Supporting Online Information

Gold Nanoparticles Self-similar Chain Structure Organized by DNA Origami

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Materials and Methods

Materials:
All unmodified helper strands were purchased from Integrated DNA Technologies, Inc. (www.idtdna.com) in a 96-well plate format, resuspended in ultrapure water and used without further purification. All 3’ thiol-modified DNA strands were also purchased from IDTDNA and purified using denaturing PAGE gel electrophoresis. Tris(carboxyethyl) phosphine hydrochloride (TCEP) was purchased from Sigma-Aldrich, USA. Bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP) was purchased from Strem Chemicals Inc.. Colloidal solution of 5nm, 10nm and 15nm AuNPs was purchased from Ted Pella Inc.

Experimental Methods:

Phosphination and concentration of AuNPs. AuNPs (5, 10 and 15 nm, Ted Pella Inc.) were stabilized with adsorption of BSPP. BSPP (15 mg) was added to the colloidal nanoparticles solution (50 mL,) and the mixture was shaken overnight at room temperature. Sodium Chloride (solid) was added slowly to this mixture while stirring until the color changed from deep burgundy to light purple. The resulting mixture was centrifuged at 3000 rpm for 30 min and the supernatant was carefully removed with a pipette. AuNPs were then resuspended in 1mL solution of BSPP (2.5mM). Upon mixing with 1mL methanol, the mixture was centrifuged, the supernatant was removed and the AuNPs were resuspended in 1 mL BSPP solution (2.5 mM). The concentration of the AuNPs was estimated from the optical absorbance at ~ 520 nm. Phosphine coating increases the negative charge on the particle surface and therefore stabilizes the AuNPs in high electrolyte concentrations at a higher particle density.

Preparation of AuNP-DNA conjugates. The disulfide bond in the thiol modified oligonucleotides was reduced to monothiol using TCEP (20mM, 1h) in water. The oligonucleotides were purified using size exclusion columns (G-25, GE Healthcare) to
get rid of the small molecules. Monothiol modified oligonucleotides and phosphinated AuNPs were then incubated with DNA to Au molar ratio more than 200:1 in 0.5 × TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0) containing 50 mM NaCl for 40 hours at room temperature to make sure the AuNPs were fully covered by thiolated DNA. AuNP-DNA conjugates were washed with 0.5 × TBE buffer in (Millipore, Billerica, MA) to get rid of the extra oligonucleotides. The concentration of these AuNP-DNA conjugates was estimated from the optical absorbance at ~ 520 nm. Freshly prepared, fully covered AuNPs did not precipitate in 1× TAE-Mg2+ buffer (Tris, 40 mM; acetic acid, 20 mM; EDTA, 2 mM; and magnesium acetate, 12.5 mM; pH 8.0) which is preferred for the formation of DNA origami. This high salt resistance property of fully covered AuNPs makes it possible to assemble them on the DNA origami template.

**Self-assembly of DNA origami template.** Triangular shaped origami template was formed according to Rothemund (Rothemund, P. W. R. Nature 2006, 440, 297-302). A molar ratio of 1:5 between the long viral ssDNA and the short unmodified helper strands (unpurified) was used. The modified helper strands that will hybridize with the thiolated DNA strands on AuNP-DNA conjugate were used in 1:1 ratios to that of the viral DNA (5nM). DNA origami was assembled in 1× TAE-Mg2+ buffer (Tris, 40 mM; Acetic acid, 20 mM; EDTA, 2 mM; and Magnesium acetate, 12.5 mM; pH 8.0) by cooling slowly from 90 °C to room temperature. DNA origami was then filtered with 100 kDa MWCO centrifuge filters to remove extra DNA helper strands. Purified DNA origami was mixed with different size AuNP-DNA conjugates with 1:1 ratio and annealed from 37 °C to room temperature.

**Purification of origami-AuNPs complex.** Annealing product of DNA origami and AuNPs mixture was loaded to 1% EtBr stained agarose gel (running buffer 0.5 × TBE, loading buffer 50% glycerol, 15 V/cm). Selected bands were cut out and the DNA Origami-AuNPs complexes were extracted from the gel with Freeze-Squeeze column (Bio-Rad) at 4 °C.

**SEM and TEM characterization of origami-AuNPs complex.** A silicon wafer with about 150nm thick oxide layer was treated with oxygen plasma to make the surface hydrophilic. The sample of origami and AuNP complex (4 µL) was left to adsorb on wafer surface for 5 minutes then washed with water. The sample was scanned by Zeiss XB 1540 Focused Ion Beam/SEM system or Zeiss Ultra 55 Field Emission SEM/STEM system with EHT 2.00 kV to 5.00 kV. High-resolution transmission electron microscopy (HRTEM), and energy-dispersive X-ray spectroscopy (EDS) were performed on a JEOL JEM 2010F electron microscope operating at 200 kV.
**Supporting figures and legends**

**Figure S1.** Additional SEM images of one DNA origami carrying 6 well-aligned AuNPs. Images were taken on samples extracted from band e in Fig. 1b. The triangle shape of DNA template is visible as a darker color.
Figure S2. Sample SEM image of two DNA origami linked by multiple AuNPs. Images were taken on samples extracted from band f in Fig. 1b. The triangle shape of DNA template is still visible. The top complex contains two DNA origami with one 5 nm AuNP missed. The bottom complex contains two DNA origami and well aligned six Au particle chain.

Figure S3. Sample SEM image of an agglomerate of multiple DNA origami and multiple AuNPs. Images were taken on samples extracted from band g in Fig. 1b.
**Figure S4.** Sample SEM image of the DNA origami template without AuNPs. The triangle shape can be clearly seen.

**Figure S5.** Agarose gel electrophoresis of different size AuNPs and their corresponding conjugates with thiolated DNA strands.
Figure S6. Schematic drawing of triangle DNA origami showing internal features with staple strands marked with numbers. The viral ssDNA is colored in red and the staple strands are in blue and each individually numbered. The whole complex consists of three major domains which are labeled as A, B and C. Thick colored strand extensions show the sites of sticky ends. Three same color sticky ends will localize one AuNP. Superimposed orange circles show the position of organized AuNPs.
**Figure S7.** SEM images of control experiments with only two DNA linkages for each AuNP. There are missing particles and inaccurate positioning and mis-alignment of AuNPs on the DNA origami template indicating that three linkages are necessary and important for precise assembly.
**Figure S8.** TEM images and EDS analysis of AuNPs chain structure. The EDS data shows that the nanostructure contains the elements of Au, N, O, P. It is consistent with our AuNPs/DNA origami nanostructures.
Sequences used in the assembly of DNA origami template

Sequence of single stranded circular M13mp18 viral DNA (purchased from New EnglandBiolabs) can be found at:
http://www.neb.com/nebecomm/tech_reference/restriction_enzyme/sequences/m13mp18.txt

Sequences of staple strands containing sticky ends.
There are totally 18 staple strands are modified with sticky ends to organize six AuNPs. Sequence of the staple strands (left to right: 5’-3’):

B04-SE, AGA CTC TAA TGC AGT CAC CAA CGC TTTT
   TTCGAGCTAAGAGCTTCAATATCGGGAACGAG
B07-SE, AGA CTC TAA TGC AGT CAC CAA CGC TTTT
   AAGCCCGATCAAAGCG ACCAGACGTTTACGTATATTTTTCTTCTACTA
B08-SE, AGA CTC TAA TGC AGT CAC CAA CGC TTTT
   GAATACCACATTTCAACTTAAAGAGG
B16-SE, AATAAATAAAT AATAAATAAAT TTTT
   GCCCAAAAGGAATTACAGTCGAAGCAAGCGAGTCAG
B20-SE, AATAAATAAAT AATAAATAAAT TTTT
   TAATGCTTTTACCCTGACTATTAGGCGATAGTAAGACG
B23-SE, AATAAATAAAT AATAAATAAAT TTTT
   AACACTACTATAACCCATCAAAAAATCAGTCTCCTTTTTGA
B28-SE, AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA TTTT
   GATAAAAACAAATATTTAAAAAGTTACAGTTACAAATTTAGAGCT
B30-SE, AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA TTTT
   TGCTGTAGATCCCCCTCAAAAAATCAGTCTCCTTTTTGCA
B31-SE, AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA TTTT
   AAAGAAGTTTTTGCCAGCATAATAATTTCAATGACTCAACATTT
B36-SE, CGCATCAGGAT TCTCAACTCGTA TTTT
   GGCAGCTCCATGTTACTTAGGCGGTCTTAA
B37-SE, CGCATCAGGAT TCTCAACTCGTA TTTT
   ACAGGTAGAAAAATCTCAGTCTGAGATTTAG
B41-SE, CGCATCAGGATTCTCAACTGTA TTTT
   CGACCTGCCTCAATCTCAAGGGAACGGAACACATTCT
B45-SE, AATAAATAAAT AATAAATAAAT TTTT
   TTAATATACCGAATAACGGAACTTGACCAAACCTCTCTTTAAC
B49-SE, AATAAATAAAT AATAAATAAAT TTTT
   TATCATCGTTGAAAGGACAGATGGAAGAAAAATCTACG
B53-SE, AATAAATAAAT AATAAATAAAT TTTT
   ACCAGTCAGGACGTGGAACGGTGACAGACGAAACAAA
B59-SE, AAAAAAAAAA AAAAAAAAAAAAAA TTTT
   ACCTTATGCGATTATATGCCTTCATCAAGAGCATCTTTT
Sequences of thiolated strands that covered the AuNPs. These thiolated strands are all modified with monothiol on the 3’.
For 5 nm AuNP: S5a: TACGAG TTGAGA ATCCTG AATGCG TTT
S5b: GCG TTG GTG ACT GCA TTA GAG TCT TTT
For 10 nm AuNP: S10: ATTATT ATTATT ATTATT ATTATT TTT
For 15 nm AuNP: S15: TTTTTT TTTTTT TTTTTT TTTTTT TTTT

Sequences of unmodified staple strands: Sequences of all the staple strands used in the experiments are listed below in a continuous fashion. Note that for preparing the origami template to organize AuNPs, particular unmodified staple strands were replaced by modified DNA strands containing sticky ends and annealed along with remaining staple strands.

| A01 | CGGGGTTCCTCAAGAGAAGGATTTTGATTA, |
| A02 | AGGCGTATGTCATCCTGAAATTTACCAGACTACCTT, |
| A03 | TTCATAATCCCCCTATTACGCTTTTTCTTACC, |
| A04 | ATGGTTTATGTCACAATCAATAAGATATTAAAC, |
| A05 | TTTGTAGTAAGAGGCGGTAGACTTGCTACGTACCCAGGC, |
| A06 | CCGGAACCACAGAATGGAAAGGCGAACACATGGCT, |
| A07 | AAAGACAAACATTTTTCGTCATAAGCCCAAAATCA, |
| A08 | GACGGGAGAATTAACTCGGAATAAGTTTTATTTCCACGGCC, |
| A09 | GATAAGTGCAGTCAGCTGGAACCATGAAAGATACGAGGAGGAG, |
| A10 | TTGACTGGAATCTCATTAAAAGACAGGCCAC, |
| A11 | CACCGGAAAAGGGCGTTTTTCACGGAAGGGCGA, |
| A12 | CATTTACAAACAGCGAAAGGCACACACCACCTGAAACC, |
| A13 | TTTAAACGGGTCGGAACCTATTATGAGGTGGATATATAGTA, |
| A14 | TCAGAGCATATTCACAAACAAATTAATAAATAGT, |
| A15 | GAGAGGAATTACAGCGTCACGACTGTCGGCCTCC, |
| A16 | GTCAGAGGGGTAATTGATGGCCAACATATAAAAAGGGATTGAG, |
| A17 | TAGCCCGGAAATAGGTTGATGCCCCCTGCTATTGTCAGTG, |
| A18 | CCTTGAGTCAGACGATTGCGCTTTCGCGACC, |
| A19 | TCAGACCCAGAAATCAGGTTGCGATTTGCGGCCGAAATA, |
| A20 | TTGACGGGAATACATACATAAAAAGGCGCTATATACAGA, |
| A21 | CAGGCGGAGAGTGGCCACGTAACAGGTGCCCC, |
| A22 | ATAAAGGGCCGAATCAGGTAGGAGCGACCCACCTC, |
| A23 | GATAAAACCAAGAAATGTTAGCAACAGTGAATAATTC, |
A24, GCCGCCAGCATGTGACACCCACCCCTC,  
A25, AGAGCGGCGCCTATCGAGGAGAGTGAATTAT,  
A26, CACCGTGACCTCAGCTGACGATATGAGTGAATGCCCCTATA,  
A27, AGACCCATTTAACGCTGAATGAGGACAACCCAGACAAG,  
A28, AAGAGAAGGAAGATAGCAGATGAGACTCCTGACTT,  
A29, CCATTAGCAAGGCCGCGGGGGAAT,  
A30, GAGGCCAGCAGAATACCCAAAAGAATCCCTAGTAGTAATGC,  
A31, TATCTCCAGAGCCCAAACGCAATATAAAGCAGAAATCCACCAG,  
A32, CAGAAGGAAACCGAGGTTTTTAAAAGAATAGCAGGAGATT,  
A33, CTTTTTTTCATTTTACAAATTTTCTAGGATAG,  
A34, TTATTACCATGATGGTGCAAGTCTCCAGTA,  
A35, AGTATAAATATGTACGTTATACAAAGGCATCTT,  
A36, CAAGTGACCTCAGCGACTGAAATTTTCTAGTAGATAAT,  
A37, AGAGGAAAGAAGATAGCATAGCAAGAGCTAGTGTGAGC,  
A38, AAAACAAAATCAAATTTTGGAAAAAGCTATAACCTGCTAGTAAT,  
A39, TTATCAAAACGGCCTAGTTGTTGGAATGATGCTGGT,  
A40, TTAGTATGCAACGGCTCAACAGTACGCTGGCTGC,  
A41, TTTCTTACAGACTCATCGAGAGAATTTTCTAGTACAG,  
A42, AGAGGAAAATCAAAATACATGCTGAGAATAGGAA,  
A43, ACTAGAAATATATATAATATGTACCGCTAGA,  
A44, TCAATAATAGGGCTTAATGGAATCTAAATT,  
A45, AACGTCAAAATGAAAAGCAAGCGGCTTTTATGCAAACCAA,  
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A56, ACAAGAAGGAAAGAAATCAAGAAAAAGCAGCTATTATTTA,  
A57, GTTTTGAAAATCTAATAATATTTTAT,  
A58, AATAGATAGAGCCGAGTAATAAGAGATTAATTG,  
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A65, TGCTATTTTGCACCCAGCTACAAATTTGTTTGAAGCTTAAAA,  
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B02, GTGAGAAAAATGTGTAGGTAAGATACAACTTT,
B03, GGCATCAAATATGGGGCGCGAGCAGTATTAAAG,
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B06, ATAGTATAGTCAATGCCTGAGTGGCCGGAG,
B07, AACAGAGCAGTTCATATTATTTTCTTCTAAT,
B08, GAATTACCAATCAATTTAAGAGGAAGCCCCGATCAAAGCG,
B09, AGAAAAAGCCCCAAAAAGTCTGGAGCACAACAAATCACCAT,
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