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Controlled immobilization of peptide nanotube-templated metallic wires on Au surfaces

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Abstract. Peptide nanotubes were immobilized on Au substrates functionalized with self-assembled monolayers of 4-mercaptobenzoic acid in a pH 6 citric acid solution *via* hydrogen bonds between the peptide nanotubes and the monolayers. Subsequently, the immobilized nanotubes were metallized by nickel *via* the electroless coating process.

PACS. 81.16.Fg Supramolecular and biochemical assembly

1 Introduction

Controlling organic molecular self-organization has been an important challenge in order to design nanometer-scale devices [1,2]. Organic-inorganic hybrids appear to be creative alternatives for developing materials with new properties [3]. Biological and organic self-assemblies have been used as templates to form coatings with various metals and inorganic molecules [4–7]. Growing organic assemblies on metal surfaces is another important fabrication for nanotechnology applications. For example, carbon nanotubes were grown at Co-doped sites *via* pyrolysis of acetylene and assembled as carbon nanotube arrays for an electron field emission application [8]. Organic assemblies may have potential to be organized with controlled placements by introducing functional groups that exploit molecular recognition. Introduction of specific functional groups to the assemblies is relatively flexible by modifying organic monomers. Recently peptide molecules were selectively bound to semiconductor surfaces with specific crystallographic orientations [9]. Molecular recognition may even be used for site-selective immobilization of particular organic nanotubes. In other words, specific nanotubes will be immobilized to specific sites by molecular recognition without any micromanipulations. Molecular recognition sites can be introduced to organic nanotubes and/or immobilized surfaces. This approach may be very useful for economical and simple production of microelectronics. To examine the potential use of molecular recognition to order organic nanotubes, we immobilized tubular assemblies of bis(N-α-amido-glycylglycine)-1,7-heptane dicarboxylate (Figure 1-a), one of the peptide bolaamphiphiles, onto Au substrates modified by self-assembled monolayers of 4-mercaptobenzoic acid (Figure 1-b). The bolaamphiphile peptide molecules are self-assembled into tubular structures in an acidic condition, whose diameters are in the range of 20 nm-1 μm (Figure 2) [10,11]. The peptide nanotubes were probed to contain free amide groups and carboxylic acid groups by X-ray and Raman studies [10], and the amide sites intercalated metal ions such as Pt, Pd, Cu, Co, and Ni to form square planar complexes and the further electroless plating results in stable metallic coatings [12]. These amide groups and carboxylic acid groups were also utilized to absorb carboxylic acid-thiol capped Au nanocrystals *via* hydrogen bonds [13]. These sites on the peptide nanotubes may be utilized to recognize surfaces that contain amide or carboxylic acid groups *via* the hydrogen-bonding interaction (Figure 3). In this paper, the carboxylic acid groups of 4-mercaptobenzoic acid were examined to bind amide groups and/or carboxylic acid

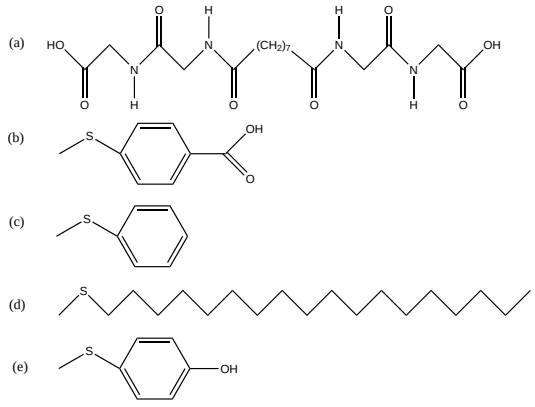

Fig. 1. Chemical structures of (a) monomeric peptide bolaamphiphile, bis(N-α-amido-glycylglycine)-1,7-heptane dicarboxylate, (b) 4-mercaptobenzoic acid, (c) benzene thiol, (d) 1-octadecane thiol, (e) 4-mercaptophenol.

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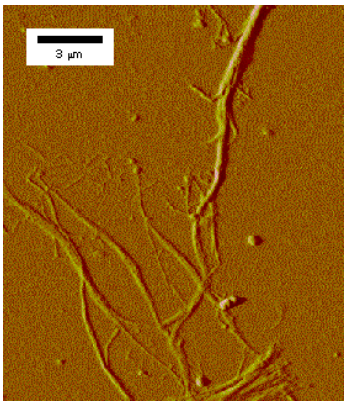

Fig. 2. AFM image of the peptide nanotubes, whose diameters were in the range of 20 nm-1 μm.

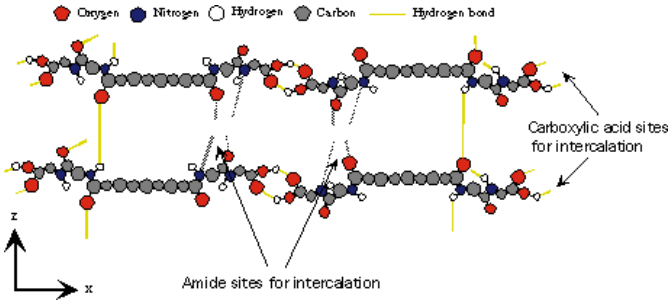

Fig. 3. Assembled structures of the peptide bolaamphiphile in the tubule: A pair of peptide bolaamphiphiles are connected by hydrogen bonds between two COOH groups *via* acid-acid dimer interactions in the x-direction. An intermolecular amide-amide hydrogen bond is formed along the z-direction and along the $(1/2)y + (1/2)z$ directions, respectively. Possible intercalating sites are shown with arrows.

groups of the peptide nanotubes. The 4-mercaptobenzoic acid was used as a model carboxylic acid SAM due to the stability as monolayers on Au surfaces [14].

2 Experiment

2.1 Peptide nanotube immobilization on Au surfaces

The self-assembled peptide nanotubes were immobilized on Au substrates functionalized with self-assembled monolayers (SAM) of 4-mercaptobenzoic acid (Figure 1-b). Bis(N-α-amido-glycylglycine)-1,7-heptane dicarboxylate was synthesized from glycylglycine benzyl ester and 1,7-heptane dicarboxylic acid in dimethylformamide (DMF) (both were purchased from Sigma Co.) [15]. The peptide nanotubes were assembled in a pH 6 citric acid/sodium citrate buffer solution. In this solution, the tubule structures of the peptide bolaamphiphiles appeared after two weeks at room temperature. Further details of the tube assembly method are described elsewhere [10,15]. The peptide nanotubes were grown as bundles in suspension, and those were sonicated for one hour to break up to short pieces of the tubes. For the gold surface modification, Au substrates were immersed in a solution of 10 mM 4-mercaptobenzoic acid (Aldrich Co.) for 48 hours after washing with methanol and drying under a stream of nitrogen. Monolayer formation of the 4-mercaptobenzoic acid molecules on the Au substrates was confirmed by Raman microscopy [14]. After being rinsed with methanol and deionized water several times, the 4-mercaptobenzoic acid self-assembly monolayer (SAM)/Au substrates were placed into citric acid solution (pH 6) with the peptide nanotubes. After one day, the immobilization of the peptide nanotubes was confirmed by sonication.

The peptide nanotubes could also grow on the SAM directly. The rinsed SAM/Au substrates were placed into the peptide bolaamphiphile solution (pH 6). In this process, peptide nanotubes was observed to grow on the SAM after two weeks. For both processes, the attachments were held even after sonicating for 30 seconds.

2.2 Metallization of immobilized peptide nanotubes

Electroless coatings of Ni on the peptide nanotubes were developed by loading the nanotubes in a Ni bath (0.08 M $NiCl_2$-$6H_2O$) for two hours. After metal ions were reduced by hypophosphite (0.1 M), the nanotubes were rinsed exhaustively with water several times. Ni coated the nanotube with and without the use of a Pd catalyst.

2.3 Microscopies

Transmission electron microscopy was conducted using JOEL 2000FX TEM (120 kV) after dried metal-coated peptide nanotubes were fixed on TEM grids. The metallized peptide nanotube surfaces were analyzed using an energy-dispersive X-ray spectrometer (EDS, Noran Voyager) with 10 kV of electron accelerate voltage on Si substrates. A confocal Raman microscope (LabRam, Jobin Yvon/Horiba) was used to obtain two-dimensional Raman images. A 632.8-nm line of an air-cooled He-Ne laser was injected into an integrated Olympus BX 40 microscope, and focused on a spot size of approximately 0.7 μm by a 80 $\times$ long working distance objective. A combination of an 1800 g/mm holographic grating and a slit size of 250 μm provided the spectral resolution at 1.8 cm^{-1}.

3 Results and discussion

The peptide nanotubes were observed to grow on the carboxylic acid SAM/Au surface after two weeks under room temperature with the direct growth procedure. Figure 4 is a light micrograph of the peptide nanotubes grown on the carboxylic acid SAM/Au surfaces. The dark region in the bottom of this figure is the SAM/Au substrate. The lighter background in the upper region of this figure is the citric acid/sodium citrate solution. The positions of the peptide nanotubes are shown with white arrows.

To demonstrate the surface-specific immobilization of the peptide nanotubes, we connected two carboxylic acid SAM/Au surfaces with the peptide nanotubes, grown separately in the citric acid solution (Figure 5). Two bright squares of the Au layers (10 μm $\times$ 10 μm $\times$ 0.2 μm) were

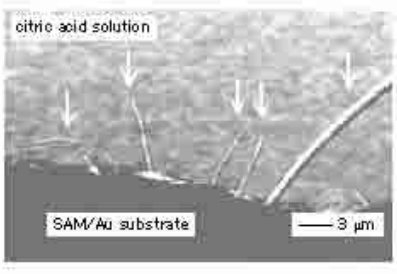

Fig. 4. A light micrograph of the peptide nanotubes immobilized on the 4-mercaptobenzoic acid SAM/Au substrate. The peptide bolaamphiphile molecules were assembled to tubular structures on the SAM/Au substrate in a pH 6 citric acid/sodium citrate solution. The location of the peptide nanotubes was shown with white arrows.

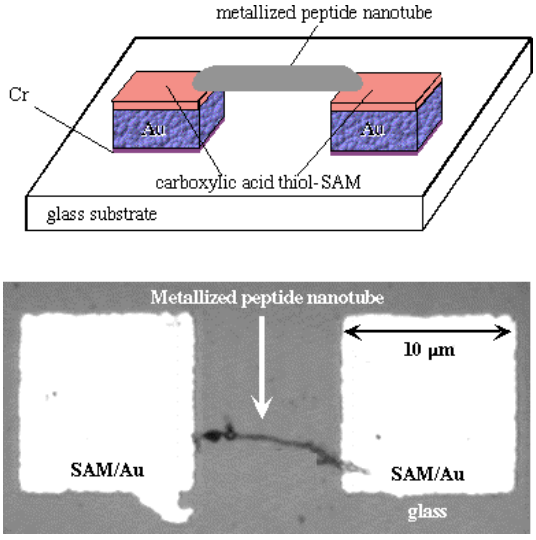

Fig. 5. A light micrograph of the peptide nanotubes immobilized onto two separated 4-mercaptobenzoic acid SAM/Au substrates. Two bright squares of the Au layers (10 μm × 10 μm × 0.2 μm), patterned on a glass substrate by photolithography, were deposited by the 4-mercaptobenzoic acid SAM. The peptide nanotubes, assembled in suspension, were placed on the carboxylic acid SAM/Au substrate and then complete the immobilization in a pH 6 citric acid/sodium citrate solution.

patterned on a glass substrate by photolithography and then the 4-mercaptobenzoic acid SAM was deposited on these Au surfaces as described above. The peptide nanotubes were grown in suspension first, and then placed on the carboxylic acid SAM/Au substrates. To complete their immobilizations, the carboxylic acid SAM/Au substrates with the peptide nanotubes were immersed in a pH 6 citric acid/sodium citrate solution. After one day, the immobilizations between the nanotube and the substrates were confirmed by sonication. While Raman microscopy was applied to probe the binding mechanism by monitoring vibrational frequency shifts of the amide and carboxylic acid groups in the peptide nanotube at the interface, there are no spectral difference between the neat peptide nanotube and the connected spot. The spectrum at the interface seems not to be affected very much by the

Table 1. Summary of immobilization conditions between the peptide nanotubes and various SAMs.

Self-assembly monolayer	Immobilization of the peptide nanotubes
4-mercaptobenzoic acid (Fig. 1b)	○
benzene thiol (Fig. 1c)	×
1-octadecane thiol (Fig. 1d)	×
4-mercaptophenol (Fig. 1e)	○

interaction since only a very small fraction of the peptide bolaamphiphile and 4-mercaptobenzoic acid molecules interact at the interface, compared with the whole probed volume by the excitation laser.

To understand the binding mechanism between the peptide nanotubes and the carboxylic acid-thiol SAM, we attempted four control experiments. First, the peptide nanotubes, prepared in suspension, were placed on the neat Au surface without any SAM depositions in the pH 6 citric acid/sodium citrate solution, and no attachment was observed. We also examine self-assembly monolayers that do not contain functional groups capable of forming hydrogen bonds with the peptide nanotubes to test hypothesis for the immobilization mechanism. A benzene thiol SAM (10 mM in methanol, Figure 1-c) was prepared in the same manner as the 4-mercaptobenzoic acid SAM and mixed with the peptide nanotubes in the acidic condition, but the tubes did not attach on the benzene thiol SAM/Au surfaces. A 1-octadecane thiol SAM (10 mM in methanol, Figure 1-d) also did not anchor the peptide nanotubes onto the surface. These control experiments indicate that SAM needs to contain functional groups, such as carboxylic acid group, to form hydrogen bonds with the peptide nanotubes for the immobilization. A 4-mercaptophenol (10 mM in methanol, Figure 1-e) anchored the peptide nanotubes in the pH 6 solution and this immobilization is due to hydrogen bonds formed between hydroxyl groups of the SAM and the amide/carboxylic acid groups of the peptide nanotubes. These results are summarized in Table 1.

While the functional groups of the peptide nanotubes involved in the immobilization have not been identified as the amide or carboxylic acid groups yet, carboxylic acid groups of the 4-mercaptobenzoic acid SAM contribute to anchor the peptide nanotubes. Since the bindings were observed to be very stable, we consider that both amide and carboxylic acid groups of the peptide nanotubes may be involved in this immobilization mechanism. This proposed scheme is illustrated in Figure 6.

Figure 7 shows a transmission electron micrograph of individual Ni-coated peptide nanotubes. Before coating, the nanotube was observed to have a tubular structure [10,15]. A hole was observed at the end of the tubule structure after the Ni coating in Figure 7. These results show that the metallized nanotube preserve a hollow structure.

The peptide nanotubes, which has been immobilized between two carboxylic acid SAM/Au substrates, were

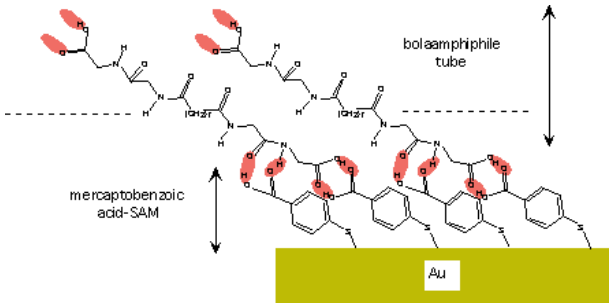

Fig. 6. Illustration of proposed binding mechanism between the peptide nanotubes and the 4-mercaptobenzoic acid SAM. Hydrogen bonds are highlighted by red circles.

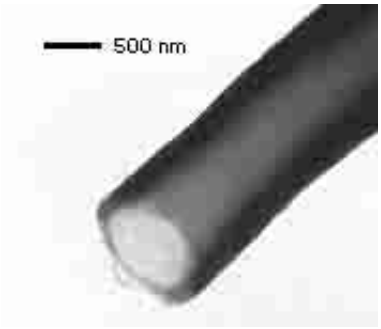

Fig. 7. A transmission electron micrograph of the peptide nanotube, coated by Ni.

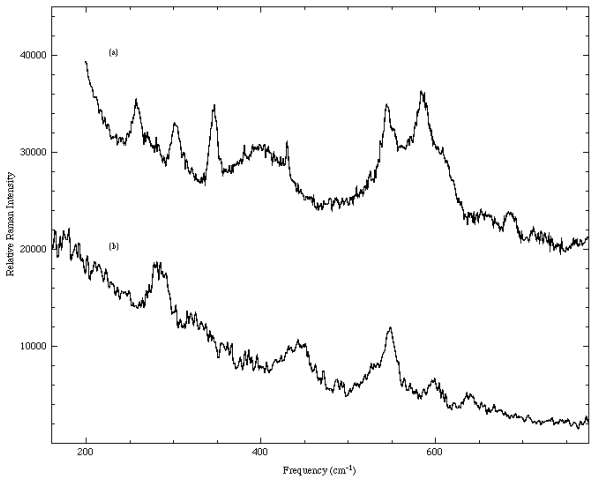

Fig. 8. Raman spectra of (a) non-coated peptide nanotube (b) Ni-doped peptide nanotube.

metallized *via* the Ni electroless coating process. The Ni coatings on the immobilized nanotubes were confirmed by elemental analysis of the nanotube surfaces using an energy-dispersive X-ray spectrometer with 10 kV of electron accelerate voltage, while uniformity of the Ni coverage on the nanotube surfaces was not yet evaluated.

Vibrational modes of the Ni-peptide nanotube complexes between two carboxylic acid SAM/Au substrates were probed by a Raman microscope (Jobin Yvon/Horiba, LabRam) with 632.8 nm excitation. Figure 8 shows Raman spectra of the non-coated nanotube, Ni-nanotube, and $NiCl_2$ solution. The Ni-O asymmetric stretch mode and the Ni-N symmetric stretch mode appear at 284 cm^{-1} and 442 cm^{-1} respectively. These assignments are consistent with the vibrational frequencies of Ni-N and Ni-O stretching modes in bis(glycino) Ni complex, 289 cm^{-1} and 441 cm^{-1} [16]. This result indicates that the Ni ions are captured by the non-hydrogen bonded amide groups of the nanotubes. It should be noted that nickel-nitrogen vibrations appear in the Raman spectra only when Ni ions bind nitrogen atoms of the amide groups in the nanotubes. These sites seem to serve as nucleation sites for further metallization. Peaks at 545 cm^{-1} and 595 cm^{-1} are assigned as the vibrational modes of the peptide bolaamphiphile from the comparison between spectra (a) and (b) in Figure 8.

4 Conclusion

The peptide nanotubes were immobilized onto Au substrates functionalized by the 4-mercaptobenzoic acid SAM *via* hydrogen bonds. This achievement may become very useful for nano-electronics fabrications since the peptide nanotubes can be coated with various metals to form metallic nanowires *via* electroless metallic coatings.

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