Synthesis of Long-template DNA Using Enzymatic Reaction for Regular Alignment of Au-nanoparticles

Shin-ichi Tanaka,*[1] Wolfgang Fritzsche,[2] Yasushi Sako,[3] and Toshio Yanagida[1]
[1]*Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita, Osaka 565-0871*
[2]*Institute for Physical High Technology, A.-Einstein-Str. 9, Jena, Thueringen 07745, Germany*
[3]*Cellular Informatics Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198*

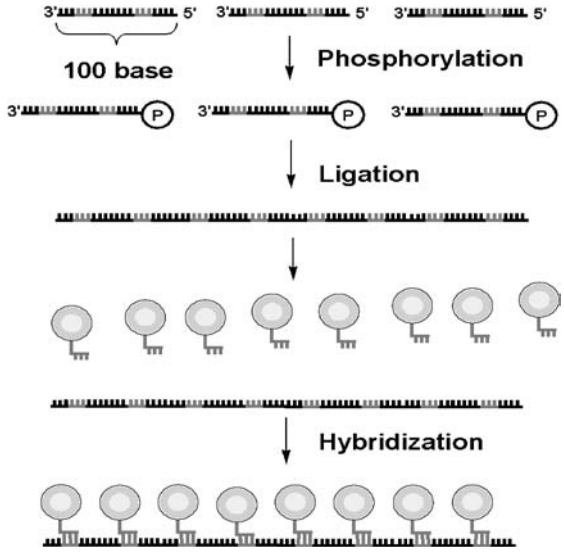
(Received August 17, 2006; CL-060946; E-mail: s_tanaka@phys1.med.osaka-u.ac.jp)

We have successfully synthesized more than 1000 bases of DNA template with a repeat unit of 100 bases and have prepared regular alignments of Au-nanoparticles. This technique will allow to realize multifunctional biosensor, molecular nanodevice, and long-range photonic wires.

A number of molecular recognition systems are being used in living organisms, such as the avidin–biotin system, antigen–antibody reaction, and complementarity of DNA. One of the key goals of nanotechnology is the realization of complex nanoscale structures by utilizing these systems. Particularly, since DNA has a well-defined geometry and highly predictable structure, is diverse and programmable for intra/intermolecular interactions, Seeman et al.[1] and other researchers[2] have constructed a variety of nanoscale structures by utilizing the complementarity of DNA. Furthermore, numerous researchers have used DNA as a scaffold in DNA-directed organization of nanoparticles[3,4] or proteins.[5] While these experiments have succeeded in creating a regularly short range or a randomly long-range alignment of nanoparticles and proteins, construction of regular longer nanoscale structures have not been successful due to technical difficulties in the synthesis of long template DNA with a specific sequence. Thus, in order to construct long one-dimensional molecular arrays, it is necessary to synthesize long template DNA.

In a recent communication, we reported the synthesis of long homopolymeric DNA for the first time by using T4 RNA ligase.[6] RNA ligase is a bacteriophage T4 enzyme that is capable of covalently jointing single-stranded RNA or DNA molecules containing 5′-phosphate and 3′-hydroxy terminals, and ligation is well-suited for the synthesis of long repetitive template DNA. This method was applied to the synthesis of long template DNA, and we now successfully produced extended Au-nanoparticle alignment in which the interval between Au-nanoparticles was controlled. In this communication, we report the synthesis of template DNA with more than 1000 bases with a repeat unit of 100 bases and the regular alignment of Au-nanoparticles along this template DNA.

The template DNA with a repeat unit of 100 bases was synthesized by application of the previously described procedure.[6] Scheme 1 depicts the synthetic route and details of synthesis are described in the Supporting Information.[8] Briefly, 100 bases of single-stranded DNA were used as the starting material. This DNA possessed two 18 bases of a specific sequence, which can hybridize with complementary DNA in order to align the Au-nanoparticle. Purified starting DNA was dissolved in ultra-pure water to a concentration of 0.5 to 1 mM and the 5′-end of this DNA was phosphorylated using T4 polynucleotide kinase at

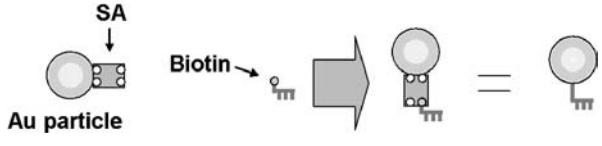


Scheme 1.

37 °C for 60 min in order to facilitate ligation. Next, this reaction mixture was allowed to react with T4 RNA ligase at 5 °C for 5 days in order to obtain long-chain template DNA. After these reactions, the length of synthesized DNA was evaluated by electrophoresis (in Figure S1).[8] It shows that the synthesized DNA was extended every 100 bases and possessed various lengths between 200 and 1000 bases. Then, in order to separate, refine, and purify each product DNA, electroelution, and electrophoresis were carried out.

In preparing the (Au-nanoparticle)–DNA conjugate, the specific interaction between streptavidin and biotin was utilized. (Au-nanoparticle)–DNA conjugates were prepared by incubating streptavidin (SA) modified 5-nm Au-nanoparticles with biotinated oligonucleotides (18-mer) in phosphate-buffered saline (PBS) (pH 7.4) at 37 °C for 60 min. (Scheme 2). Synthesized long template DNA was hybridized with (Au-nanoparticle)–DNA conjugate by annealing in SSPE buffer (6×) including 1% of sodium dodecyl sulfate (SDS), and Au-nanoparticles were aligned on the template DNA.

The prepared Au-nanoparticle alignment was studied by using atomic force microscope (AFM) (Figures 1, S2,[8] and S3[8]).



Scheme 2.

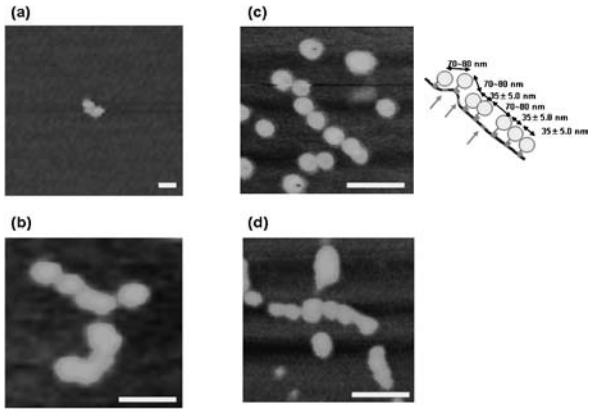


Figure 1. AFM images of Au-nanoparticle alignment on mica substrate. Scale bar in each images are 100 nm. Au-nanoparticles were ordered on (a) 200 bases, (b) 500 bases, and (c), (d) 1000 bases of template DNA. In AFM image (c) and (d), the measured height of Au-nanoparticle and the interval between Au-nanoparticles were 5.5 ± 0.5 nm and about 35 ± 5.0 nm, respectively, and were roughly in accordance with the height of the used Au-nanoparticles and the length of 100 bases of DNA. This measurement indicated that Au-nanoparticles were arrayed every 100 bases on template DNA, and demonstrated our experimental design.

When the sample without template DNA was checked, a number of isolated Au-nanoparticles were observed on the mica surface as shown in Figure S2.[8] However, by adding template DNA, 5-nm Au-nanoparticles were arrayed along DNA, as shown in Figure 1. Since Au-nanoparticles can be arrayed on template DNA every 100 bases, two and five Au-nanoparticles were found to be aligned on 200 and 500 bases of template DNA, respectively (Figures 1a and 1b). However, when 1000 bases of template DNA were used, less than ten Au-nanoparticles were arrayed while ten (Au-nanoparticle)–DNAs could theoretically hybridize to this DNA. Then, seven Au-nanoparticles alignment in Figures 1c and 1d and several (six to eight) Au-nanoparticles alignments in Figure S3[8] were observed. Although the resolution in AFM images depends on the Tip curvature and the horizontal diameter of visualized Au-nanoparticle is bigger than that of the actual Au-nanoparticle, the interval between the Au-nanoparticles and the height of Au-nanoparticle in Figures 1c and 1d were measured in order to characterize these Au-nanoparticle assembly. Then, the measured height of the Au-nanoparticles was 5.5 ± 0.5 nm and was equivalent to that of used Au-nanoparticles. The distances measured between Au-nanoparticles in Figures 1c and 1d were about 35 ± 5.0 nm, and so roughly in accordance with the length of 100 bases of DNA. This measurement leads to the conclusion that Au-nanoparticles were arrayed every 100 bases on template DNA. Furthermore, we found three points where the interval between Au-nanoparticles was from 70 to 80 nm in Figure 1c and was approximately equivalent to the length of 200 bases of ssDNA. Since this interval was about twice the length of 100 bases of DNA (40 nm), we assumed that these could be attributed to a vacant binding site without (Au-nanoparticle)–DNA conjugate. In general, single-stranded DNA is less rigid and bends easily so that the longer ssDNA tends to become tangled. Thus, it is difficult to array Au-nanoparticles on the longer DNA template because of the tangling of longer template DNA and the steric hindrance between Au-nanoparticles. This issue has been discussed in the literature.[4] However, we have successfully produced Au-nanoparticle alignments on long template DNA.

In conclusion, we describe a synthesis method for long template DNA using ligase and the controlled fabrication of a regular Au-nanoparticle alignment. The synthesized DNA had a length of more than 1000 bases in spite of the low yield. In order to produce longer template DNA, we are currently studying experimental conditions such as reaction time, temperature, and enzyme concentration. Moreover, in this study, we have successfully produced Au-nanoparticle alignment on long template DNA. As an application, two kinds or two sizes of metallic nanoparticle can be arrayed on template DNA by using two distinct sequences. Currently, we are working on the fabrication of wires with alternating material composition. Since metallic nanoparticles or semiconductor quantum dots have size-tunable photoluminescence and allow the simultaneous excitation of several particle sizes at a single wavelength, it is conceivable to realize multifunctional biosensor, molecular nanodevice, and long-range photonic wire using fluorescent resonance energy transfer (FRET)[7] in the future.

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8 Supporting Information is available electronically on the CSJ-Journ Web site, http://www.csj.jp/journals/chem-lett/.

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