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ScienceDirect
Journal of Physics and Chemistry of Solids 68 (2007) 1211–1214
JOURNAL OF PHYSICS AND CHEMISTRY OF SOLIDS
www.elsevier.com/locate/jpcs

Patterned self-assembly of magnetic biomolecules on semiconductor substrates

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Abstract

The paper reports on the methods of preparing and patterning magnetic metallothionein (Mn,Cd-MT-2) molecules on semiconductor surfaces. The molecules are placed into nanopores prepared on silicon (0 0 1) substrates. We observe the self-assembled growth of those MT molecules on the patterned Si surface. Dense arrays of molecular rods are demonstrated on templates with small pores and pitch sizes. Then the structures of the self-assembled protein are studied by atomic force microscopy (AFM) and magnetic force microscopy (MFM). Our measurements indicate that molecular self-assembly has a magnetic dipole moment which interacts with the applied magnetic field.

Keywords: A. Nanostructures; A. Semiconductors; C. Electron microscopy; D. Magnetic properties

1. Introduction

Molecular clusters are small objects characterized by a small spin compared with single-domain particles, but are significantly larger than the largest spin observable in atoms. The macroscopic magnetization of these clusters shows bi-stability, hysteresis effects, and super-paramagnetic behavior [1] similar to the more classical single-domain particles; hence, they are also known as “single molecule magnets”. Molecular magnetic clusters have attracted great interest for their potential applications such as single molecule memory units and spin sensors. In fact, improving the magnitude of the critical temperature is a necessary condition for any potential application utilizing single molecule magnets [1]. Another major problem for their use in applications is how to handle a single molecule. Attempting to deposit the clusters on substrates (metallic, insulating, or semiconductor) is a necessary step toward the utilization of these molecular clusters.

Surface modification and patterning at the nanoscale is a frontier in science with significant possible applications in biomedical technology and nanoelectronics. A thin film of diblock copolymers can self-assemble into ordered periodic structures at the molecular scale ($\sim 5-50\,\mathrm{nm}$), and have been used as templates to fabricate quantum dots [1,2], nanowires [3–5], and magnetic storage media [6]. More recently, in an epitaxial assembly of block-copolymer films, molecular level control over the precise size, shape, and spacing of the order domains was achieved with advanced lithographic techniques [7]. The development of methods for patterning and immobilizing biologically active molecules with micrometer and nanometer scale control has been proven to be integral to a wide range of applications such as basic research, diagnostics, and drug discovery. Some of the most important advancements have been in the development of biochip arrays that present either DNA [8], protein [9], or carbohydrates [10]. Modification of a surface to anchor protein molecules is an important strategy for obtaining the construction of new biocompatible materials with smart bioactive properties.

In recent years, there has been substantial attention focused on the reactions of organic compounds with silicon

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doi:10.1016/j.jpcs.2007.01.035

surfaces. In this work, we demonstrate the methods of fabricating the magnetic molecules produced from metallothionein (MT-2) and patterning the Mn,Cd-MT-2 molecules on silicon surfaces with advanced electron-beam lithography techniques. The magnetic properties of the molecular self-assembly and the possible applications in nanodevices and spintronics were also investigated.

2. Synthesizing magnetic Mn,Cd-MT-2 molecules

The steps for synthesizing the magnetic molecules are described as follows. A total of 5 mg of MT-2 was dissolved in 5 ml denatured buffer at pH 11 to strip away all metal ions. The free metal ions were removed by dialysis against the same buffer solution. The denatured MT-2 was refolded through the quasi-static procedure. In addition to the basic constituents of the buffer solution at pH 11, 10 μM Mn^{2+} and 10 μM Cd^{2+} were added during the dialysis for removing urea. These two metal ion concentrations were increased to 1 mM when the pH was lowered to 6.8. The excess Mn^{2+} and Cd^{2+} in the solution were removed after MT-2 was again folded back to its native form through dialysis to ensure that no free metal ions were present.

The metal content was determined using the graphite furnace atomic absorption spectrophotometer at 20 °C. The UV and circular dichroism (CD) spectra were recorded on a UV-VIS spectrophotometer and a spectropolarimeter, also at 20 °C. The size of synthesized Mn,Cd-MT-2 was measured by dynamic light scattering (DLS), and its effective diameter was measured at 2.86 ±0.29 nm, which is identical to the effective size of the native MT-2. Both the UV absorption and the CD spectra of Mn,Cd-MT-2 were similar to those of the native MT-2. These results indicate that the Mn,Cd-MT-2 has been refolded into its native conformation.

The magnetic moment of Mn,Cd-MT-2 was measured on a 1.8 mg lyophilized powder sample by a commercial SQUID magnetometer in a sealed capsule from 10 to 330 K. By applying a cyclic external magnetic field between 3 and −3 T, a clear magnetic hysteresis cycle was observed. Fig. 1 shows the result at temperature 10 K after subtracting a linear diamagnetic background. The magnetic moment of Mn,Cd-MT-2 was saturated at ±0.2 T, and the value of magnetic moment was about 311.4 emu/mol (emu, electron magnetic unit), the remanence was about 5% of the saturation, and the coercive field was around 40 Oe (Oersteds). The observed hysteresis behavior of the magnetic moment indicates that Mn,Cd-MT-2 is ferromagnetic. Its magnetic moment changed within 4% (of 311.4 emu) between 277 and 330 K with a fluctuation of 0.3%. The Mn,Cd-MT-2 not only holds a significant and detectable magnetic moment at low temperatures but also remains stable at room temperatures.

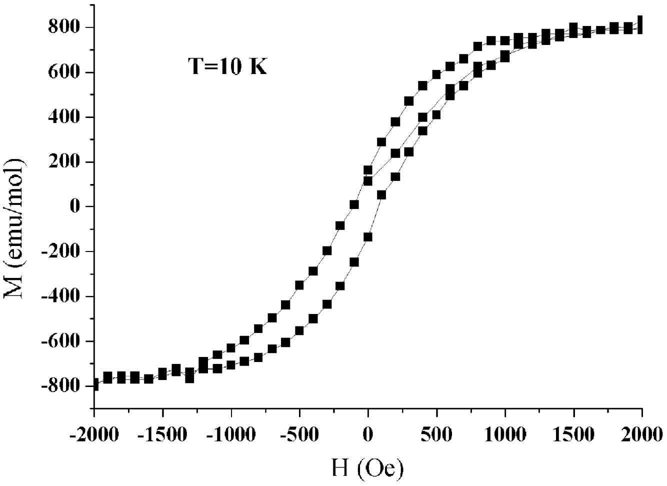

Fig. 1. Magnetization measurements at 10 K for Mn,Cd-MT-2.

3. Pattern formation on Si surface

An Si (0 0 1) wafer was diced into 1 cm^2 substrates, which were then cleaned with a modified RCA cleaner and were dried at 150 °C for 1 h to get rid of excess moisture. The fabrication process commenced by spinning a thin layer of photoresist (ZEP-520) onto the Si wafer using a LAURELL spin coater and then prebaking at 180 °C for 30 min. Photoresist layers with a thickness between 100 and 400 nm were deposited onto the Si substrates by varying the spin rates of the coater. The samples were then exposed to the electron beam with a fixed acceleration voltage of 50 keV. Nanopore patterns were directly written by the electron beam with a square area of about 100 × 100 μm. The exposed samples were developed at 25 °C in a MIBK:IPA = 1:3 solution, followed by residue photoresist descumed through an ULVAC ozone system. By using the patterned ZEP-520 as the mask, the samples were etched onto to the Si substrates with a depth of approximately 120 nm via reactive ion etching (RIE). Samples with different pitch sizes and different pore sizes ranging from 40 to 100 nm were fabricated. In Fig. 2, we show the scanning electron microscope (SEM) image of the nanopores after the RIE process on one of the templates. The size of the nanopore increases by only 1 or 2 nm after the etching process. Their sizes and distributions are highly uniform and can be controlled with high precision.

4. Self-assembled growth of MT-2 on the patterned substrates

The above-mentioned templates were first immersed in a Tris.HCL buffer solution with an Mn,Cd-MT-2 concentration of 1 mg/ml. An electric field, approximately 20 V/cm, was applied for 5 min to drive the MT molecules into the nanopores. The sample was then washed twice with DI water to remove the unbounded MT molecules and salts on the surface.

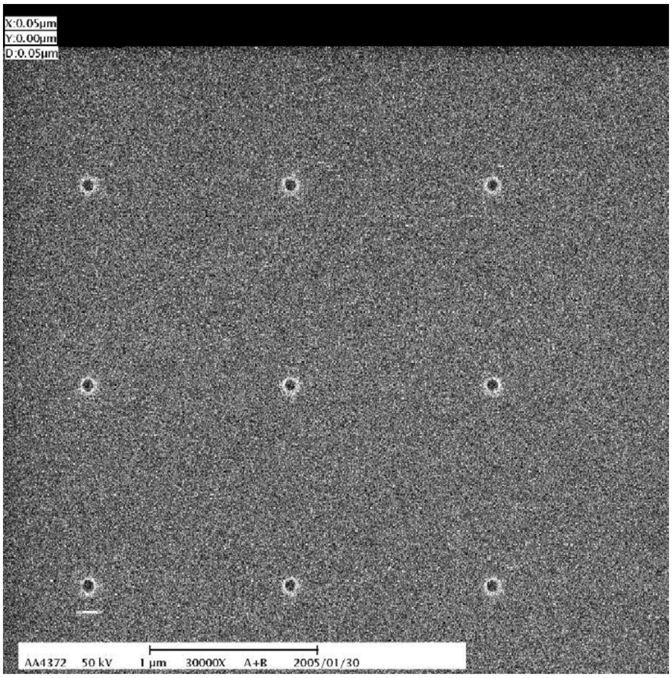

Fig. 2. SEM image of the nanopores on an Si (0 0 1) substrate after reactive ion etching process.

After the deposition of the MT molecules, the profile and phase images of the templates were measured with an atomic force microscope (AFM) operated in taping mode at room temperature. Fig. 3(a) shows the AFM image of the template surface with 80 nm nanopores and a pitch size of 600 nm after they were filled by the MT molecules. Keep in mind that the top of the Si surface was still covered by photoresist after the RIE, which prevented the MT molecules from bonding with the Si surface underneath. In contrast, the surfaces inside the nanopores after the RIE processes inevitably contained dangling bonds. Therefore, the electrical field-driven MT molecules were all anchored on a bare surface inside the nanopores. The MT molecules in each pore were found to self-assemble into a rod shape from the bottom of the pore and extended above the Si surface. Fig. 3(b) shows the AFM image of a single molecular nanorod. The molecular nanorod has an average height of ~70 nm above the template surface and a diameter equal to the size of the pore. Dense molecular nanorod arrays were prepared on the template with a density as high as $10^{10}\,\mathrm{cm}^{-2}$.

For templates with pore sizes larger than 100 nm, the MT molecules that landed inside the larger pores were able to self-assemble; however, the molecular structures were unable to extend above the template surface even with a much longer deposition time. Judging from the AFM phase images, the MT molecules have self-assembled into a more dense structure in the larger pores compared with the templates with smaller pores. We also discovered that the molecules which self-assembled in the larger pores can diffuse laterally toward the neighboring pores.

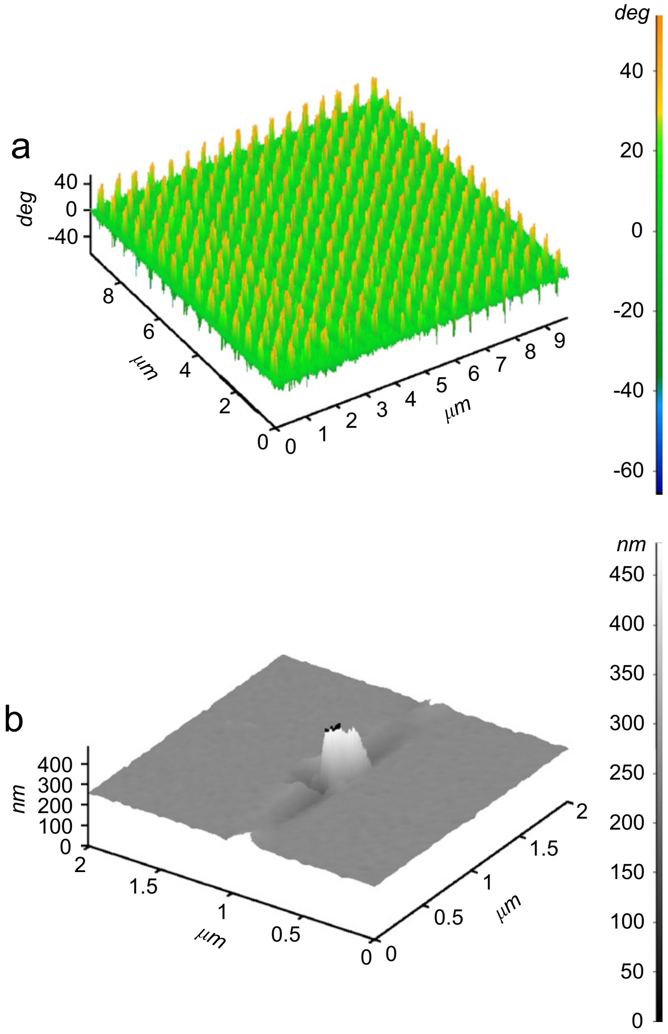

Fig. 3. (a) AFM image of the dense array of molecular nanorods, (b) AFM image of single molecular nanorod.

The magnetic properties of the self-assembled molecular nanorods were investigated with a magnetic force microscope (MFM). We monitored the contour change of a particular nanorod on the template when an external magnetic field was applied. Fig. 4(a) shows the MFM image of the nanorod without the external magnetic field. In Fig. 4(b), a magnetic field of 500 Oe was applied during the measurement with a field direction from right to left. The strength of the field was kept at minimum in order not to disturb the magnetic tip on the instrument. In Fig. 4(b), we can clearly see that the contour of the nanorod has changed in shape as compared to the sample with no applied field. This indicates that the molecular self-assembly carries a magnetic dipole moment which interacts with the external magnetic field.

5. Summary

The assembly of a biological system with complex structures is dictated by highly selective, non-covalent

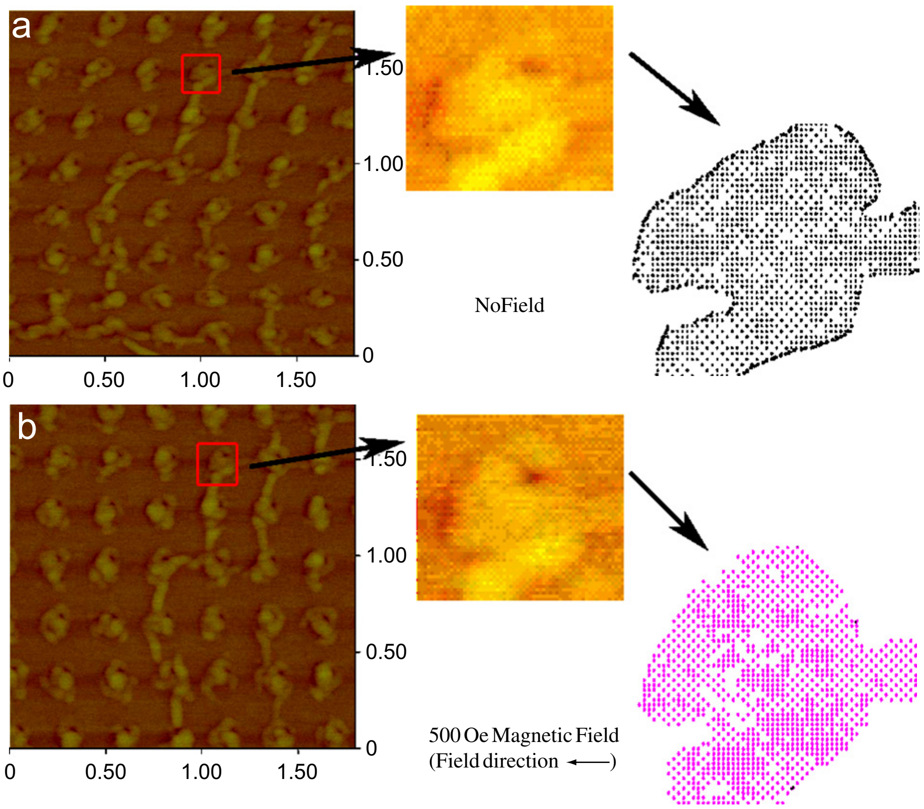

Fig. 4. MFM images of nanorods: (a) without the magnetic field, (b) with a 500 Oe magnetic field applied with a field direction from right to left.

interactions, such as hydrogen bonding and van der Waals attractions. We believe that the first layer of proteins anchored inside the nanopores bonded with the Si surface dangling bonds. They provided building blocks for proteins that arrived at a later time. With the assistance of spatial confinement from the patterned nanostructures, the rest of the proteins were able to self-assemble via van der Waals interactions and form a molecular self-assembly.

In conclusion, we successfully synthesized magnetic Mn,Cd-MT-2 molecules and demonstrated methods for patterning this particular molecule on nanostructured semiconductor surfaces. The MT molecules were shown to self-assemble into 3D nanostructures. Although the details of the self-assemble mechanism and its magnetic properties need further study, the unique features of molecular magnetization and biocompatibility make it an excellent candidate for biological applications and sensing sources of nanodevices.

Acknowledgment

This study was supported in part by grants from the National Science Council of the Republic of China under contract NSC 95-2112-M-009-046 and the MOE-ATU program.

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