Heterogenous DNA Self-Assembly Nucleated on Solid Surfaces

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Abstract – DNA self-assembled nanostructures coupled with solid surfaces have been utilized as a tool for constructing various nanodevices. However, DNA self-assembly directly nucleated by immobilized DNA strands has not been realized, and also the surface effect on the DNA self-assembly has not been investigated. Here we developed a method to construct DNA nanostructures nucleated by DNA strands bound on gold surfaces. By a scanning electron microscopy technique specialized for observation of organic molecules, DNA-assembled objects were observed on gold surfaces without any staining, and difference in growth of the objects between on solid surface and in solution phase was revealed.

1. Author information

H. Hata: Specialty is DNA nanotechnology. In this study, he is responsible for design and construction of DNA nanostructures, and atomic force microscopy (AFM) measurements.

J. Yamada: Specialty is chemical modification of solid surfaces, and observation of organic molecules using electron microscopy. In this study, he is responsible for modification of gold surfaces, and scanning electron microscopy (SEM) measurements.

The authors found the idea of this joint research in conversations when the authors got together at a MERIT special lecture. At that time, H. H. had been interested in DNA self-assembly with coupled with other materials. J. Y. had been interested in SEM observation for DNA nanostructures.

2. Introduction

DNA self-assembly has been found to be available to construct various complex nanostructures, and so is one of the most promising candidates for bottom-up nanotechnology. [1] Especially, DNA nanodevices coupled with solid surfaces are useful in biosensing, material science, and cell biology.[2] However, DNA self-assembly nucleated by immobilized DNA strands has not been realized, and so, surface effects on the self-assembly has not been investigated. The surface-nucleated DNA self-assembly would be utilized not only to construct novel nanodevices, but also to gain more insights for self-assemble processes through the interaction between DNA and surfaces. Such experimental insights of DNA self-assembly are highly desired for comparison with recent theoretical observations.[3]

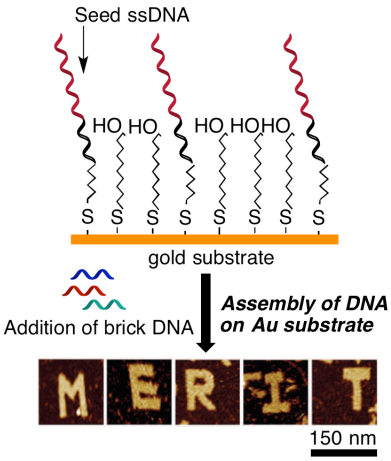

Figure 1. Concept and purpose of this study. AFM images have been adopted from ref. 4.

Herein we designed a method to construct DNA nanostructures nucleated by DNA strands immobilized on solid surfaces. Figure 1 shows a perspective of our method. First, thiol-modified single-stranded DNA (seed ssDNA) was bound to gold surface with S-Au bonding. Next, to prevent nonspecific binding of DNA to the surface and to make highly thermally stable seed ssDNA-mixed self-assembled monolayer (SAM), the gold surface was coated with mercaptoundecanol (MCU). Then, the substrate and a lot of ssDNA (brick DNA) were mixed together in solution and annealed to form DNA structures. Lastly, the DNA structures were observed and investigated by AFM and SEM.

3. Materials and Methods

3.1. Design of DNA nanostructure

A DNA structure was designed based on Wei et alx.[4] Figure 2 shows the structure design used in this study. Total 90 ssDNAs were used to construct the rectangular shape. One of those DNA strands was thiol-modified terminally, and immobilized on gold surfaces. The calculated size of the rectangle is 61 × 28 nm in solution.

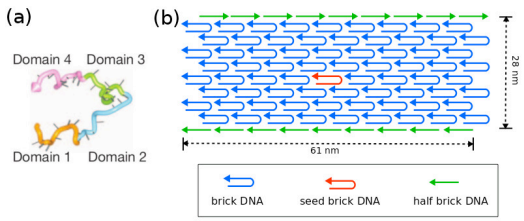

Figure 2. Seeded self-assembly of a DNA nanostructure. (a) The canonical motif of brick DNA, adopted from ref. 4. Each domain binds different brick DNA. (b) Design of the DNA nanostructure used in this study.

3.2. Surface modification on gold

Surface modifications on gold substrate were performed according to Levicky et al..[5] Single-stranded, 49-base-long DNA oligonucleotides, designated RSS-ssDNA (R = 6-*O*-di(*p*-methoxyphenyl)phenylmethylhexyl, DMTr), were adsorbed on a UV/O_3 treated Au/Si substrate from a 2 μM of solution in potassium phosphate buffer (1 M, pH 6.7) for 24 h. After rinsing with pure water, the substrate was immersed into a solution of MCU (1 mM) in ethanol for 2 h, annealed at 50 ºC for 28 h,[6] and thoroughly rinsed with ethanol and pure water. Finally, the mixed SAM was immersed into 80% acetic acid solution for 15 min to deprotect the DMTr group adsorbed on the substrate together with the seed ssDNA, and then it was thoroughly rinsed with water. In all steps, nitrogen blow was conducted to dry the substrates.

3.3. DNA structure construction

Construction of DNA nanostructures were done according to Wei et al.[4] Briefly, to construct the target shape, 89 DNA strands were mixed to make an equimolar mixture (500 nM) in 0.5× TE buffer (25 mM $MgCl_2$). The surface modified gold substrates were dropped into the mixture solution, and annealed from 90°C to 25°C over 30 hours.

3.4. Contact angle measurement

Contact angle measurements were performed using a DM-301 (Kyowa Interface Science Co. Ltd.) and analyzed by FAMAS software. The modified Au/Si substrates were placed on the stage of contact angle machine, and then water (3 μL) was dropped onto the substrates. The reported water contact angles on the substrates were obtained as an average of three measurements.

3.5. XPS measurement

X-ray photoelectron spectroscopy (XPS) was carried out using a PHI 5000 VersaProbe (ULVAC PHI Inc.). For XPS measurement, the same substrates were used as the contact angle measurement. Signals of C_{1s}, N_{1s}, and P_{2p} were detected by irradiation of X-ray beam (100 μm, 25 W, 15 kV) with charge neutralization by electron beam and Ar ion beam. To increase the S/N ratio, the measurements were repeated as 30 times and 100 times for N_{1s} and for P_{2p}, respectively.

3.6. AFM measurement

After the DNA nanostructure construction, sample substrates were rinsed with pure water, and dried naturally. Subsequently, the samples were subjected to the AFM imaging in air. Atomic force micrographs were obtained by the tapping mode on a NanoScope

IIIa (Digital Instruments) equipped with a multimode head with OMCL-TR400PSA-1 tips (Olympus Inc.).

3.7. SEM measurement

SEM was carried out using a FEI Magellan 400L (FEI Inc.). Sample substrates that have DNA nanostructures forming on the mixed SAM or DNA nanostructures post-deposited on the SAM were subjected to the SEM observation at a beam landing voltage of 1 kV under a vacuum of 5×10^{-5} Pa without any conductive coatings.

4. Results and Discussion

4.1. Surface analysis

At first, the surface modification was evaluated using water contact angle measurements. **Table** 1 shows water contact angles on gold surfaces. After the surface modification with seed ssDNA, the contact angle decreased by 30.2 °. Therefore, hydrophilic seed ssDNA was bound to gold surface via Au-S bonding, and the surface wettability was improved. After formation of mixed SAM, the water contact angle became much smaller than before. It means that MCU molecules filled vacant space on seed ssDNA-modified gold surface.

Table 1. Water contact angles on gold surfaces.

Modification	UV/O_3 treatment	w/ seed ssDNA	mixed w/ MCU
C.A. (•)	72.3 ± 2.2	42.1 ± 2.1	36.3 ± 2.3

Next, to confirm the immobilization of the seed ssDNA on the gold surface, XPS measurement was carried out. As a result of XPS measurements for gold surface modified with (i) seed ssDNA and MCU, and (ii) only MCU, signals of P_{2p} and N_{1s} including in DNA were detected only in substrate (i). Based on those results, we concluded that the mixed SAM of seed ssDNA and MCU was constructed on the gold surface.

4.2. Observation of DNA nanostructures

Using the surface modified gold substrates, the DNA structure construction was performed. Then, to observe the DNA structure, the gold surface was measured using AFM. However, the AFM measurement for the SAM surface did not work well. This would be because the surface was too flexible to monitor with AFM. Figure **3** shows an AFM image of a SAM surface of not MCU but mercapthexanol (MCH) after the annealing process (the MCU SAM surface has not been observed due to the machine trouble). The surface was not observed clearly. In addition, the difference in height was over 10 nm. Compared with the height of DNA structures used here (~2 nm), the observed surface was much rougher. Therefore, the AFM measurement was found to be unsuitable to monitor DNA nanostructures on the gold surface.

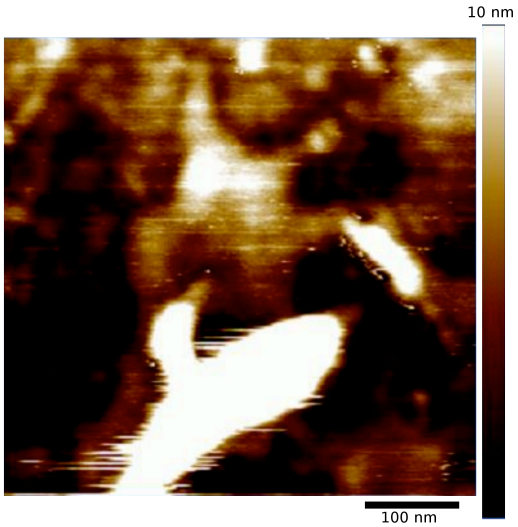

Figure 3. An AFM image of the mercaptohexanol modified gold surface.

Then, we performed SEM measurements to observe DNA nanostructures. Interestingly, the desired rectangular DNA bricks were observed without staining (Figure 4). Numbers of DNA bricks growing on (i) seed ssDNA mixed MCU SAM and on (ii) MCU SAM were counted in 30 images (2.0 μm × 1.7 μm) taken in different areas. The number on substrate (i) was 90, in contrast that on substrate (ii) was 57. Nonspecific growth of DNA bricks on MCU SAM was not completely prevented, but this result still indicates that formation of DNA bricks was induced by the seed ssDNA.

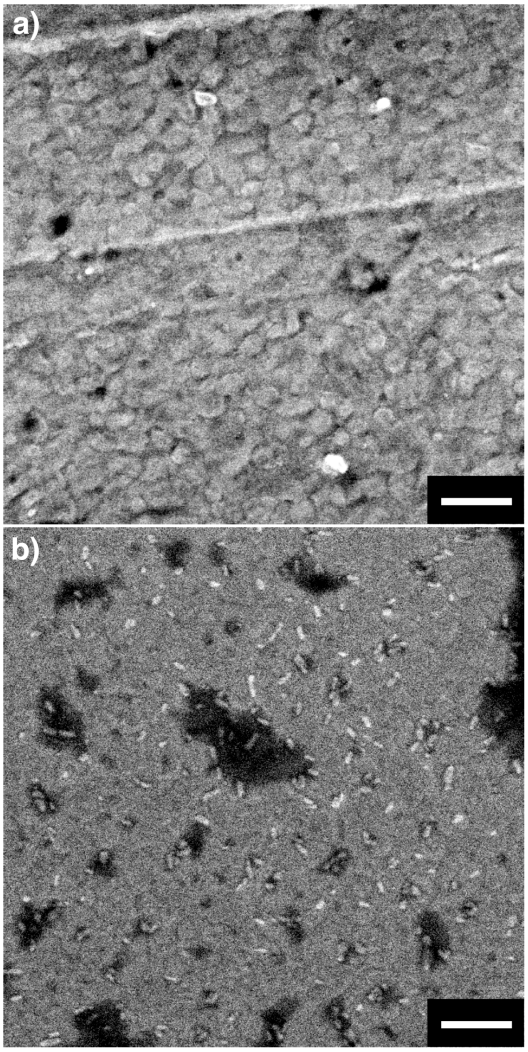

Figure 4. Representative SEM images of DNA bricks. a) DNA bricks growing on seed ssDNA mixed MCU SAM, b) DNA bricks post-deposited on the same SAM after growth in solution phase. The scale bars are 200 nm.

Next, in order to investigate the difference between growth of DNA bricks on solid surfaces and that in solution phase, size distribution of DNA bricks were evaluated from the SEM images. DNA bricks prepared in the buffer solution were drop-casted on a seed ssDNA mixed SAM-coated gold substrate and measured by the same method. A histogram about the size distribution of the long-side of rectangular DNA bricks is shown in Figure 5. Common to the three samples, most of DNA bricks did not completely grow up to the maximum size (61 nm). The similar trend was observed in previous researches using the DNA bricks. Wei et al. reported that the yield of the target structure is estimated to be less than 50%.[4] This yield is much lower than that of the DNA origami (the yield is almost 100%). This would be because the DNA bricks structure is constructed only from short ssDNA strands and DNA bricks are easily dissociated due to crash of them in the solution during growing process. These strands are easily bound via multivalent hydrogen bonding and form a lot of nuclei that reduce the size of DNA bricks under the cooling process.

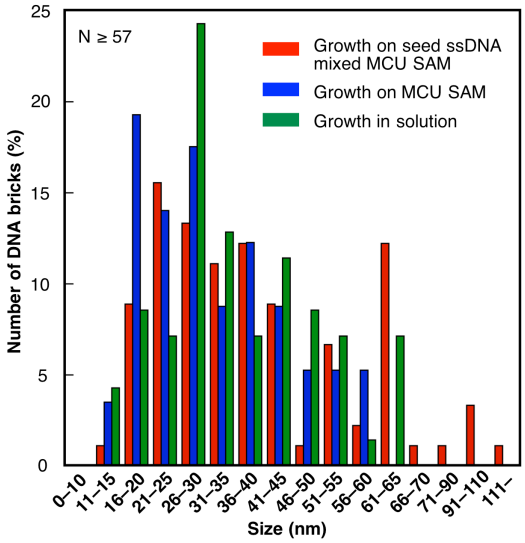

Figure 5. Size distribution of DNA bricks. DNA bricks grown on seed ssDNA mixed SAM (red), on MCU SAM (blue), in solution (green). Observed numbers of DNA bricks were above 57 in each sample.

According to the size distribution of DNA bricks, sizes of the thermodynamically stable DNA nanostructures were estimated about 26-30 nm (4 brick DNAs) in solution phase and 16-25 nm (3-4 brick DNAs) on solid surface, respectively. However, on seed ssDNA mixed SAM, 13% of DNA bricks grown up to the maximum size. And the ratio was higher than that growing in solution phase (7%). This is because that deformation of DNA bricks due to clash of DNA bricks was effectively prevented on the substrate compared to the solution process. However,

the shape of the DNA bricks was not completely rectangular. Some of them formed sphere structure due to effects of surface roughness.

Comparing two samples for self-assembly on the solid surface, larger DNA bricks were clearly observed for the self-assembly with seed ssDNA (Figure 5, red bars) than that without seed ssDNA (Figure 5, blue bars). This is because that the rinse after the annealing washed out such large unseeded DNA bricks. Thus, this also shows that the DNA bricks assembled with the seed ssDNA were strongly bond on the substrate via the seed ssDNA.

5. Conclusion

Formation of DNA nanostructures on solid surface was successfully demonstrated on the seed ssDNA modified gold substrate. And we succeeded in observing the nanostructures by SEM measurement without any staining. The observation revealed that the seed ssDNA effectively induces the growth of DNA bricks. By prevention of the deformation of DNA bricks due to crush of them, the DNA bricks grown more largely on the solid surface than in solution phase. However, the size and the shape of DNA bricks were not completely controlled because of nonspecific and irregular growth of them on solid surface induced by the surface roughness of the substrates. Smooth surface and more optimized DNA design that prevent these problems are necessary to construct DNA nanostructures on solid surfaces more precisely.

Acknowledgements

We would like to thank Prof. T. Kato, Prof. E. Nakamura, Prof. A. Suyama, and Prof. H. Tanaka for great supports and fruitful advices. We would like to show our greatest appreciation to MERIT for a chance to challenge a multidisciplinary study.

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