Tunghai Science Vol. 19: 57-63
July, 2017

Visualizing DNA Molecules on Cysteamine Modified Annealed Gold by Ambient Scanning Tunneling Microscopy

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Abstract

In order to visualize DNA molecules in ambient environment by Scanning Tunneling Microscopy (STM), it is essential to immobilize DNA on conducting substrate. Annealed gold can meet the best criteria as the solid substrate in STM. To overcome the dragged force on DNA molecules due to surface tension between solution and air, annealed gold was modified by a monolayer of cysteamine that could immobilize DNA on the substrate. A successful spreading of individual DNA molecules was obtained and reported here.

Keywords: Scanning Tunneling Microscopy, DNA, Cysteamine-modified Annealed Gold

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1. Introduction

Immobilization of DNA molecules onto substrate for Scanning Probe Microscopy is essential in visualization of DNA structure in ambient environment [1]. Progresses were made in applying Atomic Force Microscopy (AFM) into this subject [2]. Using freshly cleaved mica strips as atomically flat substrate, DNA molecules could be immobilized onto the surface of mica through divalent cat-ions, such as Mg^{2+}, or mica modified with 3-aminopropyltriethoxy silane [3]. However, information obtained by AFM about DNA molecules in ambient environment is limited to topological structures [4]. In order to further investigate DNA molecules in detail, Scanning Tunneling Microscopy (STM) is suggested to be used to visualize DNA molecules, especially in complex 3 dimensional ambient environments [5].

The substrate used for STM should be atomically flat without questionable artifacts [6]. The best choice is annealed gold chip. Yet, annealed gold surface was inert in chemical reactions and DNA molecules in solution were dragged away from gold surface due to strong surface tension between solution and air [7]. Thus, similar to 3-aminopropyltriethoxy silane to mica, cysteamine was proposed to be used as a linker between gold surface and DNA molecules [8]. The function of the cysteamine on gold and to DNA molecules was schematically illustrated in Figure 1. The thiol group of cysteamine could be immobilized onto the surface of annealed gold chip. The amino group of cysteamine could interact with and immobilize DNA molecules in solution onto solid gold surface.

Annealed gold surface with cysteamine modification was thus used here as substrate for depositing DNA molecules in order to facilitate visualization of individual DNA molecules using scanning tunneling microscopy in ambient condition.

2. Methods

2.1 Preparation of cysteamine-modified annealed gold strip

Substrate strips were made of gold foil of 0.1 mm thick cut into 2 mm × 3 mm pieces. These strips were cleansed by regular detergent solution with ultrasonic cleaner at 60°C for 1 hr. Rinsed gold strips were placed inside a furnace at a temperature close to bulk melting point 1064°C for several hours. This step may be repeated for many times as long as time allowed. Annealed gold strip was deposited with 5 μl of 0.1% cysteamine for 10 min, then rinsed with a stream of distilled water and dried with air blow. Cysteamine modified annealed gold strip was glued with silver paint onto a mount for microscope. A mount with annealed gold strip

was stored in a desiccator for later usage.

2.2 Preparation of DNA solution

DNA of bacteriophage ΦX174 was used as received from New England BioLabs, supplied at a concentration of 1,000 μg/ml in 10 mM Tris-HCl, 1 mM EDTA pH 8.0 at 25°C. DNA solution was diluted with distilled water for the final working concentration down to 4 ng/ml.

2.3 Deposition of DNA onto cysteamine modified annealed gold surface

A drop of 5 μl DNA solution was deposited onto cyseamine modified gold chip for 3 min. Rinsed with a stream of distilled water and dried with Nitrogen blow. The mount with already DNA treated annealed gold strip was stored in a desiccator till later scanning.

3. Results

The cysteamine-modified annealed gold surface was imaged by Scanning Tunneling Microscope in air. The scanning rate was 2 Hz. Figure 2 showed the annealed gold surface formed as terraces. The atomically flat gold surface did not become rough due to modification of 0.1% cysteamine. No DNA was deposited onto this gold surface yet.

Figure 3 showed many individual DNA molecules after exposing cysteamine-modified annealed gold strip to a drop of DNA solution. The individual DNA molecules seen on the surface were ΦX174RF. This distribution showed a perfect spreading. Supercoiled DNAs were in the same orientation, indicating a liquid flow. The image was not sharp, indicating the sample was wet. The tungsten tip had better be coated with nail polish oil if possible.

4. Discussion

Deposition of DNA molecules onto cysteamine-modified annealed gold surface was successful in observing individual DNA molecules by means of Scanning Tunneling Microscopy in air. The result obtained here indicated that, although DNA molecules were pulled toward a certain orientation due to the influence of surface tension between solution and air along the course of drying process, cysteamine-modified gold surface could immobilize supercoiled DNA molecules up to a certain degree. For better situation, the preparation method may be improved further by using a stream of mist containing DNA molecules instead of liquid-drop of sample solution.

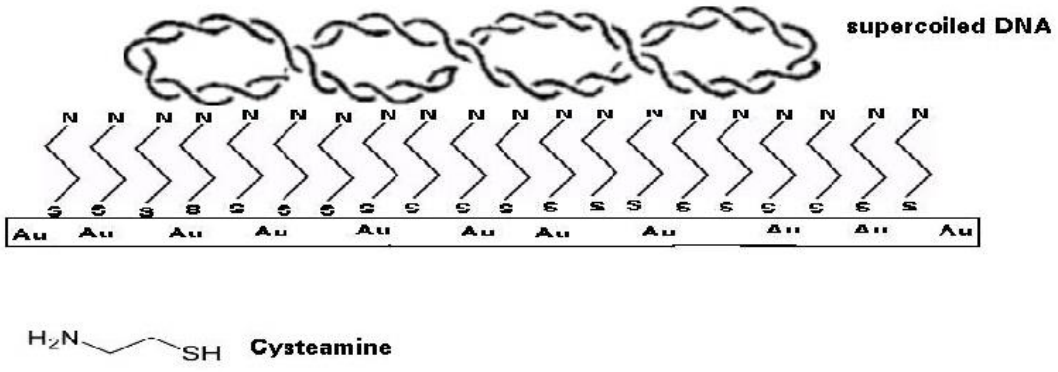

Fig. 1: Schematic diagram of cysteamine monolayer on annealed gold surface. The thiol group of cysteamine could be immobilized onto the surface of annealed gold chip. The amino group of cysteamine could interact with and immobilize DNA molecules in solution onto solid gold surface.

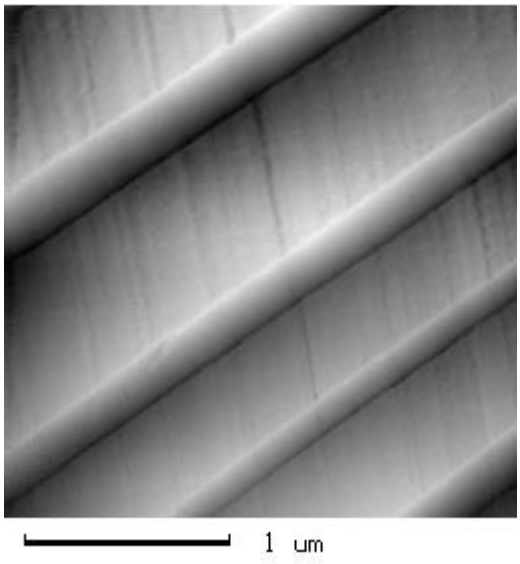

Fig. 2: The image was taken by STM in air. The annealed gold surface formed as terraces. The atomically flat gold surface did not become rough due to modification of 0.1% Cysteamine. No DNA was deposited onto this gold surface yet.

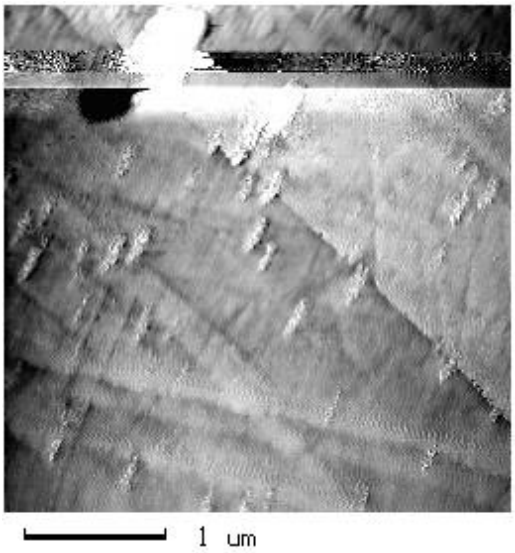

Fig. 3: The image was taken by STM in air. Exposing cysteamine-modified annealed gold strip to a drop of DNA solution, many individual DNA molecules were observed. The individual DNAs seen on the surface were ΦX174RF. This distribution showed a perfect spreading. They were in the same orientation, indicating a liquid flow. The image was not sharp, indicating the sample was wet.

5. References

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以掃描穿隧顯微術觀測分佈於半胱胺修飾之退火黃金的個別 DNA 分子

丁君毅

摘 要

為了在大氣條件下以掃描穿隧顯微術觀測 DNA 分子，必須將 DNA 分子固定於導體基質上。退火黃金符合充當導體基質的最佳要求。為克服溶液與大氣間的表面張力強行拖移 DNA 分子，退火黃金表面須以半胱胺修飾，藉以定住 DNA 分子於基質上。本實驗藉此而成功觀測到許多個別 DNA 分子的分佈影像。

關鍵詞：掃描穿隧顯微術， DNA ，半胱胺修飾之退火黃金表面