

# Atomic Force Microscopic Observation of the Molecular Orientation in Ultrathin Films of Alkanoic Acid-Derivatized Porphyrins on a Mica Surface

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The observation of the molecular orientation of alkanoic acid-derivatized porphyrins in ultrathin films deposited on mica was carried out by atomic force microscopy. It was observed that the tetraacid derivative 5,10,15,20-tetra(*N*-10-carboxydecyl-pyridinium-4-yl) porphyrin was arranged as a monolayer with the porphyrin macrocycle oriented coplanar to the mica surface. On the other hand, the diacid derivative protoporphyrin IX Zn(II) formed a bilayer with the hydrophobic part inside and the hydrophilic part in the periphery. Therefore, in this case, the porphyrin macrocycle is roughly perpendicular to the mica surface.

**Keywords:** Atomic Force Microscopy, Porphyrin, Alkanoic Acid-Derivatized Porphyrin, Monolayer, Bilayer, Molecular Orientation, Ultrathin Film, Mica Surface.

The organization of native porphyrins and their intentionally designed derivatives on solid substrates is of current interest because of the biological and practical importance of these compounds.<sup>1–18</sup> In particular, monolayer films of porphyrins may provide not only a template for their catalytical and biopolymer-binding properties, but also a component in molecular devices.<sup>5–7, 9, 10, 12</sup> Ultrathin films with a controlled orientation on solid substrates can be achieved by means of the self-assembled monolayer (SAM) technique. Two methods are usually utilized for the preparation of porphyrin SAMs. One is the axial ligation of metalloporphyrin to the ligand-derivatized surface,<sup>8, 13–18</sup> and the other is the chemisorption of alkylthiol-linked porphyrin to the metal surface.<sup>6, 7, 10, 12</sup>

Zhang and Imae<sup>19, 20</sup> have investigated the preparation and the structural characterization of SAM films of a functionalized diacid, protoporphyrin IX Zn(II) (ZnPP), by surface-enhanced infrared and UV-vis absorption spectroscopies. Axial ligation of the ZnPP was observed on a 4-pyridinethiol SAM. On the other hand, the direct formation of ZnPP SAM was verified on the Au surface. In this case, one of the two COOH groups in the ZnPP is anchored by deprotonation, and the other one is hydrogen-bonded with a COOH group of an adjacent porphyrin molecule, stabilizing the SAM film of ZnPP

anchored on the metal surface. The formation of a stable SAM, in which the molecules are chemically bound to the Au surface via COO<sup>−</sup> groups, was also reported for 5,10,15,20-tetra(*N*-10-carboxydecyl-pyridinium-4-yl) porphyrin (TCPyP) with four COOH terminal groups.<sup>21</sup> Absorption spectroscopic measurements and SAM film thickness determined by surface plasmon resonance suggested that the porphyrin macrocycle is oriented almost parallel to the Au surface. The infrared absorption spectra further supported the conclusion that three COOH groups are bound to the Au surface but not the residual one. However, the reported molecular orientation on Au surfaces was deduced from spectroscopic results and is not based on visual observations.

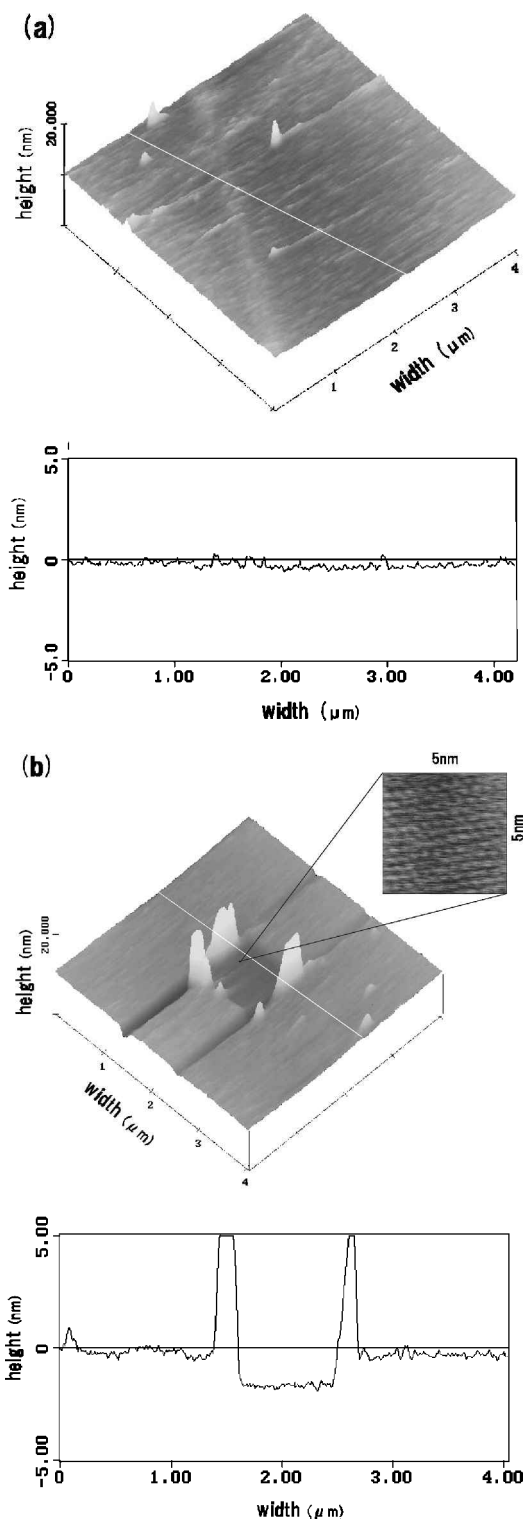
In the present study, the textures of porphyrin ultrathin films on mica are visualized by atomic force microscopy (AFM), and the molecular orientation of ZnPP and TCPyP in the films is discussed and compared.

TCPyP was synthesized by alkylation of 5,10,15,20-tetra(4-pyridyl)porphyrin (TPyP) with 10-Br-1-decanoic acid according to the literature.<sup>22</sup> ZnPP was purchased from Aldrich and used as received. Methanol was of reagent grade. An aqueous solution of TCPyP ( $1.5 \times 10^{-4}$  M) and a methanol solution of ZnPP ( $3 \times 10^{-5}$  M) were used. Freshly cleaved mica substrates were immersed in the solutions of TCPyP and ZnPP. The porphyrin-coated substrates were dried without rinsing and used for the AFM observation, which was performed

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at contact mode on a Digital Instruments Nanoscope III. All experiments were carried out at room temperature.

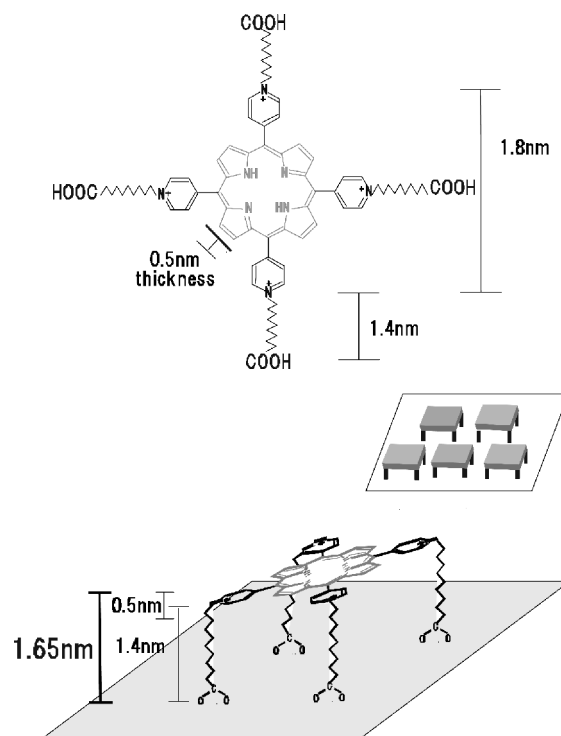
An AFM image of TCPyP film on mica after immersion for 5 min is shown in Figure 1a. This immersion time



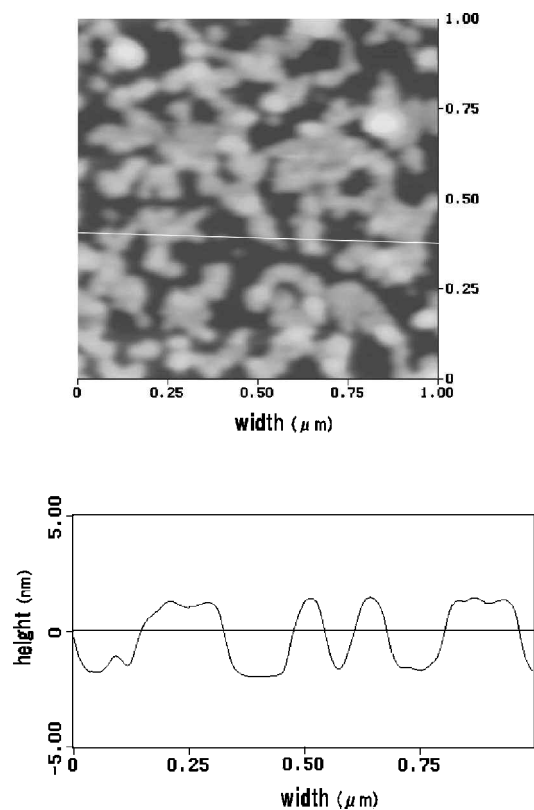
**Fig. 1.** AFM images ( $4 \mu\text{m} \times 4 \mu\text{m}$ ) of TCPyP film on mica and their section analyses. (a) Before scratching. (b) After scratching. Bars in AFM images denote the section-analyzed lines. The additional AFM image ( $5 \text{ nm} \times 5 \text{ nm}$ ) in (b) indicates the surface in the scratched area.

is longer than the time needed to reach the adsorption equilibrium, which was observed to be about 100 s.<sup>21</sup> A flat surface was observed with a roughness of  $\sim 0.25 \text{ nm}$ , and the surface of an area of  $1 \mu\text{m}^2$  was then scratched by the AFM tip. The scratched surface displayed a hexagonal lattice of mica, as can be seen in Figure 1b. The observed atom–atom distance ( $5.3 \text{ \AA}$ ) is consistent with previously reported values.<sup>23,24</sup> Then the TCPyP film thickness was determined by the height difference from the mica surface to the film surface in the image resized at  $4 \mu\text{m}^2$ . The obtained average thickness was  $1.51 \pm 0.09 \text{ nm}$ .

The length of the COOH-terminated alkyl chain ( $-(\text{CH}_2)_9\text{COOH}$ ) is about  $1.4 \text{ nm}$ , if the alkyl chain is in *trans* conformation.<sup>25</sup> Since the thickness and the diameter of the TCPyP macrocycle are roughly  $0.5$  and  $1.8 \text{ nm}$ , respectively,<sup>26,27</sup> the total molecular length of TCPyP is  $\sim 4.6 \text{ nm}$ . This length is far from the observed TCPyP film thickness. Therefore, the orientation of the porphyrin macrocycle plane in the ultrathin film must be parallel and not perpendicular to the mica surface. If the COOH groups of the porphyrin anchor on the mica surface, as illustrated in Figure 2, the estimated thickness of the TCPyP ultrathin film is about  $1.65 \text{ nm}$ . If the alkyl chain is partly in the *gauche* conformation, the film thickness should shrink, and the coplanar orientation of the TCPyP molecule on the mica surface is in agreement with the observed film thickness. This model is similar to that reported before for a TCPyP SAM film on the Au surface.<sup>20</sup>



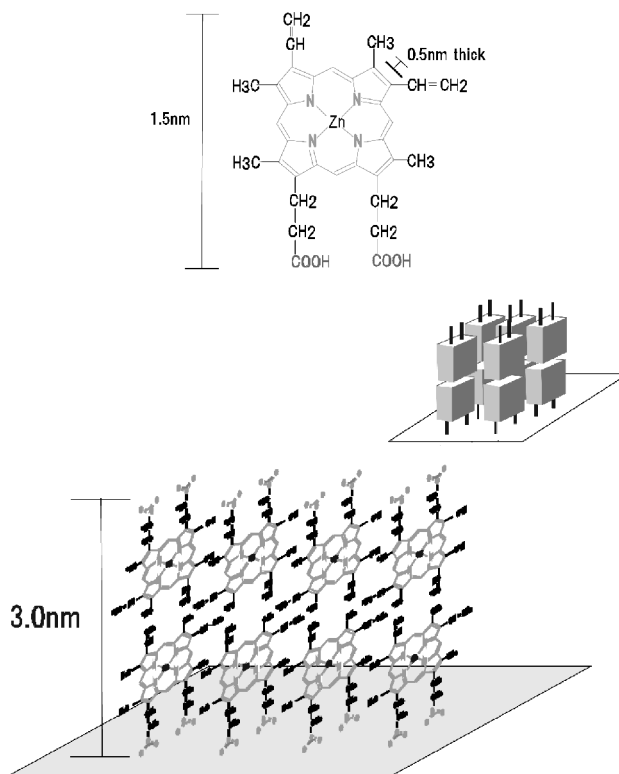
**Fig. 2.** Molecular dimensions for the TCPyP and its molecular orientation on mica.



**Fig. 3.** AFM image (top view,  $1 \mu\text{m} \times 1 \mu\text{m}$ ) of ZnPP film on mica and its section analysis. The bar in the AFM image denotes the section-analyzed line.

Figure 3 shows an AFM image of a ZnPP film on mica after immersion for 5 h, which is long enough to reach the adsorption equilibrium. The surface was not homogeneously flat, but displayed the “fractal texture” of layers with uniform thickness. It was proved that the bottom of the layer was the mica surface, because it was invariable even after being scratched. Then, the layer thickness was determined to be  $3.07 \pm 0.04$  nm. Since the extended molecular length of ZnPP was calculated to be about 1.5 nm, a monolayer arrangement of ZnPP is not enough to explain the observed layer thickness.

There are two main possible explanations for the molecular orientation of ZnPP on mica, namely parallel and perpendicular orientations of the porphyrin macrocycle plane related to the mica surface. Assuming the parallel orientation and taking into account that the thickness of ZnPP is 0.5 nm, a multilayer should be formed. But, in this case, the uniformity of the multilayer thickness must be explained. On the other hand, evidence supporting the tilted orientation of the porphyrin plane with respect to the solid surface was provided by infrared reflection-absorption spectroscopy, which indicates a selection role for the absorption bands.<sup>20</sup> Thus the formation of a bilayer is demanded by the perpendicular orientation of porphyrin macrocycle planes to satisfy the observed thickness, as can be seen in Figure 4. In the bilayer, the hydrophobic porphyrin macrocycle is inside, and the terminal COOH



**Fig. 4.** Molecular dimensions for the ZnPP and its molecular orientation on mica.

groups are in the periphery. One side of the bilayer is in contact with the hydrophilic mica surface, and the other one faces the bulk solution on the process of the ultrathin film formation. When the adsorbed layers are prepared, the substrate is dipped in the solution. In such a situation, the outermost adsorbed layer of ZnPP is exposed to aqueous solution. Therefore, having an outermost layer of carboxyl groups is favorable.

In conclusion, the molecular orientation in ultrathin films of alkanolic acid-derivatized porphyrins on mica was reported. The tetraacid derivative was arranged as a monolayer with coplanar orientation of the porphyrin macrocycle plane to the mica surface, and the diacid derivative formed a bilayer with the hydrophobic part inside and the hydrophilic part in the periphery. The orientations are consistent with those observed for porphyrins on Au surfaces.

Native porphyrins are involved in hemoglobin as oxygen carriers, in chlorophyll as photosynthetic dyes, and so on; therefore they are physiologically indispensable to our biological activity. On the other hand, the artificial organization of porphyrins is one of the main targets in biotechnology because of its utility. The present report will help in the construction of well-organized porphyrin nanodevices.

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