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Reversible Ligation of Programmed DNA-Gold Nanoparticle Assemblies

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Supporting Information

ABSTRACT: We demonstrate a new method to reversibly cross-link DNA-nanoparticle dimers, trimers, and tetramers using light as an external stimulus. A DNA interstrand photo-cross-linking reaction is possible via ligation of a cyano-vinyl carbazole nucleoside with an opposite thymine when irradiated at 365 nm. This reaction results in nanoparticle assemblies that are not susceptible to DNA dehybridization conditions. The chemical bond between the two complementary DNA strands can be reversibly broken upon light irradiation at 312 nm. This is the first example of reversible ligation in DNA-nanoparticle assemblies using light and enables new developments in the field of programmed nanoparticle organization.

Twenty years ago, it was independently shown by the groups of Alivisatos[1] and Mirkin[2] that thiol-modified oligonucleotides can bind to gold nanoparticles and direct nanoparticle organization. This was the first time that the rich properties of DNA molecules and gold nanoparticles were combined and marked the foundations of a new class of highly programmed[3−14] and functional nanomaterials.[15−17] Since then, DNA-coated nanoparticles have been explored in drug delivery and gene therapy as well as in imaging and sensing with great success.[18−21] Recently, the experience gained in the preparation and handling of DNA-gold nanoparticle colloids led to the construction of ordered plasmonic materials. These possess intriguing photonic properties such as circular dichroism in the visible domain of the electromagnetic spectrum[22−24] and plasmon-light coupling peculiarities.[25,26]

Accurate control of organization of DNA-nanoparticle assemblies is of great research interest with the main goal being the precise fabrication of exotic structures with predictable properties.[27−36] However, many of the currently reported DNA-nanoparticle structures lack stability and adaptability in different environments. The morphology, programmability, and information content of these advanced structures can be completely lost in conditions that favor DNA dehybridization and therefore nanoparticle disassembly. Currently, this drawback restricts their broad applicability.

To tackle this issue, research efforts in the field involve the utilization of biomolecular and chemical tools to manipulate the DNA on the nanoparticles enriching both the library of methods to adapt DNA-gold nanostructures in complex environments and the availability of tools to program nanostructures. For example, in 2003, the Brust group reported, for the first time, the ligation and cleavage of DNA-nanoparticle assemblies using ligases and restriction enzymes,[37] while a few years later, in 2008, the Alivisatos group utilized ligases to program the production of covalently linked DNA-nanoparticle dimers on a large scale.[38] In 2012 Yan et al. reported that azobenzene-modified oligonucleotides could be employed for the photoswitchable destabilization of DNA-gold nanoparticle assemblies.[39] Recently, in 2013, our group developed a one-pot method to rapidly and selectively ligate DNA-gold nanoparticle dimers and trimers using a copper-free click chemistry method. The key advantage of that technique was that the reactive clicking groups, a strained cyclooctyne and an azide, were directly incorporated into the oligonucleotide strands and spontaneously reacted once the strands were brought in close proximity during complementary hybridization.[40]

Here, we demonstrate a novel approach to reversibly cross-link and de-cross-link DNA in nanoparticle assemblies, using light. This is possible by the incorporation of a 3-cyanovinyl carbazole DNA nucleoside, which can react with a thymine base diagonally opposite via a [2 + 2]-photocycloaddition reaction to form a cyclobutane.[41,42] This new approach allows the efficient and reversible ligation of nanoparticles without the need for special pH conditions or the use of additional molecules or ions. The DNA cross-linking and de-cross-linking reactions take only a few minutes at room temperature, and the yield of the product can reach 100%, without any DNA damage.[42] The employment of this non-invasive approach to reversibly ligate nanoparticles unravels a new way to manipulate DNA-nanoparticle assemblies.

Scheme 1 illustrates the experimental route for the formation of the simplest nanostructure, a nanoparticle dimer. Two batches of 15 nm gold nanoparticles were functionalized with complementary thiol-modified oligonucleotides, respectively.

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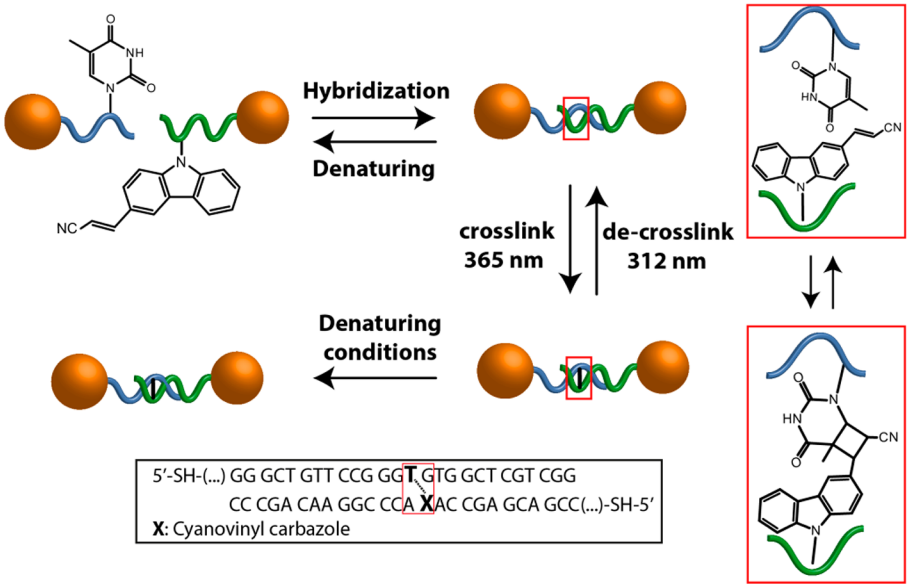
Scheme 1. Schematic Illustration of DNA Cross-Linking and De-Crosslinking on Gold Nanoparticle Dimers[a]



[a]Gold nanoparticles conjugated with complementary oligonucleotides are hybridized to form dimers. One of the oligonucleotides contains a 3-cyanovinyl carbazole group, which can react with an adjacent thymine, in the opposite strand, upon light irradiation. Irradiation with UV-A light allows the formation of a cyclobutane bridge between the opposite thymine base as indicated by the red box. After cross-linking, the dimers remain intact even under denaturing conditions. The crosslinked DNA strands can be reversibly de-crosslinked with UV-B light irradiation.

One DNA strand was chemically modified with a cyanovinyl carbazole nucleoside, and the second was designed in such a way that after hybridization a thymine group would be placed diagonally opposite the 3-cyanovinyl carbazole group (as seen in Scheme 1). For the purpose of the experiment, particles modified with only one DNA strand, following a protocol reported previously,[43] were mixed in equimolar amounts, and the sample was incubated following three heating and cooling cycles to allow the efficient hybridization of the DNA-nanoparticle dimers. The particles were then irradiated with UV-A light (centered at 365 nm, with the lamp set at 4 cm above the solution) for 15 min in order to facilitate the cross-linking of the DNA between the nanoparticles. To demonstrate the reversibility of the method, the ligated DNA was de-cross-linked by irradiation with UV-B light (centered at 312 nm, with the lamp set at 4 cm above the solution). An additional DNA cross-linking and de-cross-linking cycle was performed to validate this method.

The DNA-gold nanostructures were monitored at all different stages using various techniques. The most popular method to purify and analyze nanoparticle conjugates is gel electrophoresis where the nanoparticles migrate within a polymer matrix (e.g., agarose) at different rates, according to their size and charge. Figure 1 shows an agarose gel of the DNA-nanoparticles at different stages of the experiment. As expected, the nanoparticle dimers (lanes 2, 4) possess a significantly reduced electrophoretic mobility compared with monoconjugates (lanes 1, 3). The gel was run under denaturing conditions to ensure that non-cross-linked nanoparticle dimers would dehybridize into monomers. Indeed, lane 2 shows that the nanoparticles remain as dimers after DNA photo-cross-linking. On the other hand, once the dimers are de-cross-linked the particles dehybridize and run in the gel as monomers (lane 3). Lane 4 illustrates a full cycle of cross-linking, de-cross-linking, and re-cross-linking of dimers. The yield of DNA cross-linking and de-cross-linking approaches 100% as no other

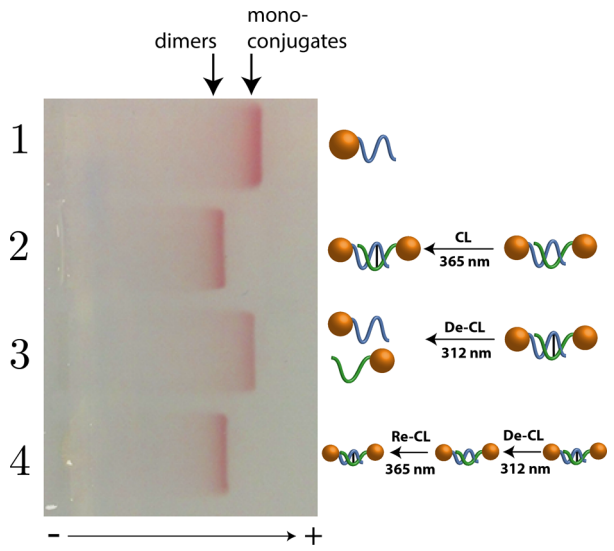


Figure 1. Agarose gel electrophoresis of gold nanoparticle dimers under denaturing conditions showing the reversibility of the photo-cross-linking reaction. Lane 1: Monoconjugates as reference; lane 2: cross-linked dimers; lane 3: de-cross-linked dimers running as monoconjugate band; and lane 4: re-cross-linked dimers.

nanoparticle bands are visible in the gel. Besides the high efficiency, no smearing appeared within the bands, which suggests that the nanoparticle size was not influenced by the UV irradiation applied for the photochemical reaction. This was further confirmed by UV−vis spectra acquired before and after UV irradiation (Figure S7, Supporting Information). The visible spectra of the gold nanoparticles after both UV-A and UV-B irradiation overlap, without a shift in the peak or a plasmon band broadening. This indicates again that neither a change in size nor morphology occurred. To gain a better understanding of the state of the nanoparticle assemblies in

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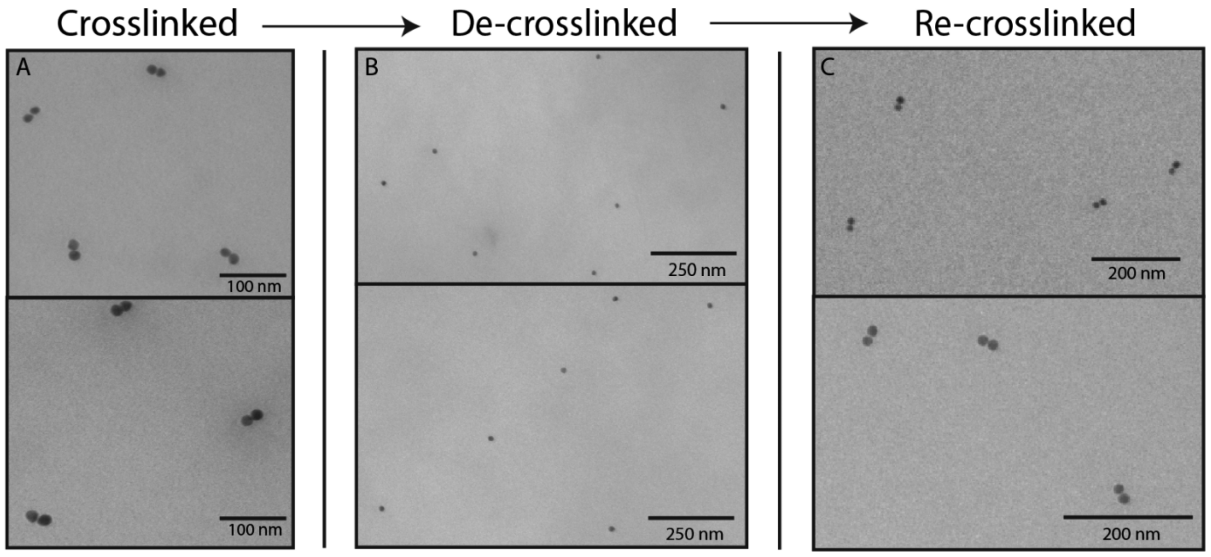

Figure 2. Representative TEM images of cross-linked and de-cross-linked 13 nm gold nanoparticle assemblies. The samples were deposited on the grids under DNA denaturing conditions. Photo-cross-linked gold nanoparticle dimers (A), de-cross-linked nanoparticle dimers, which disassemble to monoconjugates (B), and re-cross-linked dimers (C).

solution, dynamic light scattering (DLS) was employed to measure the hydrodynamic radii of the different species under denaturing conditions (Figure S18). A significant increase in size for the dimers is observed. As DLS detects the size of the ensemble in solution and is particularly sensitive to larger sizes, the measurement indicates that there are no larger aggregates present. To visualize the nanoparticles, the assemblies were studied by TEM as shown in Figure 2. All samples were deposited on the grid under conditions that favor DNA dehybridization. The DNA cross-linked nanoparticles remained as dimers (Figure 2A), while de-cross-linked nanoparticle dimers were dehybridized and appeared as monomers (Figure 2B). After a re-cross-linking step, the dimers remained intact under DNA denaturing conditions (Figure 2C).

To test the versatility of the new DNA photo-cross-linking method, we utilized it to cross-link even more complex structures such as nanoparticle trimers and tetramers. Figure 3 shows DNA-cross-linked nanoparticle trimers and tetramers deposited on the grids under DNA denaturing conditions. As in the case of dimers, nanoparticle trimers and tetramers remained intact. Figures S11−13 and S15−19 show the relevant electrophoresis gels, DLS, visible spectra, and more TEM images, which prove the successful cross-linking and de-cross-linking of these higher order DNA-nanoparticle assemblies.

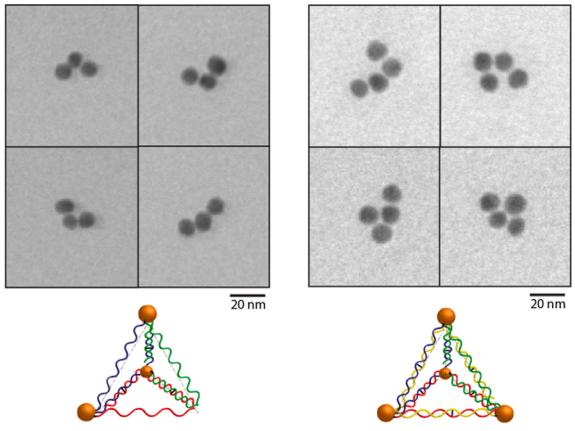

Figure 3. TEM images of cross-linked trimers and tetramers deposited on the grids under DNA denaturing conditions.

In conclusion, we have developed a new tool to manipulate DNA-nanoparticle oligomers using light. A cyanovinyl carbazole-modified nucleotide photoreacts at 365 nm with an adjacent thymine group in the complementary DNA strand to create a covalent bond between DNA strands. This bond can be reversibly broken with light irradiation at 312 nm. This new technique for cross-linking and de-cross-linking DNA-nanoparticle assemblies will be of particular interest and applicability in several research fields where nanoparticle oligomer assemblies could potentially be utilized such as catalysis, sensing, and photonics.[10,44]

■ ASSOCIATED CONTENT

Supporting Information

DNA synthesis, experimental protocols, characterization of nanoparticle trimers and tetramers. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b05683.

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Notes

The authors declare no competing financial interest.

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