Supporting information

Stepwise ligand-induced self-assembly for facile fabrication of nanodiamond-gold nanoparticle dimers via non-covalent biotin-streptavidin interactions

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EXPERIMENTAL SECTION

Materials. Thiolated and biotinylated DNA oligonucleotides with HPLC grade were purchased from Integrated DNA Technologies. Gold nanoparticles, sodium chloride, sodium dodecyl sulfate (SDS), tri(2-carboxyethyl)phosphine hydrochloride (TCEP) and sephadex G-25 medium were purchased form Sigma-Aldrich. Streptavidin magnetic beads was bought from Bangs Laboratories. Streptavidin nanodiamond was purchased from Adamas Nanotechnologies, InC. TEM grids were obtained for SPI supplies.

Instruments. Transmission electron microscopy (TEM) images were obtained using a JEOL JEM-2100 transmission electron microscope operating at an acceleration voltage of 120kV. Scanning electron microscopy (SEM) images were obtained using a Zeiss Supra-55 FESEM (Field Emission Scanning Electron Microscope). Fluorescence spectra were measured using a Princeton instruments SP2-300-i, with a Pixis 100F CCD camera. The particle size was analysis by Malvern Zetasize Nano ZS. Ultra violet-visible (UV-Vis) spectrophotometer was used for characterization of concentration of DNA and gold nanoparticles. XPS was used for the characterization of the bonding between DNA and gold nanoparticle.

Formation of gold nanoparticles AuNP-D\textsubscript{1b'2} with functionalized DNA strands:

The surface of AuNP was modified with single-stranded DNAs (ssDNAs) using Mirkin’s
Before coupling, thiol groups on DNA strands including \(D_{1b'}\) and \(D_2\) were activated by tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) and purified by desalting via sephadex G-25 column. Subsequently, excess amount of TCEP-activated \(D_{1b'}\) and \(D_2\) were added to 1 mL of citrate-protected AuNP solution and then further incubated for overnight at 4 °C. After incubation, phosphate buffer was added in a final concentration 10 mM (pH 7.4) and 0.01 % sodium dodecyl sulfate (SDS). The reaction solution was incubated for an additional 2 h before aging. In aging process, the sodium ion concentration of reaction was increased to 0.01 M with 0.01 % SDS by adding NaCl and SDS solutions (or 5 µL of 2 M NaCl was added to the aging solution and the concentration of SDS was kept at constant (0.01%)), followed by additional 30 min incubation. This process was repeated until the solution reaching up to 0.75 M of NaCl and then incubated with an additional 24 h. Finally, excess DNAs were removed by centrifugation. The successful formation of biotin-labeled DNA-encoded AuNPs was confirmed by further adding free streptavidin and then analyzed by 1 % agarose gel. The successful DNA-conjugated AuNPs (AuNP-\(D_{1b'2}\)) were stored in PBS-TAMg buffer for future use.

| DNA  | 5'-end                      | 5' → 3'                                      | 3'-end                      |
|------|-----------------------------|---------------------------------------------|-----------------------------|
| \(D_{1ab}\) | Biotin-TEG | TTT TTT TTT TTT TAA CCT AAC CTT CAT CTG TGT TC | -                           |
| \(D_{1b'}\) | Thiol Modifier C6 S-S | TTT TTT TTT ATC TAG TAG AG TAG GTG GC CGA AAC GAC AT GAG AGG ATT AGT AAG GTT AGG TTA | -                           |
| \(D_{1a'b'}\) | - | GA ACA CAG ATG AAG GTT AGG TTA | -                           |
| \(D_2\) | Thiol Modifier C6 S-S | TTT TTT TTT ATC TAG GAG AG TAG AGT GC CGA AAC GAC AT GAG AGG ATT ATC TAG GAG AGT AGA | Biotin-TEG                  |
| \(D_3\) | Biotin-TEG | AAG AGA TGT GAC AGA | -                           |

**Table S1** Sequences of DNA oligonucleotides used in this project.

| Buffers                    |                              |
|---------------------------|------------------------------|
| 1 x TAMg                  | 40 mM Tris, 7.6 mM MgCl2     |
| PBS-TAMg                  | 1 x PBS, 0.3 x TAMg, 0.3 M NaCl, and 0.01 % SDS |
| Tris-NaCl                 | 1 x Tris, 1M NaCl and 0.01 % SDS, |

**Table S2** The composition of binding buffer solutions.
DNA functionalization of streptavidin magnetic bead MB-D$_{1ab}$

The biotinylated DNA, D$_{1ab}$ was conjugated to MB-SA via streptavidin-biotin interaction. MB-SA was washed with 1 x Tris buffer with 0.01 % SDS for three times. Excess amount of D$_{1ab}$ was added to MB-SA in Tris-NaCl buffer and then shaken gently for 1 h. After magnetic separation in proximity to a neodymium magnet, the excess D$_{1ab}$ was then removed. The successful grafted D$_{1ab}$ was confirmed by adding the complementary strand with fluorescent dye, Cy3-labeled D$_{1a'b'}$, characterized by fluorescence analysis.

![Fluorescence analysis of successful formation of MB-D$_{1ab}$ by hybridizing with fully complementary Cy3-labeled D$_{1a'b'}$.](image)

**Figure S1** Fluorescence analysis of successful formation of MB-D$_{1ab}$ by hybridizing with fully complementary Cy3-labeled D$_{1a'b'}$.

**Fabrication of nanodiamond-gold nanoparticle dimers, ND-AuNP**

AuNP-D$_{1b'2}$ was added to the MB-D$_{1ab}$ in PBS-TAMg solution and shaken gently for overnight. After magnetic separation in proximity to a neodymium magnet, the excess AuNPs were discarded and the purified MB-AuNP complexes were washed with 1 x PBS with 0.01 % SDS for three times. Excess amount of ND-SA was added to the purified MB-AuNP complexes, shaken gently and incubated at 4 °C for an additional 48 h. Afterwards, the excess NDs were removed by magnetic separation. The resulting complexes were washed with 1 x PBS with 0.01 % SDS for three times. After washing, excess amount of biotin-functionalized DNA, D$_3$ was added to block the free streptavidin on the bound NDs. Finally, 1000 times excess amount of eraser DNA, D$_{1a'b'}$ was added to remove the ND-AuNP dimers from MB surface. The successful formed ND-AuNP dimers were then stored in water for further studies.
Figure S2 UV-vis analysis of the concentration of AuNP-D$_{1b_2}$ in supernatant before and after reacting with MB-D$_{1ab}$.

Figure S3. TEM images of MB-AuNP conjugates. Scale bar is 500 nm.
Figure S4 TEM images of (a) MB-D₁₁₀, (b) MB-AuNP, (c) MB-AuNP-ND and (d) adding ND-SA to MB-D₁₁₀.

Investigation of strand displacement strategy on D₁₁₀ strand
**Figure S5** 6 % native PAGE analysis confirming DNA hybridization between D$_{1ab}$ and D$_{1b'}$ (lane 1 to 3) and verifying the strand displacement approach to release the bound D$_{1b'}$ by an addition of eraser DNA strand D$_{1a'b'}$ (lane 4 to 6).

**Melting temperature experiments**
The corresponding DNA strands were assembled at room temperature and mixed with syber green. The fluorescence intensity of syber green as a function of temperature from 20 °C to 95 °C was monitored by qPCR studies.

**Figure S6.** The melting curve of a DNA pair of D$_{1ab}$ + D$_{1b'}$ and a DNA pair of D$_{1ab}$ + D$_{1a'b'}$. 
Figure S7 TEM imaging showing (a) a large nanocluster formed by mixing ND-SA with AuNP-D_{1b/2} without using the MB solid support and (b) only 30 nm AuNPs released from MB under the same synthetic strategy if AuNPs are fully coated with D_{1b'} only (without the biotin coating).
Figure S8 TEM images of 40 nm ND- 30 nm AuNP dimers.
The percentage yield of the particle synthesis was quantified by TEM imaging. Statistical analysis of the resulting nanoparticle conjugates found that ~ 50% of 40 nm ND-30 nm AuNP dimer are formed while less than 10% of trimer or other large size of particle clusters are obtained.

Figure S9. The synthetic percentage yield of different conjugates including 40 nm ND-30 nm AuNP dimer, trimers, 40 nm ND, 30 nm AuNP, and particle clusters (N = 114).

Fabrication of different combinations of particle dimers using same strategy:

Other dimer complexes were also formed under the same strategy either by replacing 30 nm AuNPs with 80 nm AuNPs or by replacing 40 nm NDs with 200 nm ND or two different sizes of AuNPs (e.g. 30 nm and 80 nm) but with various NaCl and TAMg content. This is due to the large repulsion between the large nanoparticles. From the TEM imaging, the dimer formed from two similar sizes nanoparticles with a regular shape having a higher percentage yield.
Figure S10 TEM images of different combinations of dimers: (a) 30 nm AuNP-30 nm AuNP, (b) 80 nm AuNP-80 nm AuNP, (c) 30 nm AuNP-80 nm AuNP, (d) 40 nm ND-80 nm AuNP, and (e) 200 nm ND-80 nm AuNP.

TEM imaging of dimer-treated HeLa cell slice after the high-pressure freezing procedure

1 x 10⁵ HeLa cells were seeded and cultured on the cover slip for overnight, and then treated with dimer for additional 24 h. After the incubation, cell sample was rinsed with PBS for a few times, and then immersed in primary fixation solution with 2.5 % glutaraldehyde and 2 % paraformaldehyde in 0.1 M cacodylate buffer at pH 7.2 at room temperature for 2 h. Secondary fixation was performed in dark for 2 h at room temperature with the solution of 1 % OSO₄ and 1.5 % potassium ferrocyanide in 0.1 M cacodylate buffer. After each fixation, the samples were rinsed with 0.1 M cacodylate buffer three times for 5 min, then dehydrated with ethanol from 30 % to 100 % and further with 100 % acetone. The dehydrated cell samples were infiltrated with acetone and Spurr’s resin from ratio 3:1 to 0:1. The infiltrated samples were then embedded in plastic mold and baked in oven at 70 °C for 2 days. Before TEM imaging, the baked sample were undergone ultrathin sectioning and collected on the TEM grid. The collected samples were stained with 5 % uranyl acetate solution for imaging.
For the SEM samples, a drop of ND-AuNP dimers dissolved in water was placed on a micro cover glass. After drying, the sample was coated with a 5 nm thick layer of platinum to avoid disturbances in the imaging arising from charging effects.

**MTT assay**

20,000 of HeLa cells were seeded on each well of 96-well plate and then incubated with different concentration of dimer at 37 °C for overnight. After overnight incubation, the media was removed. 100uL of fresh media with 0.8 mg MTT reagent was added to the cell samples and incubated for an additional 4 h. After incubation, media was removed and 100 µL of DMSO–ethanol (1:1) was added to each well and mixed thoroughly following by UV-Vis measurement at 570 nm absorbance using micro reader.
Reference

Sarah J. Hurst, Abigail K. R. Lytton-Jean, and C. A. M.; Hurst, S. J.; Lytton-Jean, A. K. R.; Mirkin, C. A. Anal Chem. 2008, 78 (24), 8313.