Electronic supplementary information (ESI)

DNA–gold nanoparticle hybrid hydrogel network prepared by enzymatic reaction

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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-$(CH_2)_6$-cctctagagtcgacctgcaggc-3′

For the 911 bp template,

Forward: 5′-SH-$(CH_2)_6$-cgtaagatgcttttctgtgact-3′

Reverse: 5′-SH-$(CH_2)_6$-caacgtcgtgactgggaaaac-3′

For the 1633 bp template,

Forward: 5′-SH-$(CH_2)_6$-cgtaagatgcttttctgtgact-3′

Reverse: 5′-SH-$(CH_2)_6$-gacctacaccgaactgagatac-3′

For the 6012 bp template,

Forward: 5′-SH-$(CH_2)_6$-acgagtgggttacatcgaactg-3′

Reverse: 5′-SH-$(CH_2)_6$-aggaagggaagaaagcgaaagg-3′

For the 8570 bp template,

Forward: 5′-SH-$(CH_2)_6$-cagggcgcgtcagcgggtgttg-3′

Reverse: 5′-SH-$(CH_2)_6$-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 *Eco*RI (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

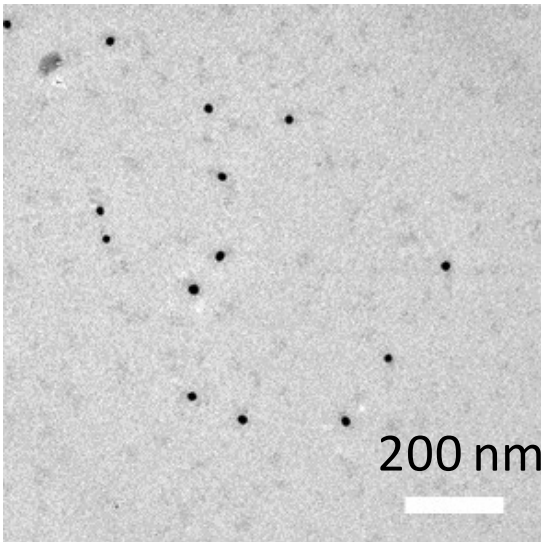

Figure S1 AuNPs in the DNA–AuNP hydrogel prepared by method 1.

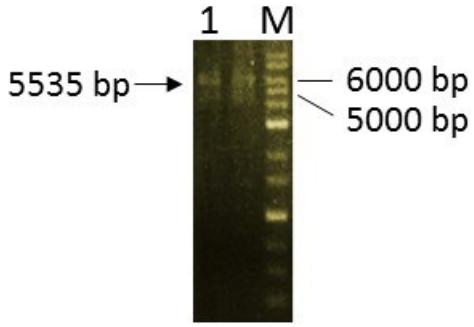

Figure S2 Agarose gel electrophoresis of the fragment (5535 bp) derived from a PCR amplicon (6012 bp) prepared by method 2. Lane M is the marker and lane 1 is the fragment (5535 bp). The fragment (5535 bp) was prepared by cleavage of the PCR amplicon (6012 bp) on the AuNPs. *Sca*I and *Not*I were used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

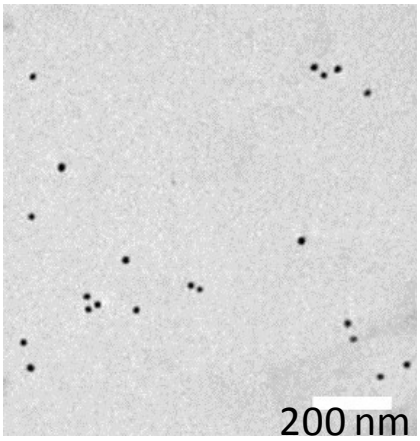

Figure S3 AuNPs in the DNA–AuNP hydrogel prepared by method 2.

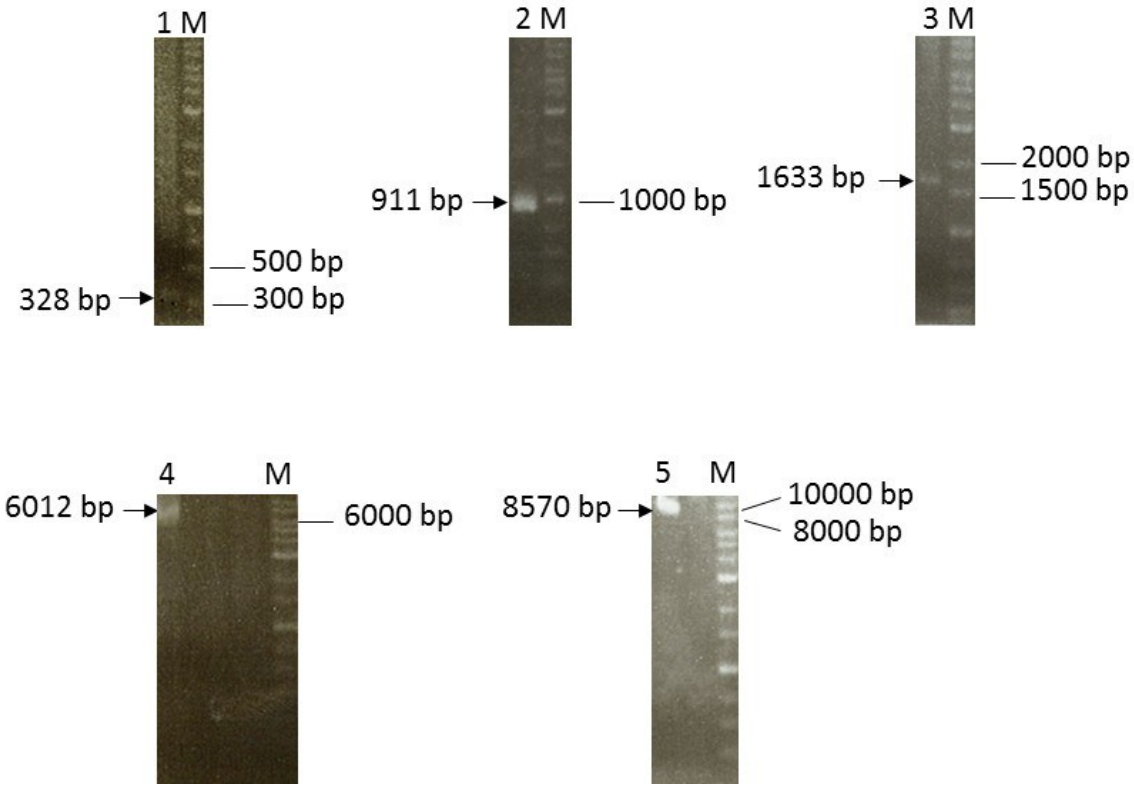

Figure S4 Agarose gel electrophoresis of PCR amplicons with various DNA lengths. Lane M is the marker, lane 1 is a 328 bp amplicon, lane 2 is a 911 bp amplicon, lane 3 is a 1633 bp amplicon, lane 4 is a 6012 bp amplicon, and lane 5 is a 8570 bp amplicon.

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

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

The AuNP concentration was 30 nM.

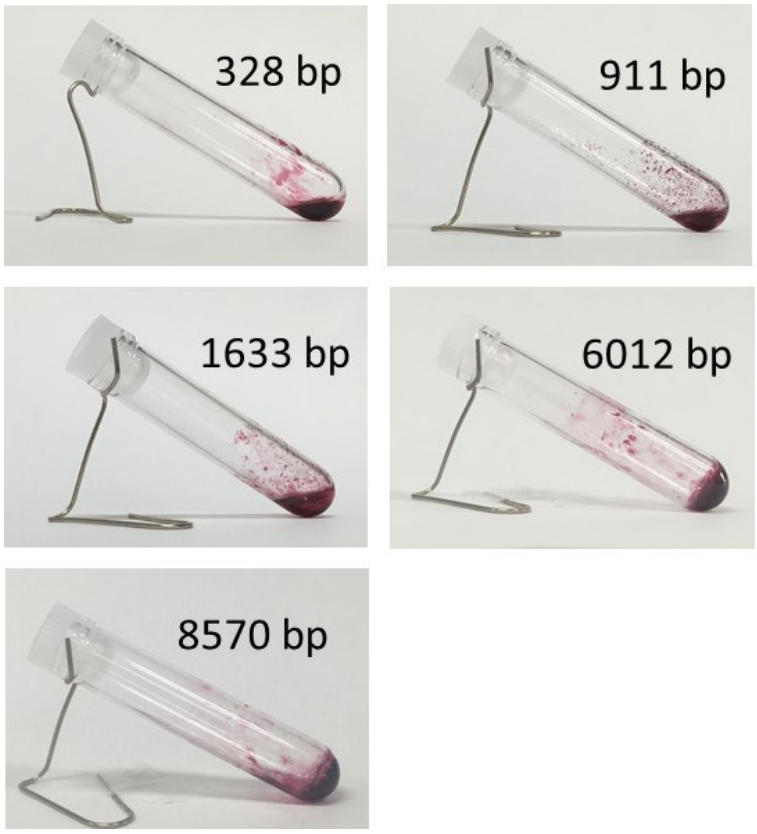

Figure S5 Gelation tests of DNA–AuNP complexes with various DNA lengths.

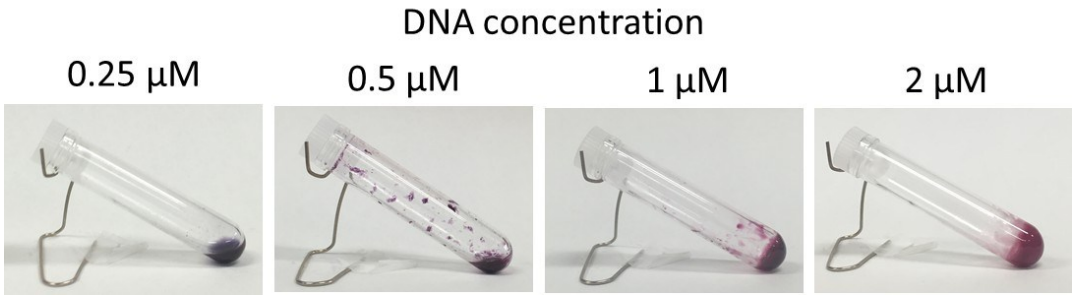

Figure S6 Gelation tests of DNA–AuNP complexes with various DNA concentrations.

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.