Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary information for

DNA-functionalized colloidal crystals for macromolecular encapsulation

Maasa Yokomori[†], Hayato Suzuki[∇], Akiyoshi Nakamura[∇], Shigeo S. Sugano[∇], Miho Tagawa[†§]

† Center for Integrated Research of Future Electronics (CIRFE), Institute of Materials and Systems for Sustainability (IMaSS), Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan

∇ Plant Gene Regulation Research Group, Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, 305-8566, Japan

§ Department of Materials Process Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan

Supplementary experimental section

Quantification of DNA loading on AuNPs

Dithiothreitol (DTT) dry powder (molecular grade; Promega, Madison, WI, USA) was dissolved in ultrapure water, which was prepared using a Direct-Q water purification system. Urea, ammonium peroxodisulfate (APS, for electrophoresis), and N, N, N', N'-tetramethylethylenediamine (TEMED, for electrophoresis) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Tris-boric acid-EDTA (TBE)/urea sample buffer, 40% acrylamide/bis solution 19:1, and 10x Buffer × TBE buffer were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). SYBR Gold nucleic acid gel stain (10,000 × concentration in dimethyl sulfoxide) (Invitrogen, Carlsbad, CA, USA) was used. DNA for standard curves, which had the same DNA sequences as Ligands A, B, and S, was purchased from Nihon Gene Research Laboratories Inc. (Sendai, Japan).

DTT solution (1 M) was added to the DNA-AuNP solution at a DTT/AuNP mole ratio of 1×10^7. The mixture was incubated for 1–2 h at room temperature (approximately 20 °C) and centrifuged at 20238 × *g* for 10 min. The supernatant was then subjected to electrophoresis.

For DNA-AuNPs coated with both Ligands S and A (or Ligand B), the supernatants were analyzed by capillary electrophoresis (Shimadzu MultiNA microchip electrophoresis system; Shimadzu, Kyoto, Japan). For DNA-AuNPs coated with Ligands A or B, 15% polyacrylamide gels containing 7 M urea and 1x TBE were prepared. Polymerization was initiated by adding 10% APS (45 µL) and TEMED (4.5 µL) to 8.5 mL of the gel. The prepared gel was applied to an electrophoresis apparatus (AE-6530P; ATTO Corp., Tokyo, Japan) and filled with 500 mL of 1x TBE running buffer at the top of the gel. Prerun electrophoresis was performed at a constant voltage of 300 V for 15 min. Then, 1.25 µL of known concentrations of DNA for the standard curve or DTT-treated supernatant was mixed with 1.25 µL of TBE/urea sample buffer. Each sample was subjected to electrophoresis at a constant voltage of 300 V for 45 min. After electrophoresis, the gel was stained with 1x SYBR Gold nucleic acid gel stain in 1x TBE at room temperature for 30 min and analyzed using an ATTO WSE-6100 LuminoGraph I to estimate the concentration of DNA in each DNA-AuNP. All experiments were repeated thrice to obtain the average DNA concentration.

Supplementary table

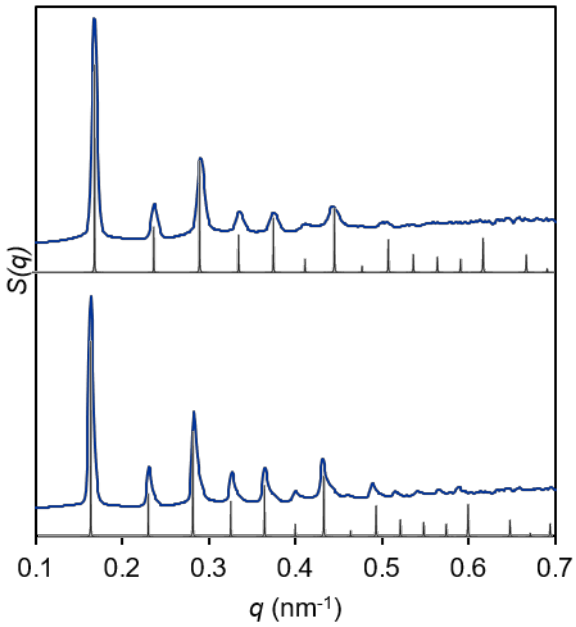
Table S1. Oligonucleotide sequences used for constructing DNA-functionalized gold nanoparticle (DNA-AuNP) crystals

Ligands A and B consisted of flexible spacer bases (adenine nucleotides) and recognition bases that bind to the complementary regions in Linkers A and B, respectively. Linkers A and B comprised three basic regions: (*i*) the recognition region, (*ii*) spacer region, and (*iii*) sticky end region. Single or three unpaired nucleotides were inserted into these regions. Ligand S contained 10 flexible spacer bases. The space sequence was complementary to the spacer region of Linkers A and B. Linkers A and B were hybridized via six sticky end bases.

Name	Sequence (5′ to 3′)
Ligand A (for 13-nm AuNPs)	TCAACTATTCCTACCTACAAAAAAAAAAAAAA-SH
Ligand B (for 13-nm AuNPs)	TCCACTCATACTCAGCAAAAAAAAAAAAAAAA-SH
Ligand S (for 13-nm AuNPs)	AAAAAAAAAA-SH
Ligand A (for 23-nm AuNPs)	TCAACTATTCCTACCTACAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAA-SH
Ligand B (for 23-nm AuNPs)	TCCACTCATACTCAGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAA-SH
Linker A (n=0) (3′-biotin modification)	GTAGGTAGGAATAGTTGAAAATTCCTT-biotin
Linker B (n=0) (3′-biotin modification)	TTGCTGAGTATGAGTGGAAAAAGGAA-biotin
Linker A (n=1)	GTAGGTAGGAATAGTTGAACTGCGCTCGGTCGTTCGGAAATTCCTT

Linker B (n=1)	TTGCTGAGTATGAGTGGAACTGCGCTCGGTCGTTCGGAAAAAGGAA
Linker A (n=1) (3′-biotin modification)	GTAGGTAGGAATAGTTGAACTGCGCTCGGTCGTTCGGAAATTCCTT-biotin
Linker B (n=1) (3′-biotin modification)	TTGCTGAGTATGAGTGGAACTGCGCTCGGTCGTTCGGAAAAAGGAA-biotin
Linker A (n=1) (^{CNV}K modification)	GTA^{CNV}KGTAGGAATAGTTGAACTGCGCTCGGTCGTTCGGAAAGCA^{CNV}KAC
Linker B (n=1) (^{CNV}K modification)	TTGCTGA^{CNV}KTATGAGTGGAACTGCGCTCGGTCGTTCGGAAAGTGTGC
Linker A (n=3)	GTAGGTAGGAATAGTTGAACTGCGCTCGGTCGTTCGGACTGCGCTCGGTCGTTCGGACTGCGCTCGGTCGTTCGGAAATTCCTT
Linker B (n=3)	TTGCTGAGTATGAGTGGAACTGCGCTCGGTCGTTCGGACTGCGCTCGGTCGTTCGGACTGCGCTCGGTCGTTCGGAAAAAGGAA
Linker A (n=3) (3′-biotin modification)	GTAGGTAGGAATAGTTGAACTGCGCTCGGTCGTTCGGACTGCGCTCGGTCGTTCGGACTGCGCTCGGTCGTTCGGAAATTCCTT-biotin
Linker B (n=3) (3′-biotin modification)	TTGCTGAGTATGAGTGGAACTGCGCTCGGTCGTTCGGACTGCGCTCGGTCGTTCGGACTGCGCTCGGTCGTTCGGAAAAAGGAA-biotin
Spacer	CCGAACGACCGAGCGCAG
Spacer (5′-Alexa Fluor 488 modification)	Alexa Fluor 488-CCGAACGACCGAGCGCAG
Spacer (5′-biotin and ^{CNV}K modification)	biotin -CCGAACGA^{CNV}KCGAGCGCAG

Supplementary figures



	Modification	
	No biotin	Biotin
Lattice constant (D_p) [nm]	53 (40)	55 (41)

Figure S1. Comparison of small-angle X-ray scattering (SAXS) patterns of non-biotinylated (top) and biotin-modified (bottom) DNA-functionalized gold nanoparticle$_{(13\ nm,\ n=1)}$ crystals. The SAXS patterns (blue curves) are shown as plots of the structure factor ($S(q)$) versus the scattering vector (q, in units of nm^{-1}). The black traces are the calculated scattering patterns for a perfect body-centered cubic lattice.

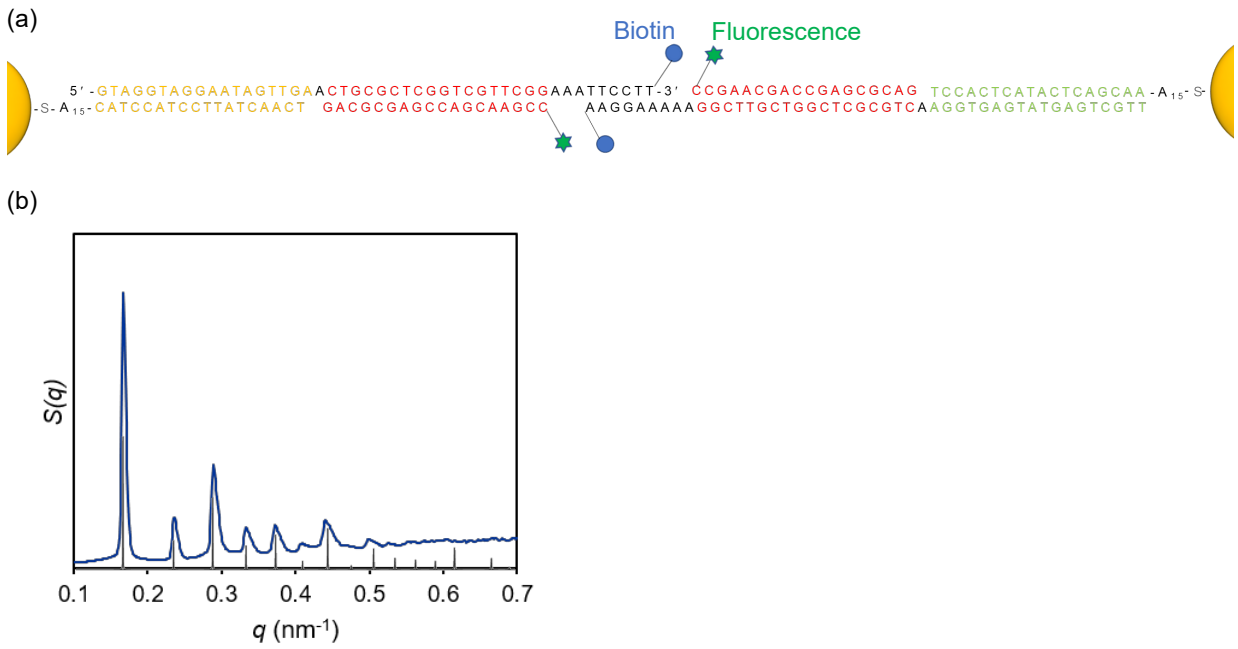


Figure S2. Small-angle X-ray scattering (SAXS) pattern for biotin-modified DNA-functionalized gold nanoparticle (DNA-AuNP)$_{(13\ nm,\ n=1)}$ aggregates, which were additionally modified with a fluorescent molecule (Alexa Fluor 488). (a) DNA design for biotin-modified DNA-AuNP crystal labeled with Alexa Fluor 488. Illustrations are not drawn to scale. (b) SAXS pattern (blue curve) shown as a plot of the structure factor ($S(q)$) versus the scattering vector (q, in units of nm^{-1}). The black trace is the calculated scattering pattern for a perfect body-centered cubic (bcc) lattice. By analyzing the diffraction peak position ratios, their crystal structures were confirmed as bcc with a 53-nm lattice constant.

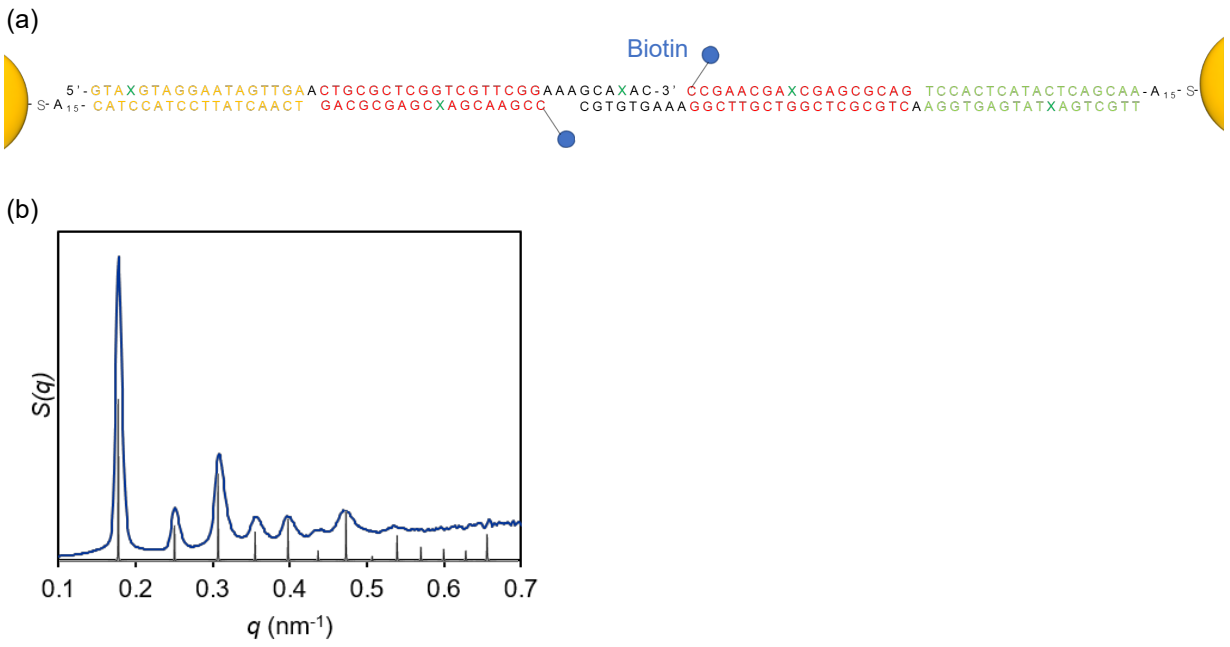


Figure S3. Biotin-modified DNA-functionalized gold nanoparticle (DNA-AuNP)$_{(13\ nm,\ n=1)}$ aggregates in which 3-cyanovinylcarbazole nucleoside (^{CNV}K) was incorporated into linker DNA. (a) DNA design for biotin- and ^{CNV}K-modified DNA-AuNP crystal, in which X represents ^{CNV}K. Illustrations are not drawn to scale. The ^{CNV}K-modified oligodeoxynucleotide was photo-crosslinked with an adjacent pyrimidine base via UV irradiation. (b) Small-angle X-ray scattering pattern (blue curve) for biotin-modified DNA-AuNP$_{(13\ nm,\ n=1)}$ aggregates with UV irradiation, which is shown as a plot of the structure factor ($S(q)$) versus the scattering vector (q, in units of nm^{-1}). The black trace is the calculated scattering pattern for a perfect body-centered cubic (bcc) lattice. By analyzing the diffraction peak position ratios, their crystal structures were confirmed as bcc with a 50-nm lattice constant.

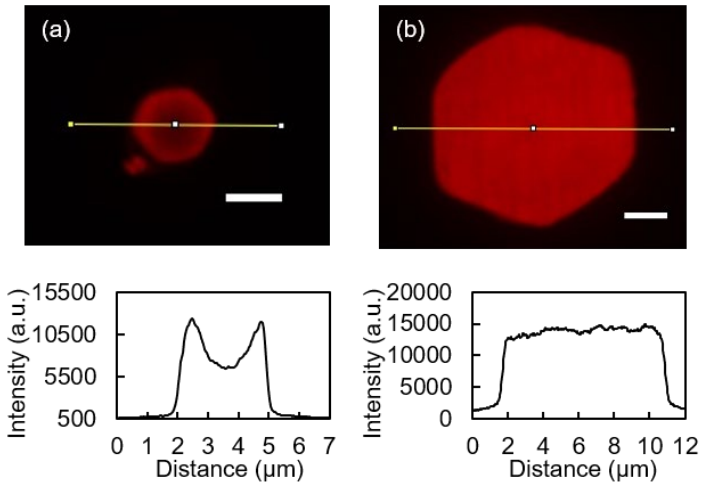


Figure S4. Confocal microscopic images and line profiles of the fluorescence intensity of streptavidin molecules labeled with Alexa Fluor 568, which were soaked with (a) DNA-functionalized gold nanoparticle $(DNA\text{-}AuNP)_{(13\ nm,\ n=0)}$ crystals (D_p =29 nm) and (b) $DNA\text{-}AuNP_{(13\ nm,\ n=3)}$ crystals (D_p =60 nm). Scale bar = 2 μm.

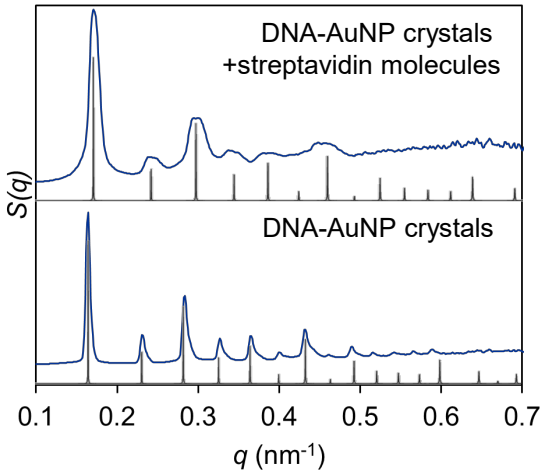


Figure S5. Comparison of small-angle X-ray scattering (SAXS) measurements of biotin-modified DNA-functionalized gold nanoparticle (DNA-AuNP)$_{(13\ nm,\ n=1)}$ crystals before and after streptavidin encapsulation. The SAXS patterns (blue curves) are shown as a plot of the structure factor ($S(q)$) versus the scattering vector (q, in units of nm^{-1}). The black traces are the calculated scattering patterns for a perfect body-centered cubic lattice.

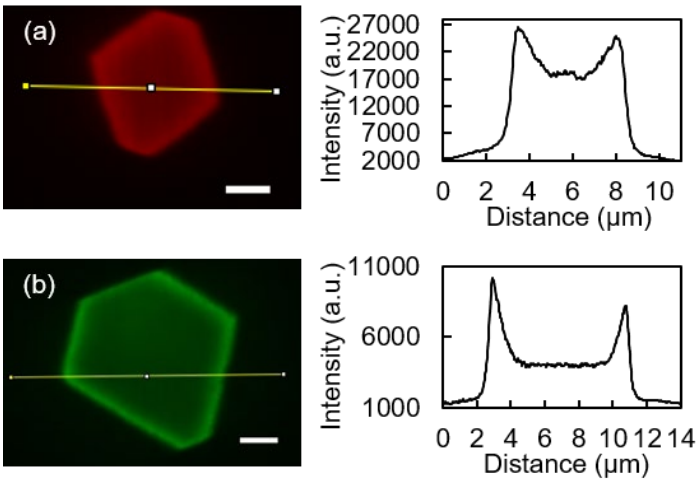
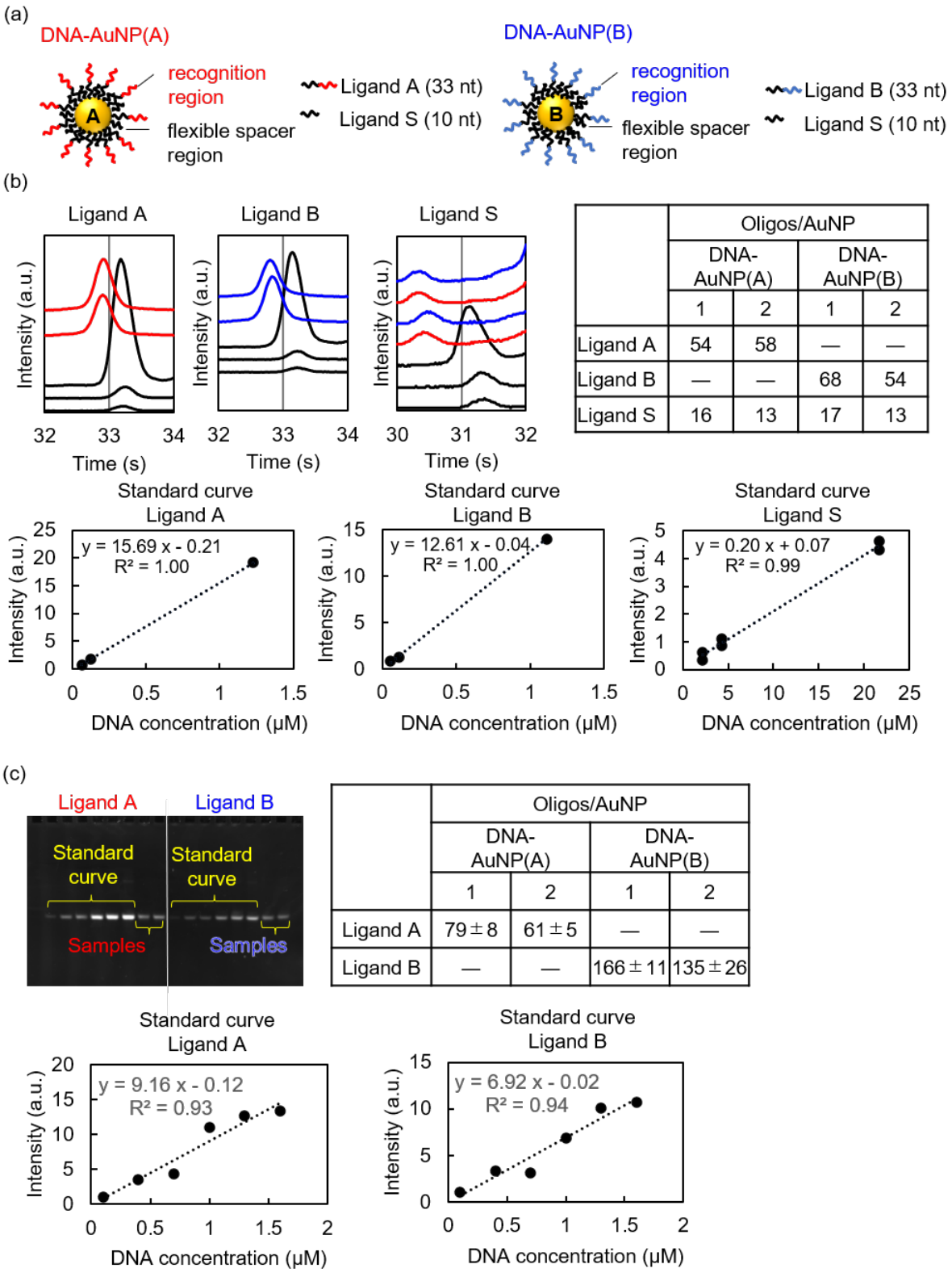


Figure S6. Confocal microscopic images and line profiles of the fluorescence intensity of streptavidin molecules labeled with (a) Alexa Fluor 568 and (b) Alexa Fluor 488, which were soaked with biotin- and ^{CNV}K-modified DNA-functionalized gold nanoparticle$_{(13\ nm,\ n=1)}$ crystals. Fluorescence intensity from the left to the right on each confocal image corresponds to the left to the right in the plot. Scale bar = 2 μm.



	Oligos/AuNP			
	DNA-AuNP(A)		DNA-AuNP(B)	
	1	2	1	2
Ligand A	54	58	—	—
Ligand B	—	—	68	54
Ligand S	16	13	17	13

	Oligos/AuNP			
	DNA-AuNP(A)		DNA-AuNP(B)	
	1	2	1	2
Ligand A	79 ± 8	61 ± 5	—	—
Ligand B	—	—	166 ± 11	135 ± 26

Figure S7. Quantification of DNA loading on gold nanoparticles (AuNPs). (a) Schematic illustration of AuNPs coated with two different lengths of ligand DNA, Ligand S (10 nt) and Ligand A or B (33 nt). Illustrations are not drawn to scale. (b) DNA amounts for AuNPs, which were coated with Ligand S (10 nt) and Ligand A or B (33 nt), were quantified using capillary electrophoresis. The assays were performed for two sets of DNA-functionalized AuNPs (DNA-AuNPs), which were prepared on different days (1 and 2). For each electropherogram, red lines represent DNA from DNA-AuNP(A), blue lines represent DNA from DNA-AuNP(B), and black lines represent DNA for standard curves. The DNA for standard curves were not modified with thiols, and the sample solutions did not contain dithiothreitol. These differences may affect the results of electrophoretic mobilities of DNA from DNA-AuNPs and DNA for standard curves. (c) DNA amounts for AuNPs, which were coated with Ligand A or B (33 nt), were quantified using polyacrylamide gel electrophoresis. The assays were performed for two sets of DNA-AuNPs, which were prepared on different days (1 and 2).

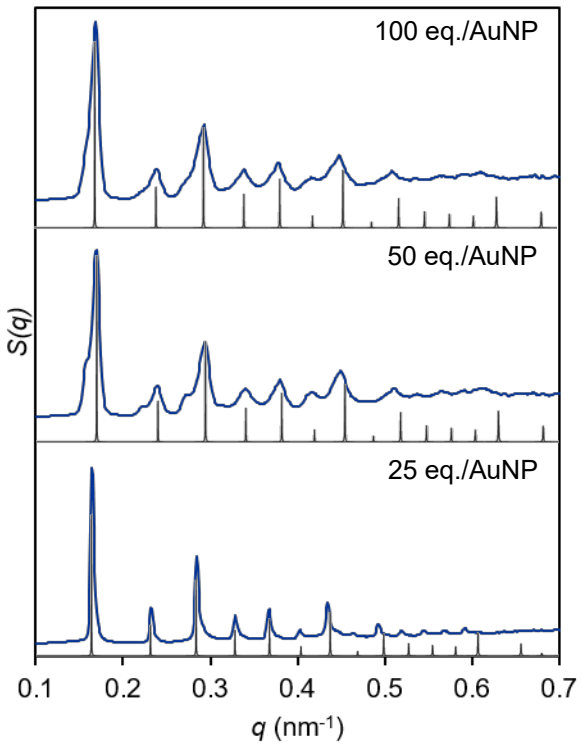


Figure S8. Small-angle X-ray scattering (SAXS) patterns for biotin-modified DNA-functionalized gold nanoparticle$_{(13\ nm,\ n=1)}$ aggregates with different amounts of linker strands (from 25 to 100 equivalents per particle). The SAXS patterns (blue curves) are shown as plots of the structure factor ($S(q)$) versus the scattering vector (q, in units of nm^{-1}). The black traces are the calculated scattering patterns for a perfect body-centered cubic lattice.

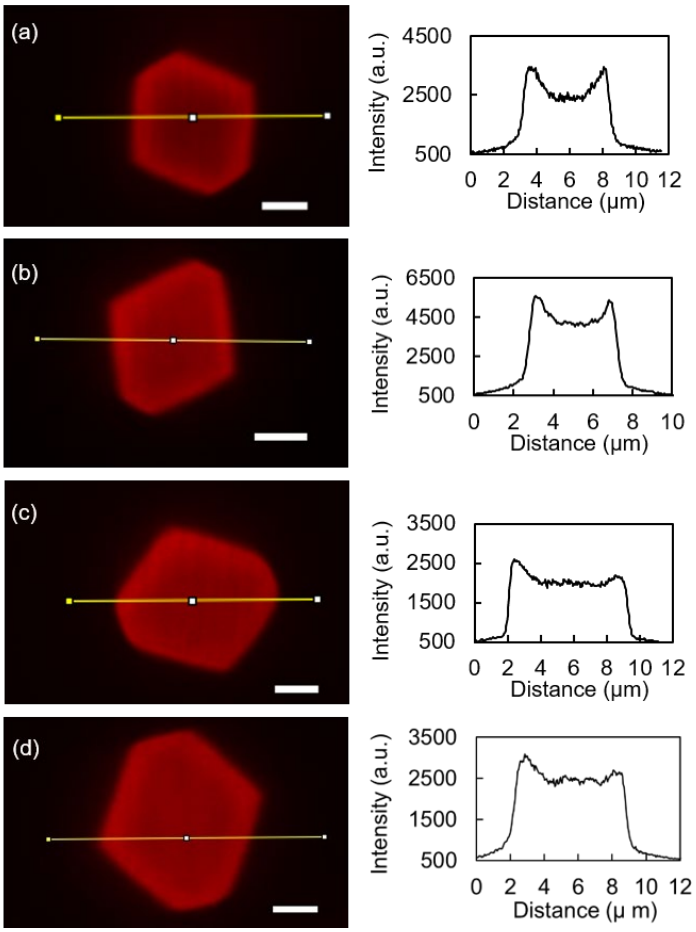


Figure S9. Confocal microscopic images and line profiles of the fluorescence intensity of streptavidin molecules labeled with Alexa Fluor 568, which were soaked with biotin-modified DNA-functionalized gold nanoparticle $(DNA\text{-}AuNP)_{(13\ nm,\ n=1)}$ crystals with different amounts of DNA. (a) Crystals that consist of AuNPs covered with 33-nt DNA and linker DNA (100 equivalents per AuNP). (b) Crystals that consist of AuNPs covered with two different lengths of ligand DNA (33-nt DNA and 10-nt DNA), and linker DNA (100 equivalents per AuNP). (c) Crystals that consist of AuNPs covered with 33-nt DNA and linker DNA (25 equivalents per AuNP). (d) Crystals that consist of AuNPs covered with two different lengths of ligand DNA (33-nt DNA and 10-nt DNA), and linker DNA (25 equivalents per AuNP). Fluorescence intensity from the left to the right on the confocal image corresponds to the left to the right in the plot. The micrographs were taken at a depth of 1 μm from the crystal surface. Scale bar = 2 μm.