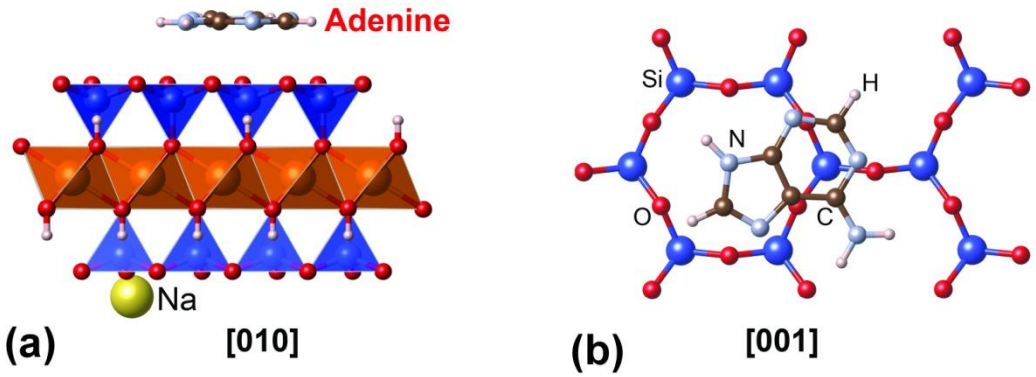

Fig. 5 Scheme of the preferential orientation of adenine on a montmorillonite surface. (a) View from the [010] direction; (b) view from [001] direction

MD methods have a timescale. MD is commonly adopted as valuable tools in proteins structure refinement (and in few cases, also prediction) alongside experimental techniques, such as XRD and nuclear magnetic resonance (NMR). We report in the following a review of QM (*ab initio* and MD) of important biomolecules interacting with mineral substrates integrated to specific experimental approaches.

3.1 Nucleic acids

The study of the affinity between DNA (or RNA) and minerals is incredibly important because they are the molecules for the storage and transport of the genetic information, and their interaction with minerals is fundamental for both basic research, such as that related to prebiotic chemistry and origin of life, and for biotechnology.

There are two main theoretical studies on this subject. In the first one, periodic plane wave *ab initio* simulations were conducted on RNA/DNA nucleobases in interaction with Na^+-Montmorillonite surface (Mignon *et al.* 2009). The authors considered the adsorption of the nucleobases on the mineral without and with the interaction with Na^+. In the former case, Mignon and co-workers (2009) observed that the preferential orientation of the nucleobase is parallel to the surface (face-to-face), because it maximizes the dispersion forces (see the scheme of Fig. 5(a) and (b)). When the interaction occurs in the presence of the Na^+, it can follow three different configurations: cation–π/ring, cation–π/ring displaced and cation/heteroatom, where the cation is Na^+, π/ring is the six-atom ring of the nucleobases and the heteroatom is N or O. Results show that for the classical cation–π/ring interaction, the electrostatic contribution is far smaller than the pure dispersive one. The opposite effect is observed for the cation–π/ring displaced configuration. The cation/heteroatom orientation, however, renders these cases less effective in exploiting dispersion interactions, which are indeed the smallest for all considered orientations. Overall, dispersive forces are essential to stabilize the face-to-face and cation/π-displaced configurations, but the relative stability may differ in presence of water due to the solvation of the metal cation.

These results were used in a subsequent work by other authors (Michalkova *et al.* 2011) in which, adopting a cluster model, considered a non-hydrated adsorption process to the tetrahedral and octahedral surfaces of kaolinite. The modelled nucleic acid bases were thymine and uracil. Both of the considered nucleic acid bases interact in a very similar way with the mineral surface.

The adsorption is classified as physisorption and the strength is proportional to the number of intermolecular interaction formed between the target molecule and the surface. Other factors, such as the type of surface, position and orientation of the nucleobase and the formation of hydrogen bonds, may provide additional stability to the complex. These results were confirmed by calculations of the electrostatic potential maps of the most stable complexes. The adsorption of thymine and uracil on kaolinite minerals was found to depend on the type of surface and its chemistry, which controls the binding strength and character of the intermolecular interactions. Such a large effect is suggested to be an indication of the catalytic properties that clay minerals may have also shown when used as substrates for biomolecules in the prebiotic chemistry.

Other computational studies (*ab initio* and MD) concerning nucleobase, RNA and DNA adsorption on mineral surfaces were related to the interaction between thymine and uracil on dickite surface and nanopatterning of nucleotides on graphite surface and confirmed the physisorption mechanism (Walsh 2008, Robinson *et al.* 2007).

The analysis of mechanisms which involve the interface DNA-mineral could prove themselves useful not only in biotechnology (like hydroxyapatite chromatography which is used to separate single strand DNA, ssDNA, from double strand DNA, dsDNA, thanks to the different binding affinity), but also to allow other branches of science to better understand the processes involved in the biochemical and structural formation of this main component of life. Details of the mechanism of ssDNA and dsDNA separation by hydroxyapatite are presented in a recent study (Chen *et al.* 2007). The authors examined the effects of binding parameters (i.e. salt concentration, temperature and pH) and the effects of ssDNA and dsDNA base composition and sequence on the binding behaviour. The affinities and binding amounts were obtained from equilibrium batch isotherm analysis, and the binding enthalpy was derived from isothermal titration calorimetry (ITC). Isotherm analysis reveals that electrostatic forces are the main driving force of dsDNA binding to HA. Due to hydrophobic bases and the negative charge of the phosphate backbone, the ssDNA molecule binds to HA with both hydrophobic and electrostatic interactions. The adsorption enthalpy of both ssDNA and dsDNA is endothermic under all of the conditions studied and the dehydration step in the binding process plays a key role.

3.2 Amino acids

Throughout the analysis of the interaction of biomolecules with mineral substrates, the importance of the interaction with amino acids is crucial for various reasons. Amino acids are not only the components of proteins, but have also a primary role in the interactions with minerals, especially for stem cells. It has been reported very recently that specific peptide sequences are used as promoters for both cell adhesions and differentiations (Lee *et al.* 2009). In their work, the researchers showed that modular peptide molecules can be strategically important in the field of bone regeneration, but also that the modular approach could be used to bind other biologically active molecules to clinically important biomaterials.

Mineral-protein interactions play also a fundamental role in biomineralization, the regulated and controlled growth of minerals that occurs in biological systems, for example in the skeletal tissues of mammals. The size and form of the crystals within mineralized tissues reflect the controls and constraints imposed during tissue morphogenesis. However, details of protein-mineral interaction for biomineralogical study is beyond the scope of the present paper that deals mainly with SPM and QM (*ab initio* and MD) simulations of the mineral interaction with single amino acids.

3.2.1 Interaction with hydroxyapatite

Since any interaction between protein matrix and mineral crystals must be mediated via association with the crystal surfaces, physico-chemical characterisation of crystal surfaces is a key requirement to the understanding of the matrix-mineral relationship. The knowledge of the principles that govern the mechanisms of biomineralization would allow the development of biomimetic/prosthetic materials and therapeutics. It is known that mammal bone tissues are composed by a mineral phase, whose major component is a defective hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HA] containing carbonate, magnesium, fluoride and other ions, and an organic phase, in particular tropocollagen fibrils, arranged in a staggered way at the nanoscopic length scale. Interaction between the two phases critically affects the strength of the material, both biological and synthetic. The former concern essentially the prosthetic field, where the key clinical objective is to develop implantable materials which achieves rapid fixation with the surrounding bone.

Corno and co-workers (2010) performed *ab initio* quantum mechanics simulations of glycine, lysine and glutamic acid adsorbed on pure hydroxyapatite (HA) surfaces, with and without the presence of water molecules (see the scheme of Fig. 6(a) and (b)). The authors initially optimized and characterized from a computational point of view the bulk and surface structures of hydroxyapatite, obtaining results that were in good agreement with experimental data. Then, it was studied the adsorptions of glycine, lysine and glutamic acid on the (001) and (010) surfaces of HA under strict-gas phase conditions. This model have been previously proved adequate, because it is possible to experimentally sublimate proteins without decomposition. The glycine interacts with the surface in its zwitterionic state, with the carboxyl group (COO^-) that electrostatically interacts with two calcium ions and the NH^{3+} group making hydrogen bonds with the oxygen atoms of the HA surface. The adsorption of the lysine involves mainly the interaction of the N lone pair with the most exposed Ca^{2+} ion of both the surfaces considered. For the glutamic acid, the adsorption is dependent of the surface: on the (001) there is a spontaneous proton transfer from the COOH group to a P=O oxygen and the instauration of a hydrogen bond COO^- --- H-O=P. This does not occur easily on the (010) surface, because there are only POH groups that are poorer H-bond acceptors than P=O groups. It has been evidenced that the interplay between electrostatic forces, hydrogen bond interactions and dispersive contribution occurring at the hydroxyapatite interface stabilizes either the zwitterionic or the deprotonated forms of the amino acids, the latter being stabilized by the ion/pair interaction. The authors found their results in line with spectroscopic and classical molecular modelling-based works (Corno *et al.* 2010).

We would like just to mention one case of MD simulation between HA and an important protein. Considering a larger length scale, MD was in fact recently conducted on two complexes of HA and tropocollagen (TC) fibrils exposed perpendicularly to or along the (001) surface of the mineral (Dubey and Tomar 2009). The aim was to test the strength of the biomaterial resistance, applying transversal and longitudinal stretching to the system. The authors evaluated Young's modulus and tensile stress-strain curves of the complexes taking into account different parameters, such as the presence of water, salt, pH, the collagen volume fraction, etc. It was evidenced that, if applying a tensile stress, the dominant failure mechanism of the biomaterial is due to cleavage of TC after its full stretching capability and in shear breakage of the mineral platelet. In the case of compressive stress, the failure is primarily shear driven. The presence of water and calcium ions provides stabilizing effect on the triple helix of tropocollagen and strengthen the TC-TC interactions, delaying the failure of the overall system.

A different approach in surface selectivity was adopted by Tiselius *et al.* (1956). They

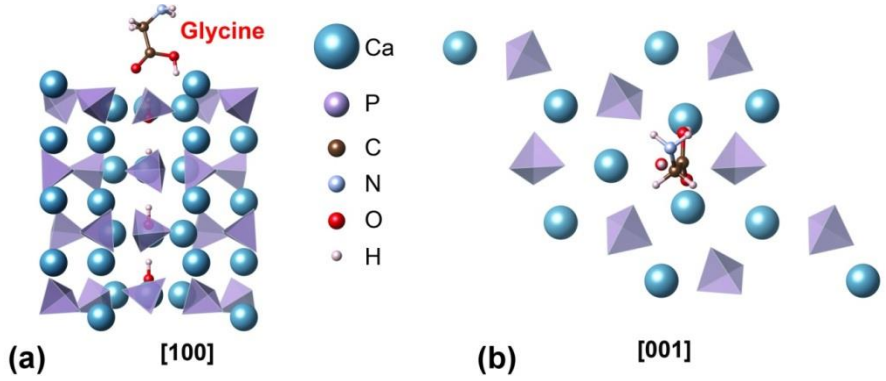

Fig. 6 Scheme of a glycine molecule adsorbed on a hydroxyapatite crystal. (a) View from the [100] direction; (b) View from the [001] direction

investigated the chromatographic separation of amino acids, where the column used was obtained from hydroxyapatite (HA) powder. It is well established that this mineral is a multifunctional material that contains simultaneously both negatively (P-sites) and positively (C-sites) charged binding sites. This leads to an unique selectivity and affords the ability to separate complex mixtures. As a consequence, basic proteins in their cationic form are adsorbed mainly on the P-sites with electrostatic interactions and can be eluted with salts like NaCl in a step-wise or gradient fashion. Acidic proteins, on the other hand, are adsorbed primarily on the C-sites and their desorption often requires competitive eluents, such as phosphate or citrate, which themselves form complexes with calcium. A limitation of hydroxyapatite is its modest stability at acidic conditions. Experimental and phenomenological models of the HA in chromatography were very recently proposed (Bankston *et al.* 2010).

The interaction of serine in its zwitterionic form on hydroxyapatite was also observed (Spanos *et al.* 2001).

3.2.2 Interaction with layer silicates

Amino acids might have played a fundamental role in the development of complex structure in prebiotic chemistry. Several hypotheses concerning amino acids on a mineral substrate and their way to assemble in oligopeptides show how the study of the interaction of these biomolecules on substrates is of great importance not only in biomedical application, but also in different fields, such as amino acids polymerization processes in industrial technology. Two recent reviews reported the state of art in this field, comparing both experimental and theoretical results (Hazen and Sverjensky 2010, Lambert 2008).

It was discovered that amino acids polymerization is favoured by adsorption, at least if the latter is followed by drying at moderately high temperatures to lower the water activity (a_{H2O}), which prevents the peptide bond formation (de Duve and Miller 1991). Three possible types of interaction between amino acids and a mineral surface have been proposed. First, a non-specific adsorption mechanism involves electrostatic bonding. Charged amino acids can be retained in the vicinity of mineral surface bearing the opposite charge. This situation is common for most oxide and silicate minerals. For example, Churchill *et al.* (2004) used an SPM to measure interaction forces between amino acids and surfaces of quartz, calcite and albite over a range of pH. In order to correlate mineral surface charges with ionic characteristics and corresponding isoelectric points

(pI) of amino acids, the researchers immersed quartz and calcite in solutions of six different types of amino acids. Quartz adsorbs lysine (isoelectric point, pI = 9.74) more strongly than amino acids with lower pI. In contrast, calcite (point of zero charge, pH_{pzc} = 9.5) adsorbs a variety of amino acids with a range of pI.

Second, another proposed mechanism is the formation of covalent bonds between the amino acid and the surface, a process known as "formation of an anhydride", or surface ester (Collins *et al.* 1988). However, quantum mechanics simulations indicate a positive, thus unfavourable, free energy formation for this adsorption mechanism (Rimola *et al.* 2006a, b). The authors stated that the "anhydride" on the surface may occurs by reaction with strained siloxane cycles of the SiO_2: this means that the surface specie would be produced by coupling the covalent bond formation with a thermodynamically favourable siloxane ring opening.

The third type of interaction involves the formation of hydrogen bonds between the amino acids and the surface, as recently observed in the works of Ikhsan *et al.* (2004) and Vlasova and Golovkova (2004). In the first paper the authors evidenced an aspartic acid adsorption on kaolinite, in the latter arginine, lysine and ornithine were adsorbed on silicon oxide. The adducts can be formed both with protonated and deprotonated surface groups; from the point of view of the amino acids, the adsorption may involve their cationic or anionic form. Combinations of molecular modelling and spectroscopic data confirmed the formation of H-bonded adducts and allowed evaluations of their structures. Simulations of the adsorption of glycine from the gas phase showed cooperative bonding between the carboxylic group of a neutral glycine molecule and an isolated silanol from the (001) surface of an all-silica edingtonite (Rimola *et al.* 2006b). The simulated IR spectra of the system exhibited a shift to shorter wavelengths of the glycine fingerprint signal (C=O) and a bathochromic shift of SiO – H peak due to the occurred adsorption. The simulation results were in good agreement with previous experimental data, especially for the fingerprint peak shift (Gromovoy *et al.* 1991). A molecular modelling technique was applied to L-lysine on a hydroxylated quartz surface (Gambino *et al.* 2004). Investigations were performed with both quantum and classical mechanics theories to study the static properties of the adsorbing molecules. Molecular dynamics techniques were used to address the time evolution of the adsorption process. Calculations demonstrated that the main contribution to the L-lysine – surface interaction is given by the electrostatic forces between the amino acid NH^{3+} group and the SiO- groups of the surface. An important contribution to the adsorption is due to the hydrogen bonds between the H of the amino groups and the oxygen of the silanol groups. Furthermore, it has been observed an increased density of the water layer in proximity to the surface due to the hydrophilic nature of the substrate, an effect that indirectly and cooperatively contributed to the adsorption process. A similar result was obtained later, where a glycine molecule in interaction with a silica surface was stabilized by two water molecules (Stievano *et al.* 2007). In a hypothetical quartz surface functionalized with methyl groups, rather than silanol groups, the adsorption process does not take place (Gambino *et al.* 2006).

3.2.3 Interaction with quartz and calcite

Another important aspect that deserves attention is mineral surface selectivity, that has not been systematically and exhaustively analysed so far. The most successful works were conducted by Hazen and Sverjensky (2010). In their studies, the authors pointed out that, if the mineral played an important role in the prebiotic synthesis of biomolecules, they also selected and concentrated a small group from the others. A key attribute of life, and an important consideration in origins-of-life models, is life's molecular handedness, or chirality. Quartz is the only common chiral rock-

forming mineral, but all centric crystals also have the potential to display chiral fracture or growth surfaces (Downs and Hazen 2004). Many common minerals exhibit surface chirality when the exposed face presents high Miller indexes. From theoretical models it has been evidenced that chiral molecular selection by a crystal requires three, noncolinear interaction points between the molecule and mineral surface. For example, it has been analysed, both from experiments and simulations, the separation of L- and D-aspartic acid by calcite (214) surface (Asthagiri and Hazen 2007a, b). The amino acid presents two carboxyl groups with an O-O separation very close to the calcite surface Ca-Ca separation: this leads to the formation of two Ca-O bonds that fix the spatial orientation of the biomolecule. The different adsorption energy of the two chiral aspartic acid molecules is given by a third O-H bond that is readily formed by the D-aspartic acid and not formed by the other enanthiomer. This property can be used to separate racemic solutions of L- and R- amino acids.

3.3 Phospholipids

Phospholipids are biological, amphiphilic molecules that self-assemble into different types of structures: planar, cylindrical, spherical, lamellar, and bi-continuous three-dimensional networks. Planar lipid bilayers are the basic building blocks of biological cell membranes, defining the cell, nucleus, and organelles. Closed shell bilayers (vesicles), whose primary function is cellular transport, abound in the cytoplasm and are involved in complex processes such as adhesion, fusion and budding. Despite the simplicity of bilayer and vesicle structures, there are complex intra- and inter-molecular forces that determine their size, shape, and stability, and their interactions with other bilayers, vesicles, and surfaces, both biological and non-biological (Muscatello *et al.* 2000, 1996).

Phospholipid bilayers supported on solid substrates are of interest for understanding cell-substrate interactions, for developing chemical and biochemical sensors, catalytic surfaces, or immobilized protein arrays, and as insulating layers with thickness of few nanometres on conductive surfaces. They are also of general fundamental interest for understanding complex colloidal systems, including nanoparticle dispersions. Notions and equations about lipid membranes formation can be found in a detailed and specific review (Sackmann 1996).

There are very few computational studies on phospholipids, both as single molecules and bilayers, in interaction with surfaces. A model that tries to simulate and analyse geochemical reactions at the outer side of the membrane of Gram-negative bacteria has been developed some years ago (Lins and Straatsma 2001). Lipopolysaccharides (LPSs) form the major constituent of the outer membrane of these bacteria and are believed to play a key role in processes that govern metal binding, adsorption to mineral surfaces, and mediated oxidation/reduction reactions at the bacterial exterior surface. Molecular dynamics simulations of the LPS membrane of *Pseudomonas aeruginosa* with Ca^{2+} ions in contact with the sugars of LPS were carried out. Inner and outer water layers were also accounted. Trajectories were analysed for the energetic and structural factors that determine the role of LPS in processes at the cell surface. The membrane displayed little flexibility as a whole. Calcium cations are uniformly distributed across a small region in the inner core, as they are needed to stabilize the membrane structure because of the huge negative charge, and complement the distribution of the negatively charged functional groups. A well-defined structural arrangement is found for the Ca^{2+} ions that interact with inner core phosphate groups. A highly negative electrostatic potential surface on the LPS membrane was calculated, which corresponds very well with experimentally determined transmembrane potentials. The fast

dynamics of the patches of negative charge on the surface and the large flexibility of the analysed structure insertion points suggest that the LPS membrane of *Pseudomonas aeruginosa* is able to adapt to approaching environmental cations or mineral surfaces.

Interesting experimental studies have been made with different techniques to understand the adsorption mechanisms of these biomolecules.

Supported phospholipid bilayers are commonly produced either by Langmuir-Blodgett (LB) deposition or by vesicles adsorbing or "self-assembling" from solution. LB deposition is quantitative and controllable, but slow and unscalable. Adsorption from solution is not well-understood nor well-controlled, but it could be promising for fast, large-scale preparation of bilayer-coated surfaces. Despite the considerable work on vesicle adsorption during the 20 years since the pioneering work of McConnell *et al.* (1986), there is still no clear picture of the forces and various stages that a vesicle goes through as it adheres, fuses, and spreads on a solid surface. Understanding and controlling the transformation of vesicles in solution into a continuous and stable single bilayer on a surface would provide a potentially important tool for functionalizing surfaces, both planar and porous.

Gromelski *et al.* (2009) conducted such a study, where they showed how the formation of a complete lipid bilayer by vesicle adsorption and rupture depends on the type of the surface used. Fairly smooth synthetic SiO_2 surfaces and much rougher polyelectrolyte cushions – poly(sodium 4-styrenesulfonate) and poly(allylamine hydrochloride) – were used to study this process. Depending on the chemical structure of the lipids, two different pathways were found on SiO_2 surfaces: 1) vesicle adsorption occurs in a first step until a critical coverage is reached, followed by vesicle rupture or bilayer formation (or adsorption), and 2) vesicle rupture occurs almost at the same time. In the case of polyelectrolyte cushions, both neutron reflectivity and quartz crystal microbalance experiments showed that the formation of homogeneous phospholipid bilayers is significantly better on the negatively charged poly(sodium 4-styrenesulfonate) surface compared to the positively charged poly(allylamine hydrochloride).

A detailed and interesting review on membrane assembly assisted by a mineral surface was recently presented by Hanczyc and co-workers (2007). A solution containing RNA, fatty acids and clay produces structures (vesicles) that contain a potentially catalytic surface (clay) and a potential informational biopolymer (RNA) encapsulated within a membrane. A great variety of supporting materials and minerals were used, such as aluminium silicate, glass microspheres, montmorillonite, quartz powder, talc, *etc*. The authors showed that the formation of vesicles in the presence of minerals occurs proximal to the mineral surface and it is favoured by the available surface area of the mineral and by the curvature of the surface. Also, a negatively charged mineral surface may stimulate membrane formation, while an intrinsically positive surface is coated by adsorbed phospholipids, which may serve as catalysts for subsequent vesicles formation. It seems that soluble factors, such as Mg^{2+} or Ca^{2+} ions, play a negligible role in the formation process.

Several resources are spent by the scientific community studying phospholipids as biological membranes. For example, Cagnasso *et al.* (2010) used Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy to probe the interaction of phospholipids comprising phosphodiester or terminal phosphate functionalities with α-FeOOH (goethite) and α-Fe_2O_3 (hematite) surfaces, and to assess the impacts of zwitterionic, basic and acidic polar head groups. The visualization of the phospholipid-substrate system was also provided by transmission electron microscopy analysis. Interaction with iron (oxyhydr)oxides involves electrostatic attraction, phosphodiester H-bridging interactions, and P–OFe covalent bond formation. The relative strength of these interactions depends on the composition of the polar head groups and on

the type of iron oxide surface. From the results, a hypothesis suggests that these domains may mediate an initial bacterial cell interaction with iron oxides and iron hydroxyoxides through electrostatic attraction, followed by the formation of inner-sphere phosphate complexes on oxide surfaces.

In the field of prosthetic materials, early studies have suggested the use of lipid vesicles as models for the study of the biological calcification process (Goldberg and Boskey 1996, Vogel and Boyan-Salyers 1976). Extracellular membrane vesicles (matrix vesicles) contain phospholipids associated with mineralized material. These matrix vesicles seem to be involved in initial formation of hydroxyapatite crystals via the interaction of calcium and phosphate ions with phosphatidylserine (PS), an acidic phospholipid found in high concentration in the membrane bilayer. These investigations have demonstrated that PS-based formulation can promote calcification *in vitro* (Eanes 1989). Also, it has been shown that, when used as coating materials, PS-rich phospholipids can induce a fast mineralisation of the implant surface during incubation in simulated body fluids (Santin *et al.* 2006, Merolli *et al.* 2006). In recent studies, porous synthetic titanium was used as substrate for PS-based coatings (Bosetti *et al.* 2005, 2007). Results show that this model has no cytotoxicity, good cell vitality and osteoblast collagen type I synthesis for all the phospholipid coatings.

4. Conclusions

The investigation of the bio/substrate interaction mechanisms is important to determine the main forces ruling adsorption and binding, to understand how they operate and if there are multiple forces acting cooperatively to create the overall interaction.

New SPM investigations on ribonucleic acids in interaction with layered minerals have been reported and the most relevant studies concerning the interaction between nucleic acids, nucleotides, phospholipids, amino acids and mineral substrates have been here reviewed.

The understanding of the interactions at the nanoscale of fundamental biological molecules with mineral surfaces is of paramount importance for studies of biomolecules condensation from dispersed solutions, patterning and self-assembly, enzyme-biomolecules interactions, nano-biotechnology, biomedical and biomaterial sciences, soils and environmental sciences, and prebiotic chemistry.

Inter-disciplinary research complementing SPM-based methodologies and quantum mechanics simulations are suggested and fostered as effective approach for future scientific advances in the knowledge on biomolecule-mineral interaction. The present work is a first step towards a database of the main forces ruling adsorption and binding on minerals.

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