A DNA-gold nanoparticle hybrid hydrogel network prepared by enzymatic reaction

Eguchi, Yuka

Kato, Tomoharu

Tanaka, Tsutomu

Maruyama, Tatsuo

Journal Name

COMMUNICATION

DNA–gold nanoparticle hybrid hydrogel network prepared by enzymatic reaction

Yuka Eguchi,[a] Tomoharu Kato, [a] Tsutomu Tanaka[a] and Tatsuo Maruyama*[a]

We report a DNA–gold nanoparticle (AuNP) hybrid hydrogel in which the AuNPs crosslink enzymatically synthesized DNA to form a gel network. PCR-elongated DNA and AuNPs act as a one-dimensional polymer and cross-linkers, respectively. The DNA–AuNP hydrogel has the functional properties of both long DNA and the AuNPs.

A number of fascinating functional gels, especially hydrogels, have been studied for diverse applications.[1-3] Many gels consist of three-dimensional (3D) networks immobilizing a liquid. The 3D networks are prepared with cross-linked polymers, nanostructured inorganic frameworks, nanostructured molecular self-assembly or composites of organic and inorganic materials. The cross-linking between one- or two-dimensional structures plays an essential role in gelation. However, it is still challenging to precisely control the crosslinking positions in the networks, and consequently control the network length and structure. A few groups have reported that chemically synthesized DNA oligonucleotides can form well-defined gel networks.[4-6] These and other reports show that DNA is an intelligent building block owing to its designable sequence, its strong binding to its complement with high specificity, its translatable genetic information, it being a substrate for various enzymatic reactions, and it being replicable by polymerase.[7-15]

Polymerase-chain reaction (PCR) with thermal cycles can elongate DNA primers (short single-stranded DNA oligonucleotides) to precisely replicate a DNA template in an exponential manner.[16] The sequence and length of a PCR product (enzymatically synthesized DNA) can be designed for a particular purpose. PCR very effectively produces a number of copies of a DNA template, which cannot be achieved by chemical synthesis, and its amount can be simply controlled by thermal cycles. PCR is a highly useful and practical approach to
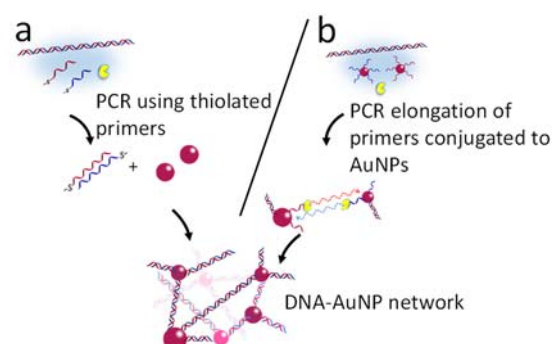

Fig. 1 Schematic illustrations of the two approaches used to prepare the DNA–AuNP network. (a) Conjugation of thiolated DNA with AuNPs after PCR elongation (method 1). (b) PCR elongation of thiolated DNA primers conjugated to AuNPs (surface-initiated PCR, method 2).

synthesize well-defined DNA building blocks.

Combining metal nanoparticles and hydrogels offers the possibility of multifunctional soft materials. Many reports have described physical mixing of metal nanoparticles and hydrogels to integrate different functional properties.[6, 17-22] However, there is the risk that the nanoparticles leach out of the hydrogel. To overcome this drawback, Stark et al. covalently crosslinked cobalt nanoparticles into a polymer backbone to produce a hydrogel with stable integration of the functional properties of the polymer and nanoparticles.[23]

Since the report by Mirkin in 1996,[24] DNA–AuNPs have emerged as powerful and versatile functional nanomaterials in nanotechnology and biotechnology.[25, 26] The applications of DNA–AuNPs include sensing, bioimaging, diagnostics, therapeutics, and electronics. The Au–S bond, which is used for preparation of DNA–AuNP conjugates, readily forms in aqueous solution and is relatively stable. In the present study, we used Au–S chemistry to incorporate metal nanoparticles into the polymer backbone (DNA) of a gel network. DNA with controlled lengths was enzymatically synthesized using PCR and crosslinked with AuNPs to create a novel class of well-defined hydrogels. The hydrogels composed of DNA and AuNPs possess a variety of the functional properties of DNA and AuNPs.

a. Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, 1-1 Rokkodai, Nada-ku, Kobe 657-8501, Japan.
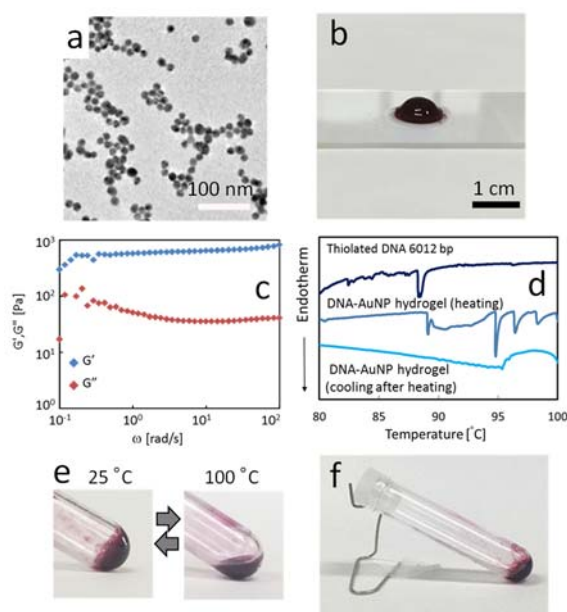E-mail: tmarutcm@crystal.kobe-u.ac.jp

Fig. 2 (a) TEM image of AuNPs synthesized by the citrate method. (b) DNA–AuNP hydrogel prepared by method 1. (c) Rheological measurement of the DNA–AuNP hydrogel at room temperature. (d) DSC measurement of the DNA–AuNP hydrogel. (e) Gel–sol transition with temperature. (f) DNA–AuNP hydrogel prepared by method 2.

There are two possible approaches to prepare the DNA–AuNP network: (1) conjugation of PCR-synthesized DNA with AuNPs (method 1, Fig. 1a) and (2) PCR elongation of DNA primers conjugated to AuNPs (surface-initiated PCR, method 2, Fig. 1b). First, we used method 1. AuNPs (15 nm) were synthesized using the citrate method[27] and characterized by transmission electron microscopy (TEM) (Fig. 1a). PCR was performed using 5'-end thiolated DNA primers and a DNA template (6012 base pairs (bp)) to prepare elongated DNA (6012 bp) with thiol groups at both 5'-ends. After desalination, the elongated DNA was conjugated with AuNPs by mixing. The final concentrations of the elongated DNA and AuNPs were 2 μM and 30 nM, respectively. The mixture was heated at 94 °C for 2 min and cooled to room temperature. Successful hydrogel formation occurred within several minutes after cooling (Fig. 1b). The hydrogel was characterized using a rheometer. Figure 2c shows that storage modulus (G') is significantly higher than loss modulus (G'') over the entire frequency range, which is typical of a gel. TEM observation also showed that the AuNPs in the DNA–AuNP hydrogel were not aggregated (Fig. S1), suggesting that the hydrogel did not formed by aggregation of AuNPs.[28]

The present DNA–AuNP hydrogel is suggested to consist of AuNPs interconnected by thiolated DNA. To validate this hypothesis, we added an excess amount of 2-mercaptoethanol to the hydrogel to cleave the Au–S bonds, and we found that it turned into a solution. As a control, we also mixed AuNPs and non-thiolated DNA (6012 bp) that were elongated by PCR using non-thiolated primers and confirmed no gelation. These results suggest that the Au–S bond plays an important role in formation of the DNA–AuNP hydrogel.

The gel–sol transition temperature of the DNA–AuNP hydrogel was measured by differential scanning calorimetry

(DSC) (Fig. 2d). Endothermic peaks were observed at 89–98 °C for the DNA–AuNP hydrogel, while DNA (6012 bp) without AuNPs has an endothermic peak at about 88 °C. DNA was highly condensed in the DNA–AuNP hydrogel, which might affect the thermal stability of the DNA duplex in the hydrogel. Observation with the naked eye confirmed that the DNA–AuNP hydrogel turned to the liquid state above 95 °C (Fig. 2e) and reversed to the gel state after cooling to 25 °C, indicating a thermoreversible gel–sol transition. These results support our hypothesis that the DNA–AuNP hydrogel network is composed of the DNA duplex crosslinked with AuNPs.

We also attempted to prepare the DNA–AuNP hydrogel network using another approach (method 2, Fig. 1b). Shen et al. reported that DNA primers immobilized on AuNPs can be elongated by PCR.[29, 30] We immobilized the 5'-end thiolated forward and reverse primers onto the surfaces of different AuNPs by Au–S bonds. PCR was performed using a 6012 bp DNA template. To prove DNA elongation from the AuNP surfaces by PCR, restriction enzymes (*Sca*I and *Not*I) were added to the PCR solution to prepare a 5535 bp fragment, and the fragment was then subjected to agarose gel electrophoresis. Agarose gel electrophoresis (Fig. S2) showed that the immobilized DNA primers were successfully elongated from the AuNP surfaces, which agrees with previous reports.[29, 30] However, no gelation was observed even after 35 PCR cycles. This is because the concentration of DNA–AuNPs (0.02%) was much lower than the minimum gelation concentration (see later discussion). Marx et al. also reported a 3D DNA network produced by PCR, although it did not form a hydrogel, which agrees with the present result.[31] The PCR solution containing DNA–AuNPs was then condensed to give 2 μM thiolated DNA and 30 nM AuNPs, heated at 94 °C for 2 min, and cooled to room temperature. A hydrogel successfully formed (Fig. 2f). TEM observation confirmed that the AuNPs maintained their size and shape after the surface-initiated PCR (Fig. S3). These results prove that the DNA–AuNP network can also be produced by surface-initiated PCR, which is available for hydrogel formation.

The present study proposes two methods to prepare the DNA–AuNP hydrogel and each method has different advantages and disadvantages. For instance, method 1 can precisely control the ratio of AuNPs and elongated DNA. Method 2 can avoid the risk that both forward and reverse primers are conjugated to a single AuNP. Method 2 can also control the network density by varying the PCR cycle.

One of the attractive features of PCR is that is can easily and precisely control the DNA length. To examine the effect of the DNA length on gelation (method 1), DNAs with six different lengths (328, 911, 1633, 6012, and 8570 bp) were synthesized by PCR with varying template length and designing appropriate primer sequences (see Supporting Information). The PCR amplicons were identified by agarose gel electrophoresis (Fig. S4). Each elongated DNA was mixed with a AuNP solution to give 2 μM DNA and 30 nM AuNPs, followed by heating and subsequent cooling (method 1). The results of the gelation tests are summarized in Table S1 (photographs are shown in Fig. S5).
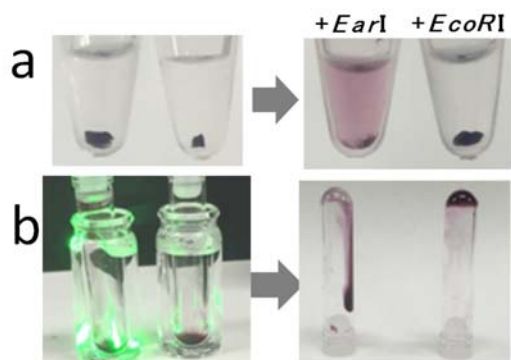
Fig. 3 Stimuli-induced gel-sol transition of the DNA-AuNP hydrogel prepared by method 1. a) Addition of restriction enzymes (*Ear*I and *Eco*RI). b) Light exposure (532 nm laser pointer).

The mixture containing DNA longer than 6000 bp and AuNPs formed a hydrogel, while the mixtures containing DNA shorter than 1633 bp did not form a hydrogel. The gelation tests suggest that the gelation ability is greatly affected by the DNA length, indicating the importance of the interconnecting distance between AuNPs. The mean distance between the AuNPs in the 30 nM solution was estimated to be 380 nm. Because the linear length of 1633 bp DNA is comparable with the estimated inter-nanoparticle distance, it would be difficult for DNA shorter than 1633 bp to interconnect all of the AuNPs and encapsulate all of the water molecules in the resultant DNA–AuNP network. The length of DNA with more than 6000 bp is sufficient to interconnect all of the AuNPs in this solution and result in hydrogelation.

The effect of the DNA concentration on gelation (method 1) was investigated at a fixed AuNP concentration (30 nM). Table S2 shows that hydrogelation occurred with ≥1.0 μM DNA, while 0.25 and 0.5 μM DNA did not result in hydrogelation (Fig. S6). The DNA concentration indicates the mean number of DNA molecules conjugated to a single AuNP (DNA/AuNP ratio). If the DNA/AuNP ratio is too low (low DNA concentration), it might not sufficiently interconnect the AuNPs, resulting in no gelation. Although there was a study that DNA-hydrogel (prepared with X-shaped oligo DNAs) containing AuNPs,[6] the DNA concentration was approx. 100 times higher than ours. The accurate design of the crosslinkage of elongated DNAs with AuNPs contributed to the remarkably low DNA concentration required in the present DNA-AuNP hydrogel.

We then investigated the effect of the AuNP concentration on gelation (Table S2). An increase of the AuNP concentration means a decrease of the DNA/AuNP ratio. DNA–AuNP hydrogels were successfully prepared at AuNP concentrations above 7.5 nM, while no hydrogel was obtained at AuNP concentrations less than 4 nM. These results indicate that if the DNA/AuNP ratio is too high then DNA duplex formation is inhibited because of electrostatic repulsion between DNA molecules.[32] These investigations reveal that the minimum gelation concentration of the DNA–AuNP complex is 0.4 wt%, where the concentrations of DNA and AuNPs are 1 μM and 7.5 nM, respectively.

The DNA–AuNP hydrogel is supposed to have properties derived from both DNA and the AuNPs. As described above,

the DNA–AuNP hydrogel exhibited a thermoreversible gel–sol transition, which was because of thermoreversible formation of a DNA duplex. DNA is cleaved by restriction enzymes in a sequence specific manner. The DNA (6012 bp) contained three cleavage sites for the restriction enzyme *Ear*I but none for *Eco*RI. We added the DNA–AuNP hydrogel to *Ear*I and *Eco*RI solutions. The hydrogel was dissolved and the solution turned red when added to an *Ear*I solution (Fig. 3a). When added to an *Eco*RI solution, there was no change in the hydrogel. These results indicate that the DNA–AuNP hydrogel is responsive to specific nucleases and it can be designed by varying the nucleobase sequence of the DNA. It should be noted that an *Eco*RI solution with DNA–AuNP hydrogel was not dyed with red at all, indicating negligible leakage of AuNPs from the hydrogel probably due to the incorporation of AuNPs to the polymer backbone.

AuNPs absorb visible light and exhibit photothermal heating.[33, 34] We next investigated the photothermal property of the DNA–AuNP hydrogel (prepared by method 1). The present study used 15 nm AuNPs, whose plasmonic absorption peak matches the wavelength of green laser (532 nm). After 1 min laser irradiation at 70 °C, the DNA–AuNP hydrogel collapsed (Fig. 3b), and the photothermal heating of the AuNPs induced melting of the DNA duplex or the release of thiolated DNA from AuNPs.[35, 36] It should be noted that we also confirmed photothermal heating of AuNPs upon laser irradiation in the absence of DNA.

The genetic information of the DNA–AuNP network can be read for protein synthesis in vitro because PCR-synthesized DNA can be designed as long as protein encoding. We attempted cell-free production of a protein from the DNA–AuNP hydrogel.[37] A DNA–AuNP hydrogel containing DNA (6012 bp) encoding green fluorescent protein (GFP) was prepared by method 1 and incubated with cell lysates made from *E. coli*. As a control, linear DNA (6012 bp) without AuNPs was used for cell-free production of GFP in solution. Fluorescence measurements revealed that the DNA–AuNP hydrogel (4 μL, 2 μM DNA) produced 0.6 μg GFP in a 50 μL reaction volume within 1 h. Linear DNA (4 μL, 6 nM) produced 0.9 μg GFP. The low efficiency of the protein synthesis can be explained as follows. The GFP-encoding DNA is present in the backbone of the DNA–AuNP network. The mobility of the DNA molecules and the accessibility of transcription enzymes to the DNA are limited by the gel network. These results mean that the genetic information of DNA in the backbone of the DNA–AuNP hydrogel network is accessible and can be read for protein synthesis.

The DNA–AuNP hydrogel can provide a platform for immobilization of biomolecules. We investigated immobilization of horseradish peroxidase (HRP) as a model protein on the AuNPs in the DNA–AuNP hydrogel by formation of Au–S bonds. The amino groups of HRP were thiolated using 2-iminothiolane.[38] The DNA–AuNP hydrogel (5 μL) was placed in the well of a microtiter plate and thiolated HRP solution (50 μL) was added to the well (Fig. 4a). A fluorogenic substrate for HRP was added and incubated for 1 h at 37 °C. Fluorescent measurements showed the enzymatic activity of immobilized
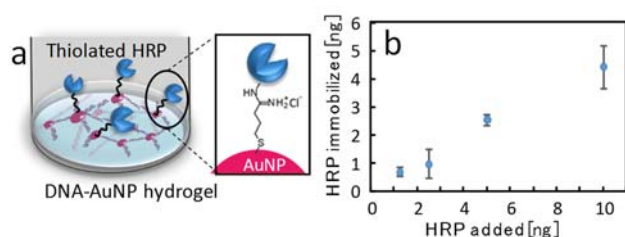
COMMUNICATION

Fig. 4 (a) Schematic illustration of covalent immobilization of HRP on AuNPs in the DNA–AuNP hydrogel. (b) Relationship between the added HRP amount and the amount of immobilized HRP.

HRP and also revealed the amount of HRP immobilized in the well. With increasing addition of HRP, the amount of HRP immobilized in the DNA–AuNP hydrogel increased (Fig. 4b). When 10 ng of HRP was added, 44% of the added HRP was immobilized while maintaining its activity. The HRP/AuNP molar ratio was 0.73, indicating that the HRP molecules penetrated into the DNA–AuNP hydrogel probably because of the low density of the gel network. These results demonstrate that the present DNA–AuNP hydrogel can be used as a "semi-wet" platform for covalent immobilization of biomolecules.[39]

In conclusion, we succeeded in hydrogelation by covalently interconnecting AuNPs with enzymatically synthesized DNA. In the hydrogel, the enzymatically synthesized DNA acts as a well-defined linear polymer, which was long enough to encode protein genes. The AuNPs act as cross-linking sites in the 3D gel network. The DNA–AuNP hydrogel shows a gel–sol transition in response to various types of stimuli, which is derived from the properties of DNA and the AuNPs. The genetic information in the DNA–AuNP hydrogel network can be read and translated into a protein. We also succeeded in covalent immobilization of an enzyme on the AuNPs in the hydrogel. The present study shows that combining metal nanoparticles and enzymatically synthesized DNA creates a novel class of inorganic/biopolymer hydrogels that possess a variety of the functional properties of both biopolymer and the metal nanoparticles.

Notes and references

1   J. P. Cohen Addad, *Physical properties of polymeric gels*, J. Wiley, Chichester ; New York, 1996.
2   T. Okano, Biorelated polymers and gels : controlled release and applications in biomedical engineering, Academic Press, San Diego, 1998.
3   A. Tanaka, Y. Fukuoka, Y. Morimoto, T. Honjo, D. Koda, M. Goto and T. Maruyama, *J. Am. Chem. Soc.*, 2015, **137**, 770-775.
4   S. H. Um, J. B. Lee, N. Park, S. Y. Kwon, C. C. Umbach and D. Luo, *Nat. Mater.*, 2006, **5**, 797-801.
5   E. Cheng, Y. Xing, P. Chen, Y. Yang, Y. Sun, D. Zhou, L. Xu, Q. Fan and D. Liu, *Angew. Chem. Int. Ed.*, 2009, **48**, 7660-7663.
6   J. Song, S. Hwang, K. Im, J. Hur, J. Nam, S. Hwang, G. O. Ahn, S. Kim and N. Park, *J. Mater. Chem. B*, 2015, **3**, 1537-1543.
7   N. C. Seeman, *Annu. Rev. Biophys. Biomol. Struct.*, 1998, **27**, 225-248.
8   P. W. Rothemund, *Nature*, 2006, **440**, 297-302.
9   N. Park, S. H. Um, H. Funabashi, J. Xu and D. Luo, *Nat. Mater.*, 2009, **8**, 432-437.
10 Y. H. Roh, R. C. Ruiz, S. Peng, J. B. Lee and D. Luo, *Chem. Soc. Rev.*, 2011, **40**, 5730-5744.
11 J. Lee, S. M. Peng, D. Y. Yang, Y. H. Roh, H. Funabashi, N. Park, E. J. Rice, L. W. Chen, R. Long, M. M. Wu and D. Luo, *Nat. Nanotechnol.*, 2012, **7**, 816-820.
12 H. Kuang, W. Ma, L. Xu, L. Wang and C. Xu, *Acc. Chem. Res.*, 2013, **46**, 2341-2354.
13 L. Y. T. Chou, K. Zagorovsky and W. C. W. Chan, *Nat. Nanotechnol.*, 2014, **9**, 148-155.
14 T. Yata, Y. Takahashi, M. Tan, K. Hidaka, H. Sugiyama, M. Endo, Y. Takakura and M. Nishikawa, *Sci. Rep.*, 2015, **5**.
15 B. Xiang, K. He, R. Zhu, Z. Liu, S. Zeng, Y. Huang, Z. Nie and S. Yao, *ACS Appl Mater Interfaces*, 2016, **8**, 22801-22807.
16 R. K. Saiki, D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis and H. A. Erlich, *Science*, 1988, **239**, 487-491.
17 N. A. Peppas, J. Z. Hilt, A. Khademhosseini and R. Langer, *Adv. Mater.*, 2006, **18**, 1345-1360.
18 N. S. Satarkar, D. Biswal and J. Z. Hilt, *Soft Matter*, 2010, **6**, 2364-2371.
19 J. Li, C. Y. Liu and Y. Liu, *J. Mater. Chem.*, 2012, **22**, 8426-8430.
20 H. P. Cong, X. C. Ren, P. Wang and S. H. Yu, *ACS Nano.*, 2012, **6**, 2693-2703.
21 W. Guo, R. Orbach, I. Mironi-Harpaz, D. Seliktar and I. Willner, *Small*, 2013, **9**, 3748-3752.
22 Y. X. Che, A. Zinchenko and S. Murata, *J. Colloid. Interface. Sci.*, 2015, **445**, 364-370.
23 R. Fuhrer, E. K. Athanassiou, N. A. Luechinger and W. J. Stark, *Small*, 2009, **5**, 383-388.
24 C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, **382**, 607-609.
25 M. R. Jones, N. C. Seeman and C. A. Mirkin, *Science*, 2015, **347**.
26 A. Samanta and I. L. Medintz, *Nanoscale*, 2016, **8**, 9037-9095.
27 G. Frens, *Nature. Phys. Sci.*, 1974, **241**, 20-22.
28 I. U. Arachchige and S. L. Brock, *J. Am. Chem. Soc.*, 2006, **128**, 7964-7971.
29 H. B. Shen, M. Hu, Y. B. Wang and H. Q. Zhou, *Biophys Chem*, 2005, **115**, 63-66.
30 W. Chen, A. Bian, A. Agarwal, L. Liu, H. Shen, L. Wang, C. Xu and N. A. Kotov, *Nano. Lett.*, 2009, **9**, 2153-2159.
31 S. Keller, J. Wang, M. Chandra, R. Berger and A. Marx, *J. Am. Chem. Soc.*, 2008, **130**, 13188-13189.
32 A. W. Peterson, R. J. Heaton and R. M. Georgiadis, *Nucleic Acids Res.*, 2001, **29**, 5163-5168.
33 D. Boyer, P. Tamarat, A. Maali, B. Lounis and M. Orrit, *Science*, 2002, **297**, 1160-1163.
34 H. Erdogan, H. Sakalak, M. S. Yavuz and G. Demirel, *Langmuir*, 2013, **29**, 6975-6982.
35 L. Poon, W. Zandberg, D. Hsiao, Z. Erno, D. Sen, B. D. Gates and N. R. Branda, *ACS Nano.*, 2010, **4**, 6395-6403.
36 36. F. Li, H. Zhang, B. Dever, X. F. Li and X. C. Le, *Bioconjug. Chem.*, 2013, **24**, 1790-1797.
37 J. M. Betton, *Curr Protein Pept Sci*, 2003, **4**, 73-80.
38 R. Jue, J. M. Lambert, L. R. Pierce and R. R. Traut, *Biochemistry*, 1978, **17**, 5399-5406.
39 S. Kiyonaka, K. Sada, I. Yoshimura, S. Shinkai, N. Kato and I. Hamachi, *Nat. Mater.*, 2004, **3**, 58-64.

DNA–gold nanoparticle hybrid hydrogel network prepared by enzymatic reaction

Yuka Eguchi, Tomoharu Kato, Tsutomu Tanaka and Tatsuo Maruyama*

Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, 1-1 Rokkodai, Nada-ku, Kobe 657-8501, Japan.

Materials and Methods

Materials

Hydrogen tetrachloroaurate (III) was purchased from Strem Chemicals, Inc. (Newburyport, MA, USA). Thiol-modified DNA primers were purchased from Tsukuba Oligo Service Co., Ltd. (Ibaraki, Japan). PCR reagents were purchased from Toyobo Co., Ltd. (Osaka, Japan). Ethidium bromide, DNA ladders for DNA gel electrophoresis, and 2-iminothiolane hydrochloride were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). DNase I was purchased from Sigma (St Louis, MO). 2-Mercaptoethanol, RNase A, and HRP were purchased from Wako Pure Chemicals (Osaka, Japan). The restriction enzymes were purchased from New England Biolabs Inc. (Ipswich, MA, USA). Tween 20 was purchased from Chemical Industry Co., Ltd (Tokyo, Japan). QuantaBlu peroxidase fluorogenic substrate was purchased from Thermo Fisher Scientific (Waltham, MA). Water was produced by a Millipore water purification system and sterilized by autoclaving prior to use.

Primer sequences

When using the 328 bp template,

Forward: 5′-SH-(CH$_2$)$_6$-cagggcgcgtcagcgggtgttg-3′

Reverse: 5′-SH-(CH₂)₆-cctctagagtcgacctgcaggc-3′

For the 911 bp template,

Forward: 5′-SH-(CH₂)₆-cgtaagatgcttttctgtgact-3′

Reverse: 5′-SH-(CH₂)₆-caacgtcgtgactgggaaaac-3′

For the 1633 bp template,

Forward: 5′-SH-(CH₂)₆-cgtaagatgcttttctgtgact-3′

Reverse: 5′-SH-(CH₂)₆-gacctacaccgaactgagatac-3′

For the 6012 bp template,

Forward: 5′-SH-(CH₂)₆-acgagtgggttacatcgaactg-3′

Reverse: 5′-SH-(CH₂)₆-aggaagggaagaaagcgaaagg-3′

For the 8570 bp template,

Forward: 5′-SH-(CH₂)₆-cagggcgcgtcagcgggtgttg-3′

Reverse: 5′-SH-(CH₂)₆-cctctagagtcgacctgcaggc-3′

Templates

The DNA templates (328, 911, and 1633 bp) used to synthesize thiol-modified double-stranded DNA were amplified by PCR with non-modified primer pairs, which were the same sequences as the above thiol modified primers from pUC 18 (Takara bio Inc., Shiga, Japan).

The gene encoding GFP was obtained by PCR with 5′-acagcccagatctgggtaccattgaaggccgtggcggcggcggcagcgtgagcaagggcgaggag-3′ as the forward primer and 5′-tgctcgagtgcggccgcttagccgccggtttccggcagcttgtacagctcgtccat-3′ as the reverse primer from pEGFP-N1. The amplified fragment was sub-cloned into the KpnI/NotI sites of the pET32b vector (Merck Millipore, Billerica, MA) to give a DNA template (6012 bp).

The DNA template (8570 bp) was amplified by PCR with 5′-cagggcgcgtcagcgggtgttgccacaattttgggaatagcgcaagc-3′ as the forward primer and

5′-cctctagagtcgacctgcaggcccacacaacatacgagccggaagca-3′ as the reverse primer from pDUAL-FFH1 (RIKEN BRC, Tsukuba, Japan).

Synthesis of AuNPs

The AuNPs (15 nm in diameter) were prepared by citrate reduction of $HAuCl_4$. A $HAuCl_4$ aqueous solution (10^{-2} wt%, 100 mL) was vigorously boiled with stirring in an Erlenmeyer flask, and 1 mL of trisodium citrate (1 wt%) was added. The solution was boiled for another 5 min, during which time the solution changed from pale yellow to brilliant red. The solution was allowed to cool to room temperature with continuous stirring. The synthesized AuNPs were centrifuged at 10,000 × g for 1 h and the supernatant was removed to purify and condense the AuNPs. The AuNP concentration was determined by atomic absorption spectrophotometer (Z-2310, Hitachi, Ltd., Tokyo, Japan). The AuNP diameter was determined by TEM observation. TEM observation was performed with a JEM2100-M microscope (JEOL, Ltd., Tokyo, Japan) at 200 kV acceleration voltage.

Preparation of gels using the usual PCR (method 1)

PCR conditions (method 1)

PCR solution: 30 pmol of each primer, 100 μg DNA template, 40 nmol dNTPs, 2.0 U of KOD FX Neo, 1 × PCR buffer for KOD FX Neo, and water were mixed to give a total volume of 100 μL.

PCR program (method 1)

When using the 328 bp template, denaturing (5 min at 94 °C) and 35 cycles (30 s at 94 °C, 30 s at 55 °C, 1 min at 68 °C).

For the 911 bp template, denaturing (5 min at 94 °C) and 35 cycles (10 s at 98 °C, 1 min at 68 °C).

For the 1633 bp template, denaturing (5 min at 94 °C) and 35 cycles (10 s at 98 °C, 1 min at 68 °C).

For the 6012 bp template, denaturing (5 min at 94 °C) and 35 cycles (30 s at 94 °C, 30 s at 60 °C, 6

min at 68 °C).

For the 8570 bp template, denaturing (5 min at 94 °C), 5 cycles (15 s at 94 °C; 8 min 40 s at 74 °C), 5 cycles (15 s at 94 °C; 8 min 40 s at 72 °C), 5 cycles (15 s at 94 °C; 8 min 40 s at 70 °C), and 25 cycles (15 s at 94° C; 8 min 40 s at 70 °C).

Preparation of DNA–AuNP gels (method 1)

A PCR solution was applied to a NAP-10 column (GE Healthcare, Little Chalfont, UK) using 0.05 × SSC buffer (7.5 mM NaCl in 0.75 mM sodium citrate, Nacalai Tesque, Inc., Kyoto, Japan) to purify elongated DNA. The DNA solution was then mixed with a AuNP solution and shaken for 1 h, and the resulting mixture was freeze-dried. An appropriate amount of water was added. The final concentrations of elongated DNA and AuNPs were 2 μM and 30 nM, respectively.

The concentration of elongated DNA synthesized by PCR was determined using a Qubit DNA assay kit (Thermo Fisher Scientific).

Agarose gel electrophoresis was performed in a 1.0% agarose gel containing 1.0 × TAE. The DNA samples were separated at 135 V for 30 min.

Preparation of DNA–AuNP gel using surface-initiated PCR (method 2)
Preparation of AuNP–DNA primer conjugates

Thiolated DNA (DNA primer) was purified by a NAP-5 column (GE Healthcare) using 0.05 × SSC buffer. Each primer solution (thiolated DNA) was mixed with a AuNP solution and shaken for 1 h. The final concentrations of thiolated DNA and AuNPs were 1.15 μM and 6.4 nM, respectively.

PCR conditions (method 2)

The PCR conditions were the same as those for method 1 with the 6012 bp template.

Agarose gel electrophoresis (method 2) (Figure S2)

*Sca*I, *Not*I (20 U each), and 10 × CutSmart buffer (1 μL) were added to the PCR solution (7 μL) and the solution was gently shaken at 37 °C overnight. The DNA fragment that was produced by cleaving elongated DNA with restriction enzymes was 5535 bp. After restriction enzyme treatment, agarose gel electrophoresis was performed in a 1.0% agarose gel containing 1.0 × TAE. The DNA samples were separated at 135 V for 30 min.

Preparation of DNA–AuNP gel (method 2)

A PCR solution was dialyzed against 0.05 × SSC buffer using a Slide-A-Lyzer dialysis cassette (Thermo Fisher Scientific, Waltham, MA) with a molecular-weight cutoff of 2.0 kDa for 2 h at room temperature and freeze-dried. An appropriate amount of water was added. The final concentrations of thiolated DNA and AuNPs were 2 μM and 30 nM, respectively.

Rheology measurement

An MCR301 rheometer (Anton Paar GmbH, Graz, Australia) with a parallel plate fixture was used to measure the mechanical properties of the DNA-AuNP hydrogels in rotating mode. Dynamic frequency sweeping mode was used with the stain fixed at 1%.

Evaluation of the laser-responsive gel–sol transition

The laser-triggered gel–sol transition of the DNA–AuNP hydrogel was investigated using a green-laser pointer at a power density of 100 mW/cm^2 with a wavelength of 532 nm.

Evaluation of the enzyme-catalyzed gel–sol transition

DNase I was dissolved in acetic acid buffer (pH 5, 0.1 M) containing 5 mM MgSO$_4$. RNase A was dissolved in Tris-HCl buffer (pH 7.5, 0.1 M). Each nuclease concentration was 10 mg mL^{-1}. Each

nuclease solution (2 µL) was added to 2 µL of the DNA–AuNP hydrogel.

*Ear*I or *EcoR*I (each 80 U) was dissolved in 1 × CutSmart buffer. DNA–AuNP hydrogel (2 µL) was added to 50 µL of the enzyme solution and the solution was gently shaken at 37 °C overnight.

Cell-free protein synthesis

Coupled transcription and translation kits (Musaibokun Quick) were purchased from Taiyo Nippon Sanso Corporation (Tokyo, Japan), and the reactions were carried out by the following procedures suggested by the manufacturer. For protein expression, DNA–AuNP hydrogel (6012 bp coding for GFP) or linear DNA (6012 bp) was directly added to the reaction solution. The protein expressions were conducted in a 96-well microtiter plate at 37 °C. After incubation for 1 h, the reaction solutions were cooled on ice. The protein amount (GFP) was evaluated by measuring the fluorescence. The fluorescence intensity of the produced protein was measured at room temperature using a microplate reader (SH-9000, Corona Electric Co., Ltd., Ibaraki, Japan). The excitation and emission wavelengths were 488 nm and 515 nm. The excitation and emission band widths were 12 nm. The molecular weight of GFP was 42 kDa.

Thiolation of HRP

A 20 times molar excess of 2-iminothiolane hydrochloride was added to HRP in phosphate buffer (pH 7.2, 0.1 M) containing 5 mM EDTA and the solution was incubated for 1 h at room temperature. The unreacted 2-iminothiolane hydrochloride was separated from the iminothiolated HRP by a PD-10 column (GE Healthcare) using phosphate buffer (pH 7.2, 0.1 M) containing 5 mM EDTA.

Immobilization of HRP on AuNPs

Immobilization of HRP on the DNA–AuNP hydrogel (DNA 1 µM, AuNPs 30 nM) and measurement of the HRP activity were performed in a 384 microtiter plate. DNA–AuNP hydrogel (5

μL) and a thiolated HRP solution (50 μL) were added to each well and incubated for 1 h at room temperature. The supernatant solution was removed. Each well was washed three times for 5 min on a shaking platform in 90 μL phosphate buffer (pH 7.2, 0.1 M) containing 0.15 M NaCl and 0.05% Tween 20. QuantaBlu solution (40 μL) was placed in each well and incubated for 1 h at 37 °C. The active HRP amount was evaluated by measuring the fluorescence. The fluorescence intensity was measured at 37 °C using a microplate reader. The excitation and emission wavelengths were 315 nm and 407 nm, respectively. The excitation and emission band widths were both 12 nm.
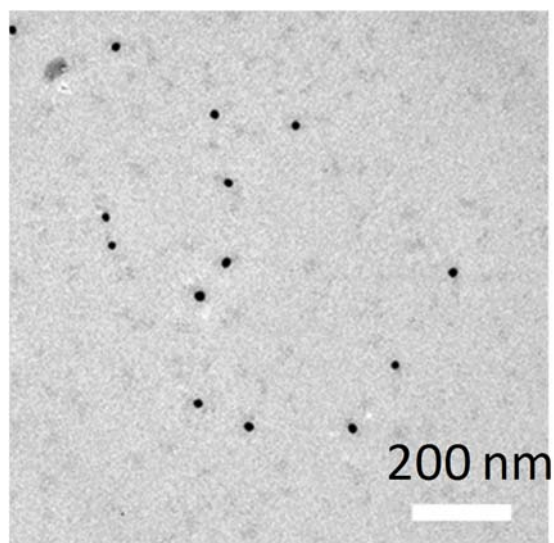
Results

Figure S1 AuNPs in the DNA–AuNP hydrogel prepared by method 1.
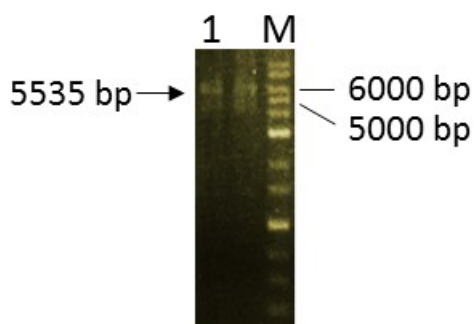

Figure S2 Agarose gel electrophoresis of the fragment (5535 bp) derived from a PCR amplicon (6012 bp) prepared by method 2. Lane M is the marker and lane 1 is the fragment (5535 bp). The fragment (5535 bp) was prepared by cleavage of the PCR amplicon (6012 bp) on the AuNPs. *Sca*I and *Not*I were used for the cleavage.
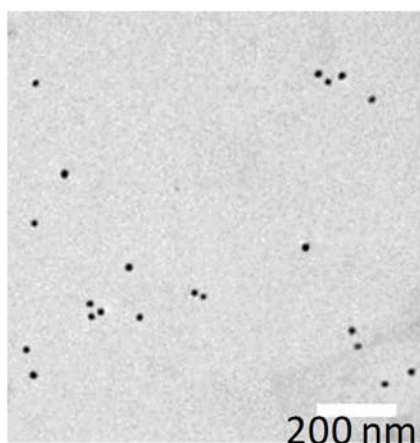
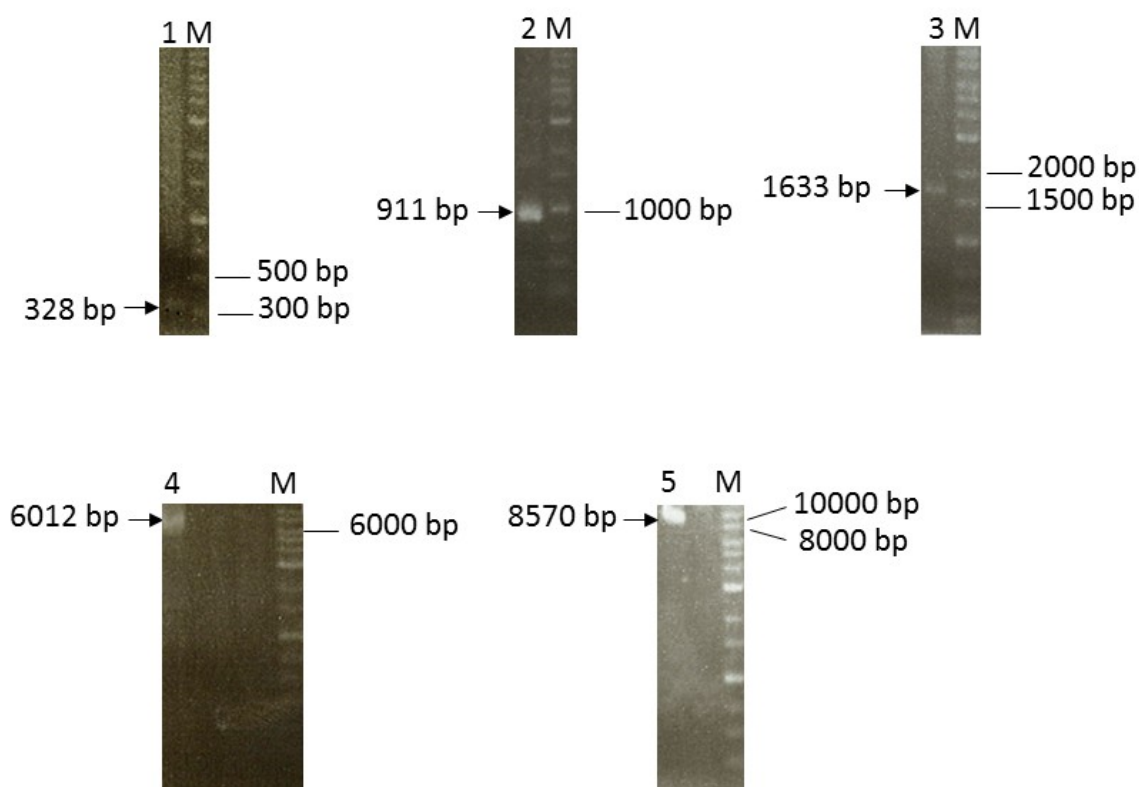**Figure S3** AuNPs in the DNA–AuNP hydrogel prepared by method 2.

Figure S4 Agarose gel electrophoresis of PCR amplicons with various DNA lengths. Lane M is the marker, lane 1 is a 328 bp amplicon, lane 2 is a 911 bp amplicon, lane 3 is a 1633 bp amplicon, lane 4 is a 6012 bp amplicon, and lane 5 is a 8570 bp amplicon.

Table S1 Effect of the DNA length and concentration on gelation.

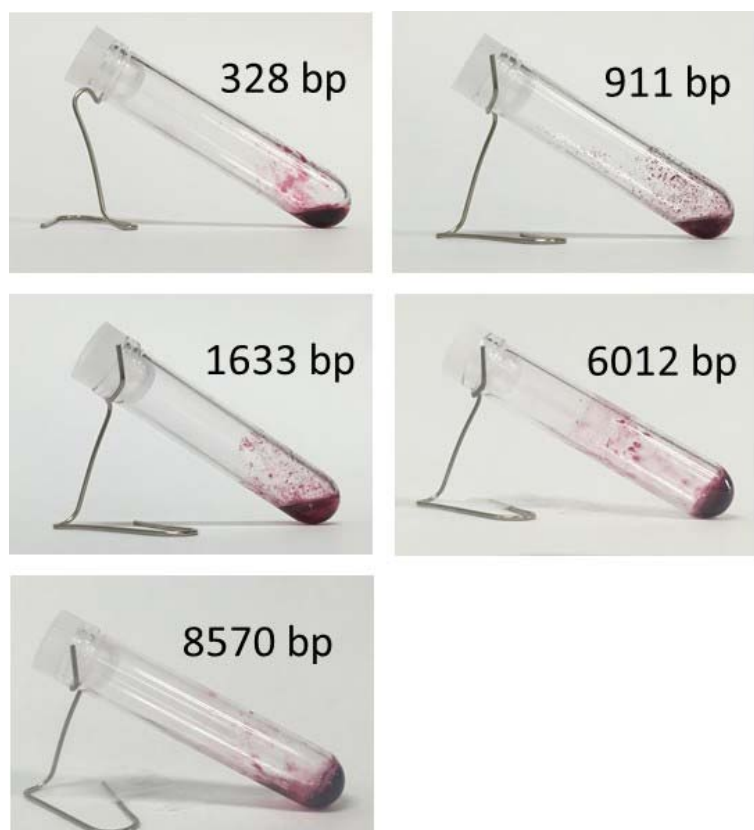| DNA length	DNA concentration	Gel or Sol
328 bp		Partial gel
911 bp		Sol
1633 bp	2 μM	Sol
6012 bp		Gel
8570 bp		Gel
	0.25 μM	Sol
	0.5 μM	Partial gel
6012 bp	1.0 μM	Gel
	2.0 μM	Gel

The AuNP concentration was 30 nM.

Figure S5 Gelation tests of DNA–AuNP complexes with various DNA lengths.
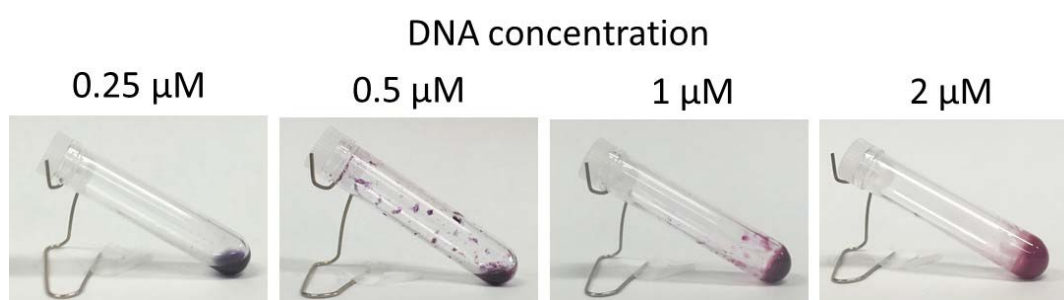

Figure S6 Gelation tests of DNA–AuNP complexes with various DNA concentrations.

Table S2 Effect of the AuNP concentration on gelation.

AuNP concentration [nM]	DNA/AuNP molar ratio [-]	Gel or Sol
2.0	1000	Partial gel
4.0	500	Partial gel
7.5	267	Gel
15	133	Gel
30	67	Gel

The DNA concentration (6012 bp) was 1 μM.