

**Research Article** 

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# In Situ Scanning Tunneling Microscopy Study of Interfacial Structures of Self-Assembled Monolayers of 3-(2-Pyridyldithio)Propionyl Hydrazide on Au(111)

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## Abstract

Self-assembled monolayers of 3-(2-pyridyldithio) propionyl hydrazide (PDPH) on Au(111) in perchloric acid solution were studied by in situ scanning tunneling microscopy (STM). The two-component PDPH molecules adsorbed uniformly on the Au(111) surface without phase separation in the monolayer. The 2-pyridine and propionyl hydrazide moieties of PDPH appeared as distinct features in the STM image of the PDPH monolayer and were successfully imaged in solution.

# Introduction

Self-assembled monolayers (SAMs) of organic molecules on Au(111) are becoming increasingly important not only in the areas of fundamental surface science and interfacial electrochemistry but also in other areas [1-2]. For example in biomedical sensing, various modification of carbon substrate to introduce functional group has been reported [3-7]. It is well known that alkanethiols adsorbed on gold are closely packed via thiolate-gold covalent bonds and van der Waals forces between the alkyl chains [8-12]. However, the high density packing of alkanethiols causes decline of surface reaction efficiency between functional group to target molecule. To avoid constitution of high density packing, the mix SAM method [13,14] or the use of selfassembled synthesis polymer was adopted [6-7]. 3-(2-Pyridyldithio) propionyl hydrazide (PDPH, Figure 1) is a heterobifunctional compound containing a hydrazide group and a pyridyl disulfide residue which is often used as protein crosslinker. PDPH is a good candidate for a cleavable cross-linker for a biomaterial. The PDPH's hydrazide group selectively reacts with carbohydrates oxidized by sodium meta-periodate; while the pyridinyldisulfide moiety is reactive toward reduced thiols. Moreover, PDPH can be expected to form monolayers on gold through interaction of the pyridyldithio group with the gold surface as well as the use of crosslinker. If functional groups of PDPH efficiently expose on Au substrate, the hydrazide group and pyridyl disulfide residue can react with carbohydrates and built a space to avoid too high density packing on Au substrate surface, respectively. Scanning tunneling microscopy (STM) is a promising tool for direct determination of interfacial structures and orientations and of internal structures of organic molecules adsorbed on electrodes in solution [8,15,16]. Here, we used in situ STM to investigate selfassembled monolayers of PDPH on Au(111) in perchloric acid solution to elucidate the interfacial structure and estimated the adsorption ratio



of the hydrazide and pyridyl disulfide moieties of PDPH through comparison with mixed SAMs of 2-pyridinethiol and cystamine.

## Experimental

3-(2-Pyridyldithio)propionyl hydrazide (PDPH), 2-pyridinethiol (2-PySH), and cystamine were used without further purification. The 0.05 M  $\text{HClO}_4$  electrolyte solution was prepared by adding an appropriate amount of ultrapure water (18.2  $\Omega$ , Milli-Q SP, Millipore Co.) to a stock  $\text{HClO}_4$  solution (ultrapure grade, 61.2%, Cica-Merck).

A single-crystal gold bead was prepared by crystallizing a molten ball of gold at the end of a gold wire (99.999% purity, 0.8 mm in diameter) heated in a hydrogen–oxygen flame. One of the (111) facets, which were arranged in an octahedral configuration on the singlecrystal bead, was directly used for the in situ STM experiments. The Au(111) substrate was annealed in a hydrogen–oxygen flame and then quenched in ultrapure water saturated with hydrogen. Self-assembled monolayers of PDPH or a 1:1 mixture of 2-PySH and cystamine were formed on the Au(111) substrate by soaking the clean Au(111) electrode in a freshly prepared aqueous solution containing 50  $\mu$ M PDPH or a mixture of 50  $\mu$ M 2-PySH and  $\mu$ M cystamine for 10 min. After immersion, the Au substrate was thoroughly rinsed with pure water to remove excess solution from the surface.

# **Results and Discussion**

Figure 2A shows a high-resolution STM image (200 nm  $\times$  200

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nm) of the self-assembled monolayers of PDPH on Au(111), acquired in 0.05 M HClO, under electrochemical control. Two small, distinct features, labeled I and II, were evident on the molecularly ordered surface. Feature I consisted of two spots, and feature II was a small ambiguous area homogeneously covering the Au (111) terraces. Figure 2B shows the in situ STM image of a 15 nm  $\times$  15 nm area of the monolayer. We previously reported [17] that the 2-PySH molecule appeared as two spots in the STM image, presumably due to the adsorption through the nitrogen and sulfur atoms of 2-pyridine (2-Py) with a vertical orientation on the Au(111) surface. This monolayer can be characterized by a  $p(4 \times \sqrt{7R} - 40.9^{\circ})$  structure. In the present study, we similarly observed two spots (III in Figure 2B). The size of each spot was 4 Å, in good agreement with the theoretical size of 2-PySH. The spots, however, were irregularly shaped, unlike those of the earlier study. Area IV in Figure 2B seems to correspond to the propionyl hydrazide (PH) portion of PDPH. This area was not sharply defined, probably because the hydrazide group of pH is hydrophilic and the 3-carbon chain of PH is not completely constrained in solution.



Figure 2: In situ STM images of the PDPH monolayer on Au(111) in 0.05 M HCIO<sub>4</sub>. The potentials of the sample and tip were 0.9 V and 0.35 V vs. RHE, respectively. The tunneling current was 1.5 nA. (A) Image area: 200 nm × 200 nm; (B) image area: 15 nm × 15 nm. The dashed black line shows the 2-Py molecule.

The height difference between the 2-Py and PH domains (0.05 nm) was smaller than anticipated. Considering the molecular lengths of PH and 2-Py (ca. 0.72 nm for PH and ca. 0.4 nm for 2-Py), the actual height difference would be about 0.32 nm when the molecules are adsorbed normal with respect to the surface. Because STM images generally involve both electronic and topographic features, the height corrugation would be affected not only by the electronic properties of the surface but also by interactions between the STM tip and the adsorbates. The discrepancy in the height profile is thought to originate from different tunneling mechanisms at domains consisting of different molecules with hydrazide and benzyl functional groups. Therefore, we determined that areas III and IV in Figure 2B correspond to the 2-Py and PH domains, respectively.

Page 2 of 3

Figure 2B shows that the PH groups formed small domains (2–3 nm) and that 2-Py existed in small circular areas within these domains (white square area). This indicates that PDPH did not form phase-separated domains like those in two-component monolayers [18,19]. During adsorption of PDPH molecules on Au(111), the hydrophilic PH groups of adjacent PDPH molecules formed small domains. Therefore, 2-Py formed a ring-like structure that encircled the PH domain (dashed black line in Figure 2B).

We have reported that adsorption of molecules from a twocomponent mixture on Au(111) resulted in SAMs with phaseseparated domains [16]. This result means that molecules that are more strongly adsorbed on gold will be arranged preferentially on the surface, even for 1:1 mixtures. Here, we studied adsorption from a 1:1 cystamine: 2-PySH mixture. Figure 3A shows in situ STM image of the monolayers of cystamine and 2-PySH on Au(111). Two domains were clearly observed (large, bright islands (I) and slightly darker areas (II)), suggesting that the monolayer formed a phase-separated domain structure on the Au(111) surface. The bright islands were 3–17 nm wide and usually irregularly shaped. The average heights of the islands (2.8 Å) and darker areas (1.4 Å) were not in agreement with the theoretical values, probably due to the effect of different tunneling mechanisms as described previously. Therefore, domains I and II consist of cystamine and 2-PySH molecules, respectively.

The STM image of PDPH (Figure 3B) shows that the 2-Py and PH moieties of PDPH were uniformly adsorbed on Au(111). This result suggested that molecules adsorbed on the surface did not exchange with molecules in solution. To evaluate the fractions of 2-PySH and PH in the monolayers, the STM images in Figures 3A and 3B were further analyzed by depth profiling. For two-component SAMs with 2-PySH and cystamine, the estimated fractions of 2-PySH and cystamine were 0.30 and 0.70 (values estimated on the basis of peak profiles (data not shown). For SAMs with PDPH, the estimated fractions of 2-Py and PH were 0.50 and 0.50 (values estimated on the basis of peak profiles (data not shown). This result means that the 2-Py and PH moieties of PDPH were homogeneously adsorbed and did not form phase-separated domains like those in mixed SAMs of 2-PySH and cystamine.

In conclusion, in situ STM was used to demonstrate that PDPH adsorbed uniformly on Au(111) without formation of phase-separated domains. SAMs with PDPH have potential for biotechnology applications, because PDPH contains a hydrazide group that can react with carbonyls. SAMs with PDPH are currently being used to develop biochips such as DNA chips and protein chips.



Figure 3: In situ STM images of (A) cystamine and 2-PySH and (B) PDPH monolayers on Au(111) in 0.05 M HCIO<sub>4</sub>. The potentials of the sample and tip were 0.9 V and 0.35 V vs. RHE, respectively. The tunneling current was 1 nA. (A) and (B) Image area: 200 nm × 200 nm.

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Page 3 of 3

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