공학석사 학위논문

Multivalent DNA-Traptavidin Bioconjugates for Programmable Nano-Assembly

프로그램 가능한 나노조립체 기반이 되는

다 기능 DNA-Traptavidin 복합체

2013 년 8 월

서울대학교 대학원

융합과학부 나노융합전공

김 영 엽

Multivalent DNA-Traptavidin Bioconjugates for Programmable Nano-Assembly

지도교수 송 윤 규

이 논문을 공학석사 학위논문으로 제출함

2013 년 8 월

서울대학교 대학원

융합과학부 나노융합전공

김 영 엽

김영엽의 석사 학위논문을 인준함

2013 년 8 월

위 원 장 김 연 상 (인)

부위원장 송 윤 규 (인)

위 원 박 원 철 (인)

Abstract

Multivalent DNA-Traptavidin Bioconjugates for Programmable Nano-Assembly

Youngyoub Kim

Program in Nano science and Technology

The Graduate School

Seoul National University

DNA and Protein are programmable matter for fabricating complicated nanostructures. Despite of their great potential as building-block, they have some disadvantages from their own properties. Therefore, well-designed DNA-protein hybrid material

can be a synergistic tool by complementing their limitations.

We choose a tetrameric protein, Traptavidin(TV), protein and four different biotinylated DNAs to conjugate identical tetrahedral binding site of TV. By conducting four magnetic separation steps through Watson-Crick base-paring interaction, we fabricated multivalent protein-DNA complexes. It is novel subunit having four different probes on one protein body which enables selective and simultaneous functionalities. From this complex, we reported a DNA-directed approach for accurate spatial positioning to make metallic plasmonic structures with high yield. We easily demonstrated asymmetrical symmetrical plasmonic nanostructures assisted by addressability specified binding sites in a TV-DNA complex. In order to show the possibility of the complex as templates for highly-ordered metallic plasmonic structures, we also demonstrated extended assembly by connecting one complex to another with well-designed DNA sequences. Thus, we proposed a conceptually simple method for

predictable and complicated nanostructures. We expect that this multivalent TV-DNA complex will offer excellent platform as a buildingblock in nano-scale with addressable interaction of four different DNAs and three dimensionally symmetriccal structure of TV. As such, this novel class of buildingblocks can not only provide multifunctional,programmable and simultaneous but also platform to make 2D nanoarray and 3D nanoarchitecture.

Keyword : DNA-Protein hybrid material, Buildingblock, Traptavidin, Magnetic separation, Plasmonic structure, Nanoarchitecture

Student Number : 2011-22753

Contents

List of table, schemes and figures

1. Introduction

1.1 Building-Block of DNA and Protein

DNA and Protein are programmable matter for fabricating highly-ordered nanostructure[1-16]and bio-detection platform [17-22] with nanometer-scale precision. Despite of their great potential as building-block, they have some disadvantages from their own properties. Therefore, well-designed DNA-protein hybrid material can be a synergistic tool by complementing their limitations. First, DNA as a linker can offer great potentials with nanoparticles and biomolecules, because it can be easily synthesized with desired length and functionality, and also connects one subunit to another in a controllable and predictable manner due to the sequence specific recognition mechanism known as Watson-Crick base pairing. Although the controllable and predictable characteristics of DNA are extremely useful to

fabricate nanostructure, DNA has several limitations. Compared to chemically and mechanically more rigid materials, high-ordered DNA nanostructures cannot resist dramatic pH change or high-temperature conditions because of its denaturation and unzipping properties of DNA double helix. Also, DNA has a degree of negative charge from phosphate group, which can lead to unwanted electrostatic interactions with other positively charged molecules.

On the other hand, protein molecules are much larger, more rigid and more three-dimensional than other molecules in nature, and their symmetrical quaternary structures and biological recognition properties enable a powerful tool as building block [13, 23-26]. Furthermore, if protein core have binding sites with multiple different DNA sequences, those DNA linkers enable various structural designs at nanometer-scale by controlling length and sequence of DNAs. Therefore, DNA-protein hybrid materials as

a building-block can provide a novel method to make an application in nanotechnology. So, we can simultaneously and selectively multiplex various functions such as localized field enhancement with plasmonic nanoparticles, site-specific fluorescence labeling with nanoparticles or organic dyes, and also fabricate symmetric/asymmetric well-ordered nanostructures in multivalent DNA-complex networks.

1.2 Avidin – Biotin System

Strept(avidin) is a 52800 dalton and tetrameric protein purified from the bacterium Streptomyces. (Strept)avidin has four binding sites for biotins (also known as vitamin B7) with tetrahedral symmetry and unusual high affinity($Ka \sim 10^{14}\ M^{-1}$), which is one of the strongest non-covalent interaction known in nature. Also, biotins can be synthesized with biomolecules by chemical modification. These avidin and biotin system are powerful tool from simple purification steps and labeling methods to complex pre-targeting drug delivery and nano-buildingblock for high-ordered nanostructures. These properties provide (strept)avidins to an outstanding tool for the biomedicine, bioscience and other fields of nano-technology. In the (strept)avidins and biotin bind, 9 hydrogen-bonds network (Asn23, Tyr43, Ser27, Ser45, Asn49, Ser88, Thr90 and Asp128) and 25 Vander Waals interaction have

been influenced with a strong affinity inside of a binding site. Then, under physiological conditions at 37° C and pH 7.4, the system suggests that the (strept)avidins and biotin interaction can be considered to be stable and irreversible. However, the association of the two parts could be a limitation under harsh conditions such as high temperature, low pH, or shear stress in the presence of attached nanoparticles.

To spread out the range of the applications and to enhance chemical and mechanical properties, avidin has been genetically and chemically engineered. To overcome these limitations, Dr Howarth's group in Oxford University engineered a streptavidin(SA) mutant, TV, which has 10-fold decreased dissociation rate and a dramatic increase in thermal and mechanical stabilities compared to the wild-type SA counterparts. [27]

A different structure between TV and SA was observed in the loop connecting beta strands 3 and 4 (L3/4). L3/4 is disordered conformation in SA without biotin binding. However, all L3/4 of the tetramer was already in the 'closed' conformation in the case of TV. Also, SA does not indicate cooperatively manner in biotin association between the four subunits. However, there is a conformational change, concerning the more tight wrapping of the beta barrel structures, changed dimer–dimer packing and loop arranging. Thus, thermal stability of TV and the biotin–bound form of TV increase with conformational change of its structure.

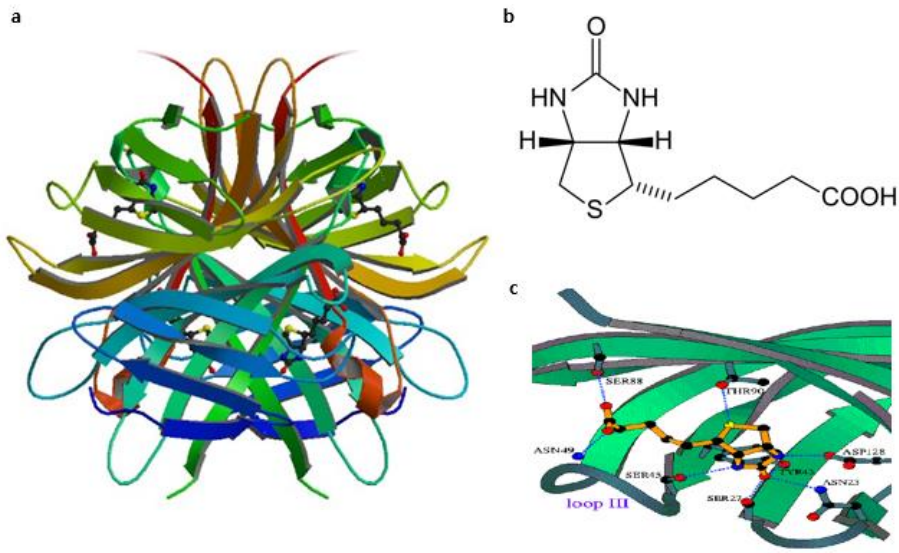


Figure 1. (Strept)avidin Structure and binding with biotin molecule. a. Tetrameric Structure of (Strept)avdin b, Biotin molecule (Vitamin 7) c, Hydrogen network with biotin molecule of individual subunit

1.3 Plasmonic structure

Plasmons are a quantum of rapid oscillation of electron cloud density, which arise from a nano sized metal in response to external electromagnetic wave. Especially, the interest of metallic nanoparticle in the optical properties has been increasing because of their unique surface plasmon resonances of the collective oscillation of electrons. Plasmons of metallic nanoparticle are quantized to the oscillations of electron clouds by a finite geometry, material type and the dielectric environment. Therefore, this optical property have been growing fields of research such as optical and electronic devices, nanophotonic lasers, and biochemical sensing etc.[28-30]

To develop these applications, it will be needed to discrete plasmonic structure with well-defined number and spatial addressability. Then, many scientists have been suggested many

approaches developed from Chad. Mirkin and Alivisatos. They reported the new strategy of DNA modified gold nanoparticles in 1996. With advantages of DNA such as Watson-Crick base pairing and easy controllability, various plasmonic nanostructures have been reported and studied with accurate positioning of plasmonic nanoparticles into complicated two-dimensional and three-dimensional nanostructures. However, easy controllable and reproducible methods need to provide desired functionalities on metallic nanoparticle due to limitation of stoichiometric control of DNA for plasmonic application. Then, utilizing DNA origami strategies and other template molecules, advanced approaches have allowed us to fabricate desired shape and function of plasmonic nanostructure. Compare with non-template method to fabricate plasmonic structure, template method offer the facilities such as reliable platform and accurate biding site. Therefore, new approach which incorporates easy controllable DNA and template

molecule with metal nanoparticles should be proposed for the fields such as biosensing, bioimaging and surface-enhanced Raman scattering (SERS) applications etc.[31-33]

2. Experimental Process

2.1 Materials, Fabrication and Characterization

Gold nanoparticles(AuNP) were purchased from Sigma-Aldrich (St. Louis, MO, USA) HPLC-purified thiolated and biotin modified oligonucleotides were purchased from IDT Inc. (Coralville, IA, USA) and reduced by using dithiothreitol (Sigma-Aldrich) The reduced oligonucleotides were then purified through a desalting NAP-5 column (Sephadex G-25 medium, DNA grade) NANOpure H2O (>18.0 MΩ), purified using a Milli-Q water purification system. Streptavidin coated CdSe/ZnS quantum dots were purchased from Invitrogen (Qdots Invitrogen) The carbon coated copper grid (Ted Pella, Inc. Redding, CA, USA) and HRTEM (JEM-2100F, Japan, 200 keV)

ssDNA for Functionalization of Magnetic Bead

A' 5′-NH2-A10-SP18-TACAGTCACATTCCGAGTACT -3′

B' 5′-NH2-A10-SP18-GTCTGTTAGTAATCAGAAGTA- 3′

C' 5′-NH2-A10-SP18-CTTACCTCCTCCAACACTATG- 3′

D' 5′-NH2-A10-SP18-CATATTCAACTATTCCTATGG -3'

ssDNA for TV-DNA conjugates

A 5′-A10-AGTACTCGGAATG -3′

B 5′ -A10-TACTTCTGATTAC -3′

C 5′ -A10-CATAGTGTTGGAG -3′

D 5′ -A10-CCATAGGAATAGT -3′

ssDNA for functionalization of AuNP

A' 5′-SH-PEG18-CATTCCGAGTACT -3′

B' 5′-SH-PEG18-GTAATCAGAAGTA- 3′

C' 5′′-SH-PEG18-CTCCAACACTATG- 3′

D' 5′-SH-PEG18-ACTATTCCTATGG-3'

Exchange DNA

A" 5′-AGTACTCGGAATGTGACTGTA-3′

B" 5′-TACTTCTGATTACTAACAGAC-3′

Table 1. The ssDNA used in this study.

2.2 Preparation of Traptavidin protein

In order to produce and purify TV protein, we purchased the plasmid encoded TV sequence (Addgene plasmid 24753, http://www.addg ene.org /24753/) at Addgene Corp, which is a non-profit plasmid sharing organization. Addgene offers its plasmids (gene of interest) as transformed bacteria in stab culture format in Luria Broth (LB) agar.

In our case, the plasmid is encoded with TV protein DNA sequence and Ampicillin resistance gene sequence. In order to select single colony from LB Agar Stab format, we streaked the bacteria with a sterilized micro-pipette tip onto LB Agar plate containing100ul/ml ampicillin. The streaked LB Agar plate was incubated for 16 hours at 37°C. When single bacterial colony was observed on the plate, the single colony was picked with a sterilized micro-pipette tip. Then, we put this tip into 15ml conical tube, which contained LB media and 100ul/ml ampicillin. It was

incubated for 12 hours at shacking condition of 225rpm and 37℃.

The conical tube cap was slightly open for air to come into the tube for bacterial growth. After the incubation, TV plasmid was purified from the bacteria by using DNA-spinTM plasmid DNA purification Kit (iNtRON biotechnology)..

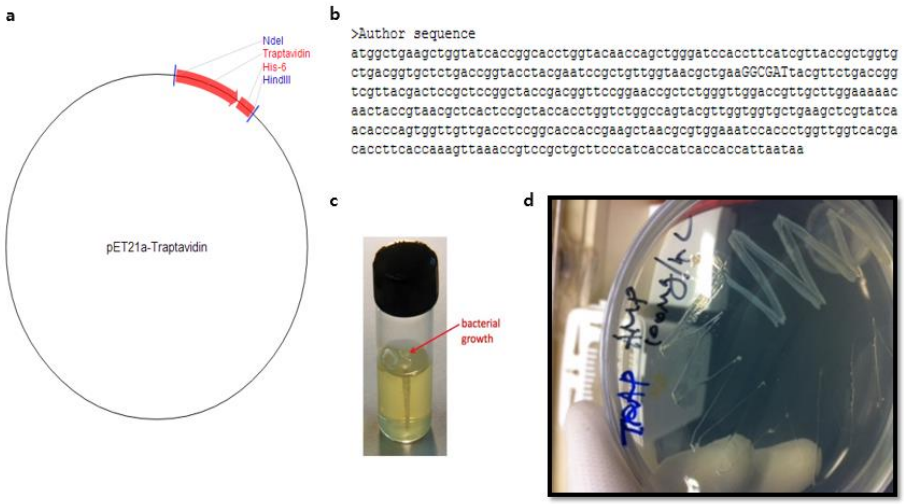


Figure 2. Preparation for Traptavidin Production and Purification

a, Traptavidin Plasmid Map b, Traptavidin DNA Sequence c, Traptavidin Transformed Bacteria in LB Agar Stab d. Single Colony on Streaked Plate

2.3 Synthesis of DNA modified Gold Nanoparticle

Oligonucleotide modified AuNP were synthesized from Chad.Mirkin group's procedures[34]. AuNP 1ml(OD1) stabilized suspension in citrate buffer was washed by centrifugation for 60min at 4℃ and 16,000rpm. Discard supernatants and resuspend in 1ml of 10mM phosphate buffer at pH7.0. Lyophilized oligonucleotides were resdispersed in a cleave solution(0.1 M dithiothreitol in 0.17 M phosphate buffer at pH8) to cleave the disulfide bond of oligonucleotides. After incubating 2hours, the oligonucleotides were purified by a NAP-5 column (GE Healthcare). The cleaved oligonucleotides (4 nmol) in Nanopure water (500ul) were added to AuNP solutions(1ml). The solution was incubated with gentle mixing for 16h before salting step. The oligonucleotides and AuNPs solution was mixed to with a final concentration of 10 mM PB at pH 7.0 and 0.01% SDS. The solution

was allowed to equilibrate for 30 min to reach a final NaCl concentration to 0.3 M over in 6 step. The solution was sonicated for 10s during the salting step. After salting steps, the particles were incubated with gently mixing for 40h at RT. Finally, to remove unreacted oligonucleotides from the oligonucleotides and AuNPs, the solution was washed by centrifugation for a total of four times and discard supernatant removal and resuspend PBS buffer solution.

2.4 Synthesis of DNA Modified Gold Magnetic Beads

Oligonucleotides modified magnetic beads (MBs, Invitrogen Dynabeads M-270 Amine) were synthesized from Chad.Mirkin group's procedures[34]. Amin-functionalized MBs were placed on a magnetic stand and discarded supernatant and washed the MBs three times with 1.5 ml anhydrous DMSO. After washing step, the MBs were resuspended in 50 mg SMPB in 1 ml anhydrous

DMSO and incubated with gentle mixing for 4h. Next, the MBs were washed with anhydrous DNA for three times and then with coupling buffer(0.1M sodium phosphate buffer,pH7.0 with 0.2M NaCl) for two times. The MBs were resuspended in coupling buffer with thiol modified oligonucleotides(25nmole) after disulfide bond of oligonucleotides were cleaved. The oligonucleotides and MBs were incubated with gentle mixing for 4hr at RT wrapped with foil and parafilm. After incubation, the supernatant was removed and MBs were washed with coupling buffer for three times and passivation buffer (0.15M sodium phosphate buffer, pH 8.0 with 0.15 NaCl) for two times. Next, And the MBs were resuspended in 100 mg sulfo-NHS-acetate in 35 ml of passivation buffer for 1h at RT. After passivation, the MBs were washed three times with 20 ml passivation buffer, and resuspend to a final concentration of 10 mg ml-1.

2.5 Fabrication of Multivalent Traptavidin-DNA complex

Figure3 illustrates schematically the approach for the multivalent TV-DNA complex. Our strategy for multivalent TV - DNA complexes is that four different sequence DNA probes are randomly mixed with four binding site of TV. To separate multivalent TV-DNA complex among all random mixed samples, stepwise filtering methods is used by complementary probes encoded magnetic separation with thermal control at four times. Four different biotinylated DNAs A,B,C,D (10uM,12ul) are randomly mixed with four binding site of TV(10uM,10ul) for 15min at RT. To separate multivalent TV-DNA complex among all random mixed samples, stepwise filtering methods is used by complementary probes encoded magnetic separation with thermal control at four times. In first magnetic separation step, TV-DNA complexes having DNA A of randomly distributed samples in PBS

hybridize with DNA A complement modified magnetic beads (0.5mg/ml) in PBS for 3hr at RT. After DNA A conjugated complexes with magnetic beads are captured by an external magnetic field, non-target DNA complement modified TV-DNA complexes was removed. The separated complexes were washed with PBS buffer solution three times and released from the magnetic beads by heating at melting temperature(50° C) for 5min. From first magnetic separation, complexes without DNA A are not existed. The sample obtained from the first magnetic separation step was dispersed in magnetic bead modified with DNA B complements and progressed to the identical magnetic separation step for DNA C and D in consecutive order.

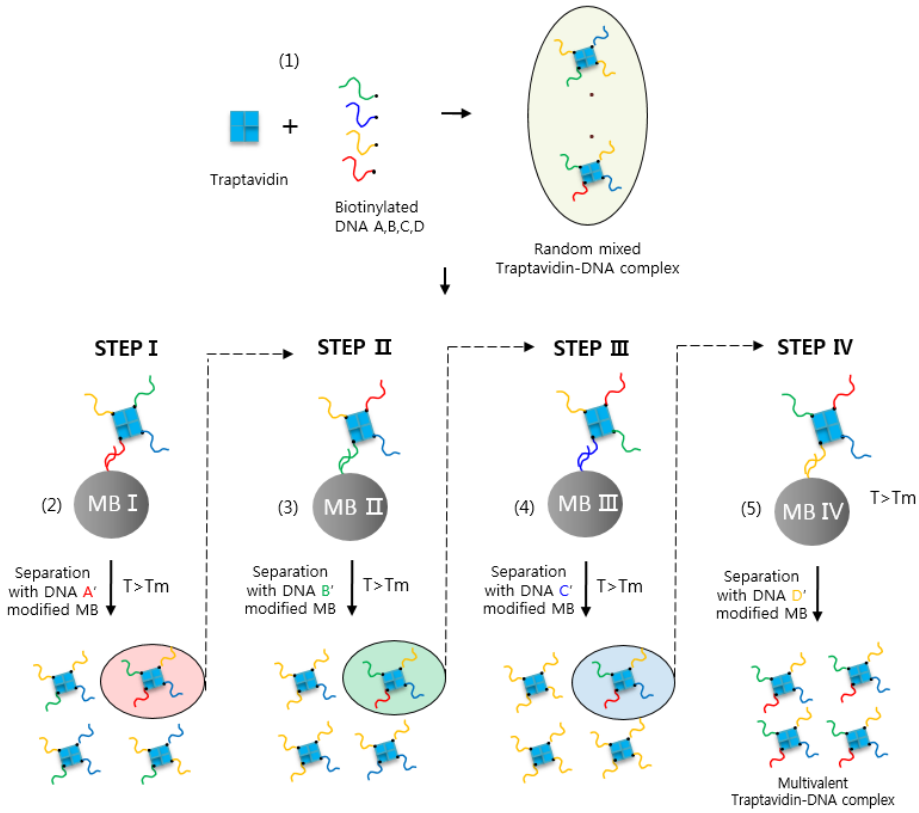


Figure 3. Scheme illustrating stepwise process for multivalent TV–DNA complex using DNA modified MBs : (1) random mixing of TV and four different biotinylated DNA (2) separation of TV–DNA complex having DNA A with DNA A' modified MB I (3) separation of TV–DNA complex having DNA B with DNA B' modified MB Ⅱ (4) separation of TV–DNA complex having DNA C with DNA C' modified MB Ⅲ (5) separation of TV–DNA complex having DNA D with DNA D' modified MB Ⅳ

2.6 20% Polyacrylamide Gel Electrophoresis analysis of Traptavidin-Biotinylated DNA complex at various ratio

Non-Denaturing PAGE analyses for TV-DNA complex at various ratio were performed in 20% acrylamide (37.5:1, acrylamide/bisacrylamide) in 45mM Tris, 45 mM boric acid, and 0.5 mM EDTA (0.5× TBE buffer). TV and biotinylated DNA mixed solution was mixed with an equivalent amount (10ul) of loading buffer containing 0.5× TBE, 0.03% bromophenol blue, and 0.03% xylene cyanol FF and then loaded onto each gel.

Loading concentration : [TV]=1uM [bDNA]=0.125, 0.25, 0.5, 1 (from lane 1 to 4, respectively) [bDNA]=1uM [TV]=0.5, 0.25, 0.125 (from lane 5 to 8), lane 9=sample after 4 magnetic separation steps

Non-Denaturing PAGE analyses for yield through 4 magnetic

steps were performed in the same conditions. While random mixed TV(1uM,100ul) and ABCD biotinylated DNA(1uM,220ul X 4) were separated with magnetic beads, each samples were collected. Loading concentration : [DNA]= 0.55uM , 0.42, 0.28, 0.16, 0.14 (from lane 1 to lane 5, respectively)

After running (37.5 V/cm, constant voltage) for 4hr, the gel was stained with GelStar, and each band was confirmed with Gel Doc Ez system.

2.7 Fabrication of Plasmonic Gold Nanostructuers

We fabricated plasmonic nanostructure based on multivalent TV -DNA complex. Figure4 illustrate schematically the approach of gold dimer structure. In first step (1), target-DNA complementary AuNPs(10nM,200ul) for probe A and B of multivalent TV -DNA(40nM,25ul) were selectively hybridized in PBS buffer for 3 hour at RT. To conjugate one AuNP to one site

of multivalent TV-DNA complex, molar ratio between binding site and AuNP was 1:2.

Next (2), non-hybridized AuNPs was eliminated to purify dimer structure through two magnetic separation step. First, DNA A′ modified magnetic beads (0.5mg/ml) were allowed to capture DNA A modified AuNP and dimer structure by external magnetic field. The captured samples were washed with PBS buffer three times to remove probe B′ modified AuNPs. After the samples were released from the magnetic beads, dimer structure was captured with probe B modified magnetic beads. In the same manner, DNA A modified AuNP was eliminated from DNA B′ modified magnetic beads. After hybridization step, we released the dimer structure by exchanging 13bp DNA modified AuNP to excess 21bp complementary DNA on magnetic bead (100uM,20ul). As 23bp dsDNA interaction is more stable than 13bp dsDNA interaction

thermodynamically, AuNP is easily released at room temperature. Because only the TA-DNA complex coexisted in probe A′ and B′ modified AuNP can hybridize, we could purify dimer structure.

2.8 Incorporated System of complex and Quantum Dot

We fabricated quantum dot (QD) and AuNP hybrid system. Figure5 illustrate schematically the approach of QD and AuNP. After STV coated QDs (1uM, 1ul) were mixed with biotinylated DNA (1uM 2ul) for 15min at RT considering with the number of STV per one QD, QDs and A′ modified 20nm AuNP (1nM, 1ml) were conjugated to TV-DNA complex (0.1uM 10ul) for 3hr at RT.

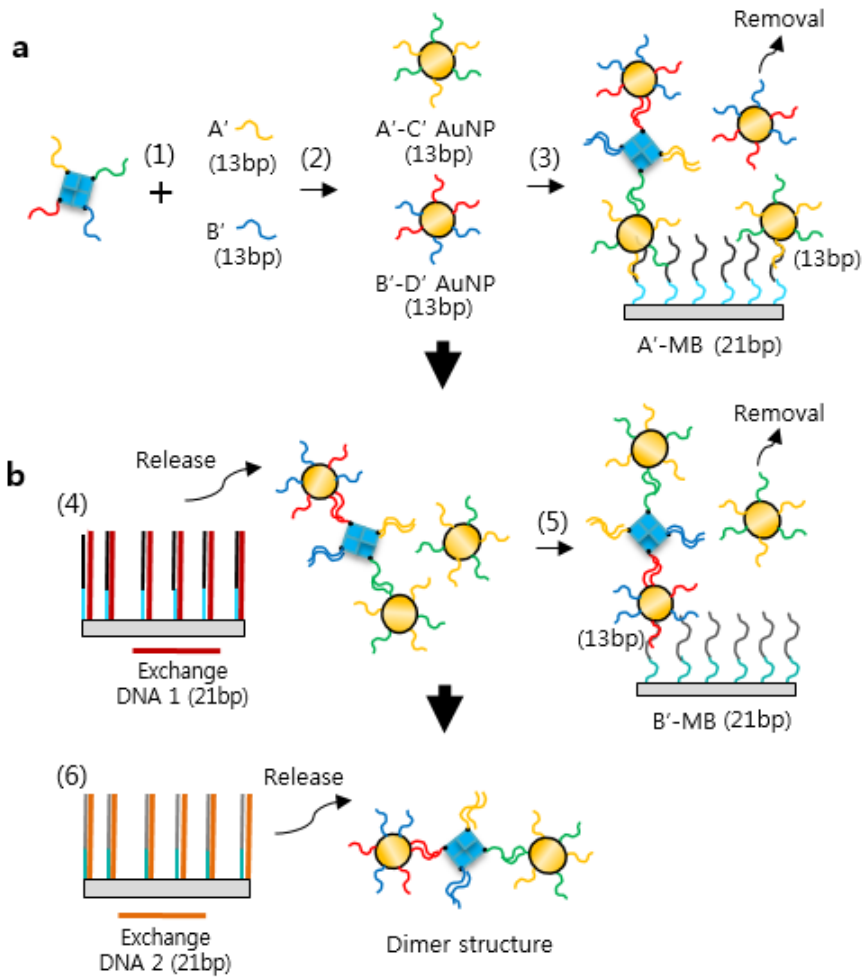

a
(1)
+
A′
(13bp)
B′
(13bp)
(2)
A′-C′ AuNP
(13bp)
B′-D′ AuNP
(13bp)
(3)
Removal
(13bp)
A′-MB (21bp)
b
Release
(4)
Exchange
DNA 1 (21bp)
(5)
Removal
(13bp)
B′-MB (21bp)
(6)
Release
Exchange
DNA 2 (21bp)
Dimer structure

Figure 4. Scheme illustrating stepwise process for fabricating plasmonic dimer structure. a, (1) passivation non-binding site(A,B-ssDNA) of TV-DNA complex with A' -ssDNA and B' -ssDNA (2) conjugation A' -C' DNA modified AuNPs and B' -C ' DNA modified AuNPs to TV-DNA complex (3) hybridization of dimer/AuNPs to A ' DNA modified MBs, followed by first removal step of non-conjugated B' -D' AuNPs by washing **b,** (4) release of dimer and B' -D AuNP from A ' -MB by substituting the exchange DNA 1 (5) hybridization of dimer to B' DNA modified MBs, followed by second removal step of non-conjugated A' -C' AuNPs by washing (6) release of dimer from B' -MB by substituting the exchange DNA 2

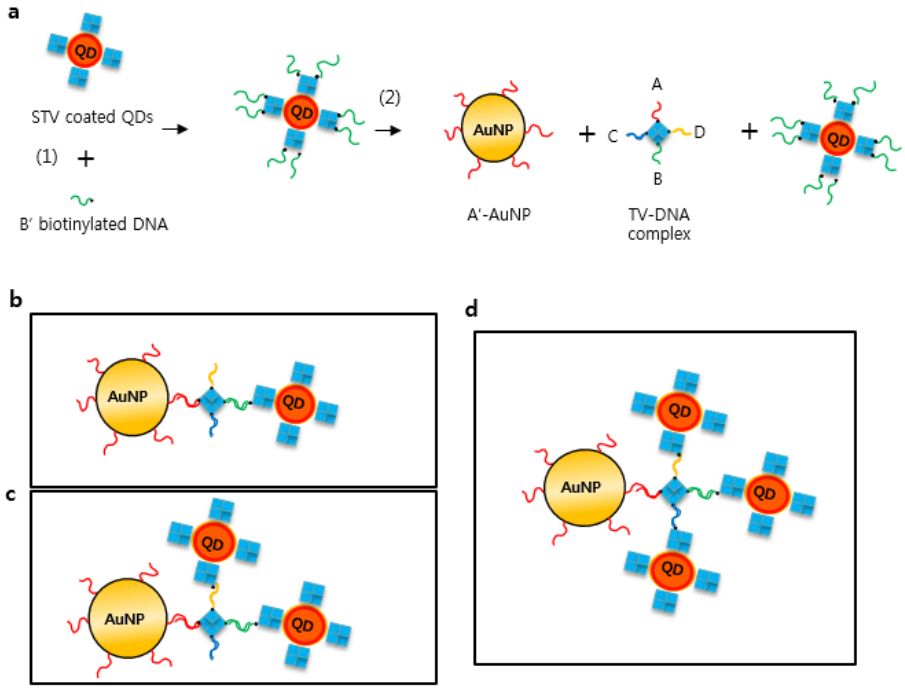


Figure 5. Scheme illustrating stepwise process for fabricating AuNP-QD hybrid system **a,** Scheme for fabricating AuNP-QD hybrid system (1) conjugation STV coated QD to biotin molecule of TV-DNA complex (2) hybridization A' DNA modified AuNP and B' bitinylated DNA to TV-DNA complex **b,** B′ biotinylated DNA of single STV coated QD and AuNP binded onto TV-DNA complex **c,** B′C′ biotinylated DNA of double STV coated QD and AuNP binded onto TV-DNA complex **d,** B′C′D′ biotinylated DNA of triple STV coated QD and AuNP binded onto TV-DNA complex

2.9 Extended Plasmonic Nanostructure from Traptavidin–DNA complex Ⅰ and Ⅱ

We showed the possibility of our TV–DNA complex to extend plasmonic structure by connecting two pre–designed TV–DNA complexes in figure 6. These complexes were fabricated using the stepwise magnetic separation we conducted in figure 3. After complex Ⅰ with DNA sequence ABCD and Complex Ⅱ with DNA sequence ABC and D complement was hybridized in the same condition of magnetic separation method, A′B′C′ DNA modified 10nm AuNPs were conjugated to extended complex Ⅰ and Ⅱ in PBS buffer for 3hr at RT. To confirm chirality of TV structure, A′B′ DNA modified 10nm AuNPs and C′ DNA modified 20nm AuNPs were conjugated to complex Ⅰ and Ⅱ in the same manner.

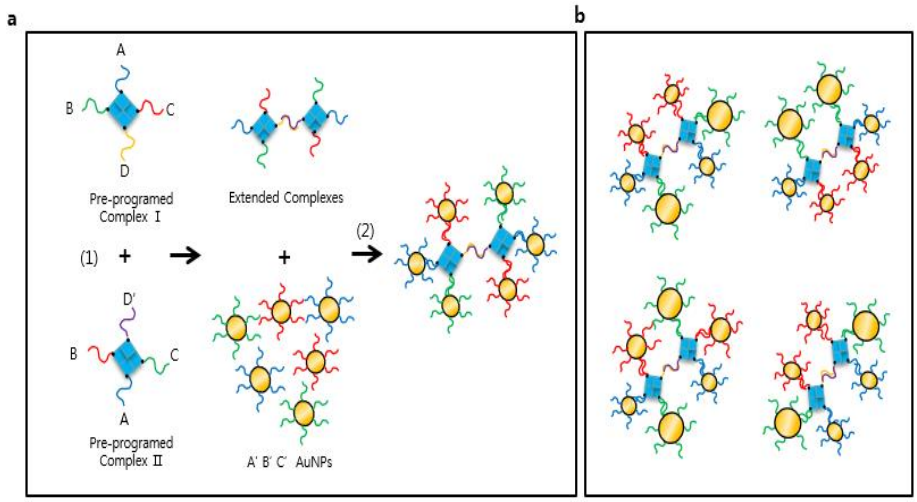


Figure 6. Scheme illustrating stepwise process for fabricating extended plasmonic structure chirality of tetrahedral structure of TV **a,** Scheme for fabricating extended plasmonic structure (1) hybridization D and D' DNA of pre-programed TV-DNA complex Ⅰ&Ⅱ(2) conjugation A′ B′ C′ AuNPs to Extended TV-DNA complex **b,** Chirality of extended Plasmonic structure with 10nm A′ B′ DNA AuNPs and 20nm C′ DNA AuNPs

3. Result and Discussion

3.1 Production and Purification of Traptavidin

In order to confirm the size of the purified TV plasmid, elect-rophoresis experiment was conducted on 1% agarose gel in 0.5X TBE buffer. Hind III marker (lane #1: 5ul, lane #2: 2ul) and 100bp DNA ladder marker (lane #4: 2ul) were loaded separately to analyze the TV plasmid band size. Lane 5, 6, 7 were loaded with 3 single colonies from LB Agar plate #1. Lane 8, 9, 10 were loaded with 3 single colonies from LB Agar plate #2. Lane 11, 12, 13 were loaded with 3 single colonies from LB Agar plate #3. The size of TV plasmid is 5848 bp including Trap-tavidin protein sequence which is 405 bp. The locations of purified TV plasmids in each lane were identical and also consistent with the marker size of 5848 bp. Furthermore, the sequence test for recovered TV plasmid was conducted by Cosmogenetech Company comparing it to author's sequence. Asterisk marks on

each sequence in Figure 7(b) shows the identical match with author's TV original sequence.

This meant that the recovered plasmid from Addgene company were to use for TV protein production and purification. Protein production and purification service provide by Ab Frontier Company was used for TV protein.

3.2 20% Polyacrylamide Gel Electrophoresis analysis with Magnetic Separation Yield in 4 Consecutive orders

To confirm and analyze the final products, we accomplished 20% native polyacrylamide gel electrophoresis. Figure8 (a) shows the result of TV –DNA complexes in a various ratios of two components. It is consistent with our hypothesis that excess TV help DNA mostly binds one site of four binding sites of TV. Also,

Lane 7 shows the quaternary structure is prominent due to high excess molar ratio of DNA. As DNA molar ratio is increased, the results correspond to increasing band intensity of quaternary structure. Then, band 1,2,3 and 4 indicate are the primary, secondary, tertiary and quaternary complexes. Electrophoretic analysis of band mobility depends on conjugates size, molecular weight, net charge and conformation. The high mobility of TV – DNA complexes mainly relies on low molecular weight. Lane 8 represents complexes following four magnetic separations. Compare to lane 7, tertiary structure does not almost exist, which means final products are well filtered from the end of 4 magnetic separation steps

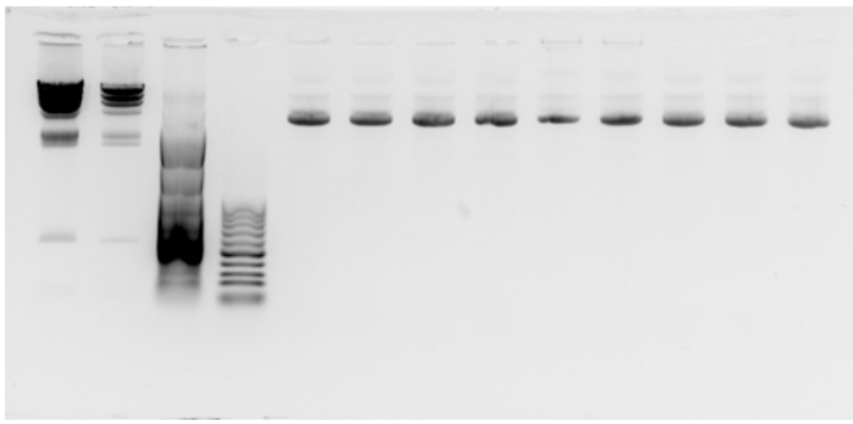
Figure8.(b) is electrophoretic images, which shows quantification of separation yields progressing each four magnetic steps. At this time, the probability to conjugate four different probes to four reactive sites of TV is about 1/11. Although this

simple method enables a new approach for making multivalent TV-DNA complexes, the yield of final products is derived from initial sample quantity with mathematical limitation. Therefore, during releasing step of double strand DNA from magnetic beads, the loss of yield should be minimized. However, streptavidin-bition system has a risk about dissociation of two couple in the condition of above melting temperature(53 ° C) for dehybridization between capture DNA encoded magnetic beads and TV-DNA complex probe. At separation temperature, the binding of streptavidin and biotin must not be separated but capture DNA on magnetic beads and target DNA of TV-DNA complex only. So, High yield can be obtained by reducing off rate of streptavidin and biotin during four magnetic separation steps. Consequently, the high thermal stability of TV has an important role in the development of our strategy with high yield. Besides, the streptavidin-biotin off rate at 37 ° C and pH 7.4 is more than

tenfold higher, in other hand, TV represents the ~2% dissociation from the start point to subsequent time.[27] Also, if biotinylated DNA on streptavidin body is changed in binding position to other binding position during magnetic separation step, multivalent protein–DNA complex could not be reliable. Then, the strong binding between TV and biotin means that it is very suitable for application with other nanoparticles for long incubation time as building block.

Lane1 indicates band image of initial random mixed sample. The lane from 2 to 5 is the results of magnetic separation in four consecutive order. As proceeding to each steps, final quantity of multivalent TV–DNA complex was 7pmole (about 9%). The yield of multivalent TV–DNA complex (in lane 5) is comparable value to initial random mixed sample considered with saturation for fabricating quaternary complex (about 75%).

a



b

```
Author          ----------------ATGGCTGAAGCTGGTATCACCGGCACCTGGTACAACCAGCTGGGA  45
Result          GAAGGAGATATACATATGGCTGAAGCTGGTATCACCGGCACCTGGTACAACCAGCTGGGA  420
                                 ********************************************

Author          TCCACCTTCATCGTTACCGCTGGTGCTGACGGTGCTCTGACCGGTACCTACGAATCCGCT  105
Result          TCCACCTTCATCGTTACCGCTGGTGCTGACGGTGCTCTGACCGGTACCTACGAATCCGCT  480
                ************************************************************

Author          GTTGGTAACGCTGAAGGCGATTACGTTCTGACCGGTCGTTACGACTCCGCTCCGGCTACC  165
Result          GTTGGTAACGCTGAAGGCGATTACGTTCTGACCGGTCGTTACGACTCCGCTCCGGCTACC  540
                ************************************************************

Author          GACGGTTCCGGAACCGCTCTGGGTTGGACCGTTGCTTGGAAAAACAACTACCGTAACGCT  225
Result          GACGGTTCCGGAACCGCTCTGGGTTGGACCGTTGCTTGGAAAAACAACTACCGTAACGCT  600
                ************************************************************

Author          CACTCCGCTACCACCTGGTCTGGCCAGTACGTTGGTGGTGCTGAAGCTCGTATCAACACC  285
Result          CACTCCGCTACCACCTGGTCTGGCCAGTACGTTGGTGGTGCTGAAGCTCGTATCAACACC  660
                ************************************************************

Author          CAGTGGTTGTTGACCTCCGGCACCACCGAAGCTAACGCGTGGAAATCCACCCTGGTTGGT  345
Result          CAGTGGTTGTTGACCTCCGGCACCACCGAAGCCAACGCGTGGAAATCCACCCTGGTTGGT  720
                ********************************** ***************************

Author          CACGACACCTTCACCAAAGTTAAACCGTCCGCTGCTTCCCATCACCATCACCACCATTAA  405
Result          CACGACACCTTCACCAAAGTTAAACCGTCCGCTGCTTCCCATCACCATCACCACCATTAA  780
                ************************************************************

Author          TAA-------------------------------------------------------- 408
Result          TAAAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCAACA 840
                +++

Author          ------
Result          AGCCCG 846
```

Figure 7. Purification results of plasmid DNA a, Agarose gel analysis for Traptavidin plasmid DNA b, DNA sequencing result of Traptavidin plasmid DNA

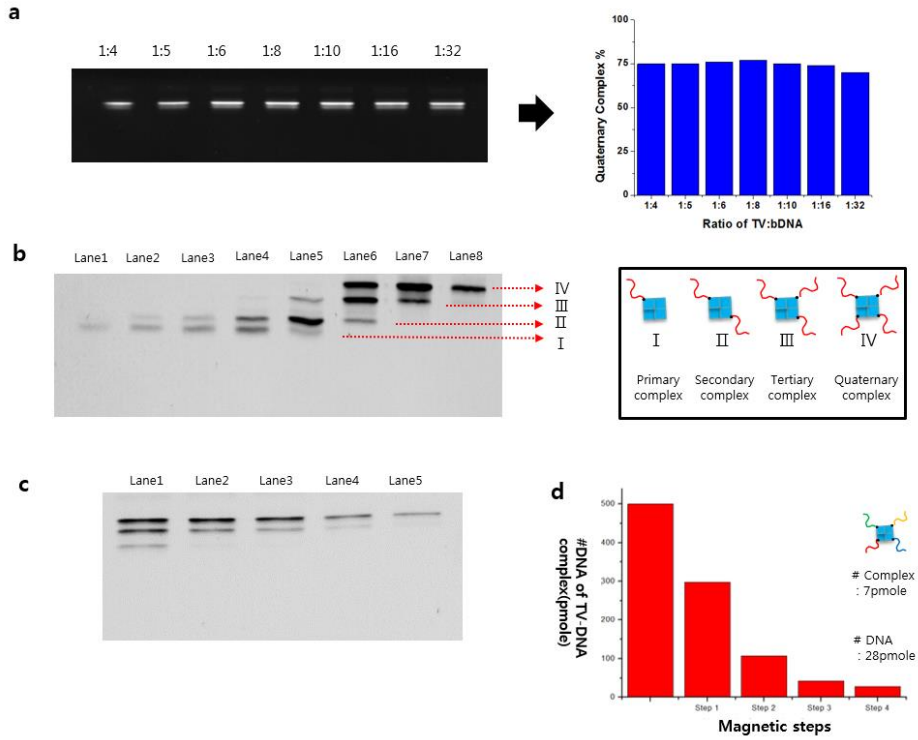


Figure 8. PAGE analysis of TV-DNA complex and 4 stepwise magnetic separation for multivalent TV-DNA complex : **a,** PAGE image of TV and DNA complex with excess DNA and saturated quaternary complex yield by UV-vis measurement of DNA **b,** PAGE image of TV and DNA complex at various molar ratios 8:1 / 4:1 / 2:1 / 1:1 / 1:2 / 1:3 / 1:4 / after 4 stepwise magnetic separation <TV : DNA> **c,** PAGE image of TV-DNA complex through 4 magnetic separation steps. **d,** Separation yield through 4 magnetic separation steps by UV-vis measurement of DNA, revealing ~7% multivalent TV-DNA complex of random mixed TV-DNA complex

3.3 TEM analysis of Symmetric and Asymmetric Dimer Gold Nanoparticle

Figure 9 (a), (b) are TEM morphologies of symmetric and asymmetric dimer nanostructure from protein-DNA template. We could attain accurate and high yield plasmonic nanostructure compared with other methods from one DNA to AuNP, stoichiometric control and geometrical restriction.

At this point, we used appropriate method where captured AuNP was released from magnetic beads through DNA exchange method.[35] Some researcher used DNA-modified magnetic beads as the template for various metal nanostructures.[32, 36] In this method, captured metallic nanoparticle on the magnetic bead was released by high temperature. However, melting temperature between DNA modified metallic nanoparticle and its complementary DNA is higher than double strand DNA. So, its method causes dissociation between TV and biotinylated DNA

above 70° C, decreasing the yield of plasmonic nanostructures. Therefore, for dehybridization, we used the DNA exchange method to exclude thermal drawback. Magnetic bead was synthesized to 21bp DNA and 13bp sequence in 21bp sequence was allowed to capture AuNP.

It should be pointed out that we used TV in place of STV. The binding association between TV and biotinylated DNA is higher than STV, which leads to have good resistance for shear stress of AuNP. Besides, Low dissociation rate help Trapdavidin-DNA complex maintain four different DNA. Also, DNA exchange method at room temperature could exclude TV-biotin dissociation during magnetic separation. If TV-DNA complex has same sequence of biotinylated DNA in 4 binding sites, identical plasmonic nanostructure could not been purified with high yield. Then, our simple and efficient assembly method with TV-DNA complex as template and magnetic separation method enables to

easily fabricate dimer nanostructure with high yield and scalable products compared with AE-HPLC and electrophoresis technique.[37, 38] Figure 9 (c) shows various shapes of plasmonic nanostructures composed with different size of AuNP and accurate addressability.

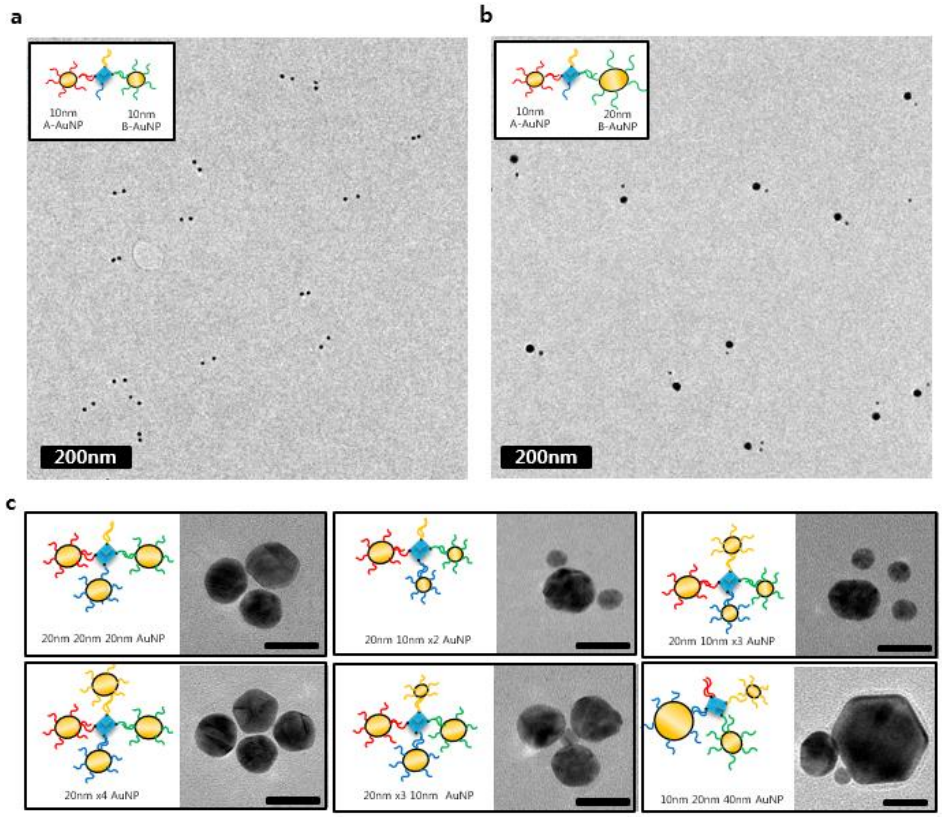


Figure 9. TEM images of symmetric/asymmetric dimer structure and various plasmonic structures. a, Morphologies of 10nm-10nm dimer structure, selected by two binding sites of TV-DNA complex b, Morphologies of 10nm-20nm dimer structure, selected by two binding sites of TV-DNA complex c, Various plasmonic structures with desired sizes and number of AuNP (c,Scale bar : 20nm)

4. TEM images of Incorporated System of Traptavidin-DNA complex and Quantum Dot

The assembly of metallic nanoparticle can be incorporated to heterogonous material system with Quantum dots, fluorophores and carbon materials, which system needs accurate binding sites and easy controllable.[33] Then, we could incorporate heterogonous material system because our platform has four binding sites and availability with complementary DNA modified any nanoparticles. Figure10 is TEM image of AuNPs and Quantum Dot hybrid materials. AuNP was conjugated to one binding site of multivalent TV-DNA complex and other binding sites were used for conjugating quantum dot by pre-programmed biotinylated These system could apply the plasmonic coupling of a quantum emitter with desirable metal nanoparticle by tailoring the length and sequence design of DNA.

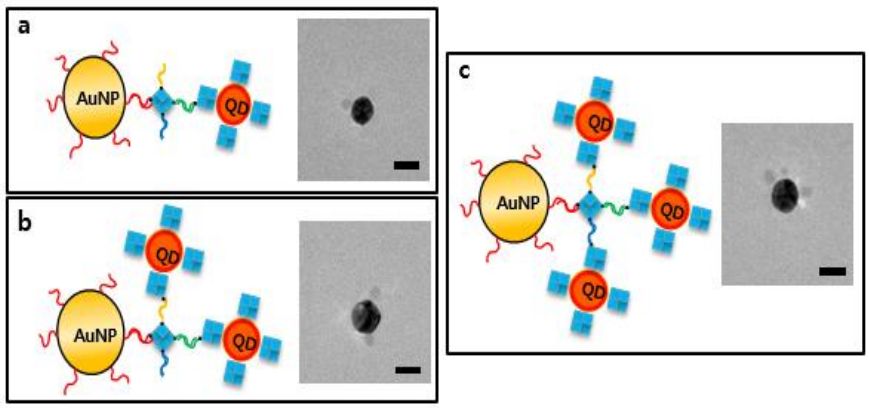


Figure 10. TEM images of AuNP-QD hybrid system

a, Single STV coated QD and 20nm AuNP binded onto TV-DNA complex

b, Double STV coated QD and 20nm AuNP binded onto TV-DNA complex

c, Triple STV coated QD and 20nm AuNP binded onto TV-DNA complex

(Scale bar : 20nm)

3.5 TEM image of Extended Plasmonic structure from Traptavidin-DNA complex Ⅰ and Ⅱ

Programmed nanoparticles functionalized with DNA should head for not only constructing two-dimensional periodic structures but also three-dimensional colloidal crystals to achieve novel properties.[39-41] To be extendable of nanoparticles, the diversity and general approach need to fabricate high-ordered structure in controllable manner. Then, we showed the possibility of our TV-DNA complex to extend plasmonic structure by connecting two pre-designed TV-DNA complexes. These complexes were fabricated using the stepwise magnetic separation as in Figure 3. After complex 1 with DNA sequence ABCD and Complex 2 with DNA sequence ABC and D complement was hybridized, we showed TEM morphologies of extended plasmonic structure. Chirality is an important issue of colloidal system due to its different properties from right handed direction

and left handed direction. The tetrahedral symmetry of TV allowed us to explore chiral plasmonic structures. Then, our platform could offer general study of chirality with easy controllable and predictable fabrication.

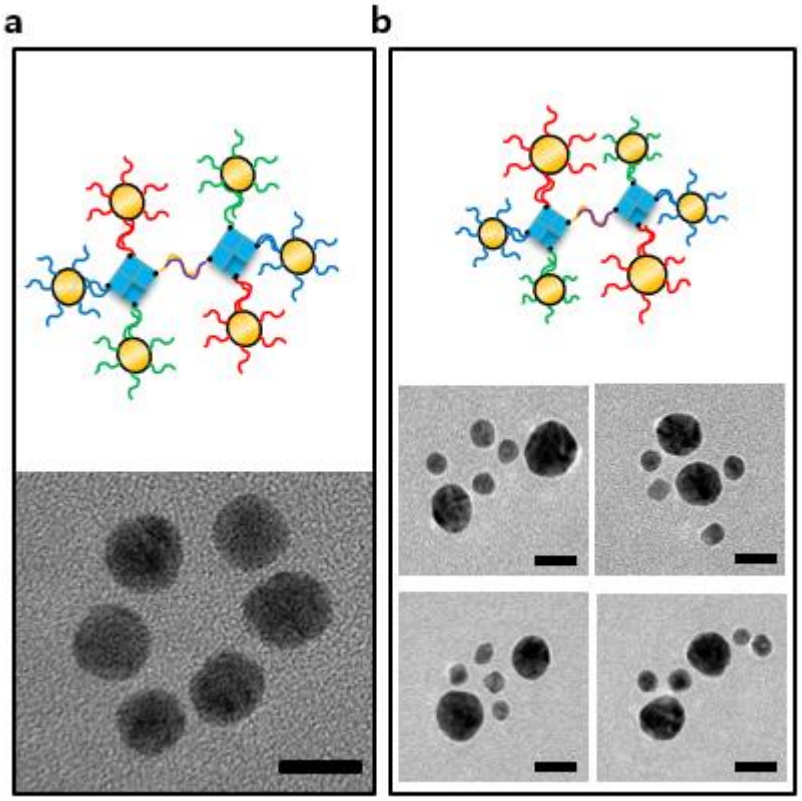


Figure 11. TEM Image of Extended Plasmonic Structure and chirality of tetrahedral structure of TV a, Hexagonal plasmonic structure of 20nm AuNPs by pre-programed complex Ⅰand Ⅱ. **b,** Different tetrameric configuration of 10nm AuNPs and 20nm AuNPs by pre-programed complex Ⅰand Ⅱ

(Scale bar : 20nm)

6. Conclusion

In this letter, we presented new possibility as a building block of multivalent, multifunctional, and easy controllable TV –DNA complex through unique and simple method. From this building block, we fabricated discrete plasmonic gold nanostructures by tailoring specific probes of complexes and showed accurate size, shape and spatial addressability. Unlike previous research in this field, plasmonic gold nanostructures is not directly conjugated with thiol modified AuNP to others but with intermediates of protein cored structures. Its outstanding advantage of this strategy is easy predictable and controllable by selecting two probes or three probes with four–valent of protein core. Also, various structure can be simply fabricated by modifying length of probes and its complementary probes. In addition, this building block enables the incorporation of each individual complexes that can be extendable to many different nanoparticle super–lattices.

Finally, the protein−DNA hybrid building block established herein with chirality of TV and well−defined number of probes can be tunable to design the geometry assembly, allowing for the construction of new crystallographic structures.

Reference

1. Chen J, Seeman NC. Synthesis from DNA of a molecule with the connectivity of a cube. *Nature* 1991,**350**:631-633.
2. Winfree E, Liu F, Wenzler LA, Seeman NC. Design and self-assembly of two-dimensional DNA crystals. *Nature* 1998,**394**:539-544.
3. Yurke B, Turberfield AJ, Mills AP, Simmel FC, Neumann JL. A DNA-fuelled molecular machine made of DNA. *Nature* 2000,**406**:605-608.
4. Ringler P, Schulz GE. Self-Assembly of Proteins into Designed Networks. *Science* 2003,**302**:106-109.
5. Rajagopal K, Schneider JP. Self-assembling peptides and proteins for nanotechnological applications. *Current Opinion in Structural Biology* 2004,**14**:480-486.
6. Colombo G, Soto P, Gazit E. Peptide self-assembly at the nanoscale: a challenging target for computational and experimental biotechnology. *Trends in Biotechnology* 2007,**25**:211-218.
7. Grueninger D, Treiber N, Ziegler MOP, Koetter JWA, Schulze M-S, Schulz GE. Designed Protein-Protein Association. *Science* 2008,**319**:206-209.

8. Yin P, Choi HMT, Calvert CR, Pierce NA. Programming biomolecular self-assembly pathways. *Nature* 2008,**451**:318-322.
9. Andersen ES, Dong M, Nielsen MM, Jahn K, Subramani R, Mamdouh W*, et al.* Self-assembly of a nanoscale DNA box with a controllable lid. *Nature* 2009,**459**:73-76.
10. Dietz H, Douglas SM, Shih WM. Folding DNA into Twisted and Curved Nanoscale Shapes. *Science* 2009,**325**:725-730.
11. Douglas SM, Dietz H, Liedl T, Hogberg B, Graf F, Shih WM. Self-assembly of DNA into nanoscale three-dimensional shapes. *Nature* 2009,**459**:414-418.
12. Woolfson DN, Mahmoud ZN. More than just bare scaffolds: towards multi-component and decorated fibrous biomaterials. *Chemical Society Reviews* 2010,**39**:3464-3479.
13. Sinclair JC, Davies KM, Venien-Bryan C, Noble MEM. Generation of protein lattices by fusing proteins with matching rotational symmetry. *Nat Nano* 2011,**6**:558-562.
14. Derr ND, Goodman BS, Jungmann R, Leschziner AE, Shih WM, Reck-Peterson SL. Tug-of-War in Motor Protein Ensembles Revealed with a Programmable DNA Origami Scaffold. *Science* 2012,**338**:662-665.
15. Douglas SM, Bachelet I, Church GM. A Logic-Gated Nanorobot for Targeted Transport of Molecular Payloads. *Science* 2012,**335**:831-834.
16. Ke Y, Ong LL, Shih WM, Yin P. Three-Dimensional Structures Self-Assembled from DNA Bricks. *Science* 2012,**338**:1177-1183.
17. Chen JI, Durkee H, Traxler B, Ginger DS. Optical detection of protein in complex media with plasmonic nanoparticle dimers. *Small* 2011,**7**:1993-1997.
18. Han MS, Lytton-Jean AK, Oh BK, Heo J, Mirkin CA. Colorimetric screening of DNA-binding molecules with gold nanoparticle probes. *Angew Chem Int Ed Engl* 2006,**45**:1807-1810.
19. Lee JS, Han MS, Mirkin CA. Colorimetric detection of mercuric ion (Hg2+) in aqueous media using DNA-functionalized gold

nanoparticles. *Angew Chem Int Ed Engl* 2007,**46**:4093-4096.

20. Liu J, Lu Y. Fast colorimetric sensing of adenosine and cocaine based on a general sensor design involving aptamers and nanoparticles. *Angew Chem Int Ed Engl* 2005,**45**:90-94.
21. Xu X, Han MS, Mirkin CA. A gold-nanoparticle-based real-time colorimetric screening method for endonuclease activity and inhibition. *Angew Chem Int Ed Engl* 2007,**46**:3468-3470.
22. Yan H, Park SH, Finkelstein G, Reif JH, LaBean TH. DNA-templated self-assembly of protein arrays and highly conductive nanowires. *Science* 2003,**301**:1882-1884.
23. Lai Y-T, Cascio D, Yeates TO. Structure of a 16-nm Cage Designed by Using Protein Oligomers. *Science* 2012,**336**:1129.
24. Padilla JE, Colovos C, Yeates TO. Nanohedra: using symmetry to design self assembling protein cages, layers, crystals, and filaments. *Proc Natl Acad Sci U S A* 2001,**98**:2217-2221.
25. Ringler P, Schulz GE. Self-assembly of proteins into designed networks. *Science* 2003,**302**:106-109.
26. Stenkamp RE. Protein Quaternary Structure: Symmetry Patterns. 2009.
27. Chivers CE, Crozat E, Chu C, Moy VT, Sherratt DJ, Howarth M. A streptavidin variant with slower biotin dissociation and increased mechanostability. *Nat Methods* 2010,**7**:391-393.
28. Kumar A, Hwang JH, Kumar S, Nam JM. Tuning and assembling metal nanostructures with DNA. *Chem Commun (Camb)* 2013,**49**:2597-2609.
29. Tan SJ, Campolongo MJ, Luo D, Cheng W. Building plasmonic nanostructures with DNA. *Nat Nano* 2011,**6**:268-276.
30. Zhang T, Yang Z, Liu D. DNA discrete modified gold nanoparticles. *Nanoscale* 2011,**3**:4015-4021.
31. Lim D-K, Jeon K-S, Hwang J-H, Kim H, Kwon S, Suh YD*, et al.* Highly uniform and reproducible surface-enhanced Raman scattering from DNA-tailorable nanoparticles with 1-nm interior gap. *Nat Nano* 2011,**6**:452-460.

32. Lim D-K, Jeon K-S, Kim HM, Nam J-M, Suh YD. Nanogap-engineerable Raman-active nanodumbbells for single-molecule detection. *Nat Mater* 2010,**9**:60-67.
33. Yoon JH, Lim J, Yoon S. Controlled Assembly and Plasmonic Properties of Asymmetric Core–Satellite Nanoassemblies. *ACS Nano* 2012,**6**:7199-7208.
34. Hill HD, Mirkin CA. The bio-barcode assay for the detection of protein and nucleic acid targets using DTT-induced ligand exchange. *Nat. Protocols* 2006,**1**:324-336.
35. Maye MM, Nykypanchuk D, Cuisinier M, van der Lelie D, Gang O. Stepwise surface encoding for high-throughput assembly of nanoclusters. *Nat Mater* 2009,**8**:388-391.
36. Xu X, Rosi NL, Wang Y, Huo F, Mirkin CA. Asymmetric Functionalization of Gold Nanoparticles with Oligonucleotides. *Journal of the American Chemical Society* 2006,**128**:9286-9287.
37. Lan X, Chen Z, Liu B-J, Ren B, Henzie J, Wang Q. DNA-Directed Gold Nanodimers with Tunable Sizes and Interparticle Distances and Their Surface Plasmonic Properties. *Small* 2013:n/a-n/a.
38. Claridge SA, Liang HW, Basu SR, Fréchet JMJ, Alivisatos AP. Isolation of Discrete Nanoparticle−DNA Conjugates for Plasmonic Applications. *Nano Letters* 2008,**8**:1202-1206.
39. Leunissen ME, Christova CG, Hynninen A-P, Royall CP, Campbell AI, Imhof A*, et al.* Ionic colloidal crystals of oppositely charged particles. *Nature* 2005,**437**:235-240.
40. Zhang C, Macfarlane RJ, Young KL, Choi CHJ, Hao L, Auyeung E*, et al.* A general approach to DNA-programmable atom equivalents. *Nat Mater* 2013,**advance online publication**.
41. Nie Z, Petukhova A, Kumacheva E. Properties and emerging applications of self-assembled structures made from inorganic nanoparticles. *Nat Nano* 2010,**5**:15-25.

요 약 (국문초록)

DNA와 Protein은 응용가능한 나노구조체를 만들 수 있는 프로그램 가능한 물질이다. 비록 DNA와 Protein는 프로그램가능하며 쉽게 조작용이한 나노구조체를 제작할 수 있는 장점이 있지만, 이들은 재료 자체의 특성에 기인한 몇몇 단점을 수반하고 있다. 따라서 잘 디자인 된 DNA-Protein 혼성 물질은 각각의 장점을 이용하면서 서로의 단점을 보완해 줄 수 있는 강력한 도구가 될 수 있다. 우리는 D2 대칭성을 가지는 Traptavidin protein과 이와 높은 친화력을 가지는 서로 다른 4개의 Biotinylated DNA를 기본 재료로 사용하였고, DNA의 base-paring 상호작용을 이용하여 4번의 자성 분리방법을 통해 다수의 결합 가능한 protein-DNA 복합체를 제작하였다. 이

는 4nm의 한 protein몸체에 4개 중 선택적으로 기능성을 부여할 수 있는 probe를 가진 새로운 기본단위이다. 이 복합체를 바탕으로, 우리는 DNA를 직접적으로 결합하는 방법을 이용하여 정확한 공간적 선택성으로 높은 수율의 금속성 plasmonic 구조체를 제작하였다. 기존의 DNA-mediated 방법과 달리 Traptavidin의 결합위치가 정해진 특성을 이용하여 정확하고 선택적인 addressability을 통해 asymmetrical/symmetrical plasmonic structure도 쉽게 제작하였다. 또한, 복합체를 periodic metallic nanostructure 를 만들 기 위한 template로서의 가능성을 보여주기 위하여, 우리는 복합체간의 연결을 통해 확장 된 조립체를 구현하였다. 그러므로, 우리는 예측가능하고 복잡한 구조체를 설계하기 위한 개념적으로 간단한 방법을 제시했다. 다수의 결합가능한 protein-DNA 복합체는 서로 다른 4개의 DNA의 선택성과 3차원 대칭을 가지는 Traptavidin의 구조와 함

께 더 많은 조합가능성을 부여할 것이다. 이처럼, Buildingblock으로서 새로운 분류는 다기능, 프로그램가능성 그리고 동시성을 부여할 뿐만 아니라 2차원의 나노배열체 그리고 3차원의 나노구조체를 수월하게 만들 수 있도록 할 것이다.

주요어 : DNA-Protein 혼성 물질, 자가조립재료, Traptavidin, 자성분리, Plasmonic 구조, 나노구조체

학 번 : 2011-22753