Quantum mechanical modeling as a virtual microscope to understand biomaterials

PIERO UGLIENGO – Dipartimento di Chimica & NIS Centre of Excellence, Università di Torino, Via P. Giuria 7, 10125 Torino, Italy. E-mail: piero.ugliengo@unito.it

Abstract. *The knowledge of atomistic details resulting from the X-ray diffraction by bulk crystals has dramatically changed the approach to chemistry and biology, paving the way to modern biochemistry, biotechnology and material science. Nevertheless, many important chemical and biochemical processes occur at the interfaces between a surface of an inorganic crystal and (bio)molecules, as in heterogeneous catalysis, chromatography or when biomaterials contacts proteins present in body fluids. The application of diffraction techniques to elucidate surface structural features at atomistic details have characterized only a small fraction of systems compared to bulk crystals, due to loss in periodicity, presence of surface defects and limited technical sensitivity, hindering our understanding of surface processes. Fortunately, in parallel with the development of experimental techniques, molecular modelling methods coupled with modern high performance computing facilities are now an essential tool to complement experimental data for processes occurring at surfaces, widening our fundamental understanding of interfacial phenomena.*

Keywords: quantum mechanical calculations, surfaces, molecular modeling, structure prediction

Riassunto. *La conoscenza dei dettagli atomici derivanti dalla diffrazione a raggi-X di cristalli ha notevolmente cambiato l'approccio alla chimica e alla biologia, aprendo la strada alla moderna biochimica, le biotecnologie e la scienza dei materiali. D'altra parte, molti processi biochimici importanti avvengono all'interfaccia tra le superfici di cristalli inorganici e le biomolecole, come in catalisi eterogenea, cromatografia o, quando un biomateriale entra in contatto con i fluidi corporei. L'applicazione delle tecniche diffrattometriche per chiarire i dettagli strutturali delle superfici è stata applicata solo ad un modesto numero di sistemi in confronto ai cristalli, a causa della ridotta periodicità, la presenza di difetti superficiali e la limitata sensibilità delle tecniche, impedendo, di fatto, la comprensione di molti processi che avvengono alle superfici. Fortunatamente, in parallelo con lo sviluppo delle tecniche sperimentali, si è assistito alla crescita notevole del ruolo della modellistica molecolare che, accoppiata con le enormi capacità computazionali dei moderni supercalcolatori, è diventata uno strumento essenziale nell'affiancare i dati sperimentali ampliando la nostra comprensione dei fenomeni all'interfase.*

Parole chiave: calcoli quanto meccanici, superfici, modellistica molecolare, predizione strutturale, biomateriali

The revolution of the accurate molecular structure determination

Modern chemistry and molecular biology are based on the detailed knowledge of the molecular structure. By "detailed knowledge", we refer to the determination of covalent bond distances in molecules with an accuracy within or better than 0.001 Å. In biological macromolecules, this level of accuracy is somehow inferior, perhaps, by one order of magnitude; nevertheless, this is still high enough to understand the conformation of proteins and nucleic acids in atomic details. The revolution in chemistry provided by the diffraction of X-ray by crystals is enormous. It is enough to think to the process that brought to the determination of the benzene structure, now straightforwardly introduced in primer chemical courses. Before

the application of X-ray diffraction to a single crystal of $(CH_3)_6C_6$ by Kathleen Lonsdale [1] in 1929, only speculations based on indirect chemical evidence of the benzene ring structure were put forward by many eminent chemists (Claus, Dewar, and Kekulé to quote a few). However, only the "unbiased analysis" (*i.e.* without imposing specific geometrical constraints based on some chemical intuition) of the experimental diffraction data by K. Lonsdale proved the chemical equivalence of the six carbon atoms at the vertices of an hexagon, separated by a C-C bond length of 1.39 Å. Since then, crystallographers have solved a huge and continuously growing number of chemical structures, as shown by the content of the three most important structural databases. The Cambridge Crystallographic Database (CSD) [2] contains, to date, more than 630000 structures of molecular crystals including at least one carbon atom in their formula (*i.e.* the benzene crystal structure); the Inorganic Chemical Structural Database (ICSD) [3], stores more than 170000 structures of inorganic crystals (*i.e.* the classical rock salt NaCl structure). Even more impressive is the Protein Data Bank [4], whose content is devoted to macromolecules of biological interest (*i.e.* the haemoglobin structure), including more than 100000 structures and steadily growing due to technological improvement in synchrotron X-ray radiation and data collection. Linus Pauling, probably the greatest chemist of the XXth century, was proving most of its brilliant ideas on different fields of chemistry based on accurate structural information. One of the most spectacular prediction was the structure of α-helix and β-sheet secondary units in proteins, both arrived at through the careful examination of the accurate structure of amino acids, the building blocks of proteins, by Robert Corey and Pauling himself. His influential book, "The Nature of the Chemical Bond and the Structure of Molecules and Crystals: An Introduction to Modern Structural Chemistry", published in 1939, build the whole chemistry framework on structural concepts and had (and still has today) a great impact on the way many generations of chemists think about molecules. It is clear that the knowledge of structural details at atomic resolution has allowed tremendous progress in molecular biology and material science, paving the way for new fields, like molecular pharmaceutics, nanomedicine and the whole nanotechnology.

From bulk crystals to crystallographic faces

We have highlighted the great progress made by the power of diffraction techniques to provide the atomistic details from the X-ray diffraction of bulk crystals. In chemistry, however, there are processes that occur at the exterior of a crystal, *i.e.* at its crystallographic faces. Selected examples in which the surface plays a key role are chromatography, heterogeneous catalysis and the interaction between biological soft-matter and biomaterials, just to mention few of them. To make progress in understanding interfaces at atomic scale, we need the same level of structural details attained for bulk matter. Unfortunately, the passage from 3D (bulk) to 2D (surface) dramatically reduces the level of accuracy of the structural details. Loss of symmetry, surface structural/substitutional defects, limited sensitivity and difficult to grow extended surfaces all limit the accuracy of the final structural model. In a recent review paper by van Hove in 2009 [5], he graphed the number of 2D accurate structures (*i.e.* with accuracy of 0.1 Å) up to 2003 year. Even if the collection of data is almost 10 years old, the conclusions are striking: when compared to the number of the 3D crystals (see above), no more than 1000 structures were determined by low-energy electron diffraction technique. Photoelectron diffraction, ion scattering, surface extended X-ray adsorption fine structure, X-ray standing waves and X-ray diffraction added only few hundred more.

The achievements of crystallography for regular 2D systems

Despite the described limitations, a number of powerful techniques allowed significant achievements in elucidating the atomic details in heterogeneous catalysis and self-assembly of organic molecules at highly regular surfaces. The work by Mitsuno *et al* [6] (see Figure 1) is an example of accurate characterization of a Copper surface on which Li atoms have been co-deposited: single steps and kinks with atomic resolution have been highlighted, providing robust structural models for the interpretation of the physico-chemical features of the composite surface. The most famous example of clever usage of the surface atomic

characterization is the work by G. Ertl, [7] Nobel laureate in 2007, on the elucidation of chemical steps transforming molecular hydrogen and nitrogen to ammonia by the catalytic action of iron surfaces.

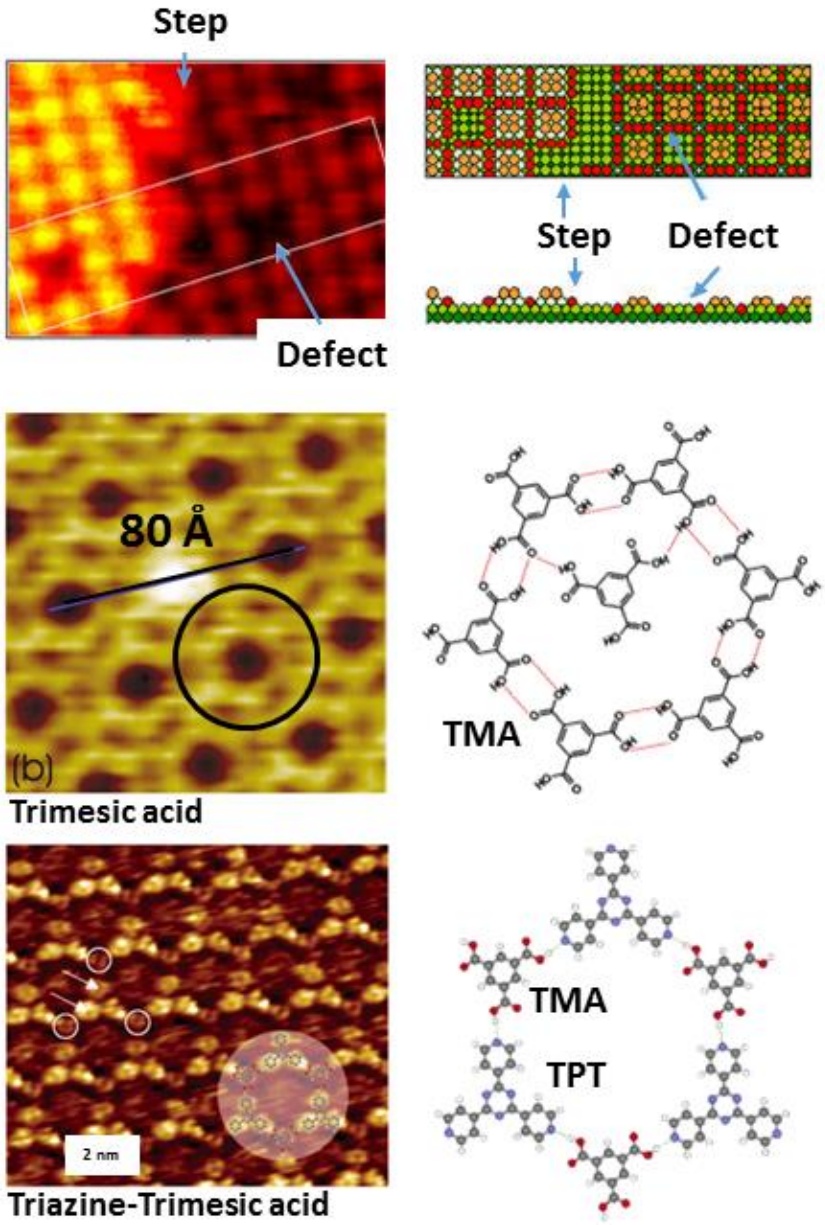

Figure 1. Top: 92 x 67 Å STM image of the Cu surface (dark color) with Li atoms (lighter colour) adsorbed on top (reproduced with permission from Ref. 6. Copyright 1997 American Chemical Society. Middle: STM image of 1,3,5-Benzenetricarboxylic TMA (Trimesic) Acid on a single crystal graphite surface under Ultra High Vacuum conditions (reproduced with permission from Ref. 8. Copyright Wiley-VCH Verlag GmbH & Co. KGaA). Bottom: STM topograph of the 2D TPT-1,3,5-tris(4-pyridyl)-2,4,6-triazine/trimesic acid-TMA cocrystal on HOPG (0001). The white circles indicate TMA molecules, and the arrows mark the center-to-center distance (~0.7 nm) between two pyridyl groups of one TPT molecule (reproduced with permission from Ref. 9. Copyright 2005 American Chemical Society).

More recent work, based on scanning tunneling microscopy, is from S. Griessl *et al* [8] on trimesic acid adsorption on graphite surfaces and L. Kampuschulte *et al* [9] on the liquid-solid nano-pattering surface obtained by co-adsorbing trimesic acids and substituted triazine molecules on a pyrolitic graphite surface. The first authors have been able to establish the cell parameters of the self-assembled trimesic acid at the graphite surface with an accuracy of ±1 Å and to arrive at the H-bond distances between the OH···O moiety

of the carboxylic groups to within 0.2 Å. In summary, these examples show that useful structural details are at hand from experiments involving 2D systems, provided that the studied surfaces exhibit high crystallographic regularity with minimum amount of structural and chemical defects.

Surfaces are key elements for the integration of biomaterials in the living body

The regularity of the ideal and clean 2D surfaces described above is entirely lost in systems contacting living matter. The difficulty in moving from a "surface science for catalysis" approach founded on techniques described above to a "surface science for biology" is highlighted in the classical work of Castner and Ratner [10]. To deepen the problem, let consider the case of a dental implant, in which a prosthesis made of a biocompatible element like metallic titanium, is embedded in bone or teeth hard tissues. Indeed, it is the implant surface (in this case a very thin TiO_2 layer) which is in direct contact with the body fluid and ultimately constitutes the layer through which immune response is regulated and mitigated (see Figure 2) [11].

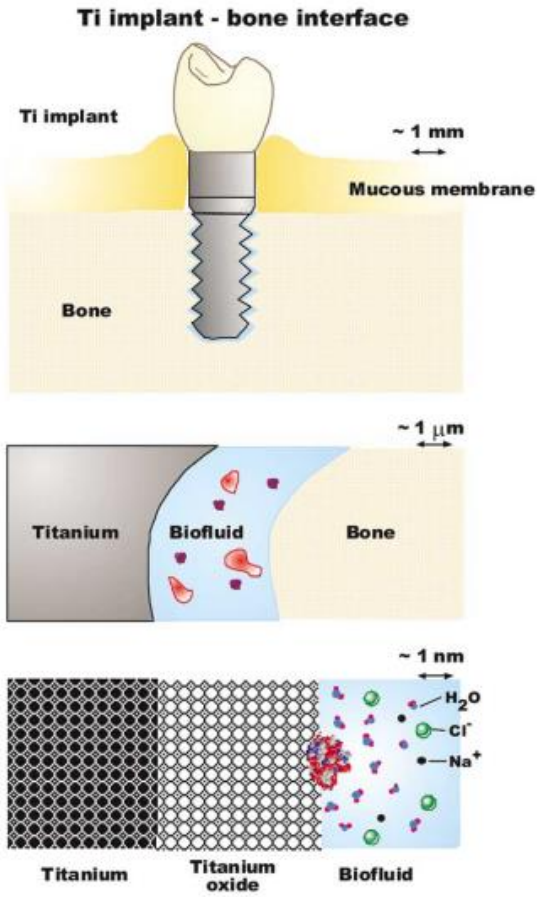

Figure 2. Schematic illustration of the interface between a dental implant and the jawbone into which it is implanted, at different magnifications. After the surgical procedure the surface is first exposed to water, then to proteins, and eventually to cells (reproduced with permission from Ref. 11. Copyright 2002 Elsevier).

The immune response triggered by an embedded biomaterial is a cascade of molecular events bringing the final implant to become part of the living body. The complexity of these events is so high that there is no hope to discover atomic and molecular details by the structural techniques described above. Even in a relatively idealized experiment, in which "simple" proteins are adsorbed on a clean and simple surface, the level of complexity remains exceedingly high for the determination of fine structural details. A beautiful example is from the work by Van De Keere *et al* [12] about the interaction of human plasma fibrinogen with commercially pure titanium as studied with atomic force microscopy and X-ray photoelectron spectroscopy.

Despite the great experimental achievement, only changes in the alignment of the protein domains are detectable (see Figure 3). A question then arises: are we stick to structural ignorance for a large fraction of phenomena, which are so important for the human health, like the development of new biomaterials?

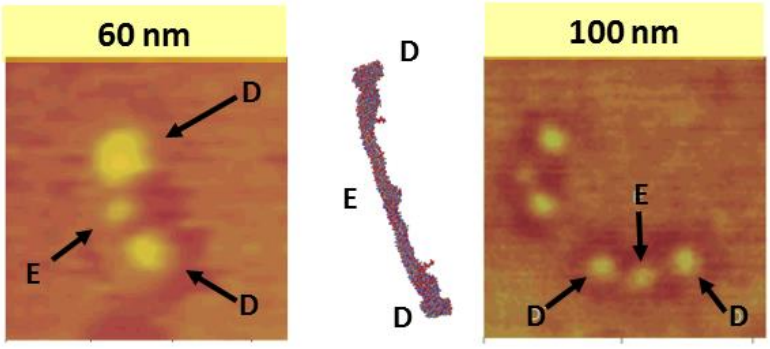

Figure 3. Left: AFM topography image of an elongated single HPF (human plasma fibrinogen) molecule adsorbed onto mica. Center: CPK model of the HPF highlighting the three domains. Right: High-resolution AFM phase image of single HPF molecules adsorbed onto the commercially pure Ti surface (reproduced with permission from Ref. 12. Copyright 2008 American Chemical Society).

Molecular modeling as a rescue for understanding complex systems

Complexity at the molecule/interface system prevents our understanding of fine details and of the processes occurring there. Basically, it the loss of long range order at the interface between surfaces and biological material that hinders the applicability of techniques based on X-ray diffraction. A possible way to tackle this problem is by computer simulation. This particular approach is becoming more and more popular thanks to the steadily increase in computer power provided by the Moore's empirical law, stating that the number of transistors in a chip doubles each 18 months. A simulation uses a *mathematical description*, or model, of a real system in the form of a *computer program*. This model is composed of equations that duplicate the functional relationships within the real system. When the model involves atoms and molecules it is the Schrödinger equation (SE), proposed in 1926, that dictates the behaviour of our model. Despite the outstanding accuracy of the SE in predicting the physico-chemical properties of simple systems, its applicability to model systems of size close to that of real systems has always been hampered by our ignorance in finding a rigorous solution. This is due to the multidimensional character of the wavefunction, which depends on the $3(n+m)$ coordinates of n electrons and m nuclei, *plus* the spin state of each electron. For instance, for the benzene molecule consisting of 42 electrons and 12 nuclei, the wavefunction depends on 162 spatial variables. Born and Oppenheimer, already in 1927, proposed a first important recipe to simplify the SE, by separating the electronic motion from that of the nuclei, in virtue of the large difference in their kinetic energy. This way, the SE reduces to electrons only, moving in the Coulomb field provided by the fixed nuclei. Another breakthrough was the Hartree-Fock (HF) approximation (around 1930), allowing to treat each electron as experiencing the mean field provided by the remaining electrons. Within this approach, the multielectron wavefunction can be factorized in simpler anti-symmetric products of monoelectron functions, called spin orbitals, the drawback being the loss of instantaneous electron-electron correlation. The venue of the electronic calculators in the early 50s allowed the solution of the SE for medium sized molecules thanks to new algorithms for solving the HF equations by Roothaan and Hall (RH). Today one can solve the RH-HF equations for a molecule with few hundred atoms on a commodity personal computer. The problem is that the RH-HF energy does not give the chemical accuracy needed to treat many important aspects of chemistry, like reactivity, conformational changes, charge-transfer complexes, weakly bound systems, excited states and many others. Progress to improve the HF solution was then achieved by the

development of post-HF methods (configuration interaction, Møller-Plesset perturbation theory, coupled cluster theory, etc.) able to recover the electron-electron correlation partly lost in the HF approximation. All these methods imply, however, a very steep increase in computing time compared to the RH-HF approach. Furthermore, their difficult implementation and the high demand of computer resources seriously hampered their applicability to real systems. Nevertheless, application to realistic systems of these post-HF methods has been recently achieved [13]. The way to escape from this impasse has been suggested in the 60s by Hohenberg and Kohn who, following early suggestions by Thomas and Fermi, proved rigorously that the wavefunction is not needed to arrive to the ground state energy of the system. Indeed, a much more simple function is needed, the electron density $\rho(\mathbf{r})$, from which, through the action of a special functional F one can get the ground state energy $E=F[\rho(\mathbf{r})]$ of the system. The simplification with respect to the SE is enormous: irrespective on the complexity of the molecular system to which it belong, $\rho(\mathbf{r})$ is a function of only three spatial variable and it is a physical observable, *i.e.* coincide with the electron density measured by crystallographers during accurate X-ray diffraction experiments. Since its discovery, the Density Functional Theory (DFT) has become the *de facto* standard in molecular modelling based on rigorous principles. The practical implementation of DFT, known as Density Functional Methods (DFM), received immense popularity and has been applied to treat molecules, solids (both crystalline and amorphous) and liquids with great success. Its success is based on recipes to find specialized functionals F, able to recover the electron correlation at the computational price lower than that of the HF calculation. Recent improvements to cope with dispersion interactions, still missing in standard DFM, has paved the way to model biological molecules rigorously as, still today, proteins and nucleic acids are mainly simulated through classical molecular mechanics and dynamics methods. Clearly, in order to run a simulation based on DFT, one needs the information about the nuclear coordinates. This information comes from structural determination through X-ray diffraction experiments, as described above. Indeed, the link between DFT and structural determination is profound and it would be almost impossible to run a simulation without some previous knowledge of the molecular or crystalline structure of the object under study.

The application of DFT to the simulation of biomaterial interfaces

Biomaterials are an ample class of materials, which may be of metallic, ceramic or polymeric nature. Irrespective on the specific class, their behaviour is such that they should be fully integrated in the living body when used to repair injured parts. As an example, bioglasses of ceramic nature are used to replace and repair injured bones. Among them, one of the most important is the Hench's bioglass, an oxide whose composition envisages mainly silicon dioxide with some content in Na, Ca and P elements. When contacting biological fluids, a complex series of reactions takes place at the surfaces of a bioglass (known as the Hench's mechanism [14]) which become covered by a thin layer of an almost crystalline hydroxyapatite mineral. Therefore, it is this material that ultimately will contact the biological material rather than a surface directly derived from the bioglass's bulk composition. Fortunately, the structure of hydroxyapatite has since long been solved by crystallographers so that DFM can be used to simulate the interaction of biologically relevant molecules with its crystal surfaces. The previous step in this kind of simulations, is to establish a ranking in the stability of each crystallographic face (indicated by its Miller indexes). This ranking is arrived at by comparing how much energy is spent by the generation of a specific (*hkl*) face when compared to the bulk crystal itself. This characterization allows choosing, on a thermodynamic ground, the most important faces exposed at real crystals, which are ultimately those in contact with the biological molecules. In the following, two kind of problems that have been addressed by the simulations will be described.

The role of water in the protein adsorption at hydroxyapatite surfaces

One of the key problems of difficult solution is to establish whether a body protein will contact the surface of hydroxyapatite of a given implant directly or through a water layer. The large size of the system complicates the modelling stage as, in principle, one should simulate a protein of thousands atoms immersed

in a large amount of explicit water molecules in contact with the hydroxyapatite surface. Techniques like *ab initio* molecular dynamics would then be used to see whether the protein would move from the water interior towards the surface. The whole process, as described above, is too complicated even for the most powerful computer and some reduction in complexity is needed. This step is an important and almost indispensable one in the practice of molecular modelling: the key point is to design a model simple enough to be tractable while maintaining the essential physical features of the original system. For the present case, the most drastic approximations are: i) adopt a single amino acid (*i.e.* glycine as the simplest one) as a model of the whole protein; ii) use the slab approach to simulate the extended surfaces of hydroxyapatite iii) use the minimum number of water molecules to micro-solvate the sites at the hydroxyapatite surface. The simulation would then establish, on an energetic ground, whether glycine will displace the pre-adsorbed water molecules from the hydroxyapatite surface to make direct contact with the surface itself or, alternatively, to get adsorbed on top of the layer of pre-adsorbed water molecules. One of the advantages of the modelling approach is that the physico-chemical properties of the hydroxyapatite surfaces can be analysed in details before studying the adsorption explicitly. For the adsorption of molecules, one relevant quantity is the electrostatic potential in vicinity of the surface.

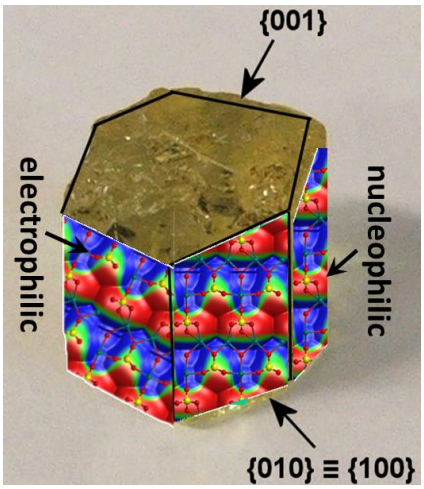

Figure 4. DFT electrostatic potential mapped on the {010} family of crystal faces of an hydroxyapatite crystal.

Figure 4 shows the corresponding maps, in which zones in red color represent nucleophilic region whereas the blue ones are with electrophilic character. Molecules would then adsorb following the principle of electrostatic complementarity, *i.e.* red region in molecular electrostatic map would pair with blue ones of the surface map. Following this clever strategy, it has been possible to simulate the whole process with DFM methods. The results are shown in Figure 5, in which the relative interaction energy tells us that glycine will prefer to make direct contact with the hydroxyapatite surface. In other words, the pre-adsorbed water molecules will be displaced by the incoming glycine molecule towards either different sites of the surface, or directly to functional groups of the glycine itself. Clearly, this result should be taken with caution, considering the drastic simplifications of the adopted model [15]. Improvements are obviously possible at increasing computational cost, by both enlarging the number of water molecules and moving from single amino acids to oligopeptides.

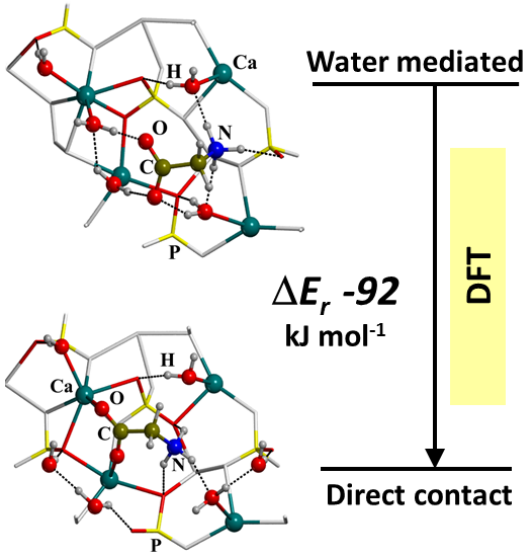

Figure 5. DFT total energy difference between glycine *plus* water adsorbed on the (001) surface of hydroxyapatite. Top: glycine adsorbed through a pre-adsorbed water layer. Bottom: glycine directly adsorbed on hydroxyapatite and displacing water molecules.

Protein conformational change due to adsorption on hydroxyapatite surfaces

The next step is to see whether the adsorption of protein at hydroxyapatite surfaces may induce important conformational changes in the adsorbed protein itself. This is an important issue as conformational changes induced by the prosthesis may modulate the biological activity of the protein which, in turn, may trigger specific biological responses of the body. A classic example is statherin, a small protein made by 43 amino acids. When in a folded conformation, its role is to hinder the precipitation of calcium phosphate in the oral fluids. However, when contacting the hydroxyapatite surface of the teeth enamel, it undergoes a partial unfolding to increase the lubricity of the enamel itself. Can the simulation shed some light on understanding the role that active sites at the hydroxyapatite surfaces may play in the folding/unfolding process? The specific case of statherin has been addressed recently by Makrodimitris *et al* [16] by combining NMR and molecular mechanics simulation. The whole system is, again, too big to be attacked by DFM as such. We simplify the system by considering an oligopeptide made by twelve (G_{12}) glycine monomers to mimic statherin [17]. Calculations revealed that its preferred conformation, when isolated, is a random coil highly folded state. This would mimic the statherin when folded in oral fluids, whereas the hypothetic extended α-helix conformation would simulate statherin in its extended conformation due to the adsorption on hydroxyapatite surface. The G_{12} as such has, however, little electrostatic affinity to the surface of hydroxyapatite, as shown in Figure 6 by the comparison between electrostatic potential maps of the two sub-systems. To increase the electrostatic affinity between the two, the oligopeptide has been mutated by substitution, respectively of two and four glycine amino acid with lysine (K) and glutamic acid (E). These residues have basic (K) and acid (E) character, respectively. Indeed the mutated $G_2KG_6EG_2$ and $G_2KG_2KEG_2EG_2$ oligopeptides sport dramatically changed electrostatic features compared to the pristine G_{12} peptide (see Figure 6). For all considered oligopeptides, the most stable conformation is the random coil folded state, for a peptide free from any kind of external interaction. The actual calculations adopt robust but relatively expensive DFM so that, to speed up the calculation, the oligopeptide is treated in gas-phase, *i.e.* in an artificial state when compared to the usual water environment. The energy penalty to pass from the random coil up to α-helix is around 90 kJ mol^{-1} for both mutants. At this point, the question to be addressed by the modeller is: "will the interaction of mutants with the hydroxyapatite surface stabilize the extended α-

helix conformation over the folded one?" A positive answer will tell us that the electrostatic affinity between surface and peptide is sufficient to gain enough energy to compensate the cost of unfolding and provide a physically sound argument to understand the role of enamel surfaces for the statherin case. Results of the calculations showed that only for the second mutant $G_2KG_2KEG_2EG_2$ the interaction with the (001) hydroxyapatite surface is strong enough to stabilize the extended α-helix conformation over the folded state. The reason is simple: the number of favourable electrostatic contacts between the mutant and the surface double when passing from $G_2KG_2KEG_2EG_2$ to $G_2KG_6EG_2$. The principles behind these results are more general than the specific case designed by the modeller and provide important clues to design peptides affine to specific surfaces.

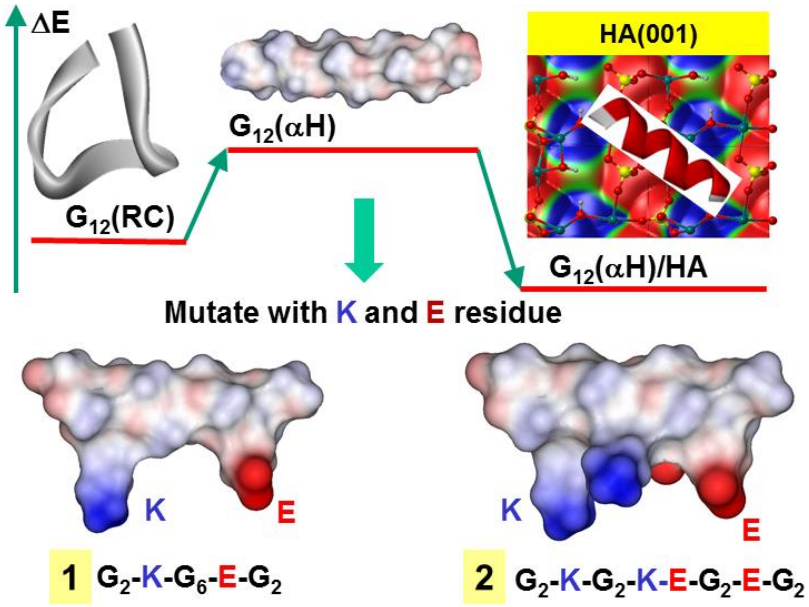

Figure 6. Random coil (RC) and α-helix (αH) of the G_{12} oligopetide. DFT electrostatic potential maped on the CPK molecular surface. Mutation with lysine (K) and glutamic acid (E) deeply change the electrostatic potential improving the affinity towards the hydroxyapatite surface. The energy graph highlights the stabilization operated on the α-helix with respect to the random coil conformation by the interaction with the hydroxyapatite surface.

Conclusions

In this work, we provide an account about the role that computer modeling based on density functional theory can provide in terms of structural and energetic features for cases where the classical experimental techniques based on X-ray diffraction are more or less blind due to the complexity of the system of interest. Two examples are discussed, both related to the characterization of the interface between biomaterials of ceramic nature and biomolecules. In the first one, it is shown how computer modeling can shed light on the long-standing problem of water-mediated interaction between biomolecules and a surface of hydroxyapatite, which is representative of the bioglasses family. Calculations revealed that the simplest amino acid glycine does prefer to be attached directly to the hydroxyapatite surface by displacing the pre-adsorbed water molecules. The second case study is the conformation change of a protein induced by the adsorption on the hydroxyapatite surface. This phenomenon is extremely relevant in living body, as the contact with a specific surface can trigger a conformational change (from random coil towards an extended conformation) with specific biochemical functionality, as it is the case of statherin in oral fluids. Computer modelling requires approximations, both for the model representing the "real system" and for the adopted

theoretical framework. Results are useful when the model is cleverly designed to capture the essential physico-chemical features of the real system, provided that the methodological approximation are well balanced to avoid systematic errors.

References

[1] LONSDALE, K., *The Structure of the Benzene Ring in C6(CH3)6*, Proc. R. Soc. London, A 123 (1929), 494-515

[2] *Cambridge Structural Database*, http://www.ccdc.cam.ac.uk/pages/Home.aspx

[3] *ICSD - Inorganic Crystal Structure Database*, http://www.fiz-karlsruhe.de/icsd.html

[4] *RCSB Protein Data Bank*, http://www.rcsb.org/pdb/home/home.do

[5] VAN HOVE, M. A., *Atomic-scale structure: From surfaces to nanomaterials*, Surf. Sci. 603 (2009), 1301-1305

[6] MIZUNO, S., TOCHIHARA, H., MATSUMOTO, Y. and TANAKA, K.-I., *STM observation of restructured Cu(001) surfaces induced by Li deposition*, Surf. Sci. 393 (1997), L69-L76

[7] ERTL, G. *Ammonia Synthesis - Heterogeneous*. In Encyclopedia of Catalysis, ed. John Wiley & Sons, Inc., 2002

[8] GRIESSL, S., LACKINGER, M., EDELWIRTH, M., HIETSCHOLD, M. and HECKL, W. M., *Self-Assembled Two-Dimensional Molecular Host-Guest Architectures From Trimesic Acid*, Single Mol. 3 (2002), 25-31

[9] KAMPSCHULTE, L., GRIESSL, S., HECKL, W. M. and LACKINGER, M., *Mediated Coadsorption at the Liquid−Solid Interface: Stabilization through Hydrogen Bonds*, J. Phys. Chem. B 109 (2005), 14074-14078

[10] CASTNER, D. G. and RATNER, B. D., *Biomedical surface science: Foundations to frontiers*, Surf. Sci. 500 (2002), 28-60

[11] KASEMO, B., *Biological surface science*, Surf. Sci. 500 (2002), 656-677

[12] VAN DE KEERE, I., WILLAERT, R., HUBIN, A. and VEREECKENT, J., *Interaction of human plasma fibrinogen with commercially pure titanium as studied with atomic force microscopy and X-ray photoelectron spectroscopy*, Langmuir 24 (2008), 1844-1852

[13] SPARTA, M. and NEESE, F., *Chemical applications carried out by local pair natural orbital based coupled-cluster methods*, Chem. Soc. Rev. 43 (2014), 5032-5041

[14] HENCH, L. L., SPLINTER, R. J., ALLEN, W. C. and GREENLEE, T. K., *Bonding Mechanism at the Interface of Ceramic Prosthetic Materials*, J. Biomed. Mater. Res. Symp. 2 (1971), 117-141

[15] RIMOLA, A., CORNO, M., ZICOVICH-WILSON, C. M. and UGLIENGO, P., *Ab initio modeling of protein/biomaterial interactions: competitive adsorption between glycine and water onto hydroxyapatite surfaces*, Phys. Chem. Chem. Phys. 11 (2009), 9005-9007

[16] MAKRODIMITRIS, K., MASICA, D. L., KIM, E. T. and GRAY, J. J., *Structure Prediction of Protein-Solid Surface Interactions Reveals a Molecular Recognition Motif of Statherin for Hydroxyapatite*, J. Am. Chem. Soc. 129 (2007), 13713-13722

[17] RIMOLA, A., ASCHI, M., ORLANDO, R. and UGLIENGO, P., *Does Adsorption at Hydroxyapatite Surfaces Induce Peptide Folding? Insights from Large-Scale B3LYP Calculations*, J. Am. Chem. Soc. 134 (2012), 10899-10910