A Facile Method Towards Cyclic Assembly of Gold Nanoparticles Using DNA template Alone

Takahito Ohshiro, Tamotsu Zako, Ryoko Watanabe-Tamaki, Takuo Tanaka and Mizuo Maeda

Supporting Information:

Experimental Section

1. Materials

All the custom oligonucleotides were purchased from Operon Japan and Tsukuba Oligo. Each of the DNA building blocks for cyclic assembly of gold nanoparticles are comprised of Ligand DNA (A, B, C, D), Template DNA (abt, bct, cat, cdt, dat), and Supporting DNA (spt). The DNA building blocks have an ability of formation for the triangular and square gold nanoparticle nanostructures (Figure S1). The DNA sequences were shown below. The concentration of each strand was estimated by measuring OD $_{260}$.

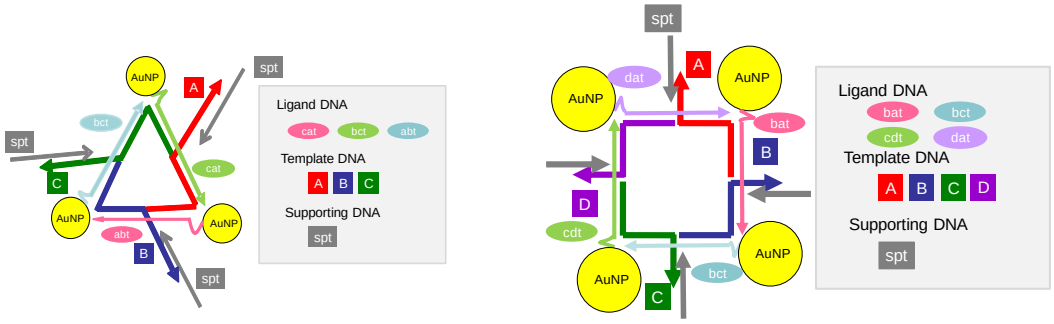

Figure S1

Ligand DNA

abt 5'-[ThiSS]TTTTTCCAGATCGAATATAATAGTATTGCCCCGATTTGTTATAGTTATTGGTC-3'
bct 5'-[ThiSS]TTTTTCAGATTGCAGTATATATGAGCAGGCTTTCCTCCTTATATCTACCATGT-3'
cat 5'-[ThiSS]TTTTTGAACTCTACCTATAAGTTAGCAAACCAAGGTGATTATAGTCATTCTGG-3
cdt 5'-[ThiSS]TTTTTTTCATACGATTATACTACTCCACACCAAGGTGATTATAGTCATTCTGG-3'
dat 5'-[ThiSS]TTTTTGAACTCTACCTATAAGTTAGCAAAGTGTGTCGACTATATATGACGC-3'

Template DNA

A: 5'-TTTGCTAACTTATAGGTAGAGTTCTTTTTGACCAATAACTATAACAAATCGGGTTTTTGTTGTAGGAGCTTGCTCGAC-3'
B: 5'-GCAATACTATTATATCGATCTGGTTTTTACATGGTAGATATAAGGAGGAAAGTTTTTGTTGTAGGAGCTTGCTCGAC-3'
C: 5'-CCTGCTCATATATACTGCAATCTGTTTTTCCAGAATGACTATAATCACCTTGGTTTTTGTTGTAGGAGCTTGCTCGAC-3'
D: 5'-TGTGGAGTAGTATAATCGTATGAATTTTTGCGTCATATATATAGTCGACACACTTTTTGTTGTAGGAGCTTGCTCGAC-3'

Supporting DNA

spt: 5'-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT GTC GAG CAA GCT CCT ACA AC-3'

Functionalization of gold nanoparticles (AuNPs) with thiolated DNA.

Citratecoated gold nanoparticles with < 10 % deviation in diameter were purchased (British BioCell Internatinal). Bis-(*p*sulfonatophenyl) phenylphosphine dihydrate dipotassium (BSPP) (Aldrich Japan) was used to stabilize the AuNPs in aqueous solutions, Sixty mg of BSPP was added to 100 mL of citrate coated AuNP solution, and the mixture was stirred at room temperature for overnight in order to allow the phosphine ligands to replace the citrate ligands. BSPP-coated AuNPs was isolated by centrifugation and suspended in 100 ul of Tris-Borate-EDTA (TBE) buffer. The aqueous solution of BSPP-coated AuNP was mixed with an equimolar of 5′-thiolated DNA (Ligand DNA) in 0.5x TBE buffer, pH 7.4, containing 30 mM NaCl solution, and then incubated with excess molar of template DNA and supporting DNA at room temperature for overnight.

Purification of "DNA building blocks" for cyclic assembly

The DNA-AuNPs mixtures comprises of AuNPs bearing different numbers of attached DNA. DNA-monoconjugated AuNPs, i.e., "DNA building blocks", were isolated by gel electrophoresis (**Figure 2a**). Agarose gels (2% (w/v) in 0.5x TBE buffer The "DNA building blocks" were extracted from the electrophoresis band as follows. The agarose gel was partially cut at the downstream of the corresponding band, and filter paper was inserted. Additional gel running allows DNA building blocks to enter the filter paper. Then DNA building blocks were eluted from the filter paper in 0.5xTBE buffer in a microtube. The concentration of the isolated "DNA-building blocks" were calculated using the manufacture's molar coefficient data (ε_{520}(20 nm) = 8.61 x 10^8 $M^{-1}cm^{-1}$).

Construction of DNA building blocks for Cyclic Assembly of DNA- monoconjugated AuNP .

In order to construct triangular and square AuNP nanostructures, an equimolar of the each "DNA building blocks" was simultaneously mixed and incubated. In a typical experiment for assembling triangular structures, 5 nM of each "building block" mixture solution was annealed slowly from 45 degree to room temperature. The trimer "DNA building block" was isolated from an aqueous solution of the mixtures, comprising a single DNA building block, dimer and trimer nanostructures of building block using gel electrophoresis as described above. Agarose gels (1.5% (w/v) in 0.5x TBE buffer were used for the gel separation. The trimer "building block" was extracted from the electrophoretic bands as described above.

UV irradiated DNA interstrand crosslinking

In order to stabilize the DNA building blocks-assembled nanostructures, UV irradiated interstrand crosslinking by using 8-methocypsoralen (8-MOP), psoralen derivative, was carried out following the previously reported procedures (**ref.6**). A saturated solution of 8-MOP in ethanol was

added into the assembled AuNPs nanostructure solution. The solution was incubated in the dark for 1 h and irradiated on ice for 45 min at 365 nm.

TEM measurements of AuNPs assemblies and their statistical analysis.

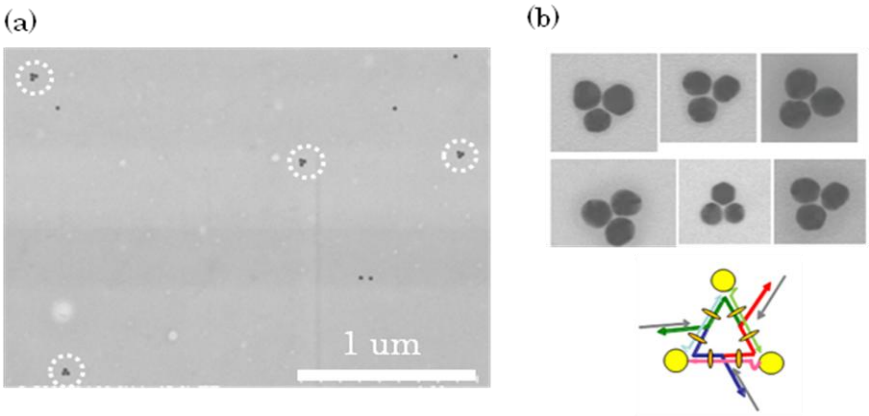

Figure S2. (a) A TEM image (2.72 um x 2.0 um) of Trimer "building block". (b) Enlarged TEM image of Trimer "DNA-AuNP building block". The white circles indicate a typical triangular assembly of AuNP

Transmission electron microscopy was performed in order to characterize the triangular and square AuNP nanostructures. Formvar film-coated cupper TEM grids were hydrophilized using a hydrophilic treatment device (JEOL DATUM, HDT-400) in advance, and then 0.5 uL of diluted aqueous sample was spotted on the surface and allowed to adsorb. 0.5x Tris-Borate-EDTA was added and excess suspension was then removed from the grid using filter paper. The grids were then air-dried prior to analysis. Figure 2c and d shows a typical TEM image of triangular and square assembly, respectively, and a typical zoom-out TEM image of triangular assembled AuNPs is shown in Figure S2 a.

Characterization of Cyclic Assembled AuNP by using Atomic Force Microscope

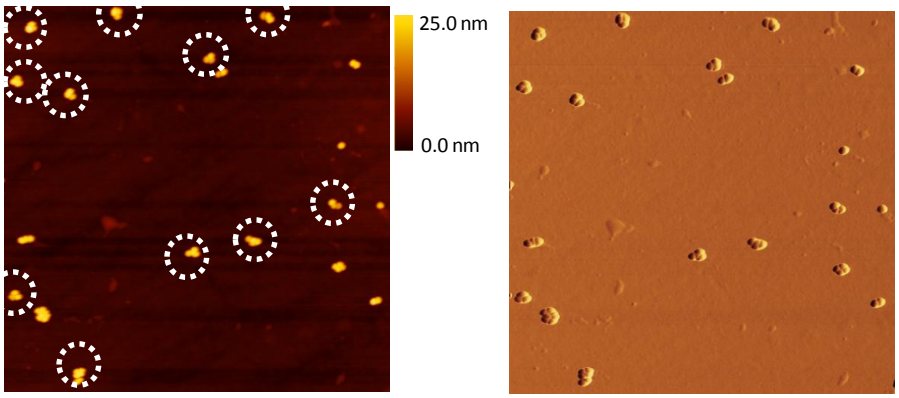

Figure S3. Topography(a) and phase (b) AFM images (3 um x 3 um) of Trimer "building block".

For the AFM observation, the assembled AuNPs were immobilized on a quartz substrate surface by following procedure. A quartz substrate was cleaned by 172 nm UV exposure for 5 min under nitrogen atmosphere by using an excimer light emission unit (USHIO Inc.). The cleaned quartz substrate was immersed into 57 mM 3-aminopropyltrimethoxysilane (Sigma-Aldrich) toluene solution for 15 min to obtain a self-assembled monolayer with positive charges. Subsequently, the substrate was washed in ultrapure water under ultrasonication for 10 min and dried by blowing. Adsorption of the assembled AuNPs on the substrate surface was done by immersion of the substrate into the solution of the assembled AuNPs for 3 h, followed by rinsing with 0.5 TBE and drying by blowing. The AFM observation was carried out by an SPA400 with SPI3800N (SII NanoTechnology Inc.) using tapping mode. The cantilever used was NCH tip (NanoWorld Inc.). Figure S2 a and b shows a typical topograph AFM image of triangular AuNPs after UV cross-linking, together with the phase AFM image.

As shown in an enlarged AFM image of trimer assembled AuNPs (**Figure S4**), the trimer of the "building block" was observed as a triangle shape, and the average sides of the triangle images was 65 ± 22 nm, which is comparable to the expected values of 47.5 nm for the triangular nanostructures. Together with the TEM image (Figure 2c upper figure), this result indicates that DNA "building block" trimer should be assembled as a triangular nanostructure.

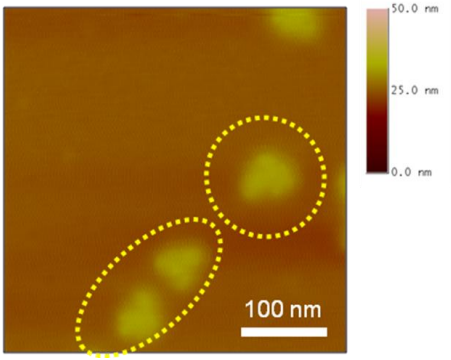

Figure S4. An AFM image (400 nm x 400 nm) of Trimer "building block". For this atomic force microscopy (AFM) imaging, freshly cleaved mica was pretreated with 1mM $MgCl_2$. A drop of the solution of the triangular assembly was spotted on the mica for fifteen seconds to allow for strong adsorption. The sample substrate was then washed off by 10 mM Tris (pH 7.6) buffer solution, and dried in a silica-gel desiccators. AFM images were obtained under ambient conditions with Nanoscope IIIa (Vecco Instruments Inc.,) by tapping mode

Statistical analysis for AuNPs assemblies was carried out using these TEM images. Trimer yields were defined as the ratio of AuNPs forming triangle per all particles. The yields of trimer purification based on gel electrophoresis were obtained from the TEM images (the number of AuNPs forming trimers/ the number of all AuNPs (%)). The yields were 52.4 % (75/143) for the crosslinked samples and 16.7 % (18/108) for non-crosslinked samples. This result indicated that the UV-induced interstrand crosslinking with psolaren significantly served to stabilize the synthesized triangular AuNPs nanostructures.

UV irradiated DNA interstrand crosslinking

We used 8-methoxypsoralen (8-MOP) as the interstrand cross-linker for the triangular and square AuNPs. The chemical structure is shown in Figure S3. It have been reported that psolaren, such as 8-MOP, is inserted into 5'-TA-3' sites in double stranded DNA, and induced interstrand-crosslink upon UV radiation (reference review paper: EMBO report, 2005, 6,551-556). We designed the

double stranded DNA containing twenty-five 5'-TA-3' sites for the triangular AuNPs (See Figure S5: 5'-TA-3' sites are colored as yellow). Therefore, the twenty-five TA sites could induce interstrand crosslink, resulting in stable formation of triangular assembles of AuNPs.

Supporting Information Figure S5