

Self-assembled Film of Cholesterol Molecules on the Au(111): An STM Study

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ABSTRACT

Self-assembled films of cholesterol were prepared on Au(111) surface and characterized by STM at nanometric level. In dependence of the concentration, two type of films were observed: one in the form of molecular monolayer and second with molecules organized in the square boxes, as a building blocks of the assembly layer. Both arrangements were characterized in great details which offers new insides into determination of molecular position, conformation and mechanism of the film growth. Accommodation of the cholesterol into the self-assembly layers was explained by help of molecular models developed in this study. The obtained finding are interesting from the fundamental as well from the technological point of view, in terms of better understanding of the process related to the functionalization of the metallic surface, and future design of the nano-scale devices (i.e. bio-medical sensors).

Keywords: cholesterol, self-assembly, scanning tunneling microscopy, molecular adsorption.

INTRODUCTION

Cholesterol is a molecule of special importance in medicine, biology and biochemistry, due to its role in the human body and relation to different diseases. In the past, the biologically important aggregates of cholesterol: LDL and HDL, were studied extensively [1]. However, there is a considerable lack of information about adsorption phenomena of a molecular cholesterol and mechanisms of interaction with other molecules and substrates, which could be a crucial point in development of new sensors. One of the primary task is a visualization and identification of the individual molecule of cholesterol at different substrates. We believe, it could be achieved by using modern high-resolution tools, as it is the Scanning Tunneling Microscopy (STM) [2-4]. STM could visualize surfaces of different materials, including materials of biological origin, at molecular and atomic scale. Imaging is based on direct, real space and real surface observations, and works in variety of media. Beside topography and atomic structure determination, it also offers characterization of the surface

electronic properties. However, the lack of experience in respect to preparation and handling of the biological material during the STM imaging is what makes this task rather difficult. Our study is based on development of new methodology for preparation and visualization of monomolecular thick films of cholesterol consisting of individual molecules or supramolecular aggregates. Films were prepared on the Au(111) substrate, using different solvents: water, methanol and ethylene glycol, in different concentration range. Imaging was carried out by STM (ex-situ, in air) at the nano-scale level.

In dependence of the preparation conditions and concentration, the STM images revealed cholesterol film in a form of closely packed monolayer of the individual molecules or at lower concentrations as the self-assembled boxes. Using the precise analysis tools of the STM software and different molecular modeling programs, we were able to determinate the exact position of individual molecules in the adsorbed layer, as well as size and form of the cholesterol box features. Detailed analysis also show that individual molecules adsorb with hydrophilic part of cholesterol, to the gold substrate.

The obtained results are interesting from several points of view. First of all, we believe it is the first report of the successful visualization of the cholesterol film at the molecular level. Note that imaging of lipids with STM technique is a rather difficult task. Formation and characterization of the cholesterol supramolecular assemblies (boxes) at the Au(111) substrate is very promising issue from the point of the surface functionalization and design of the nano-scale devices.

METHODOLOGY

The cholesterol samples (Aldrich, 99+%) for STM analysis were prepared from 2×10^{-4} M and 8×10^{-12} M solution of cholesterol in methanol (Aldrich, 99.93%). Molecular adlayer were formed directly on the gold substrate with (111) orientation (Berliner Glass Co) [5], using spin-coating technique at 37 C. Molecular films were visualized by Scanning Tunneling Microscopy (STM), Nanoscope IIIa and Nanoscope IV, Veeco, USA, at air (ex-situ conditions) and the room temperature (20 C). All glassware was cleaned in chrome-sulfuric acid and rinsed with Milli-Q water.

RESULTS AND DISCUSSION

Chemically, cholesterol is molecule (74 atoms) consisting from a cyclopentanoperhydrophenantrene core, a hydroxyl group in carbon number 3, a lateral chain of 8 carbon atoms, a double bond in C-5 position and five methyl groups in C-18, C-19, C-21, C-26 y C-27 positions, respectively. Due to its specific structure cholesterol poses amphipathic character. Most part of the molecule is hydrophobic (water insoluble), except the hydrophilic part: OH-terminal [6]. Therefore, inside the human body cholesterol is transported by lipoproteins which are a combination of macromolecules like proteins, triglycerides and phospholipids. The most common forms of lipoproteins are LDL and HDL. The excess of accumulation of lipoproteins inside arteries it is known as arteriosclerosis disease [7].

Crystallographic data (X-ray work) revealed cholesterol crystal phase with triclinic symmetry, with one of dimension: 1.71 nm [8,9], which is very close to molecular size calculated in our study by PC SPARTAN plus software: 1.7 nm (longitude), 0.4 nm width and 0.4 nm height (Fig. 1).

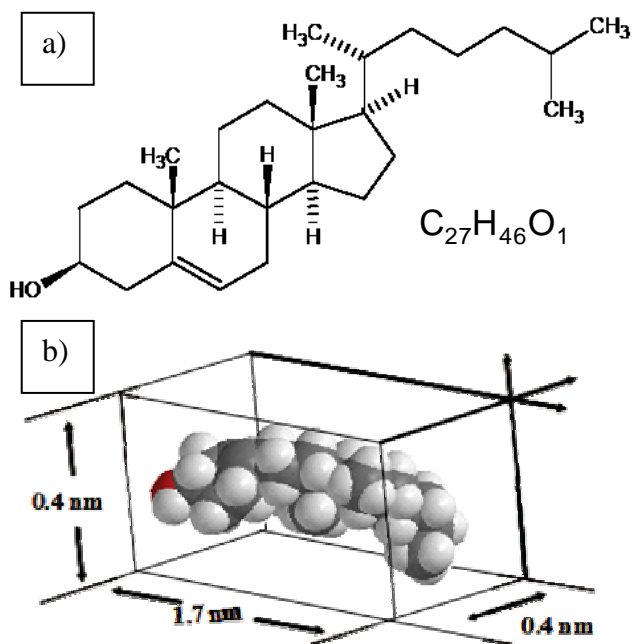


Fig. 1. Cholesterol structure (a) and dimensions (b) calculated using the PC Spartan plus molecular model.

As a first goal we focused on the cholesterol monolayer formation, from a very diluted solutions, well below the CMC point. Different solvents were used to prepare molecular monolayer, however the best results were obtained by water and methanol for cholesterol concentrations below 1×10^{-4} M. Monomolecular thick film was prepared by putting a micro size drop on the clean surface of the gold substrate. After a few minutes of drying,

at the room temperature, cholesterol film was ready for visualization by STM. Fig. 2, shows surface of the clean Au(111) substrate with characteristic large and atomically flat terraces, separated by few monoatomic steps.

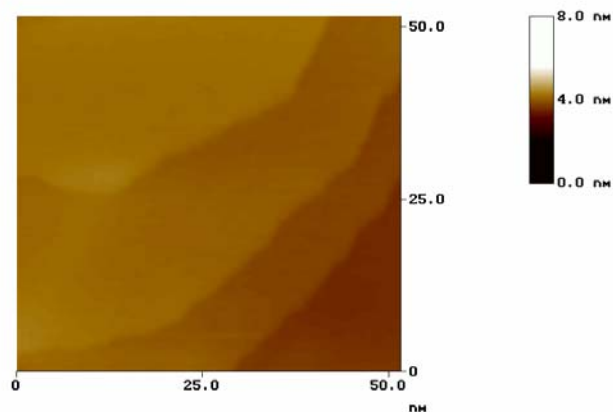


Fig. 2. STM image of the clean Au(111) substrate with characteristic flat terraces.

Contrary, the Au(111) surface covered by monolayer of the cholesterol molecules shows completely different topography. Terraces are more rounded and full of large vacancies of different size, especially in the middle of the substrate. The depth of the observed vacancies is about 0.25 nm, as a height of a gold monoatomic step. It clearly indicates that only a single monolayer of cholesterol was formed at the gold surface. For more details se Fig. 3.

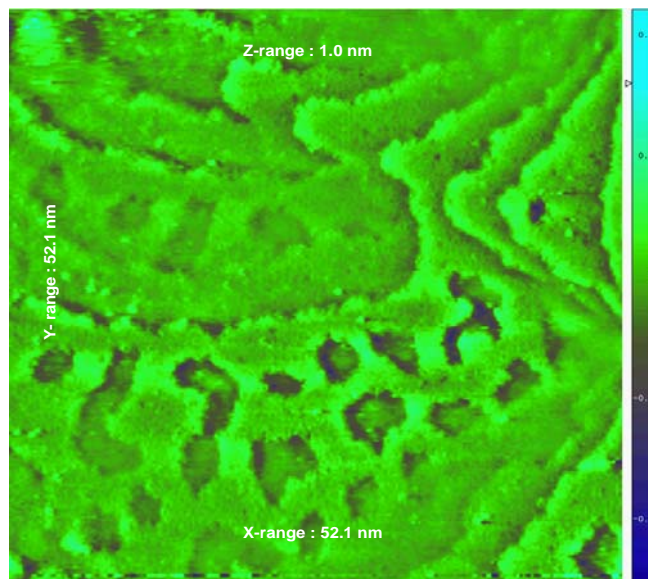


Fig. 3. STM image of the cholesterol molecular film on the Au(111) substrate, prepared from 2×10^{-4} M methanol solution.

Detailed analysis of the cholesterol free vacancies indicates that their shape is not random, what could be related to some kind of mismatch between the cholesterol size and atomic distances at the well-ordered substrate. As seems, cholesterol adlayer needs periodically break resemblance with the substrate order and continue spreading in other direction. We found that about 25% of the total surface is occupied by vacancies. More structural details can be observed at the high resolution image, at Fig. 4, which shows the compact cholesterol adlayer composed of the individual molecules.

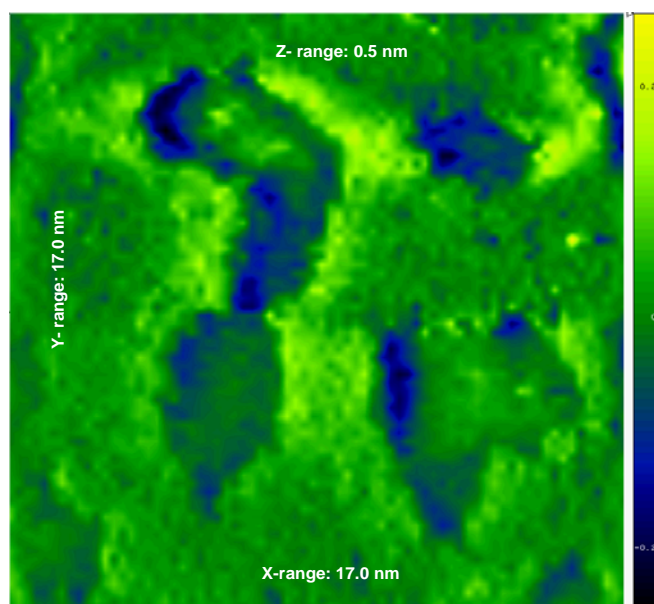


Fig. 4. High resolution STM image reveals individual cholesterol molecules in the adsorbed layer.

A graphic with cholesterol molecular model imposed over the real image, as presented at Figure 5., helps to understand detail structure of the monolayer,

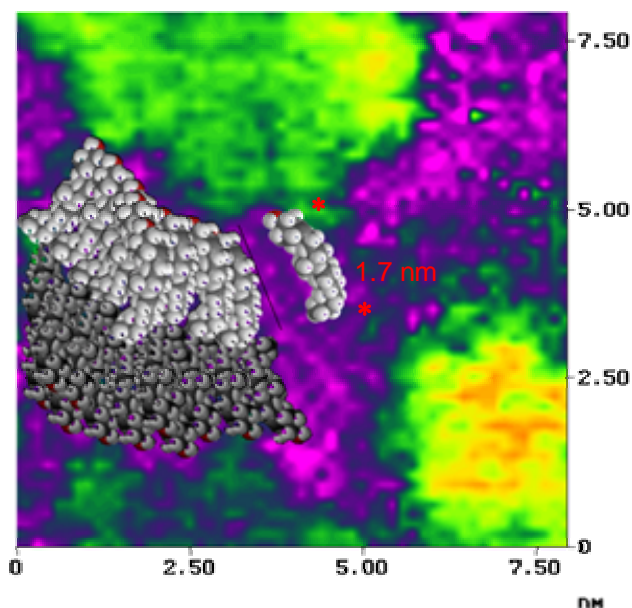


Fig. 5. A model of cholesterol adlayer imposed over the real high resolution image. Note, in this particular presentation, cholesterol free zones are brighter than rest of image.

From the same model one could also see that in order to achieve high packing density, molecules of cholesterol must be oriented with hydrophilic part towards the gold substrate. So far we could not see any resemblance with the Au(111) substrate structure, which indicates that monolayer is some kind of self-assembly structure.

This particular findings about uniform cholesterol monolayer over the metal surface, as new brand of specifically modified substrate, could be of interest for sensors design in medicine and the pharmaceutical industry. Such type of molecular layer can be important for molecular electronics, as insulator for the electron transfer, too.

When cholesterol adlayers were prepared from significantly lower concentrations, i.e. 8×10^{-12} M, the structure of the modified adlayer changed. Note, at such extremely low concentrations, there are not sufficient molecules in the solution to cover the substrate surface completely and not in the form of the closely packed monolayer. STM images, as one presented on Fig.6., show large terraces covered by cholesterol self-assembly in a form of square boxes.

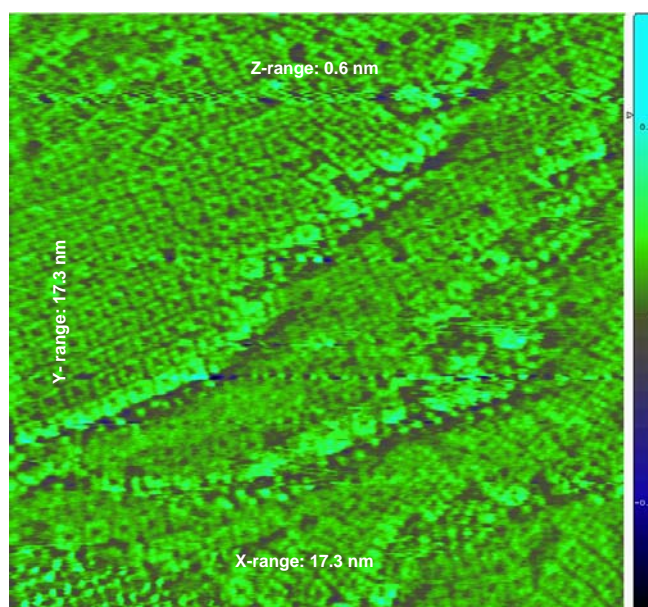


Fig. 6. Cholesterol self-assemblies in form of square boxes visualized over the Au(111) substrate, after functionalization with 8×10^{-12} M cholesterol/methanol solution.

Each self-assembly unit (box) (0.59 nm x 0.59 nm) consists of two cholesterol molecules. Note that box-assemblies are not perfectly ordered. Also, during formation, the molecular adlayer has tendency to rearrange in different domains, which indicates on rather complex mechanism of formation. So far we could not see any relation between the molecular adsorbate and the substrate structure. The high resolution images and adequate models, developed in our study, revealed that supramolecular boxes of cholesterol dimmers

are connected via interactions between the electron rich cyclic hydrocarbons. In the center of each supramolecular box a quadratic cavity with atomic space dimensions: 0.25 nm x 0.25 nm, and depth of 0.29 nm, can be found. Boxes are separated by insulating aliphatic chains. In this way the substrate surface is again completely covered by molecular film but with significantly lower density. One could have a better idea about the observed structure after checking a graphic in Fig. 7., with molecular model of the cholesterol box assembly imposed over the real, high resolution STM image.

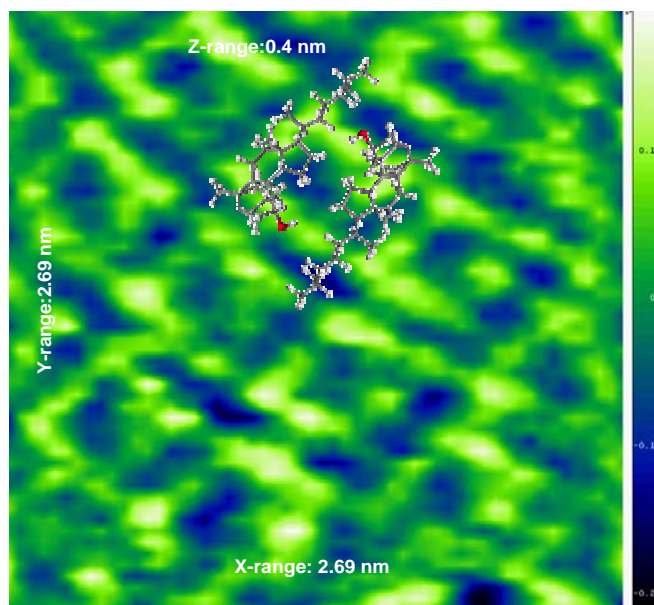


Fig. 7. The high resolution STM image with cholesterol molecules (model) imposed over the molecular layer, to illustrate structure of the self-assembly array.

However, in order to reproduce the self-assembly adlayer structure, by molecular modeling, it required to alternate molecular conformation (bend around C5 and C10 for about 30 degree), which shortened molecule for about 0.13 nm. In other words, it is telling us that in dependence of the adsorbate density (concentration) cholesterol molecules also change molecular orientation and conformation, as well as intermolecular interactions and structure of the adsorbed adlayer. Current studies are in progress to determinate relation between the self-assembled structure, preferential adsorption orientation and molecular conformation.

CONCLUSION

The cholesterol molecular films prepared from different solutions on the Au (111) substrate were characterized by STM at atomic and molecular levels. High resolution STM images revealed two type of molecular films: monolayer and self-assembly boxes of the cholesterol dimmers, which were

characterized in great details. With help of the molecular modeling, structure of the observed films were reconstructed. It offered new information over the molecular orientation in the adsorbed layer, mode of molecular attachment to the substrate and molecular conformation in the adsorbed film. The structure of the molecular adsorbate depends on the molecular concentration in the solution (methanol or water). The obtained results are interesting from several points of view. First of all, we believe, it is the first report of the successful visualization of the cholesterol film at the molecular level. Second, formation and characterization of the cholesterol supramolecular assemblies in form of boxes and monolayers at the Au(111) substrate is very promising issue from the point of the surface functionalization and design of the nano-scale sensores.

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