VI-D Synchrotron Radiation Stimulated Surface Reaction and Nanoscience

Synchrotron radiation (SR) stimulated process (etching, CVD) has excellent characteristics of unique material selectivity, low damage, low contamination, high spatial resolution and high precision, *etc*. In this project, nanolevel controlled structures are created using the synchrotron radiation stimulated process, and the reaction mechanisms are investigated by scanning tunneling microscopy and atomic force microscopy. Concerning the SR etching, we are considering to apply this technique to the microfabrication of integrated protein transistor circuits. Electric property measurement of a supported lipid bilayer membrane was achieved using a SiO_2/AgCl/Si device which was fabricated by synchrotron irradiation etching.

VI-D-1 Synchrotron Radiation Induced Si–H Dissociation on H-Si(111)–1x1 Surfaces Studied by In-Situ Monitoring in the Undulator-STM System

NONOGAKI, Youichi; URISU, Tsuneo

[*J. Vac. Sci. Technol., A* **23**, 1364–1366 (2005)]

Irradiation effects of the synchrotron radiation (SR) have been investigated on the hydrogen terminated- (H-) Si (111) surfaces by using the undulator beam and the in-situ scanning tunneling microscope (STM). The small protrusions (SPs) generated by the undulator beam irradiation were assigned to the rest-atoms with missing H (Figure 1). From the observed relation among the SP density, photon energy of the undulator beam and the total photon flux, it has been concluded that the main mechanism of the Si–H bond dissociation by the undulator beam irradiation is valence electron excitations of the Si–H bond by incident photons.

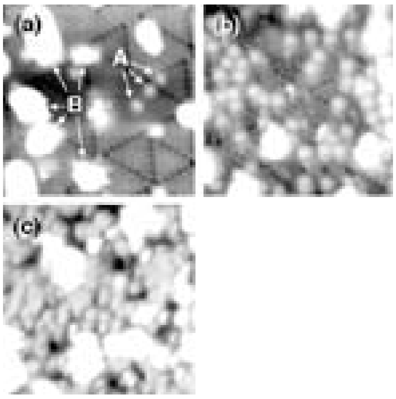

Figure 1. 10 nm × 10 nm STM images of H-Si(111) surfaces after the undulator beam irradiations with the exposure of (a) 0 mAsec, (b) 5,000 mA s and (c) 10,000 mA s. The undulator gap height was fixed at 20 mm. In (a), small protrusions (SPs) and adatom islands (AIs) are indicated by A and B, respectively. It is observed that the SP density significantly increases with increasing irradiation dose. The appeared SPs are assigned to the rest-atoms with missing H.

VI-D-2 Giant Vesicle Fusion on the Microelectrodes Fabricated by Femto-Second Laser Ablation Followed by Synchrotron Radiation Etching

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We have developed a new technique to fabricate the hole (well) with about 1 μm diameter for the microelectrode on the surface of SiO_2(600 nm)/$CoSi_2$(10 nm)/Si using synchrotron radiation (SR) stimulated etching. The Co photomask on the substrate was patterned by a femto-second-laser, and the SiO_2 layer was SR-etched. This process enabled the fabrication of the electrode holes with keeping the original nano-level flatness (R_a ~ 0.8 nm) of the SiO_2 surface. The lipid bilayer was formed by the giant vesicle fusion on these microelectrodes. Fluorescence microscopy, *in situ* AFM and electrical characteristics measurements showed that a single lipid bilayer with sufficiently high resistance (giga ohm seal) was successfully fabricated (Figure 1).

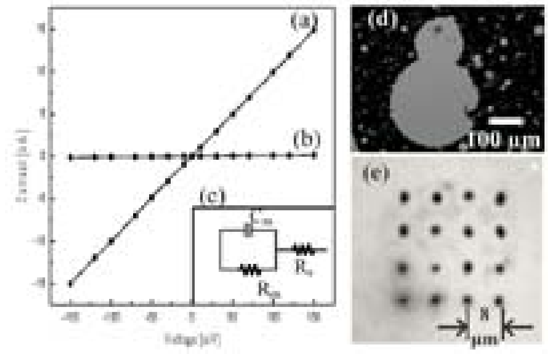

Figure 1. Current-voltage characteristics of the substrate measured under the KCl solution (10 mM), (a) before (■) and (b) after (●) the SPLB formation, and (c) the equivalent circuit of the system. The fluorescence microscopy image of the lipid bilayer formed by the rupture of the giant vesicle on (d) SiO_2/$CoSi_2$/Si surface and (e) the electrode area.

VI-E Noble Semiconductor Surface Vibration Spectroscopy

As a new high sensitive and high resolution surface vibration spectroscopy technique, we are developing an infrared reflection absorption spectroscopy using buried metal layer substrate (BML-IRRAS), which have unique characteristics of high resolution and high sensitivity at finger print regions. Several Si surface chemical reactions are investigated using this BML-IRRAS. As anew fabrication technique of BML substrate, we have almost succeeded in developing the wafer bonding technique. It is considered that BML-IRRAS is also extremely useful in the research of bio-material integration on Si substrates.

VI-E-1 Orientation of Avidin Molecules Immobilized on the COOH-Modified $SiO_2/Si(100)$ Surface

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Avidin molecules were immobilized on COOH-modified $SiO_2/Si(100)$ surfaces with subnano-level flatness ($R_a < 0.1$ nm) forming covalent bonds between COOH groups on the substrate surface and NH_2 groups of the avidin molecules. The avidin-immobilized surfaces were characterized by atomic force microscopy (AFM), infrared reflection absorption spectroscopy using buried metal layer substrate (BML-IRRAS), transmission infrared absorption spectroscopy (TIRAS), and ellipsometry. BML-IRRAS and TIRAS have the sensitivities to the perpendicular and the parallel (to the substrate surface) components of the dynamic dipole moment, respectively. In the β-sheet, the amide I transition dipole moment is oriented parallel to the C=O bond, which is oriented perpendicular to the axis of the β-strand which forms a β-barrel in avidin. Therefore, considering the molecular orientation dependence of the extended β-strand (1637 cm^{-1}) peak intensity in avidin, it will be a minimum in BML-IRRAS and a maximum in TIRAS. It is concluded from these data that the avidin molecules are immobilized with the symmetry axis of the tetramer almost perpendicular to the substrate surface.

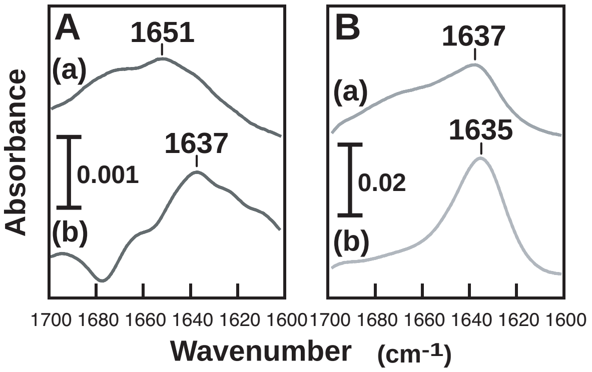

Figure 1. IR spectra of avidin molecules (A) covalently immobilized and (B) physisorbed on SiO_2/Si surfaces. The spectra (a) and (b) are measured by BML-IRRAS and TIRAS, respectively.

VI-E-2 Hydrogen-Atom-Induced Oxidation Reaction on Water-Terminated Si Surface, $2H+H_2O/Si(100)–(2x1)$: A Theoretical Study

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([1]Kyushu Univ.; [2]Nagoya Univ.; [3]AIST)

[*Chem. Phys. Lett.* **412**, 347–352 (2005)]

The reported oxidation reaction observed by BML-IRRAS spectra on the silicon surface system, 2H + $H_2O/Si(100)$ (Figure 1), has been studied by an *ab initio* molecular orbital method. The highest transition state is found at ≈+25 kJ/mol from the reactant energy level, and the oxidation occurs easily under the experimental condition (Figure 2). The present study also accounts for the reactivity deduced from the absorption bands in the IR spectra. It is noted that the quenching of the reaction by thermal relaxation is impossible because the surface is not trapped into the metastable states located much lower in energy than the reactant.

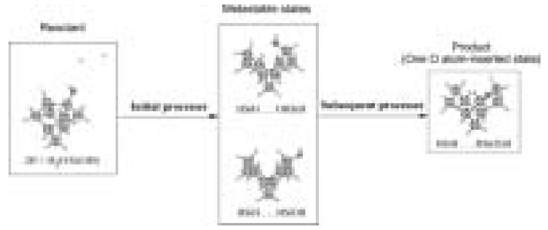

Figure 1. Reaction scheme of the H atom-induced oxidation on the H_2O-terminated Si surface system 2H + $H_2O/Si(100)–(2\times1)$, expressed by the actual conformation of the cluster model of the present work.

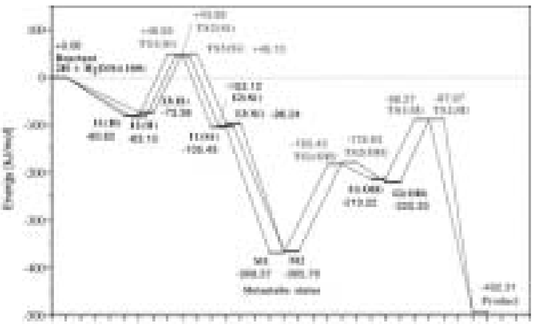

Figure 2. Energy diagram of the H atom-induced oxidation on the H_2O-terminated Si surface system. The energies (in kJ/mol) are calculated with the HF/6-31+G* method corrected for the ZPV.

VI-F Integration and Characterization of Bio-Functional Materials on Silicon Surfaces

Integration of bio-functional materials on solid surfaces is an attractive research theme and important to the development of new biosensors and screening methods in which biological reactions are directly detected on electronic circuits. We have investigated the covalent immobilization of protein, lipid bilayer membrane deposition and have characterized the characteristics of these bio-functional materials using infrared absorption spectroscopy and atomic force microscopy. We also have developed a new compact fluorescence recovery after photobleaching apparatus using a semiconductor laser, which wavelength is tuned to the absorption maximum of the fluorescence dye molecules.

VI-F-1 Fabrication of Avidin Single Molecular Layer on Silicon Oxide Surfaces and Formation of Tethered Lipid Bilayer Membranes

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([1]IMS and Kyushu Univ.)

[*e-J. Surf. Sci. Nanotech.* **3**, 237–243 (2005)]

Single molecular layer of avidin is fabricated on an atomically flat SiO_2 surface and characterized by atomic force microscopy (AFM) and infrared reflection absorption spectroscopy. Immobilization of avidin is performed as follows; i) ester-modification of the surface by silane-coupling agent, ii) carboxylation by hydrolysis in HCl and iii) amide bonding between the surface -COOH and -NH_2 in avidin molecules. Large dome structures (~60 nm height) are formed after the ester-modification, but an atomically flat surface is obtained after the hydrolysis reaction. AFM topographs and function-recognizing images (Figure 1) show that the each of avidin molecules adsorbs as a single molecule and retains the biotin-binding activity. Formation of a tethered bilayer membrane of a biotinylated phospholipid on the avidin layer is also described.

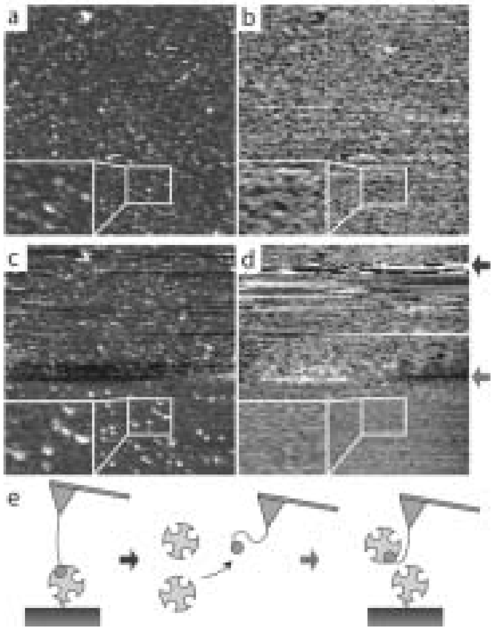

Figure 1. (a) AFM topograph (1.5 × 1.5 μm^2) and (b) function-recognizing image of the avidin on the SiO_2 surface obtained by biotinylated cantilever. (c, d) AFM topograph and function-recognizing image continuously observed after those in (a) and (b). Avidin solution was injected to the liquid phase (white arrow) and reached to the cantilever (black arrow). (e) Schematic illustration of the blocking experiment performed in (c) and (d). The size of the magnified images is 0.28 × 0.24 μm^2. All images are obtained in the buffer solution.

VI-F-2 Deposition of Lipid Bilayers on OH-Density-Controlled Silicon Dioxide Surfaces

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([1]Okazaki Inst. Integrative Biosci.)

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We investigated the effect of the SiO_2 surface hydrophilicity on the formation of lipid bilayer membranes using the vesicle fusion method with atomic force microscopy, and applied the results to constructing membrane arrays. We obtained SiO_2 surfaces with different hydrophilicity by annealing chemically oxidized SiO_2 surfaces at various temperatures under an N_2 flow. The membrane formation rate is faster on less hydrophilic surfaces after depositing a 100-nm-filtered vesicle of dimyristoylphosphatidylcholine. Desorption of the surface hydroxyl groups causes a higher affinity between the lipid membrane and the substrate. We also describe a new method to fabricate membrane arrays using “chemical patterning.” When the surface hydroxyl groups are locally removed using a focused ion beam, a bilayer membrane selectively forms on the FIB-patterned region (Figure 1).

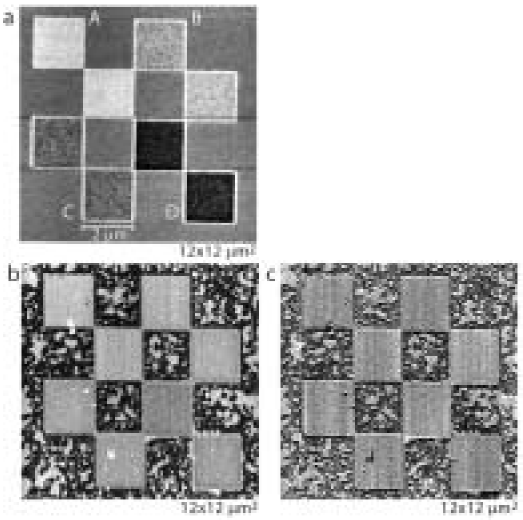

Figure 1. (a) AFM image (12 × 12 μm^2) and line profiles of 2 μm square patterns made by FIB on the chemically oxidized SiO$_2$ surface, observed in air. The Ga$^+$ ion dosages in areas A-D were 0.5, 1.5, 3 and 6 (×10^{15} atoms per cm^2), respectively. (b) AFM image (12 × 12 μm^2) and line profiles of the FIB-pattern (a) after deposition of DMPC bilayers, observed in a buffer solution. (c) Phase image (12 × 12 μm^2) obtained simultaneously with (b).

VI-F-3 Supported Lipid Bilayer Formation by the Vesicle Fusion Induced by the Vesicle-Surface Electrostatic Attractive Interaction

KIM, Yong-Hoon; RAHMAN, Md. Mashiur; ZHANG, Zhen-Long; TERO, Ryugo; URISU, Tsuneo

The effect of the electrostatic attractive force between vesicles and the substrate surface on Ca^{2+} free supported lipid bilayer formation has been investigated by using atomic force microscopy and fluorescence microscopy. When negative-charged giant vesicles were incubated without Ca^{2+}, surface coverage of lipid bilayer was extremely low on the SiO$_2$ surface (Figure 1a). It is well-known that the addition of Ca^{2+} induces the transformation from vesicle to the planar bilayer membrane on the solid surfaces (Figure 1b). On the other hand, in case of the positive-charged surface modified by aminopropyldimethylethoxysilane, the high coverage of the lipid bilayer was obtained without adding Ca^{2+} (Figure 1c). The attractive force between the negative-charged giant vesicles and the positive-charged surface is essentially useful to induce the vesicle fusion without addition of Ca^{2+}.

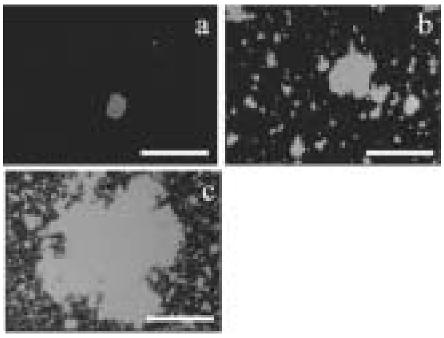

Figure 1. Fluorescence images of the lipid bilayer domain obtained under buffer solution: a) no addition of Ca^{2+} on the SiO$_2$ surface, b) addition of Ca^{2+} on the SiO$_2$ surface, and c) no addition of Ca^{2+} on the positive-charged surface. Scale bar is 50 μm.

VI-F-4 The Current Noise Characteristic of a Single Ion Channel

UNO, Hidetaka; ZHANG, Zhen-Long; NAKAI, Naohito; TERO, Ryugo; NONOGAKI, Youichi; URISU, Tsuneo

We are constructing an ion channel current measurement system using a patch clamp amplifier to investigate the lipid membrane formation, the protein reconstruction and the electrical property of membranes and proteins. The analysis of a membrane protein interaction is an important subject in the post-genome, and the development of highly efficient biosensors is demanded. We have designed a Si-based micro pore chip for ion channel current measurement (Figure 1). Teflon sheets have been generally used to make the pore, but the Si-based chip will achieve a high-speed and highly integrated biosensor.

The pore size is one of the important factors of the ion channel current measurement. The dependence of the current noise on the pore size was measured using pores with different sizes on Teflon films. The noise level single ion channel of gramicidin A in a diphytanoylphosphatidylcholine black membrane was measured in different bandwidths (Figure 2). The current noise increased with the pore size. This result shows that micronization of the pore size effectively reduce the noise level. Fabrication of Si-based chips to achieve the micron-order pore using micro-fabrication techniques is now under way.

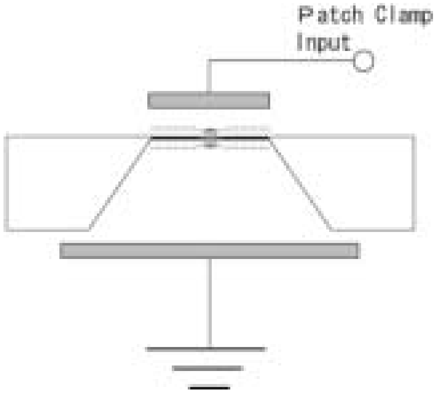

Figure 1. Schematic drawing of the Si-based micro pore chip for ion channel current measurement.

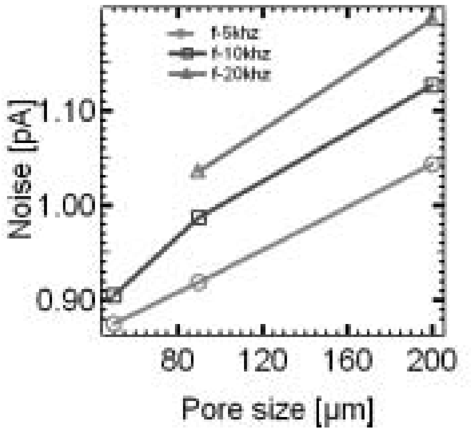

Figure 2. Channel current noise dependence on the pore size.

VI-F-5 A New Type of Fluorescence Recovery After Photobleaching Apparatus Using both Illumination Arrangements of UV Lamp and 560 nm Laser

ZHANG, Zhen-Long; MO, Yu-Jun[1]; TERO, Ryugo; AOYAMA, Masaki; YOSHIDA, Hisashi; URISU, Tsuneo
([1]IMS and Henan Univ.)

A new fluorescence recovery after photobleaching (FRAP) setup was fabricated by which the FRAP curve could be directly obtained. Figure 1 shows the FRAP setup. There are two excitation light sources in the apparatus. One is an UV lamp. The other light source is a solid state laser with a wavelength of 560 nm for photobleaching and recording the fluorescence recovery process by a photomultiplier tube (PMT). In order to avoid photobleaching during the fluorescence recovery process and improve the ratio of signal to noise, a pulse-voltage generator was designed to control the laser. The pulse induced by the generator consists of two parts. Part I is continuous for photobleaching. And part II is periodic, in which the pulse width and period are 0.01 s and 1 s, respectively. The height and duration time of the two parts could be adjusted respectively according to varied samples. Two recording devices are equipped in the setup. One is a charge coupled device (CCD) connected to a computer, by which the fluorescence of the sample could be observed and the position of the photobleached spot could be confined. The other one is the photomultiplier tube (PMT) joined with an oscillograph by which the FRAP curve could be obtained. A detection pinhole is employed to the confocal microscope before the PMT to reject the background of the fluorescence attained to PMT. Figure 2 shows the FRAP curve recorded directly by the PMT.

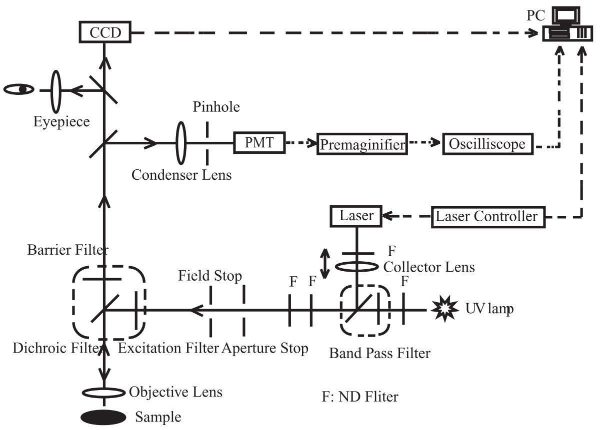

Figure 1. Schematic drawing of the new FRAP setup.

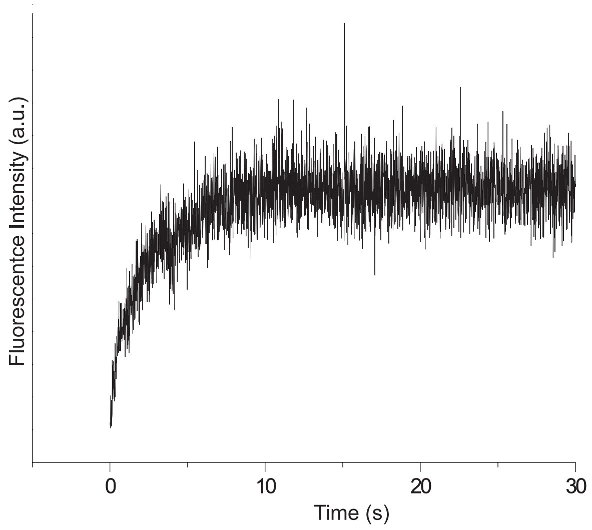

Figure 2. FRAP curve of the supported lipid bilayer (DPPC: POPS = 9:1, w/w) obtained by PMT.