NANO LETTERS

2001
Vol. 1, No. 9
461−464

Protein Tubule Immobilization on Self-Assembled Monolayers on Au Substrates

Hiroshi Matsui,*,† Precila Porrata,† and Gary E. Douberly, Jr.‡

Department of Chemistry, Hunter College of the City University of New York, New York, New York 10021, Department of Chemistry, University of Central Florida, Orlando, Florida 32816

Received June 13, 2001; *Revised Manuscript Received July 24, 2001*

ABSTRACT

Avidin-coated peptide tubules, protein tubules, were anchored onto biotin-incorporated self-assembled monolayers (SAMs) on Au substrates and assembled as bridges between the SAMs. The patterned biotin-SAM/Au substrates were placed in a citric acid solution (pH 6) with the protein tubules. After 12 h, the avidin tubules were immobilized on the biotin-SAMs and some of the tubules bridged the patterned biotin-SAMs. No avidin tubules were found to attach onto the glass region between the biotin-SAM/Au substrates. This result shows that the protein tubules have potential to serve as connecting wires for microelectronics.

While present lithographic technology can produce nanoscale two-dimensional architectures, a new generation of more densely packed integrated circuits requires three-dimensional structures. To achieve three-dimensional nanoscale device fabrication with high precision and reproducibility, it is desirable to place of nanometer sized components in exact positions. By conducting fabrication processes under simple conditions, such as in solution, three-dimensional nanoscale fabrications can be performed efficiently and economically.

Organic/inorganic nanoscale building blocks are routinely and precisely turned into complex structures for biological function with almost perfect reproducibility. Therefore, biological recognition has been introduced as a linker to design novel three-dimensional structures. For example, DNA has been used as a template to construct a nanoelectrical circuit—a conducting silver wire connecting two gold electrodes.[1] Hybridization of DNA molecules with Au surface-bound oligonucleotides was effective for targeted attachment of the silver wires coated around DNA bridges.

In this report, we mimicked biological functionality of proteins to assemble three-dimensional architectures. We assembled nanostructures consisting of a receptor protein tubule connecting two complementary acceptor self-assembled monolayers (SAMs) on Au substrates using biomolecular recognition. This geometry was chosen to study the feasibility of protein tubule-based assembly because this design can be applied to build circuit components using protein tubules as connecting wires. Our goal is to establish a fabrication method that biologically functionalized building block components turn into designed device configurations with biological recognition in solution.

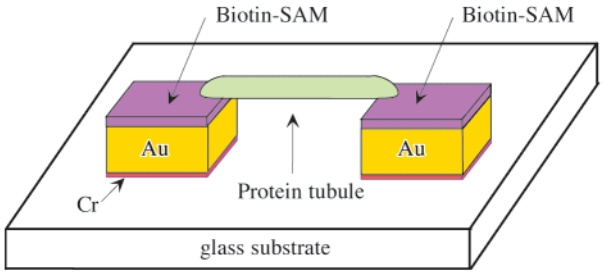

Figure 1. Illustration of an examined nanostructure, which consists of an avidin tubule bridging two biotinylated self-assembled monolayers (SAMs) on Au substrates using biomolecular recognition.

Among various available proteins, we utilized an avidin−biotin functionality to fabricate nanostructures because the avidin−biotin system has one of the largest binding free energies of association between the protein and the biotin's ligand in aqueous solution (affinity, $K_a \approx 10^{15}$ mol^{-1} dm^3) over a wide range of temperature and pH.[2] The avidin−biotin system has also been applied to construct various device configurations.[3−12] Avidin-coated peptide tubules, protein tubules, were examined to be anchored onto biotin-incorporated SAMs on Au substrates and connect two Au substrates. This configuration is illustrated in Figure 1.

Protein tubules were synthesized by binding protein molecules on bolaamphiphile peptide tubules, which act as templates.[13] Assembled structures of (*N*-α-amido-glycyl-

* Corresponding author. E-mail: hmatsui@shiva.hunter.cuny.edu.
† Hunter College of the City University of New York.
‡ University of Central Florida.

10.1021/nl015564v CCC: $20.00
Published on Web 08/14/2001

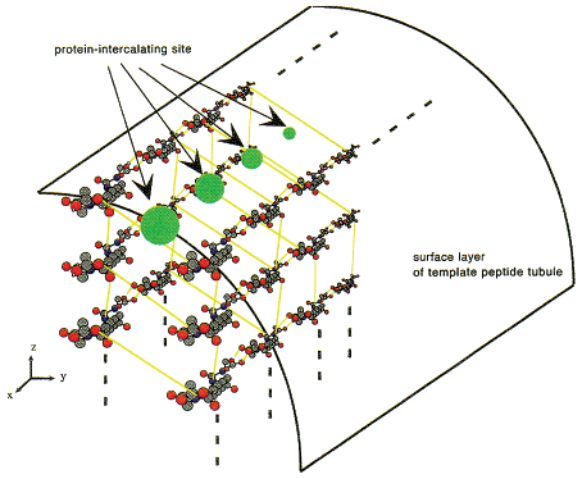

Figure 2. Assembled structure of the peptide tubule with protein molecules. A pair of peptide molecules are connected by hydrogen bonds between COOH groups as acid−acid dimer interaction toward the *x* direction. Intermolecular amide−amide hydrogen bonds are formed along the *z* direction and along the $1/2y + 1/2z$ direction, respectively. Protein molecules are intercalated between free amide groups of the peptide tubules. Hydrogen atoms are not drawn except in amide and carboxylic acid groups. The representation of atoms is in the following colors: red, oxygen; blue, nitrogen; white, hydrogen; gray, carbon.

glycine)-1,7-heptane dicarboxylate, the bolaamphiphile peptide, display sensitivity to pH, and the bolaamphiphile peptide grows to a crystalline tubule in an acidic solution.[14] The peptide tubule is assembled via intermolecular hydrogen bonds between amide groups and carboxylic acid groups of the bolaamphiphile peptide molecules (Figure 2).[14] One bolaamphiphile peptide molecule contains at least one free amide site in the tubule assembly.[15] Since all proteins possess amide functional groups capable of interacting with the tubule-free amide sites via hydrogen bonds, uniform coatings of protein molecules on the template tubules were observed.[13] The similar hydrogen bond driven coating mechanism was also observed in coatings of carboxylic acid−thiol capped Au nanocrystals onto the peptide tubules.[16]

The peptide tubules are grown in the diameter of 20 nm to 1 μm, and the peptide tubule bundles, containing various sizes of the tubes, are broken down to individual tubules via sonication in the average aspect ratio of 50.[17] Due to the current resolution limit of lithographic pattering in our facility, minimum distance between Au substrates to be fabricated is 10 μm. Therefore, we used the peptide tubules larger than 200 nm in diameter for this immobilization study. Smaller tubules were too short to bridge two Au substrates in this configuration.

Avidin was coated on the template peptide tubule with the following procedure. Bis(*N*-α-amido-glycylglycine)-1,7-heptane dicarboxylate, the bolaamphiphile peptide, was synthesized from glycylglycine benzyl ester and 1,7-heptane dicarboxylic acid in dimethylformamide (DMF) (both were purchased from Sigma Co.).[18] The peptide molecules were self-assembled into peptide tubules as templates for avidin coatings in a pH 6 citric acid/sodium citrate buffer solution. In this solution, the tubule structures were assembled after

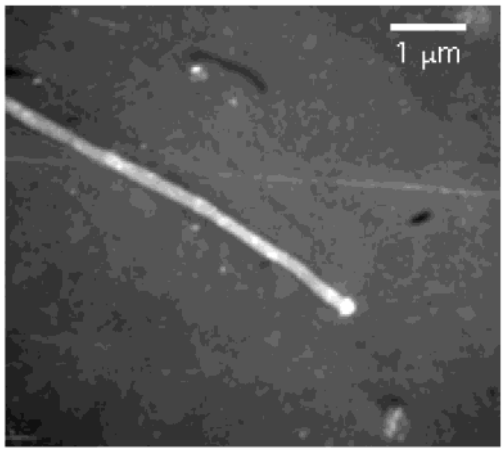

Figure 3. Fluorescence micrograph of the peptide tubule coated by FITC-labeled avidin.

two weeks at room temperature. Further details of the peptide tubule assembly method are described elsewhere.[14] The peptide tubules were grown as bundles in suspension, and those were sonicated for 1 h to break into shorter pieces of the tubes.

The peptide tubules were coated with avidin by mixing 1 μL avidin (2 mg/mL) with a washed and sonicated suspension of the peptide tubules (99 μL) in a pH 8.0 phosphate buffer solution for 12 h. The avidin coatings on the peptide tubules were confirmed by examining fluorescein (FITC)-labeled avidin-coated tubules on a Nikon Inverted Eclipse TE−300 optical microscope, equipped with a TE-FM epi-fluorescence attachment. After the FITC-labeled avidin-coated peptide tubules were washed exhaustively with phosphate buffers using a microcentrifuge, attachment of FITC on the tubules was visualized with a Nikon EF-4 B-2E/C FITC filter set (exciter 488 nm, emitter 535 nm), as shown in Figure 3. This micrograph shows uniform fluorescence on the tubule, which indicates that avidin was immobilized onto the peptide tubules and the distribution of the avidin attachment on the tubules was fairly uniform.

Squares of the Au layers (10 μm × 10 μm × 0.2 μm) were patterned on a glass substrate by photolithography, and then biotin-SAMs were deposited on the patterned Au surfaces (Figure 4a). The detailed biotin-SAM deposition procedure is described elsewhere.[19] The patterned Au substrates were cleaned in chromic acid and then rinsed with H_2O and ethanol. The cleaned Au substrates were immersed in an ethanol solution of 0.1 mM 16-mercapto-1-hexadecanoic acid and 0.9 mM 11-mercapto-1-undecanol for 16 h at room temperature, then rinsed with ethanol, and dried in nitrogen. These SAMs on Au substrates were modified by placing the rinsed substrates in an anhydrous dimethylformamide (DMF) solution of 0.1 M 1-ethyl-3-(3-dimethylaminopropyl)-carbdiimide and 0.2 M pentafluorophenol. After 20 min, these substrates were rinsed in an ethanol solution, dried in nitrogen, and then immersed in an ethanol solution of 10 mM 2,2′- (ethyhlenendiooxyl)bis-(ethylamine) to add amine functionality at the end of the SAMs. The amine groups in the SAMs immobilized biotin covalently by adding 6-(biotinamidocaproylamido) caproic acid *N*-hydroxysuc-

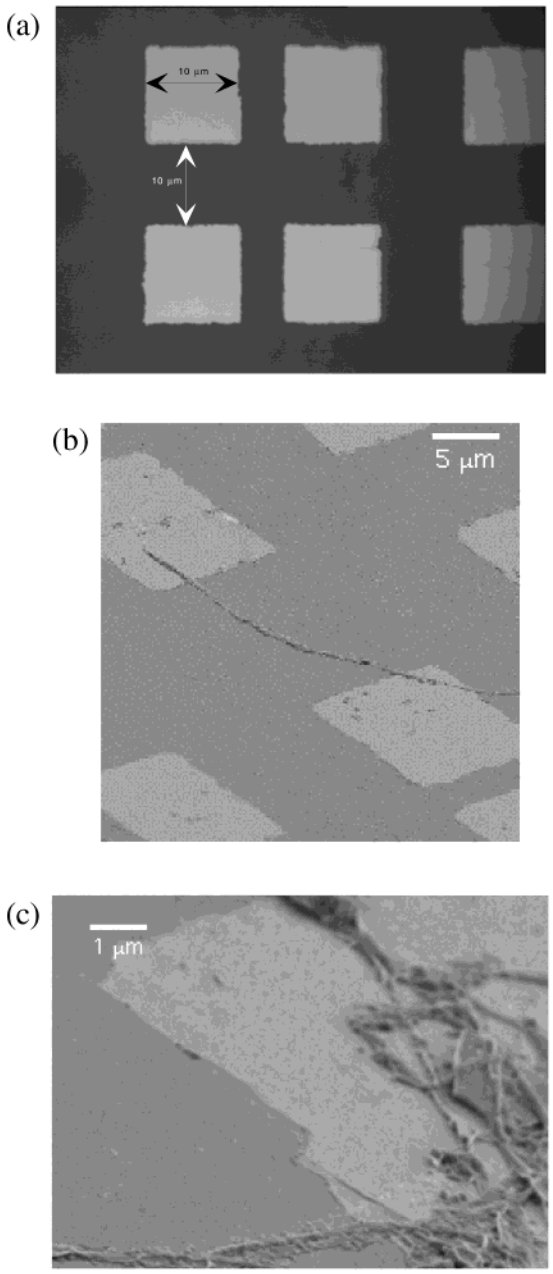

Figure 4. (a) A light micrograph of patterned biotin-SAM/Au substrates. Two bright squares of the Au layers (10 μm × 10 μm × 0.2 μm), patterned on a glass substrate by photolithography, were deposited by biotin-SAMs. The dark region between the biotin-SAM/Au substrates is the glass surface. This configuration is also illustrated in Figure 1. (b) Scanning electron micrograph of the protein tubule immobilized onto two biotin-SAM/Au substrates. The protein tubule, assembled in suspension separately, was placed on the biotin-SAM/Au substrates and then completed the immobilization in a pH 6 citric acid/sodium citrate solution. (c) Scanning electron micrograph of the multiple protein tubules immobilized onto a biotin-SAM/Au substrate.

cinimide ester in DMF (5 mg/mL) for 2 h at room temperature. Before the biotin immobilization, the substrates were rinsed in an ethanol solution and dried in nitrogen.

The biotin-SAM/Au substrates were placed in a citric acid solution (pH 6) with the avidin tubules, prepared separately. After 12 h, immobilization of the avidin tubules on the biotin-SAMs was observed and the tubule attachment was maintained even after sonication. The protein-coating mechanism on peptide tubules is described in our previous publication.[13] Observation of the avidin tubule attachment on the biotin-SAMs indicates that avidin was not denatured on peptide tubule surfaces. This observation is consistent with our previous result that avidin-coated tubules adsorbed biotin molecules on the tubular surfaces.[13] The avidin tubules that did not bind surfaces were removed by washing with water exhaustively. All of avidin tubules deposited on glass areas between the biotin-SAM/Au substrates were removed completely by this washing process. Less than 20% of tubules created the bridge configuration between two biotin-SAMs, as shown in Figure 1. The fabrication of the bridge configuration is shown in a scanning electron micrograph (SEM) image, Figure 4b. In this figure, small dots on the biotin-SAM/Au substrates are considered to be aggregation of the protein residue. We observed that 80% of the protein tubules were aggregated on the biotin-SAMs. A typical tubular aggregation pattern is shown in a SEM micrograph, Figure 4c. This observation is reasonable since the tubular aggregation geometry maximizes the contact between avidin tubules and biotin-SAMs. Therefore, majority of the biotin-SAMs was attached with multiple protein tubules. Currently, highly diluted protein tubule solution is examined to fabricate the bridge configuration on biotin-SAMs more efficiently. Assembling trace amount of tubules (or ideally a single protein tubule) on the biotin-SAMs may diminish protein tubule aggregation.

Here we demonstrated that the peptide tubules, incorporated with the complementary receptor sites, recognized and selectively bound well-defined regions on patterned substrates coated with the SAMs containing the complementary acceptor site. Because the tubules can be coated by various metals[15] and quantum dots,[16] this system has potential to be applied to construct complex electronic devices in a simple and economical manner. Patterning a smaller area of Au substrates by lithography may reduce the protein tubule aggregation, although this is not a proven solution yet. Although this result shows that the protein tubules have potential to serve as connecting wires for microelectronics, the controlled placement of a single protein tubule in the desired region still remains as a technical challenge to achieve our proposed goal at this point. In this study, we limited immobilization studies with submicron sized protein tubules due to the resolution of lithographic pattering for Au substrates in our facility. But the template peptide tubules have been observed to be assembled in diameters of 20 nm to 1 μm.[17] Therefore, this system should be competent to be applied to nanodevice fabrications by using nanometer-sized protein tubules. This biological recognition-driven assembly scheme may allow us to develop efficient, economical, and precise nanofabrication methods for microelectronics, photovoltaic cells, batteries, recording memory media, biosensors, and spin-based electronics with new properties and functionalities.

Acknowledgment. This work was supported by Florida Hospital. G.D. acknowledges Dr. Catherine Thaler for the assistance with the fluorescence microscope and for supply-

ing a portion of the materials. H.M. acknowledges Mr. Zia Ur Rahman for the assistance in the SEM and Dr. Kalpathy B. Sundaram for Au substrate fabrications.

References

(1) Braun, E.; Eichen, Y.; Sivan, U.; Ben-Yoseph, G. *Nature* **1998**, *391*, 775.
(2) Connolly, S.; Fitzmaurice, D. *Adv. Mater.* **1999**, *11*, 1202.
(3) Haussling, L.; Michel, B.; Ringsdorf, H.; Rohrer, H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 569.
(4) Spinke, J.; Liley, M.; Schmitt, F. J.; Guder, H. J.; Angermaier, L.; Knoll, W. *J. Chem. Phys.* **1993**, *99*, 7012.
(5) Perez-Luna, V. H.; O'Brien, M. J.; Opperman, K. A.; Hampton, P. D.; Lopez, G. P.; Klumb, L. A.; Stayton, P. S. *J. Am. Chem. Soc.* **1999**, *121*, 6469.
(6) Patel, N.; Sanders, G. H. W.; Shakesheff, K. M.; Cannizzaro, S. M.; Davies, M. C.; Langer, R.; Roberts, C. J.; Tendler, S. J. B.; Williams, P. M. *Langmuir* **1999**, *15*, 7252.
(7) Lahiri, J.; Ostuni, E.; Whitesides, G. M. *Langmuir* **1999**, *15*, 2055.
(8) Yang, Z. P.; Frey, W.; Oliver, T.; Chilkoti, A. *Langmuir* **2000**, *16*, 1751.
(9) Nelson, K. E.; Gamble, L.; Jung, L. S.; Boeckl, M. S.; Naeemi, E.; Golledge, S. L.; Sasaki, T.; Castner, D. G.; Campbell, C. T.; Stayton, P. S. *Langmuir* **2001**, *17*, 2807.
(10) Li, M.; Wong, K. K. W.; Mann, S. *Chem. Mater.* **1999**, *11*, 23.
(11) Lala, N.; Sastry, M. *Phys. Chem. Chem. Phys.* **2000**, *2*, 2461.
(12) Connolly, S.; Rao, S. N.; Fitzmaurice, D. *J. Phys. Chem. B.* **2000**, *104*, 4765.
(13) Douberly, G. E. J.; Pan, S.; Walters, D.; Matsui, H. *J. Phys. Chem. B* **2001**, *105*, 7612.
(14) Matsui, H.; Gologan, B. *J. Phys. Chem. B* **2000**, *104*, 3383.
(15) Matsui, H.; Pan, S.; Gologan, B.; Jonas, S. *J. Phys. Chem. B* **2000**, *104*, 9576.
(16) Matsui, H.; Pan, S.; Douberly, G. E. J. *J. Phys. Chem. B* **2001**, *105*, 1683.
(17) Matsui, H.; Gologan, B.; Pan, S.; Douberly, G. E. J. *Eur. Phys. J. D.* **2001**, in print.
(18) Kogiso, M.; Ohnishi, S.; Yase, K.; Masuda, M.; Shimizu, T. *Langmuir* **1998**, *14*, 4978.
(19) Yang, Z.; Frey, W.; Oliver, T.; Chilkoti, A. *Langmuir* **2000**, *16*, 1751.

NL015564V