# Chapter 6

# Ordering of cholesterol molecules on graphite: STM and computer simulation studies

## 6.1 Introduction

In the previous chapter, we have studied the aggregation of cholesterol (Ch) in the LB films deposited on hydrophobic substrate using AFM. Since LB films of Ch showed interesting patterns only on the hydrophobic substrate, we extended the study on the aggregation of Ch molecules in the LB films on an atomically smooth hydrophobic substrate using scanning tunneling microscope (STM). STM is a powerful tool to study the molecular assembly on the surface at the molecular resolution [1,2]. We have formed the LB films of Ch on a highly oriented pyrolytic graphite (HOPG) substrate for such studies. HOPG is a conducting and a hydrophobic substrate. The atomically smooth surface of HOPG has been well characterized. Forming LB films on such substrates and studying them with STM reveal the molecular ordering in the films.

## 6.2 Experimental

The experimental details of surface manometry are discussed in previous chapters. The chemical structure of the Ch molecule is shown in Figure 6.1. The length of the molecule is 1.6 nm and the diameter is 0.5 nm.

Highly oriented pyrolytic graphite (HOPG) of ZYB grade was obtained from Advanced

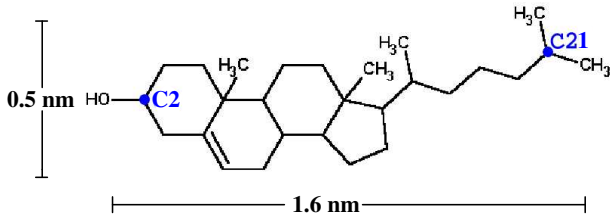



Figure 6.1: Chemical structure of the cholesterol molecule. C2 and C21 represent carbon atom number 2 and 21 of Ch molecule, respectively.

Ceramics Corporation. The LB films of cholesterol was transferred on the hydrophobic HOPG substrate at a target surface pressure ($\pi_t$) of 3 mN/m. Few layers of HOPG was peeled off using an adhesive tape (Scotch Tape) prior to the film deposition. One layer of the film can be transferred on the hydrophobic substrate during the first downstroke of the dipper. The dipper was stopped inside the subphase after the first downstroke. While holding the one layer coated substrate in the subphase, the monolayer from the interface was removed completely by a vacuum suction method. Then, the dipper was lifted up very slowly. We find this to be an effective method to obtain one layer of LB film on a hydrophobic substrate. During LB deposition of Ch on HOPG, we find a poor transfer ratio which was of the order of 0.2 to 0.3.

Scanning tunneling microscope (STM) experiments were done using a home built setup [3]. The STM was calibrated using a HOPG substrate (ZYA grade, Advanced Ceramics Corporation). We have utilized both the chemically etched and mechanically cut platinum and tungsten tips for the experiments. We find that the resolution of the images obtained through a mechanically cut tungsten tip were better than that of the chemically etched tips. The film coated substrates were transferred on the sample stage of the STM for imaging. The data were collected in an ASCII format and the images were reconstructed and analyzed using a software, Scanning Probe Image Processor (SPIP).

We obtained the minimum energy conformation of the cholesterol molecules on graphite [0001] surface by computer simulations. The simulation has been performed using a commercial software InsightII/Discover and Docker packages distributed by the Accelrys (San Diego, CA). The consistent valence force field (CVFF) was utilized for the simulation.

The CVFF describes the non-bonded interactions through van der Waals and Coulombic terms only. The bond energy was defined by the Morse potential. The conjugate gradient method was used for the energy minimization.

## 6.3 Results and Discussion

It is known from the literature that the graphite [0001] (a graphene layer) surface has two types of atoms ($\alpha$ and $\beta$) in the basis of a hexagonal surface unit cell (Figure 6.2(b)). Only $\beta$ atoms can be imaged through STM [4]. The separation between these atoms is 0.245 nm. The STM image of the HOPG is presented in Figure 6.2(a). A corrugation of 0.24 nm was

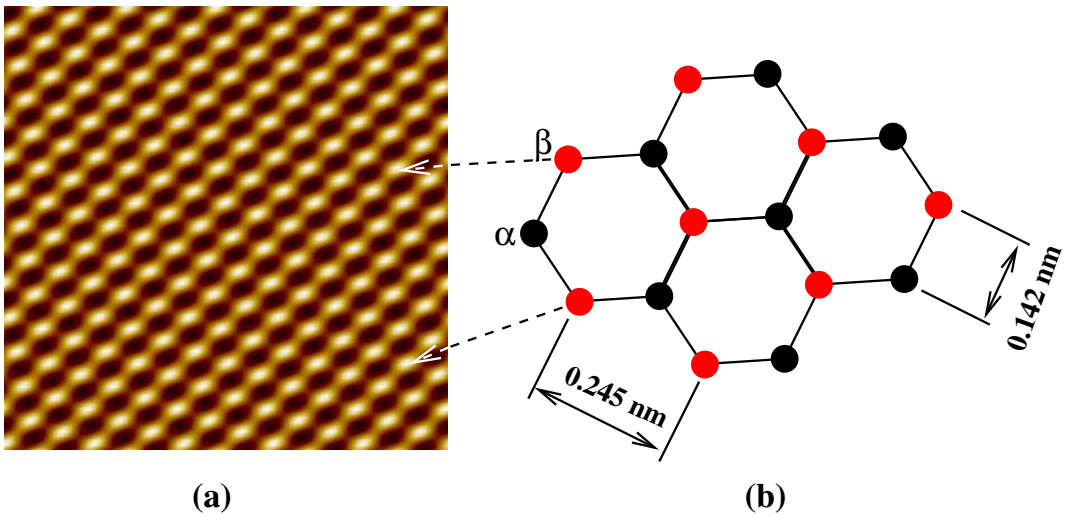
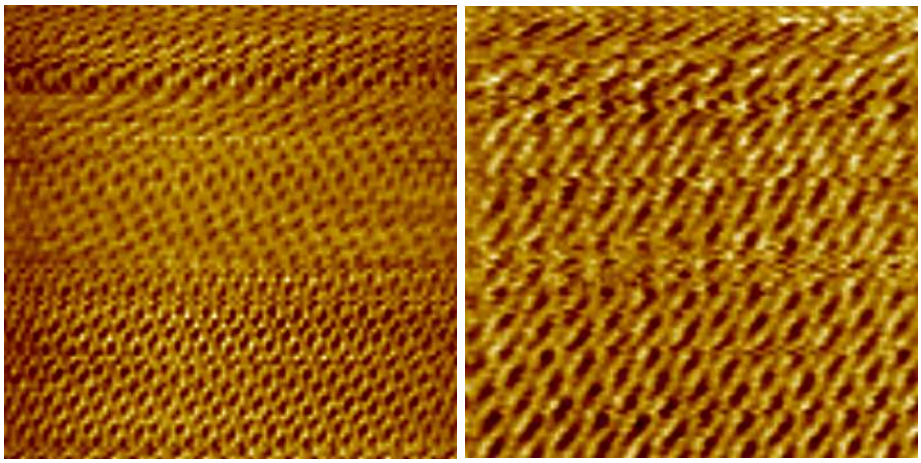


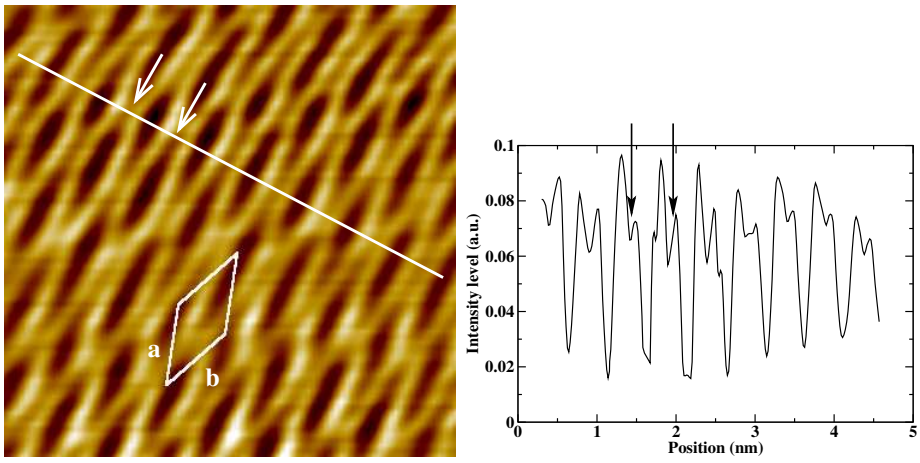

Figure 6.2: (a) shows the STM image of HOPG obtained in constant height mode at a bias voltage of 100 mV and a tunneling current of 1 nA. The size of the image is 2.38×2.38 nm$^2$. (b) shows the $\alpha$ and $\beta$ carbon atoms in a graphene layer. The arrows with dashed line indicate the bright regions in the STM image which corresponds to the $\beta$ atoms of the graphene layer.

obtained from the image. This number resembles the lateral separation of the $\beta$ atoms in a graphene plane [5]. The STM images of the LB film of cholesterol for two different scan ranges are shown in Figure 6.3. The bright regions are rich in electron density, whereas the dark regions are deficient in electron density. The images show a highly ordered wire network kind of texture. To avoid the possibility of instrumental artifacts, we have scanned the films for different scan ranges. This is shown in Figure 6.3(b). We find that the features scale according to the scan range indicating the reproducibility of the features. An image for



(a) 9.6×9.6 $nm^2$ (b) 6×6 $nm^2$

Figure 6.3: STM images of the LB film of cholesterol on HOPG. The images were taken in constant height mode at 100 mV bias voltage and 1 nA tunneling current. The size of the images are shown below the respective images.




(a) (b)

Figure 6.4: (a) shows the STM image of the LB film of cholesterol on HOPG. The image was taken in constant height mode at 100 mV bias voltage and 1 nA tunneling current. (b) shows the intensity profile along the white straight line drawn on (a). Arrows are drawn to show the corresponding positions in the image and the graph. The distance between the two arrows is 0.5 nm. An oblique rectangular shape is drawn in (a) to represent a single 2D lattice cell. Here, **a** and **b** represent the lattice parameters. The size of the image in (a) is 4×4 $nm^2$.

a scan range of 4×4 $nm^2$ with the intensity profile data along a white straight line is shown in Figure 6.4. A highly periodic texture of wire-network can be seen from the image. The intensity profile data reveal that the distance between two bright regions separated by a dark region is 0.5 nm. Such an ordered texture exhibits a two-dimensional oblique lattice. We find the magnitude of the lattice parameters **a** and **b** to be 0.705 and 0.727 nm, respectively. The angle between them was found to be 39.8 °.

## 6.4 Simulations

We have carried out computer simulations to interpret the STM images. We have attempted to pack the cholesterol molecules on the graphite [0001] plane. The structure of the system was minimized to obtain a favorable molecular conformation. Here, the graphite substrate was treated as host, whereas the cholesterol molecules were treated as guest molecules. The conformations of the molecules on the graphite were monitored as a function of the surface density. We attempted to increase the surface density by increasing the number of the guest molecules in a predefined area of 476 $Å^2$ on the graphite surface. The area per molecule (inverse of surface density) on the graphite surface was varied from 238 to 47.6 $Å^2$. For any particular value of $A_m$, 10 different random initial conformations of the Ch molecules were chosen, and the energy of the system was minimized for each individual initial conformation. The final conformation of the molecules was chosen such that it represents the system of the lowest energy among the 10 different initial conditions. The minimized energy of the system for the different area per molecule are shown in Figure 6.5. Considering an $A_m$ of 238 $Å^2$, it was observed from the curve shown in Figure 6.5 that the initial configuration number 7 is the lowest energy conformation of the system. Similarly, for the lower $A_m$, we have considered the lowest energy conformation of the system for any particular initial configuration number. The lowest energy configuration is denoted by the arrow in the Figure 6.5. It is worthwhile to note that these states may not represent the global minima. They may represent some metastable local minima. The orientation of the Ch molecules on the graphite plane was studied by determining a distribution of the tilt angle of the molecules with respect to the

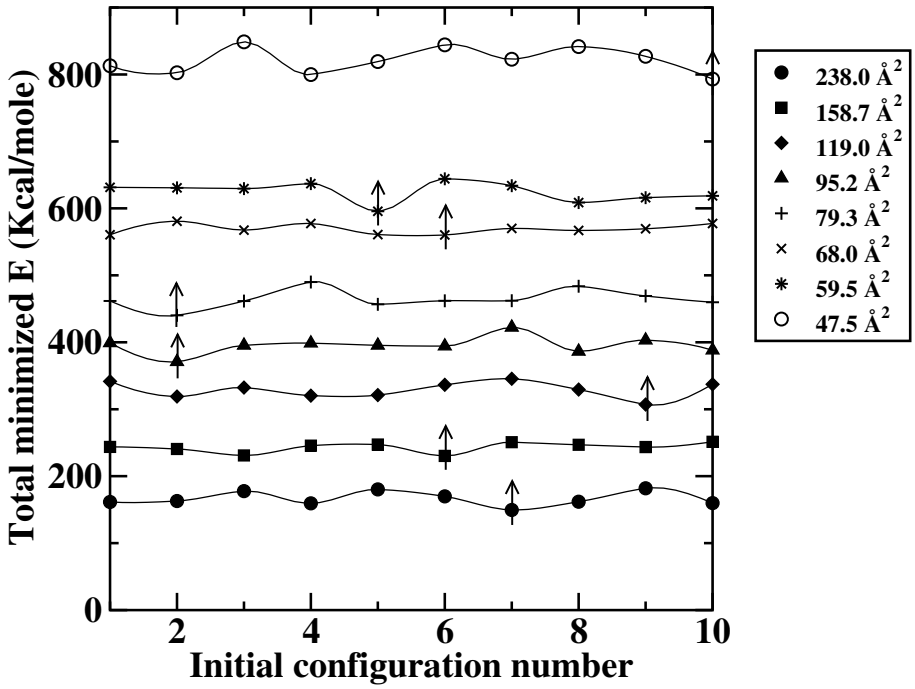



Figure 6.5: The total minimized energy (E) of the cholesterol (Ch) molecules on the graphite [0001] plane with respect to the different initial configuration number of Ch molecules. The box outside shows the labels for the area per molecule ($A_m$) for which the variation of total minimized E with respect to initial configuration number is shown. The arrow indicates the minimum energy configuration number. The minimization was performed until either 500 iterations or the maximum derivative is less than 1.000000 kcal/mole/Å.

substrate normal. We define a long molecular axis which passes between the C2 and C21 atoms of the Ch molecule (Figure 6.1). The tilt angle ($\theta$) of the molecule is defined as the angle of the long molecular axis with respect to the substrate normal (Z-axis). We have determined the $\theta$ values from the minimized conformations at various $A_m$ on the substrate. A histogram showing the distribution of $\theta$ is presented in Figure 6.6. The distribution of $\theta$ shows a peak at around 89°. This indicates a planar orientation of the Ch molecule on the [0001] plane of the graphite substrate. The minimum energy configuration of the cholesterol molecules on the graphite surface for two different $A_m$ are shown in Figure 6.7. We find that, on an average, the molecules prefer to stay with it long axis parallel to the graphite plane. Figure 6.8 represents the side view of the conformations shown in Figure 6.7. Here, the figures reveal the planar orientation of the molecules.

In the next step, initial conformations were chosen such that the molecules were placed

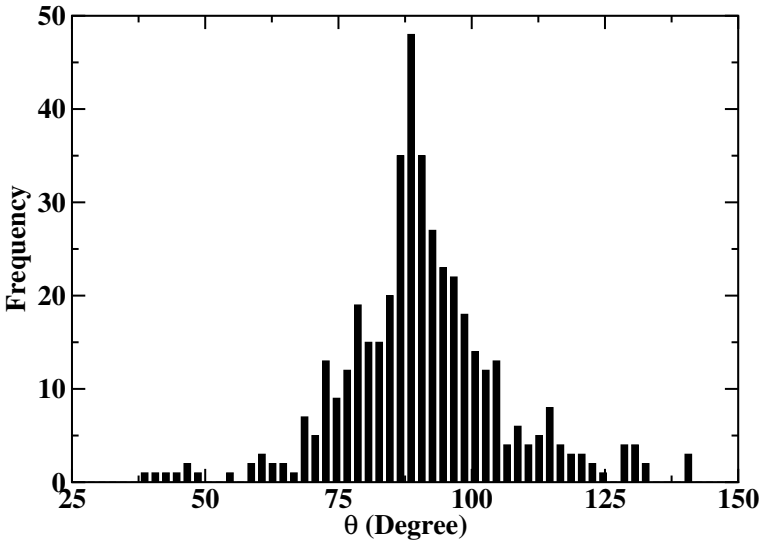
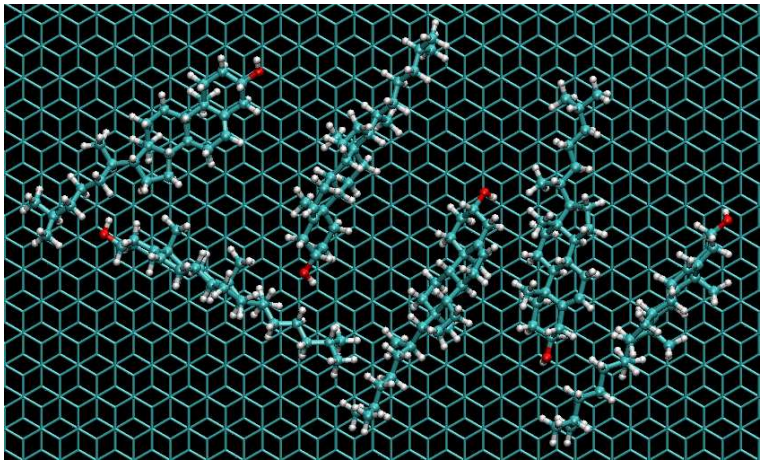


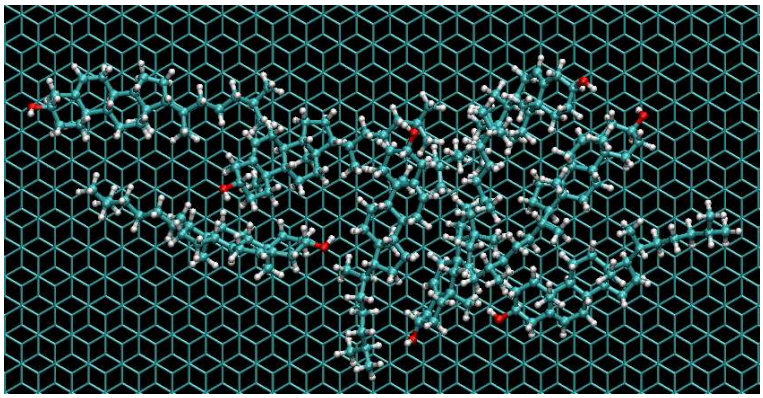

Figure 6.6: Histogram showing the distribution of the tilt angle ($\theta$) of the cholesterol molecules with respect to the substrate normal. Here, the sample points are 434 and the bin size is 2°.

planar to the graphite surface for four different pairing configurations (Figure 6.9). The pairing conformations were chosen to enhance the interaction between iso octyl chain - chain (Figure 6.9(a)), iso octyl chain - sterol skeleton (Figure 6.9(b)), skeleton - skeleton (Figure 6.9(c)) and polar head - head (Figure 6.9(d)) of the neighboring molecules. The energy of the systems was minimized using Discover. The minimum energy conformation was found for the sterol skeleton - iso octyl chain pairing conformation (Figure 6.9(b)). The minimized structure of this pairing conformation is presented in Figure 6.10.

The average diameter of a cholesterol molecule is 0.5 nm. Cholesterol is mostly a saturated compound with a single double bond between the carbon atom number 4 and 5 of the molecule. Hence, it can be considered as an electron deficient molecule. However the substrate, HOPG is very rich in electron and is atomically smooth. We expect that the bright region in the STM images are mainly due to the overlapped molecular orbitals of the graphite and the cholesterol. The contribution of the electron tunneling due to the substrate is more as compared to the electron deficient cholesterol molecule. The dark region can be assigned to the electron deficient core of the cholesterol molecule. We find from simulation that the cholesterol molecules prefer a planar orientation on the HOPG substrate. We suggest a possible arrangement of the molecules in the LB films on the HOPG substrate, as depicted

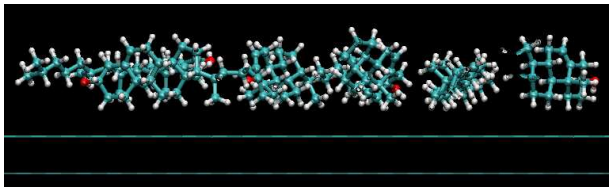


(a)

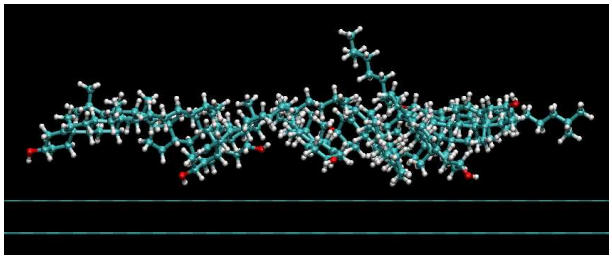


(b)

Figure 6.7: Minimum energy conformation of the cholesterol on the graphite substrate. (a) and (b) show the conformations for the $A_m$ of 79.3 and 59.5 $Å^2$, respectively. The corresponding total minimized energies are 440.3 and 595.7 kcal/mole, respectively.



(a) $A_m = 79.3\text{Å}^2$



(b) $A_m = 59.5\text{Å}^2$

Figure 6.8: Side view of the conformation of Ch molecules shown in Figure 6.7.

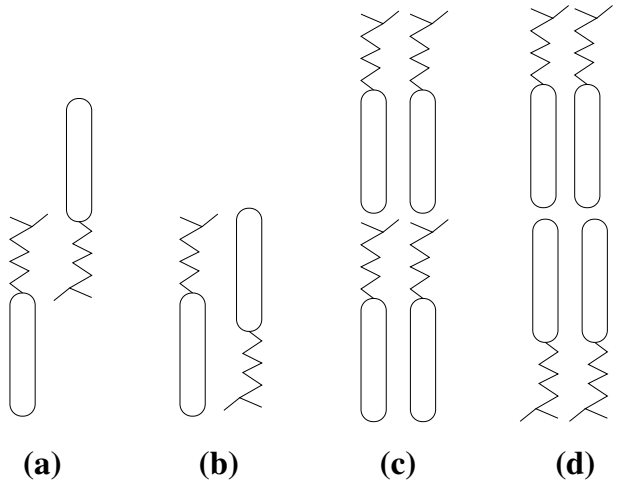



Figure 6.9: Initial planar configuration of the cholesterol molecules for minimization.

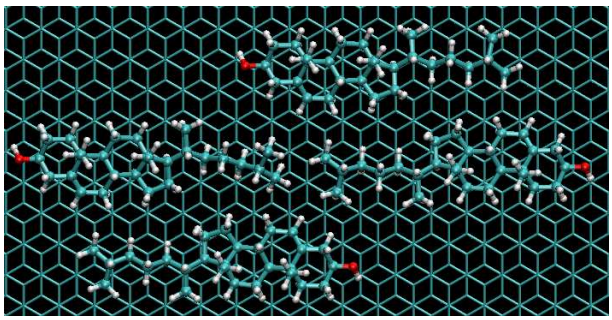


Figure 6.10: Minimized structure of the pairing conformation sterol skeleton - iso octyl chain of the Ch molecules. The energy was minimized using conjugate gradient method for 500 iterations and also until the maximum derivative becomes less than 0.001 kcal/mole/Å

in Figure 6.11. Here, we depict the dark region as the core of the cholesterol molecule, whereas the bright regions are due to the collective contribution of graphite and cholesterol. The rearrangement of the molecules on a solid substrate after LB deposition has been well documented in literature [6]. During LB deposition of Ch on HOPG, the target surface pressure ($\pi_t$) was 3 mN/m which corresponds to the untilted condensed ($L_2$) phase. The transfer ratio of LB deposition of Ch on HOPG was very small. This indicates that unlike the case of Ch on hydrophobically treated glass substrate, the normal orientation of the Ch

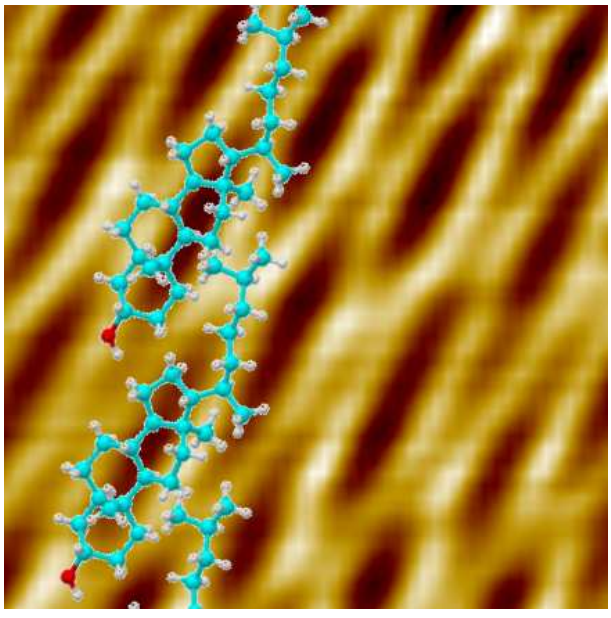


Figure 6.11: A 2.38×2.38 $nm^2$ STM image of the LB film of the cholesterol molecules on HOPG substrate. Here, the cholesterol molecules are shown to depict a possible conformation of the molecules on the substrate. The STM image is the part of that shown in Figure 6.4(a).

on HOPG substrate is unfavorable. We suggest that the cholesterol molecules after being transferred from A-W interface to the graphite substrate, rearrange themselves with a planar orientation. Such a rearrangement yields an ordered assembly of Ch molecules as observed using STM.

## 6.5 Conclusions

We find experimentally and through computer simulations that the cholesterol molecules align with its long axis parallel to the graphite [0001] plane. The STM images reveal a high ordering of the Ch molecules in the LB film.

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