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A robust nanoscale biomemory device composed of recombinant azurin on hexagonally packed Au-nano array

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ABSTRACT

We developed a nanoscale memory device consisting of signal-responsive biomaterial, which is capable of switching physical properties (such as electrical/electrochemical, optical, and magnetic) upon application of appropriate electrical signals to perform memory switching. Here, we propose a highly robust surface-confined switch composed of an electroactive cysteine-modified azurin immobilized on an Au hexagonal pattern formed on indium tin oxide (ITO) substrates that can be controlled electrochemically and reversibly converted between its redox states. The memory effect is based on conductance switching, which leads to the occurrence of bistable states and behaves as an extremely robust redox switch in which an electrochemical input is transduced into optical and magnetic outputs under ambient conditions. The fact that this molecular surface switch, operating at very low voltages, can be patterned and addressed locally, and also has good stability and excellent reversibility, makes it a promising platform for nonvolatile memory devices.

1. Introduction

Due to continuously increasing demand for ultimate miniaturization of electronic systems, molecular electronic devices are currently thriving as alternative technologies because of their promising potential in writing, reading, and processing of information at the nanoscale (Waser and Rudiger, 2004). Nanostructures exhibit optical, thermal, electrical, and magnetic properties that differ from bulk materials (Vaseashta and Dimova-Malinovska, 2005). To apply these properties to functional devices, it is often important to control their size, shape, and position on a substrate (Liz-Marzán, 2004). To achieve increasing demand for miniaturization, considerable attention has been paid to develop nanoscale devices by overcoming the technical difficulties that are currently facing the semiconductor industry. Micro-fabrication technologies are reaching fundamental diffraction limits as feature sizes approach 100 nm. Hence, there is a substantial interest in developing nanofabrication techniques to achieve both high resolution and high throughput. Several techniques have been explored with the goal of creating ordered arrays such as: (i) anodized aluminum oxide (Jung et al., 2004), which has drawbacks, such as low throughput and high cost; (ii) interference lithography (Zuppella et al., 2009), which is limited to patterning arrayed features only; (iii) self-assembly of nanoparticles, laser focused atomic deposition (Behringer et al., 1996), which has a major limitation is (a) its slowness; (b) many technologically important materials (Si, Ge, Si_3N_4, several multi-component oxides, certain metals) cannot be deposited in a cost-effective way and (c) being a chemical technique there is always a risk of residues being left from the precursors and (iv) diffusion controlled aggregation and surfaces (Roder et al., 1993), which has limitations, such as diffusion stops when an adaotm hits a stable aggregate and condenses there. Also, it is temperature dependent. Apart form these techniques, nanolithography based on scanning tunneling microscopy (STM) has received considerable attention, (Williams and Gorman, 2007) as it is useful for imaging and can operate at atomic scale (Eigler et al., 1991; Stroscio and Eigler, 1991). However, application of Scanning tunneling microscopy (STM) lithography, like electron beam lithography, has drawback as it is limited by serial processing speeds. Additonally, these techniques utilize complex procedures; thus, novel approaches to parallel nanolithography are being explored. Recently, colloidal or nanosphere lithography which is emerged as an effective and inexpensive method for patterning nanostructures over a large area (Whitney et al., 2004) in which the colloidal nanospheres act as a *mask*. Regular pattern of desired material can be obtained by after deposition and the removal of mask.

Because of high surface-to-volume ratio and tunable electron transport properties due to quantum confinement effect, nanostructures can be used for both efficient transport of electrons and

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optical excitation, and these two factors make them critical to the function and integration of nanoscale devices. In addition, the sizes of biological macromolecules, such as proteins, enzymes, and nucleic acids, are comparable to nanoscale building blocks. Therefore, any interaction between such biomolecules should induce significant changes in the electrical properties of nanostructured biointerfaces. But, one of the major goals of nanoelectronics is to make use of single molecules that function as switches on nanoscale electrode surfaces. This requires developing molecules that can reversibly switch between two conductive states in response to an external voltage/trigger. Azurin possesses two distinct electrochemical states, a reduced and oxidized states that can be reversibly controlled to develop a nanoscale switch. In our previous studies, a protein-based memory was demonstrated using a monolayer of ferredoxin, a redox protein, as an active material and an intrinsic switching mechanism (Yagati et al., 2010). Furthermore, a write-once–read-many times (WORM) type protein-based memory using a redox recombinant protein has been demonstrated (Yagati et al., 2009a). Multi-bit protein-based memory to store multiple bits electrochemically at a time and its read-out mechanism has been proposed (Yagati et al., 2009b). Also, digital memory device based on virus conjugated with nanoparticles where virus was used as a template for ordering quantum dots (Tseng et al., 2006). Further, a protein-based associative processors and volumetric memory was proposed by Birge et al., 1999. However, a method to *read* the electrochemically stored charge with optical and magnetic properties of protein molecules at the nanoscale level for realizing a memory device has not been reported yet.

Blue copper azurin (about 14.6 kDa), the protein of interest in the present study, is responsible for electron transfer in the respiratory system of several bacteria. We introduced cysteine residue into *Pseudomonas aeruginosa* azurin to improve the uniformity of the protein monolayer on the gold surface because a wild-type form of the azurin was irregularly adsorbed to the gold surface via its exposed disulfide moiety (Cys3–Cys26) (Rinaldi et al., 2002). Since azurin has copper as a key element in the electron transfer mechanisms, so this can be used as an electron acceptor in the development of molecular electronic device by mimicking biological mechanism.

Here we report, a protein based memory device, in which the recombinant protein is self assembled on Au patterned indium tin oxide (ITO) coated glass plate without the use of any additional linkers. The morphological and current measurements of azurin molecules were observed by using electrochemical scanning probe microscopy with in-situ cyclic voltammetry experiments. Current measurements were obtained by applying redox potentials to the Az/Au-ITO electrode to ensure proper memory device operation. Apart from that, clear memory device switchings for charge *storage* and *erase* functions were observed with optical and magnetic detection methods. Further, variations in the conductivity of Au thinfilm with an Au nanopattern on an ITO electrode were compared. The device reproducibility and revisbiliy was also examined with application of continuos *write* and *erase* cycles.

2. Materials and Methods

2.1. Chemicals and reagents

Polystyrene particles (0.46 μm, 1% w/w) were purchased from Thermo Scientific (Rockford, IL, USA) and were centrifuged to obtain 10% w/w. Polyoxyethylene (20) sorbitan monolaurate was obtained from Sigma-Aldrich. 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), buffer solution (10 mM, pH 7.0) was used in electrochemical experiments. All other solutions were prepared with water (18 MΩ cm) purified using by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Electrode cleaning procedure

ITO-coated glass substrates with dimensions $10 \times 10\ mm^2$ with a thickness of 400 nm were cleaned ultrasonically in successive solutions of Triton X-100/water (1:5, v/v), water and ethanol for at least 40 min each. The substrates were then treated in an oxidizing bath of NH_4OH: H_2O_2: H_2O (1:1:5) at 80 °C for 1 h to remove particulate contaminants and then washed thoroughly with DI water, and dried in N_2 stream.

2.3. Fabrication of Au hexagonal nanopattern on ITO surface

The nanosphere masks were formed by spin-coating polystyrene particles on cleaned ITO substrates using nanosphere lithography technique (Winzer et al., 1996). Particles of 0.46 μm were spin-coated at a speed of 2000 rpm for 20 s. The physical dimensions of the substrate were chosen to be $10 \times 10\ mm^2$, and the entire surface was spin-coated with nano-spheres. The nanospheres of 10 wt% dispersed in water were further diluted in Triton X-100/methanol (1:400 by volume) before spin-coating. The dilution factors for single-layer masks were ca. 1:1 (by volume) for the 460 nm particles. A surfactant was used to assist the solutions to wet the substrate. Double-layer masks were self-assembled by increasing the nanosphere concentration and dilution factors in the spin-coating solution to optimize double layer surface coverage. Thin films of Au were deposited through vacuum evaporation over the coated substrates. To obtain regular pattern, the polystyrene spheres were lifted off of from the ITO substrate by dissolution in methylene chloride (CH_2Cl_2) with the aid of sonication for 1–2 min as shown in Fig. 1.

2.4. Preparation of recombinant azurin and immobilization on Au nanopattern

Azurin was recombined with cysteine residues by site-directed mutagenesis (Lee et al., 2010; Choi et al., 2009). Cysteine modified azurin was immobilized on the patterned Au array by the strong affinity between the thiol of cysteine and Au. 20 μl of prepared recombinant azurin (0.1 mg/ml) was dropped on the Au pattern over 3 h and then treated with 0.05% solution of polysorbate20 for 2 h to remove any excess or unbound molecules on the Au triangles. Finally the azurin immobilized Au pattern was cleaned with deionized water and dried under nitrogen stream (Lee et al., 2011).

2.5. Measurements on Au hexagonal nanopattern

The surface topography of each fabrication step was investigated by Scanning electron microscopy (SEM) measurements using a JEOL JSM-7500F operating at a voltage of 15 kV having magnification 25x to 1,000,000x and resolution imaging: 1.4 nm at 1 kV, 1.0 nm at 15 kV.

The electrochemical experiments were performed on Az/Au-ITO surface by using Multimodal Nanoscope III from Veeco Instruments (Plainview, NY, USA) with an electrochemical module (EC-STM) (Digital Instruments). The in-situ cyclic voltammetry was performed using EC-STM bipotentiostat module. A Pt wire as counter and Ag electrode served as quasi-references for the EC-STM experiments. Pt/Ir tips (14 mm) were purchased from Veeco Instruments and were insulated using apiezon wax polish to measure the tunneling current and effectively blocking the leakage/background currents. Images were scanned at $E_{bias} = 100$ mV, $I_{tip} = 4$ nA with a rate of 1 Hz.