Biomolecules as soft matter surfaces

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ARTICLE INFO

ABSTRACT

Soft matter surfaces, such as organic layers or polymer surfaces, are dynamical and inhomogeneous (only in special cases crystalline). In contact with solutions, the situation becomes more complex, but still addressable with Surface Science methods. In single molecules and supramolecular assemblies, a large fraction of the molecule forms the surface. Can a single (bio) molecule be interpreted as a soft matter surface? A plant virus with a well-defined tubular structure demonstrates the problems, for which molecular dynamics simulations can give some answers.

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1. Introduction

Surface Science is dealing with ever more complex surfaces. This concerns especially the surface chemistry: self-assembled monolayers (SAMs) of all types and adsorbed layers composed of rather complex molecules can be prepared in high quality, and also analyzed with great precision and control. Moreover, biologically functional entities such as adsorbed proteins have joined this research topic. For such cases, and whenever the thickness of adsorbed organic layers on solid substrates surpasses a certain limit of some nm, we can address the adsorbate as "soft matter". Soft matter surfaces with relatively simple structure and chemical properties, such as long-chain SAMs or bulk polymer surfaces, have been analyzed for many years. Concerning application, a main driving force is the widespread use of polymers, whose interfaces with air, water and various solids are of utmost technical importance. However, a soft surface has several important drawbacks for a detailed analysis, as we know it for defined crystal planes of inorganic matter: soft matter is usually nonconductive, hence poses problems for electron-based techniques, and it has a dynamic surface. This means that we have to give up the conventional thought of experimenting with homogeneous and immobile surfaces.

2. Bulk polymer and SAM surfaces

Let us first examine the classical case of flat surfaces of macroscopic extension. This might be surfaces of bulk polymers or of SAMs (Fig. 1) immobilized on flat substrates such as single crystal

faces of gold or silicon or oxides. SAMs are one or a few molecular diameters thick, and polymers can often be prepared as thin layers [1]. For such samples, electron- and ion-based Surface Science techniques can be used advantageously (e.g. Auger spectroscopy, photoemission, ion scattering). SAMs show conformational changes in the molecular structure (Fig. 1a) [2] e.g. upon heating, while polymer and polymer surfaces become mobile above their respective glass transition temperatures, owing to the meandering motion of the chains, and aided by the available free volume. The Faupel group was able to show this elegantly by analyzing the penetration of metal particles into polymers [1]. For SAMs the penetration of metal atoms is well known [3], an effect based on lateral disorder: usually SAMs are very well ordered in the surface normal, but often this is not the case in the surface plane. This, together with the conformational defects, facilitates metal atom penetration. Thiol SAMs with amine and amide functional groups form dense layers, but exhibit high degrees of disorder in their carbon backbone; a phenomenon that depends on temperature, on surface charge (or electrochemical potential), and on the chemical environment (Fig. 1b), especially on the presence or absence of water [2,3]. Despite such structural disorder, SAMs with aine, amide and carboxylate groups can be used to construct well-defined metal islands [4] and even complete metal layers "on top", i.e. adsorbed on the chemically modified SAMs without contact to the substrate [5]. The driving force is here the interaction of the SAM end groups with either metal ions (precursors for the metal) or the metal itself.

For probing SAMs and thin organic films, a large part of classical Surface Science machinery can be used, e.g. X-ray diffraction, photoelectron spectroscopy, scanning probe techniques, or macroscopic wetting measurements (capillary forces). Ion scattering and secondary ion mass spectroscopy prove to be especially useful

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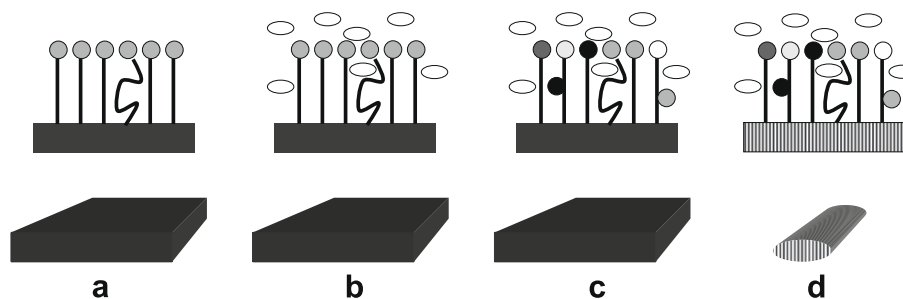E-mail address: a.bittner@nanogune.eu (A.M. Bittner).

Fig. 1. Models of soft matter surfaces. The lines represent either short methylene chains as in organic monolayers, or polymer chains, or proteins. The ends of the chains carry a head group that binds to the surface, and a free end group (dark circle). (a) SAM or polymer layer with a defect, adsorbed on solid surface. (b) As (a), but interacting with a solvent (ellipsoids) in various places. (c) Adsorbed biomolecules with a variety of chemical groups (circles), surrounded by water molecules. (d) Biomacromolecule, e.g. protein assembly, with the same surface structure as (c).

for organic layers [6,7]. However, these techniques are usually slow in the sense that they are unable to follow the appearance of defects in a SAM or in a polymer layer, e.g. during buildup of a metal structure, or during any other chemical reaction. Thus the dynamical features of soft matter under such conditions are as yet not well known. Principally, optical techniques such as infrared or sum frequency spectroscopy are applicable here [2,3]. They are able to achieve good time resolution (ps to ns scale for changes of molecular conformation), e.g. by pump-probe approaches. One can expect that research into details of structural changes will be based on optical methods.

3. Biomolecules

Principally, the same arguments as for SAMs and polymers can be invoked for immobilized biomolecules (Fig. 1c), and the same Surface Science techniques can be used. However, most biomolecules require for preserving their shape and especially their functionality water or aqueous pH buffer, which renders many Surface Science methods not useful. While substantial progress has been achieved by working in a slab of ice, or with an ultrathin water layer, generally one should use in situ methods, where the molecules are in contact with bulk water. The best developed methods are based on X-ray and on scanning probe techniques, but as yet not many systems were investigated. One of the biggest problems is the control of orientation on the surface, and hence the often observed loss of functionality – e.g. when active sites of proteins bind to the substrate, or when protein adsorption causes large structural changes. It should be mentioned that the requirement of the contact to water extends also to calculations and simulations, where the molecule in question should be surrounded by a box filled with model water molecules [8].

4. Single molecules

From above arguments we can conclude that the Surface Science of soft and biological matter in thin layers is well developed, with the discussed restrictions and requirements [7–10]. In the following let us consider how to approach a surface that is curved and extends only over very small areas: Can we probe a single "soft object", in other words **a single (large) molecule or a single supramolecular structure** (Fig. 1d), and interpret the experiments in terms of a surface? As yet, this interpretation is rarely invoked in a Surface Science context [8]; notable exceptions are carbon nanotubes and colloidal particles [10] – chemical groups and soft matter layers on these materials are of great technological relevance for obtaining stable dispersions, or for bestowing chemical functionality.

Molecules are synthesized in parallel, i.e. a very large number of identical or at least very similar objects are obtained. Hence traditionally ensemble data (averages) rather than properties of single molecules are analyzed, e.g. the chemical composition by elemental analysis, the presence of chemical groups by nuclear magnetic resonance and infrared spectroscopy, the hydrodynamic diameter by light scattering, and the mass by electrophoresis. Measuring the properties of single molecules leaves us comparatively few experimental options, often related to nanoscale science, e.g. size by electron microscopy (if water can be evaporated without destruction), diameter (height) by scanning probe microscopy, chemical composition by nano optical methods. Many of these methods require some form of adsorption of the molecule on a solid substrate – and the interaction of the substrate with the molecule can change its properties. Despite this problem, the obtained data relate largely to the molecular surface, since almost all atoms are located at the surface [10].

In the case of single biomolecules, candidates for future research are especially those that are sufficiently stable in terms of temperature, mechanics and chemistry to sustain prolonged exposure to the measurement probe (light, electrons, and mechanical contact). In this respect, nucleic acids, sugars, and proteins assembled into cages, tubes and fibers are most valuable, while many non-assembled units (especially proteins) are stable only in a small range of temperatures and pH values. Simple viruses (nucleic acid with proteins) and various biological fibers (DNA, microtubule, spider silk) are prime examples. A problematic issue is the loss of biological relevance in two cases. First, neglecting dynamical aspects means overemphasizing static structures, while all biological processes are based on dynamics in the relevant molecules. A good example is the replacement of the "key-lock" interpretation of enzymatic catalysis (molecule fitting into adsorption sites on a protein surface) by the "induced fit" view (protein changing its structure to accommodate a molecule on its surface). Second, structure and the dynamics of a biomolecule usually depend heavily on water. For example, some water molecules are so tightly linked to proteins with hydrogen bridges that they can be detected by diffraction techniques. Their average residence time is hundreds of ps, as compared to loosely bound water with 5–50 ps (a typical vibration in a chemical group requires only tens of fs) [11,12].

5. Tobacco mosaic virus

In the following we will discuss three topics on experiments with Tobacco mosaic virus (TMV), a protein–RNA nanotube. This plant virus is the first detected virus, and the one characterized in greatest detail. Moreover, it is harmless for mammals, and tolerates a range of pH values (from <3 to >8) and temperatures up to

90 °C, which facilitates handling. TMV is composed of about 2100 identical copies of the coat protein, TMVCP, in a 300 nm long helical assembly, in which a single strand of RNA is buried. The maximal outer diameter is 18 nm, but a helical groove ("constriction") runs along the external surface. The channel is only 4 nm wide and under ambient conditions filled with water. TMV adsorbs on a variety of substrates (gold, graphite, glass, oxidized silicon wafers). It will emerge that the surfaces of the tube (external surface and channel wall) can on the one hand be treated and analyzed in ways that are completely parallel to Surface Science; on the other hand the flexibility turns out to be a surprising feature.

The first example concerns the **chemical modification of TMV's external surface** achieved in the Francis group [13]: a diazonium compound (ionic and thus solvated) couples selectively to the 2100 Tyr-139 residues in TMV. Only tyrosine contains a phenol moiety which allows the diazotation. However, TMVCP contains four Tyr residues (Fig. 2), so the preference for only one of them gives this work much relevance as one of the few examples of highly selective reactions on a virus, without relying on antibody biochemistry. It is however hard to understand why only one residue is reactive. A possible reason is hindered diffusion/accessibility of three of the residues. This could protect Tyr-70 and Tyr-72, which are located deep inside the protein bulk (Fig. 2a), but Tyr-2, which is easily accessible on the outer coat of the virus (Fig. 2b), does not react either. The reason is likely that Tyr-2 is buried in a hydrophobic environment and not accessible for solvated species at its reactive ring positions. In addition, hydrophilic channels allow accessing Tyr-139 - here the flexible nature of the protein and of the protein assembly comes into play, which is not at all reflected in the static model. Indeed Tyr-139 can be accessed from the deep recess (the constriction in Fig. 2b) between the helically arranged proteins when the proteins shift their position slightly. This process is very hard to detect or to simulate; one would expect a timescale of ns to μs. We have recently started simulations on the ps time scale that can visualize minor molecular reorientations of Tyr-139 in presence of water (Fig. 2c).

The second example concerns the **metallization of TMV's 4 nm wide inner channel** [14]. The prerequisite for metallization is binding a catalyst (precursor), usually a Pd(II) complex, to the inner channel wall. As obvious from Fig. 3a, there are no basic residues in
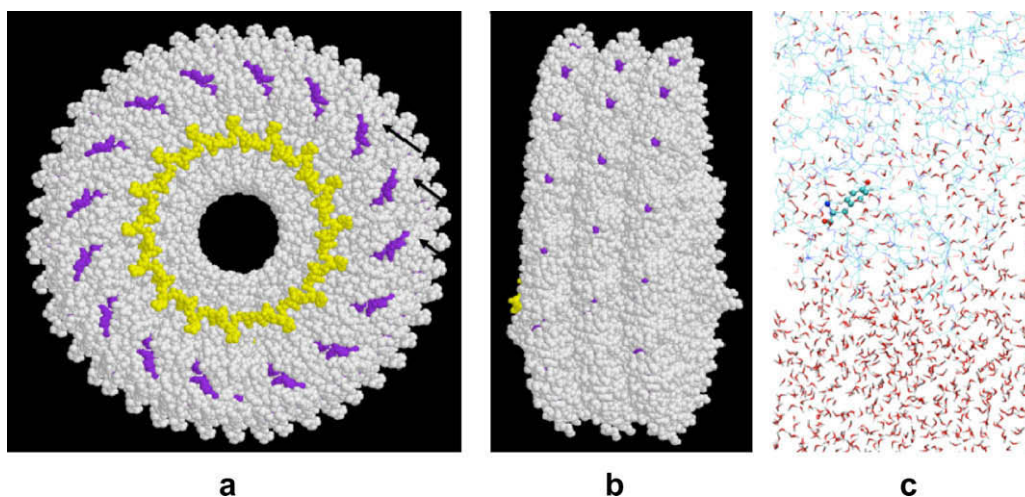

Fig. 2. Static models of Tobacco mosaic virus (TMV), showing 49 of the 2100 proteins in a van der Waals view, excluding the outermost four amino acids on the external surface. Based on X-ray diffraction, see pdb database (www.pdb.org,file2tmv, and www.ebi.ac.uk/msd-srv/pqs); MDL Chime software. (a) Axial view of the 18 nm wide cross-section, showing the 4 nm channel, the buried RNA helix (bright), and Tyrosin (Tyr) residues (dark spots) Tyr-70, Tyr-72, and Tyr-139 (arrows). (b) Side view of the external surface (18 nm diameter) showing Tyr-2 spots and the (helical) constriction, where the diameter is smaller than 18 nm. (c) Snapshot from a molecular dynamics simulation of a part of the TMV coat protein. Tyr-139 is shown as ball-and-stick model. Only very few water molecules, connected to water in the constriction (bottom part), can reach the tyrosin. (Model: 2766 atoms as TIP3P-C water molecules, 2513 atoms in the protein, 300 K, 1 bar, 3.5 nm × 6.4 nm × 3.6 nm box with reflecting sides, atoms in 0.1 nm vicinity of box boundary fixed, 0.05 fs steps, CHARMM potentials and electrostatics taken into account).
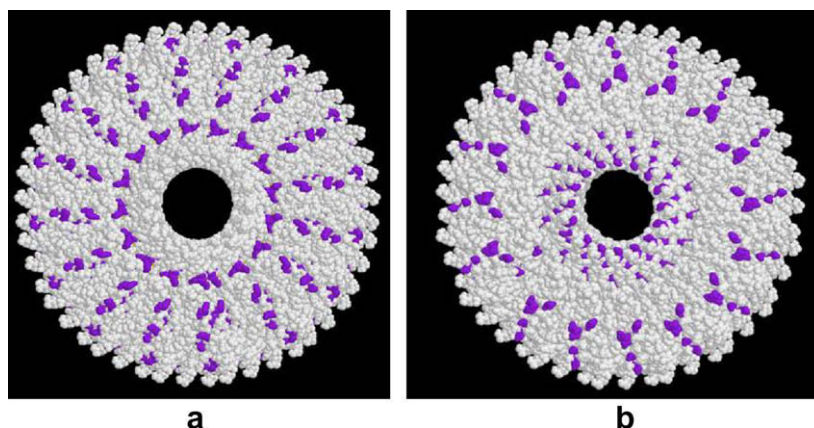

Fig. 3. (a) As Fig. 2(a), but all basic residues are marked, the innermost being Arg-90 and Arg-92. The apparently solid inner channel walls consist of flexible loops; Arg-90 and Arg-92 are not in the loop center, but still a part of the loop (b) Only acidic residues are marked; some are included in the loops and thus form the channel wall.
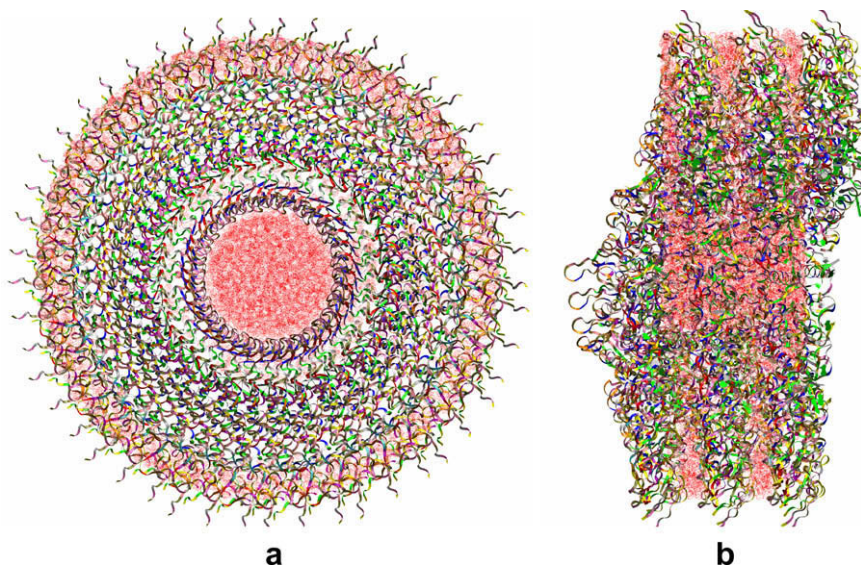
Fig. 4. Molecular dynamics simulation (526,513 atoms) of TMV and water after 3.6 ps in 0.1 fs steps (300 K, 1 bar, 22.4 nm × 22.4 nm × 14.8 nm box), comprising protein subunits with the corresponding RNA strand. Each water molecule is marked as a red cloud; most are removed to allow a view inside the box. (a) The water penetrates only into open spaces and hydrophilic patches; the hydrophobic cores of the proteins seal off the central channel. The channel is clad with slowly moving, densely packed loops of the coat protein. (b) Water penetration into the constrictions.

the channel, which would be very suitable. However, Arg-90 and Arg-92 are located at the end of the protein loop that clad the channel wall. The question is whether the dynamics of the loop could cause local defects (similar to Fig. 1), allowing Pd(II) to bind here. Alternatively, acidic groups (Fig. 3b) also show some affinity to Pd(II), and due to their location directly on the channel wall could bind Pd(II) much faster. The (static) structural model suggests a rather tight packing of the loops. Indeed our MD simulation (snapshots in Fig. 4) suggest only relatively slow and concerted movements of the loops, leaving them so densely packed that they do not expose Arg-90 and Arg-92 to the channel. Note that this information is not directly available from structural studies of TMV, in part because they require vacuum and resolve only a few very tightly bound water molecules, but mainly because they cannot provide information on defects and dynamics. Despite the short simulation time, which is still in the equilibration range, Fig 4a also shows that the water structure in the channel is not much disturbed by the channel walls (the loops), and that not all parts of the loop can be accessed by water. Fig. 4b focuses on the external surface of TMV where water penetrates the deep constrictions (see Fig. 2b and c) on the virus surface.

The third example relates to the observation that the height of TMV, found by careful noncontact scanning force experiments [15], corresponds to the expected 18 nm of the molecular model only when TMV is adsorbed on hydrophobic graphite. In this case, the interaction of the strongly polar TMV surface with the substrate is minimized. When TMV is adsorbed on hydrophilic surfaces such as glass, mica or surface-oxidized silicon, the height is below 15 nm, which is due to maximization of hydrogen bonds and dipolar interactions with COOH and OH groups on TMV. Lee et al. [16] elucidated the suspected **deformation** in detailed X-ray diffraction experiments. The flexibility of the apparently rigid TMV is so large that even the inner channel is compressed into a roughly elliptical shape. In other words, Figs. 2–4 with their circular cross-section are not applicable (too many atoms), here. Note that on this scale (>5 nm) MD is usually not applicable (too many atoms), and macroscopic elasticity theories might be invoked.

6. Conclusion

The examples show that soft matter and especially protein surfaces are prone to surprises, caused by the combination of the plethora of chemical functionalities, complex structure, and dynamics (flexibility). Even in protein assemblies such as viruses, a large fraction of the amino acids is exposed and can be addressed as soft matter surface. Rational modifications or chemical reactions are very hard to develop. Here the combination of experiments with molecular dynamics simulations on a very long time scale can help. Inspiration can be provided from the highly selective interaction of enzymes with molecular substrates (reaction partners), which relies not only on specific interactions ("key-lock"), but also on dynamical structure changes ("induced fit").

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