Assembly and electrical property of GFP/Cytochrome *b562* Fusion Protein ontothe Au Substrate

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Abstract

Transfer of an electron from one site to another in a molecular or between molecules and/or electrodes is one of the most fundamental and ubiquitous processes in chemistry, biology and physics. In this study fusion proteins composed by green fluorescent protein(GFP) and cytochrome *b562* were used in fabricating molecular array as an electron sensitizer and electron acceptor. Protein formation onto the substrate was performed by the self-assembly technique. The fusion protein film were analyzed using scanning probe microscope(SPM), Surface Plasmon Resonance(SPR) and hybrid STM/I-V. The results suggest that the proposed molecular photodiode can be used as a basic unit of the memory device.

Introduction

For fabrication of the highly ordered and close-packed molecular arrays of biological and/or organic molecules, ultra-thin film preparation techniques, such as Langmuir-Blodgett (LB) or self-assembly (SA), have been widely used [1]. Relatively simple technique for the preparation of ultra-thin film assembly, one of the SA techniques, has recently been developed using the alternate electrostatic deposition of oppositely charged polyelectrolytes, and extended to the applications to thin films fabrication of a wide range of charged molecules, such as globular proteins, enzymes, DNA, viruses, and etc.[3]. In this study, the fusion protein, composed of cytochrome *b562* and GFP which used as an electron acceptor and as a sensitizer, was deposited onto the Au substrate by self assembly technique. Scanning Tunneling Microscopy (STM) and SPR was used for confirm of formation of protein layer onto Au substrate. The

surface structure of the GFP/cytochrome *b562* fusion protein layer was analyzed using STM. And the unidirectional electron flow from GFP to cytochrome *b562* was confirmed by current-voltage characteristic with STM.

Experimental Materials

The GFP/Cytochrome *b562* fusion protein was designed by T. Nagamune *et al.* In vector construction, the linker sequence between cytochrome *b562* and GFP is Gly-Ser and the fusion protein was expressed in *E. coli* strain. The cytochrome *b562* part of fusion protein was expressed mainly the apo form, however, it could be easily reconstituted with exogenous hemin. The fusion protein was made by T. Nagamune *et al.*

Method

The film formation of fusion protein was performed by self-assembly technique. The fusion protein film was analyzed using STM. Surface morphology of fusion protein layer was obtained at a constant height mode, ambient temperature. Tunneling current and bias voltage was I=0.5nA and V=1mV, respectively. Au wire was used as a STM tip. Another method to confirm of formation of protein layer was by analysis on SPR curve. To investigate the unidirectional electron flow of fusion protein, hybrid STM/I-V measurement system was used. For the tunneling spectroscopy measurements, STM tip was used as a top electrode. During the I-V measurements, topographic images were monitored regularly to ensure that the tip did not drift apart. Electrons flow from the tip to the substrate for positive bias value. Experimental setup for the STM/I-V measurement is shown in Figure 1.

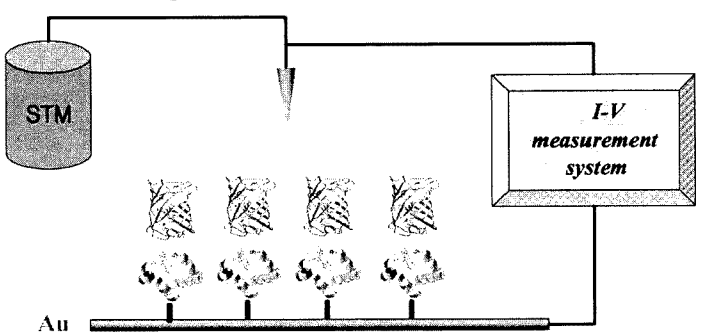

Figure 1. show the hybrid STM/I-V mesurment.

Results and Discussion

To immobilize the fusion protein onto the Au substrate, self assembly technique was used. To immobilize the fusion protein onto the Au substrate, the fusion protein was designed with thiol group insertion. Thus it can be easily adsorbed onto the Au substrate. Figure 2 shows the STM topographic images of fusion protein monolayer deposited by self assembly technique onto the surface of Au. STM observations of surface of the fusion protein layer were reproducible.

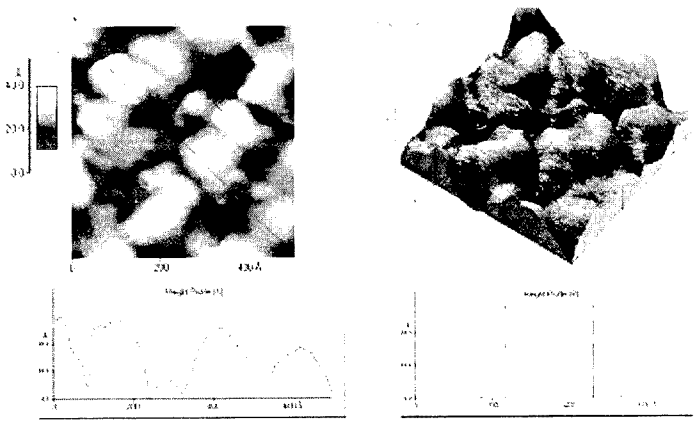

Figure 2. STM image of fusion protein adsorbed onto the Au substrate.

Figure 2 was STM image with scan size of 0.1μm and 500Å. In figure 2, protein clusters with size of 100~200Å and protein molecular with size of 40~60Å were observed. Adsorption of fusion protein onto the Au substrate was investigated by surface plasmon resonance(SPR) in steady state and flow system. In figure 4, SPR curve in steady state is shifted and it means that protein film was fomated onto Au layer.

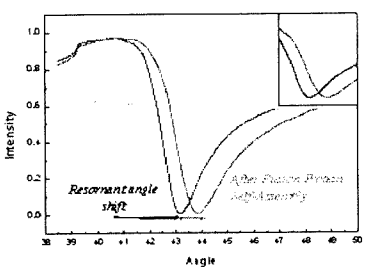

Figure 3. SPR curve of bare gold substrate and self-assembled substrate

Current-Voltage characteristics of fusion protein self-assembly layer were investigated by STM/I-V measurement. In STM/I-V measurement, rectifying properties were observed to verify the unidirectional electron transfer or current flow. It is suggested that the proposed molecular array of fusion protein can be applied to the molecular scale diode.

Acknowledgement

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