

Plasmonic Circular Dichroism Study of DNA–Gold Nanoparticles Bioconjugates

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Abstract Plasmonic circular dichroism (CD) responses of hybrid nanostructures containing noble metal nanoparticles and chiral molecules have received increasing interest with various applications in nanophotonics. Chiral biomolecules show strong CD signals typically found in the ultraviolet region, whereas, in the visible range, they produce a weak signal. Strengthening the CD signal in the visible region is of high importance, which could be achieved through fabrication of novel hybrid nanostructures. Herein, gold nanoparticles (GNPs) have been assembled via DNA linker to investigate the possibility of enhancing plasmonic CD signal in the visible range. DNA-linked assemblies with pre- and postannealed conditions were characterized by ultraviolet–visible spectroscopy, dynamic light scattering (DLS), and CD spectropolarimetry. In the presence of DNA linker with sticky ends, the aggregation phenomenon was traced by red shifts of surface plasmon resonance of nanoparticles. Time-dependent hybridization of single-stranded “sticky ends” with DNA-conjugated GNPs and increased probability of hydrogen bond formation lead to enhancement of CD signals in the ultraviolet region. Complexation of biomolecule and nanoparticle assemblies induced enhanced CD signals in the visible range, which was noticed both before and after purification. DLS characterization of the assemblies also confirmed the difference in the size of aggregates, which could be controlled by the linker molecules. This investigation encourages possibility of utilizing plasmonic CD technique as a tool for tracing fabricated nanostructure assemblies with enhanced characterization possibility.

Keywords Plasmonic circular dichroism · Hybrid nanostructures · Gold nanoparticles · DNA-linked assembly · Dynamic light scattering

Introduction

Plasmonic materials are known as the key platforms for applications that rely on the manipulation of light at small scales. Materials that possess sub-wavelength metallic features support surface plasmon phenomenon [1]. The surface plasmon resonance (SPR) characteristic arises from collective oscillations of the free electrons of the conduction band, leading to local amplification of the incident electromagnetic by orders of magnitude, at the metal surface [2]. This field enhancement is useful for extraordinary optical phenomena exhibited by metal nanostructures, such as resonant light scattering, surface-enhanced Raman scattering, superlensing, and light transmission through optically thick films [3].

Today, assembly of plasmonic nanoparticles is of particular interest, since they can be tailored to exhibit a broad range of electromagnetic properties. So far, several chemical strategies have been employed to create assemblies of nanoparticles. Among them, DNA has shown great potential for providing self-assembled electronic devices and memory components at the nanoscale level [4, 5].

Till now, conjugation of DNA nanoparticles has been extensively studied with their important applications in analytical chemistry, materials science, and nanomedicine [6]. In 1996, Mirkin and coworkers reported macroscopic aggregates of assembled colloidal gold nanoparticles (GNPs), using DNA as the linking elements. GNPs are routinely functionalized with thiol-modified DNA and polymerized with linker DNA to form large aggregates. According to the existing reports, it is possible to control the number of GNPs and the interparticle distance in each assembly precisely [7]. It is well known that

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SPR band of GNPs shifts progressively to longer wavelengths upon aggregation, depending on interparticle distance. This results in the coupling of SPR, giving distinct color change from red to purple. Such phenomenon could be exploited in designing ultrasensitive colorimetric sensors for detection of nucleic acids, presence of pathogens, metal ions, etc. [8].

The optical properties and phase transition of assembled DNA-GNPs systems have attracted great interest for the researchers, since understanding such properties would provide essential information about DNA-based nanotechnology in colloidal systems [9]. Most of the previous studies on GNPs DNA-directed assembly have been focused on the plasmonic absorption resonance of GNPs and their assembly at varying temperatures [10, 11]. Configuration of GNP-DNA hybrid systems is mostly multifaceted. However, there are only few reports about structure of these systems. Circular dichroism (CD) technique allows investigating conformational changes of complex biomolecules such as peptides, proteins, DNA, etc. Chiral biomolecules show strong CD signals, which often arise from the helical geometry of biomolecular systems, typically found in the UV range (~200–300 nm) [12]. However, the biomolecules provide CD signals of weak intensity in the visible region. Therefore, signal enhancement in the visible region could be of high significance [13]. Plasmonic CD is a new branch of science in the domain of nanotechnology, which originates from hybrid of noble metal nanoparticles and chiral biomolecules. It is generally induced by dipoles of both nanoparticles and attached molecules (peptide, DNA, and chiral fiber) [14]. Among the chiral molecules, DNA is nominated as the most attractive one because its programmability provides an excellent platform for controllable construction of hybrids with noble metal NPs [13, 14]. Previous investigations with CD have been focused on the origin and amplification of the self-assembled systems, whereas there is no experimental report on dynamics of formation. Among various techniques for investigation of configuration and transformation of nanostructures, plasmonic CD is believed to show highest sensitivity [15]. Therefore, conducting a survey on dynamics of self-assembled nanostructures by CD technique could be of high significance. Gérard et al. [16] reported plasmon-induced CD response of oligonucleotide-conjugated gold nanoparticles and noticed PCD enhancement of purified aggregates of nanobioconjugates.

Herein, a hybrid of GNPs and DNA has been fabricated using linker DNA, at a fixed concentration of salt solution. Structure and kinetics of the self-assembled system has been monitored via spectroscopic techniques such as ultraviolet–visible (UV-vis), CD, plasmonic CD, and dynamic light scattering (DLS). Enhancement of PCD signal was traced both with and without the washing process. This report introduces plasmonic CD responses as a novel detection method for ultrasensitive nanobiosensors.

Materials and Methods

Materials

All DNA samples of high-performance liquid chromatography grade were procured from Bioneer. DNA sequences and modifications are listed in Table 1. Trisodiumcitrate dehydrate, dithiothreitol, and NaCl were purchased from Merck. HEPES, tetrachloroauric acid (HAuCl₄), cetyltrimethyl ammonium bromide, silver nitrate, sodium borohydride, ascorbic acid, and other chemicals for buffer preparation were purchased from Sigma-Aldrich.

Synthesis and Characterization of GNPs

Gold nanoparticles were synthesized by the citrate reduction of HAuCl₄ according to Mirkin et al [7]. GNPs were also characterized by a UV-vis absorption spectrophotometer (Cary 100 spectrophotometer). The spectra were recorded within 400–700 nm wavelength range. Transmission electron microscopy images were recorded on a TE 2000 Ziess TEM. Prior to imaging, GNPs were centrifuged at 15,000 rpm for 15 min, and the purified sample was sonicated for several minutes. Sample was deposited on a carbon-coated copper grid and left undisturbed for TEM characterization.

Preparation and Characterization of Linker DNA

The Linker DNA (DNA 3 and 4) is a double-stranded sequence, which has one 10 base pair overlap (A-T rich) and 8-base single-stranded “sticky ends” that are complementary to the oligonucleotides attached to GNPs (DNA 1 and 2). Stock solutions of Linker DNA were prepared in hybridization buffer A (50 mM HEPES, pH 7.8, and 50 mM NaCl) according to the annealing program by a thermoblock.

Preparation and Functionalization of GNPs

For nanoparticle assembly, two batches of GNPs (with average diameter of 10 nm) were chemically modified with 3' thiol-capped 8 base DNA strands (DNA 1 and 2), as previously reported by Mirkin's group [7]. To remove unbound DNA fragments, 50 µL of both sets of functionalized GNPs

Table 1 The DNA sequences and modifications

Name	Sequences and modifications(listed from 3' to 5')
DNA1	SH-CTACTG AT
DNA2	SH-GTTGCT GA
DNA3	T ₁₀ TCA GCAAC
DNA4	A ₁₀ ATC AGT AG

was centrifuged at 15,000 rpm for 15 min. Thiol-DNA functionalized GNPs were mixed (1:1 ratio) and dispersed in 500 μL of buffer B (25 mM HEPES, pH 7.6, 300 mM NaCl). Surface plasmon resonance of GNPs conjugated with thiol-modified DNA immediately after dispersed in buffer B was monitored by UV-vis spectroscopy as a function of time.

GNPs Assembly

In order to monitor the GNPs assembly, addition of the linker DNA is necessary to initiate the process. Therefore, after 48 h, two sets of oligonucleotide functionalized GNPs were mixed at 1:1 ratio, as previously reported by Mirkin's group. Then, 1 μL of 10 μM DNA linker solution was added to give a final concentration of 200 nM [7].

GNPs assembly was traced 24 h after addition of DNA linker, when the macroscopic nanoparticle aggregates were formed, with a color change of red to purple, with recorded the SPR alterations of the assembled GNPs by UV-vis spectroscopy.

Far-UV Circular Dichroism Studies

Interaction of a chiral molecule with polarized light is very specific and can be utilized as an important technique for monitoring macromolecular structures. With the help of circular dichroism spectropolarimeter, any conformational changes that might be induced in the structure of macromolecules could be assessed [12]. To investigate alterations in the secondary structure of oligonucleotide strands in the DNA linker and assembly process (both DNA linker and thiol-modified DNA), time-dependent CD experiments were performed on a Jasco J-715 spectropolarimeter. It should be noted that concentration of DNA linker was adjusted at 4 μM (in 50 mM HEPES buffer, pH 7.8, and 50 μM NaCl).

Plasmonic CD Studies

Plasmonic CD of hybrid nanostructures containing noble metals and chiral molecules has received increasing interest in nanophotonics. In this study, conjugation of GNPs with thiol-modified DNA and assembly of GNPs with DNA-linker was traced using plasmonic CD in the UV-vis region (200–900 nm). DNA-modified GNPs were centrifuged twice to remove unreacted DNA from the solution and redispersed in 500 μL of buffer B (25 mM HEPES, pH 7.6, 300 mM NaCl). DNA linker (4 μL , 30 μM) was added to the solution of GNPs. Final spectra were obtained by subtracting the buffer contribution from the original spectra. Data were smoothed and analyzed by J-715 software, using fast Fourier transform to prevent data distortion.

Dynamic Light Scattering

Size of DNA-GNPs clusters in aqueous media was determined by DLS. This allows monitoring the hydrodynamic diameter of materials in solution. DLS measurements were performed on a Malvern Zetasizer Nano series ZS instrument, equipped with a HeNe laser operating at 632.8 nm and a scattering detector at 173°. Prior to mixing, samples of DNA-modified GNPs were filtered through a 0.22- μm acetate membrane to remove any dust particles. DNA-linked GNPs aggregate samples were also prepared as described above.

Results and Discussion

Assay Formats and UV-Visible Studies

Formation of the gold nanoparticle was characterized by transmission electron microscopy and UV-vis spectroscopy (Fig. 1). Typical surface plasmon absorption band of GNPs in the visible region appeared at 520 nm. The average diameter of GNPs was estimated to be 10 nm (see the inset of Fig. 1).

Formation of GNPs aggregate is outlined in Scheme 1. First, batches of GNPs were chemically modified with thiol-capped 8-base DNA strands (DNA 1 and 2), as mentioned in the previous section. The DNA-modified GNPs solutions are red in color, with a plasmon band positioned at 535 nm. The aggregation phenomenon starts when the DNA linker is introduced to the particles. The 8-base “sticky ends” of DNA linker sequences (DNA 3 and 4) were designed to be complementary to the DNA attached to the nanoparticles (DNA 1 and 2) with a “duplex spacer” portion of 10 bases (A-T rich).

At the first stage, formation of duplex spacer portion of the DNA linker was confirmed by monitoring its absorbance in the region at 200–300 nm (Fig. 2a). It is well known that absorbance of nucleic acid strands undergoes hypochromicity upon hybridization with their complementary strands. Since the population of single bases is remarkable compared to that of base pairs, it is not expected to observe meaningful hypochromicity in this study.

Figure 2b and c depicts formation of functionalized GNPs with thiol-modified DNA as a function of time, and DNA-linked gold nanoparticle aggregates, by monitoring the surface plasmon band in the visible region. According to Fig. 2b, upon functionalization with thiol-modified DNA, the surface plasmon band of GNPs slightly red shifted with remarkable decrease in intensity. The reason behind this is sensitivity of nanoparticles with plasmonic nature to trace changes in the refractive index of the surrounding medium. Therefore, in the vicinity of functional biomolecule, the characteristic surface plasmon absorption band of GNPs undergoes wavelength shifts and experiences decrease in intensity [17].

Fig. 1 UV–visible spectrum of GNPs with characteristic TEM image

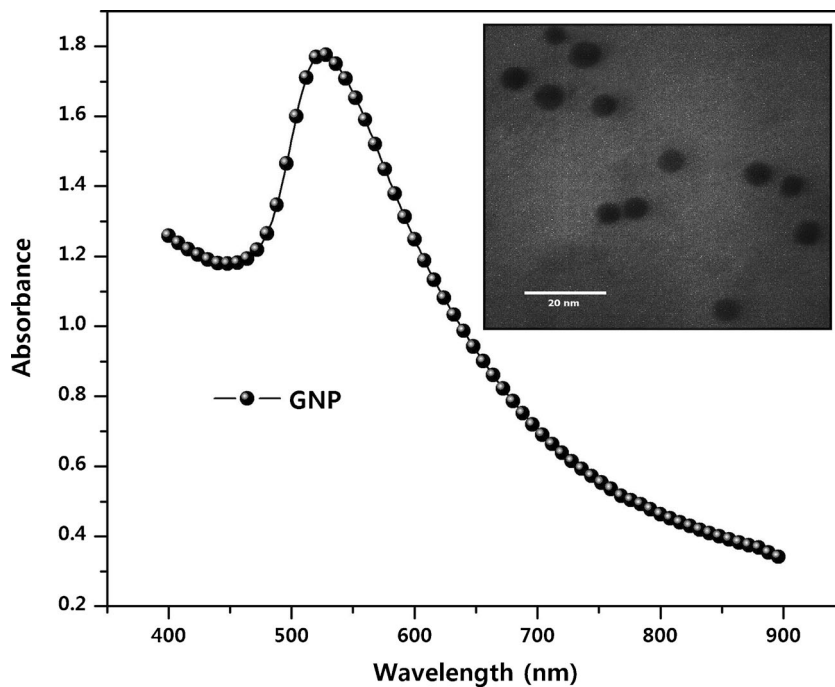
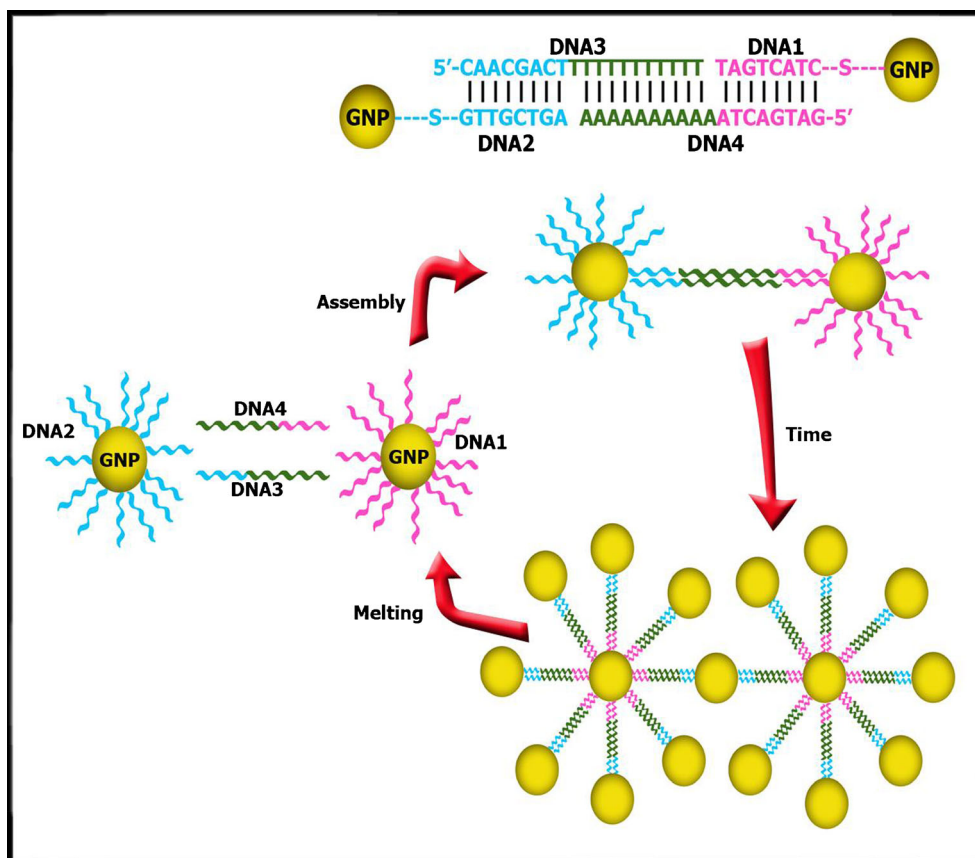


Figure 2c shows the aggregation phenomenon, which occurred via DNA hybridization in the presence of DNA linker,

leading to gradual red shift of the SPR band from 535 to 567 nm.

Scheme 1 Schematic representation of DNA-linked assembly of GNPs



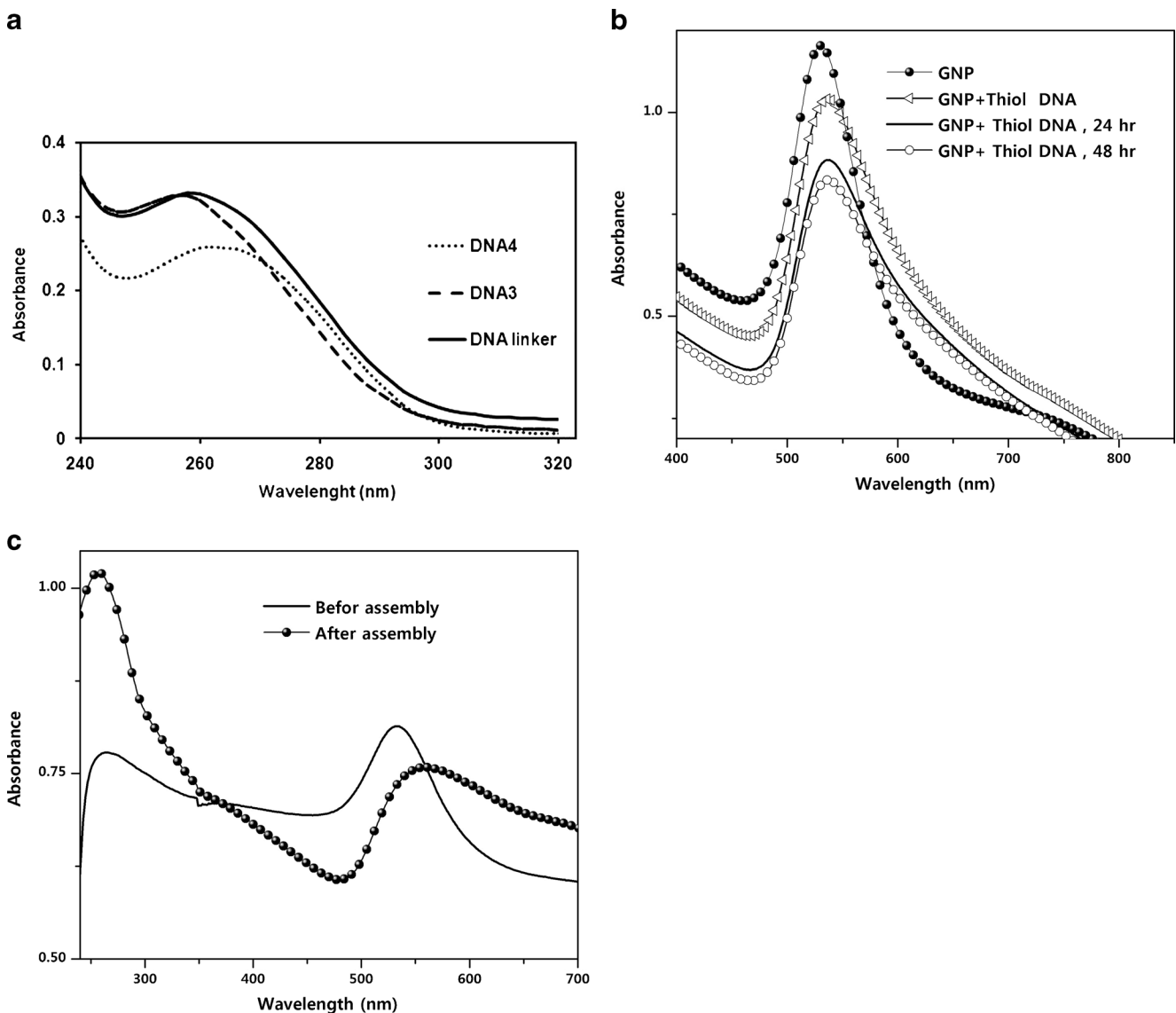


Fig. 2 UV-visible spectra of **a** DNA linker and its single strands, **b** DNA functionalized GNPs, and **c** GNP-DNA aggregates

It is worth to mention that, before the formation of macroscopic DNA-linked aggregates, the color of GNPs solution was red, whereas, after annealing, the solution develops purple color. Such optical changes could be explained as a result of DNA hybridization, leading to changes in interparticle distance and couple of plasmon waves of nanoparticles. On the other hand, quality of change in the surface plasmon band is inversely dependent on the distance between GNPs [8].

Circular Dichroism Studies

The early steps of GNPs aggregation might not be reflected by UV-vis spectroscopy. Therefore, CD spectroscopy was utilized to investigate the importance of DNA hybridization in this study. CD spectroscopy could be a useful tool for tracing the annealing process [12]. The technique can monitor signals

from nucleic acid hybridization, which is responsible for aggregation of GNPs.

At the very first step, formation of the complementary portion in DNA linker must be confirmed by investigating changes in the secondary structure of nucleic acid. Figure 3a illustrates the CD spectrum of DNA linker and its single stranded (DNA 3) in the wavelength region of 200–300 nm.

Circular dichroism revealed that DNA duplex is in a B-form conformation, as evidenced by a negative band at 245 nm and a positive band at 280 nm. After the formation of duplex portion in DNA linker, the positive peak at 280 nm increased, whereas the negative peak at 245 nm did not show any significant change in the CD signal compared to that of single stranded DNA. Such data confirmed formation of hydrogen bonds between the complementary place of two strands (AT-rich internal region).

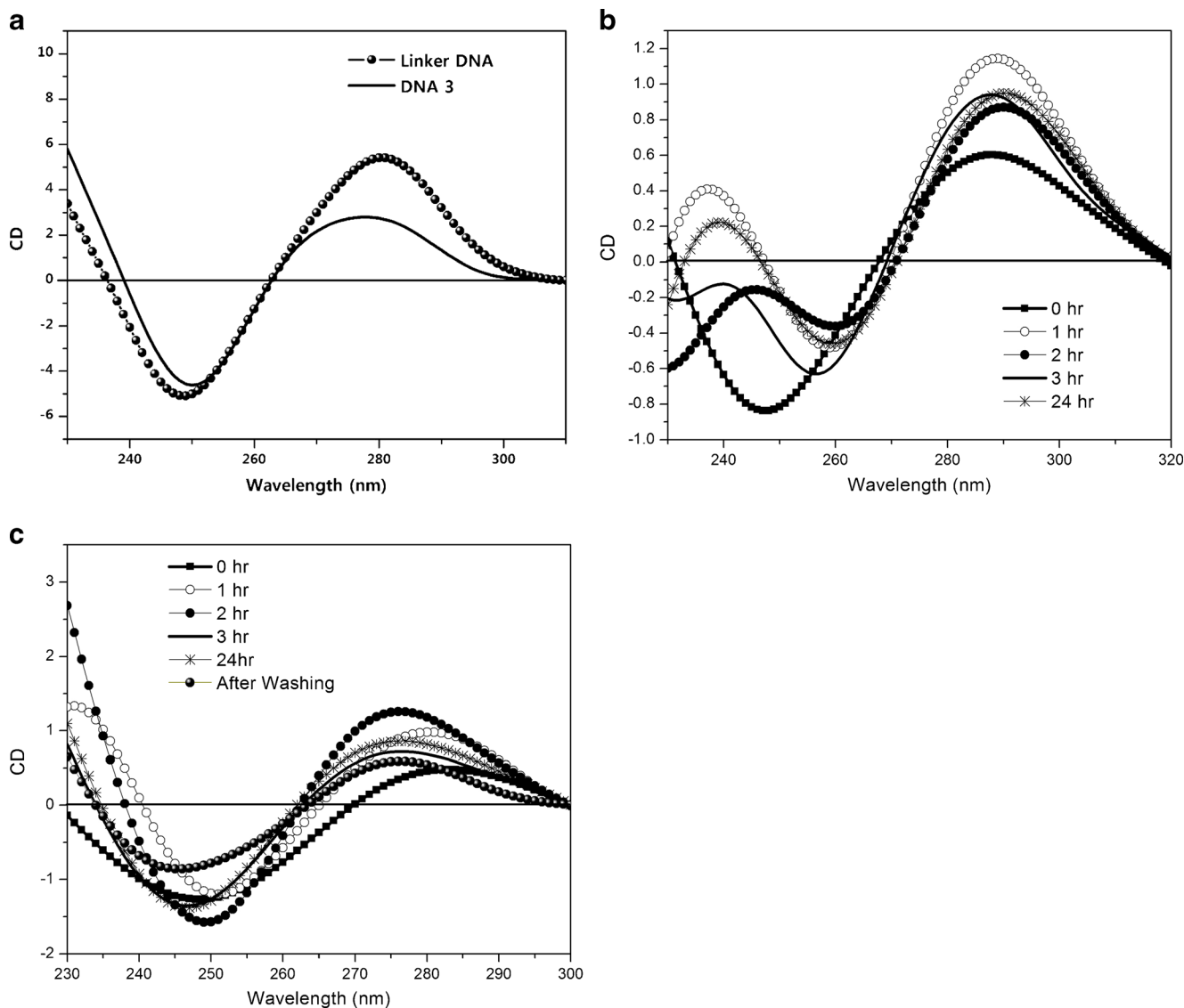


Fig. 3 CD spectra of **a** DNA linker and its single strands, **b** GNPs-DNA bioconjugate, and **c** GNP-DNA aggregates

In the next step, kinetics of GNPs functionalization with thiol-modified DNA (DNA 1 and 2) was investigated by monitoring the CD signals. The bisignated CD peaks of DNA remained the same after attachment to the surface of GNPs (Fig. 3b). The negative CD signal at 248 nm gradually decreased with a red shift after immobilization of thiol-modified oligonucleotides on GNPs, while the positive peak at 288 nm did not show any significant shift. Such alterations in CD intensity and peak position could be correlated with attachment of nanoparticles, leading to alterations in the conformation of oligonucleotides. Further time-dependent investigations (up to 24 h) showed that there is no more shift in the CD peak positions of nanobioconjugates. Appearance of shifts during different time intervals could be considered as a useful tool for tracing functionalization of GNPs with thiol-modified DNA.

Figure 3c depicts kinetics of GNPs aggregation by monitoring the CD signal of hybridization between single-stranded “sticky ends” with DNA-conjugated GNPs (DNA 1 and DNA 2). In the presence of DNA linker, the bisignated CD peaks were observed at 248 and 283 nm, with increase in the intensity of both negative and positive peaks. Kinetic studies of the aggregation phenomenon increase in CD intensity. Twenty-four hours after the assembly process, samples were centrifuged for purification. CD spectrum of the purified self-assembled nanostructures retained its characteristic peak positions with lower intensity, which could be correlated with removal of less efficient self-assembled nanobioconjugates, or loss of unhybridized DNA linkers.

This is easy to understand, since a series of dynamic geometry changes appear upon hybridization of single-stranded “sticky ends” of DNA linker with DNA conjugated

GNPs (DNA 1 and 2). Therefore, in the presence of DNA linker, CD intensity alterations could be correlated with increased number of hydrogen bonds between the complementary bases pairs and higher degree of DNA helix formation during the aggregation process. Formation of DNA helices itself induces decrease of interparticle distance and geometric alterations of GNPs, leading to stronger CD signals in the ultraviolet region [12].

Plasmonic CD Measurement

Plasmonic CD responses could be used as a new method for detection of morphological changes in DNA-GNPs assembly [14]. Typical plasmonic circular dichroism of bare DNA and GNPs are shown in Fig. 4a. GNPs show a single plasmonic signal at 530 nm, and unlike GNPs, DNA do not show any

particular CD signal in the visible region. Figure 4b illustrates immobilization of thiolated DNA onto the surface of GNPs by plasmonic CD. Such spectrum arises due to the coupling of SPR signal of GNPs and the chiral signal of DNA in the visible wavelength region (500–600 nm). Figure shows the characteristic plasmonic CD spectra of GNPs before and after functionalization with DNA. A clear change in the spectral intensity has been observed, which could be correlated with nanoparticle–biomolecule interaction.

Kinetic plasmonic CD spectra of DNA-GNP assembly are depicted in Fig. 4c with TEM image. After 2 h, the PCD signal starts to develop changes, showing the progress of the assembly process. At the beginning, GNPs shows weak CD signal in the visible region, with notable increase in intensity after 3 h. Extension of time interval to 24 h showed that PCD intensity does not change considerably after 24 h. PCD spectrum of the

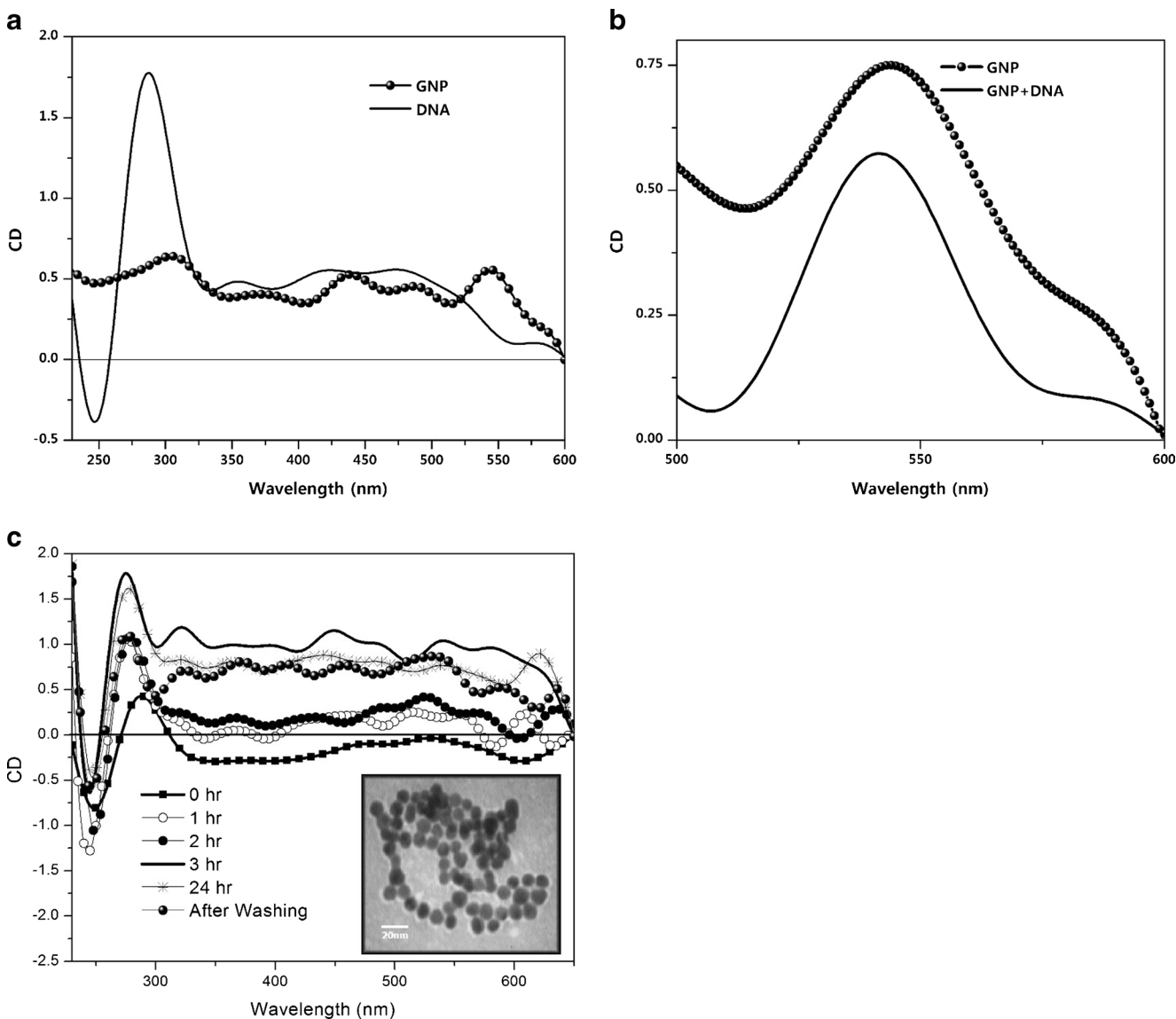


Fig. 4 Plasmonic CD of a bare DNA and GNPs, b GNP-DNA bioconjugate, and c GNPs assembly with TEM image

purified assembled samples (by washing) also showed maintenance of the signal in its characteristic region. Although results of this investigation indicate existence of plasmonic CD signals even before the washing process, Gérard et al. [16] reported appearance of such signal only after the purification process. Transmission electron microscopy image of the self-assembled nanostructures is shown in the inset of Fig. 4c.

The reason for enhancement of plasmonic CD signals is that NP–NP coulomb coupling in a chiral NP assembly splits plasmon resonances of individual NPs and creates preferentially left-handed collective plasmonic modes on the one side and right-handed modes on the other side of the plasmon resonance [13, 16]. In fact, enhancement of plasmonic CD signals arises due to the coupling of chiral signal of DNA with SPR waves of GNPs in the visible region, being very sensitive to the geometry of complex. The SPR coupling itself is mainly related to the interparticle distance of GNPs. Presence of DNA linker with sticky ends in the reaction medium results in hybridization of DNA strands, followed by decrease in

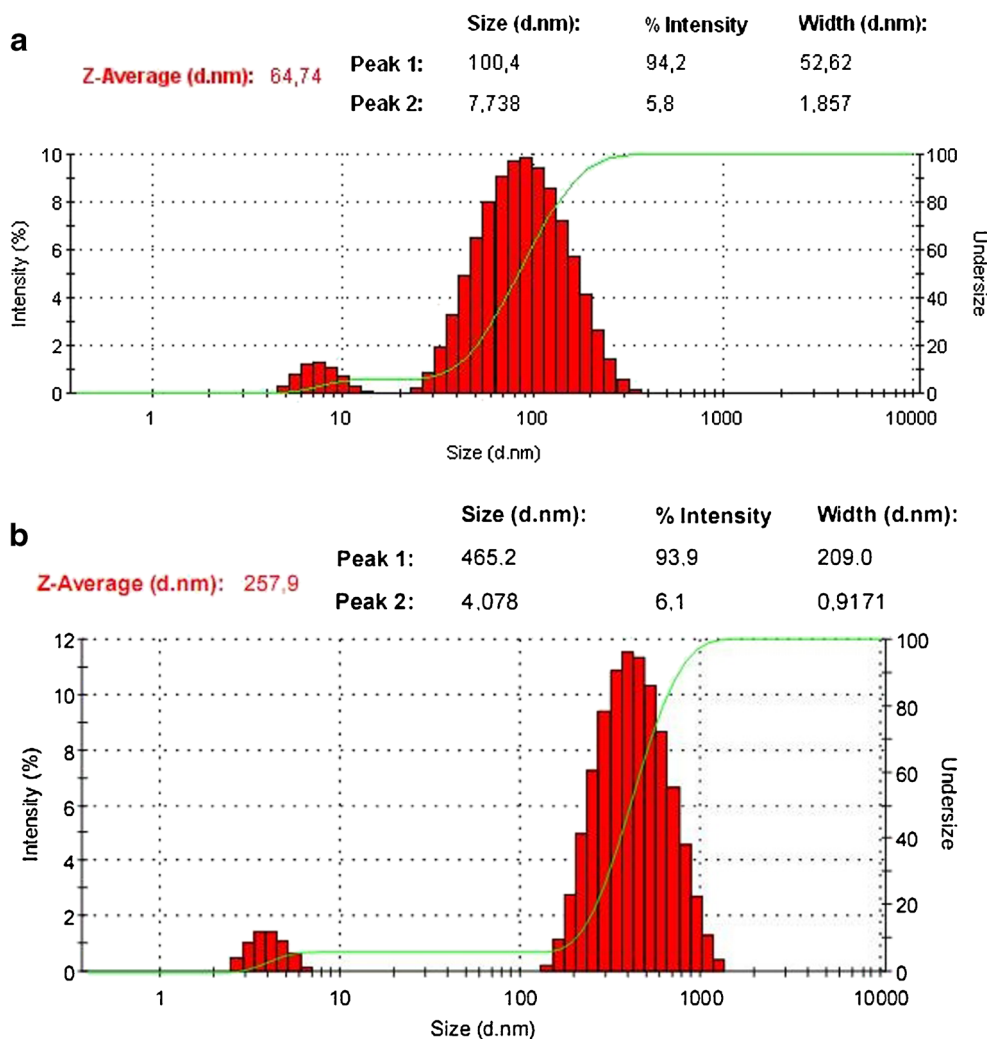
interparticle distance. This could justify formation of hotspot cross-section during the aggregation phenomenon [14–16].

Dynamic Light Scattering Measurements

Dynamic light scattering was performed to compare the size of GNPs before and after assembly (Fig. 5). Considering sensitivity of DLS technique (below 1 μm), size of the macroscopic precipitate is quite out of the range (approximately 3 μm) and cannot be detected accurately by this technique.

The initial aggregations rates suggested that sizes of the preannealed aggregates are significantly different compared to the post annealing. Results of DLS experiments showed that, in the presence of DNA linker before the annealing process, the average hydrodynamic size of GNPs was 64.74 nm (Fig. 5a). The size further increased to 256 nm when annealing was applied (Fig. 5b). The rate of aggregate growth in this system is very complex and depends upon a number of factors. The binding rate of DNA linker (k_1) and the subsequent growth of

Fig. 5 DLS results of GNPs **a** before and **b** after assembly



the aggregates (K_2) could be considered as the top most critical factors [11].

In conclusion, we have demonstrated that hybrid of DNA and GNPs produce remarkable plasmonic CD signals in the visible region of wavelength. The assembly process will increase the number of hydrogen bonds between the pair bases and formation of DNA helices, which strengthens the CD signal. Enhancement of CD signal in the visible region (plasmonic CD) occurs via the coupling of SPR waves on the surface of GNPs, when the interparticle distance decreases as a consequence of DNA hybridization. Assembly of DNA-conjugated gold nanoparticles have been widely used for construction of complex structures and detection of target molecules. Plasmonic CD responses of such assemblies will shed light on creation of intelligent materials with unique optical properties, as well as fabrication of ultrasensitive biosensors. Results of this investigation could be further extended to synthesize a new series of directed nanostructure assemblies of DNA (or any other biomolecule of interest), in conjugation with gold, silver, or alloy nanoparticle clusters. This will lead to high levels of diversity in chemical, physical, and biological applications in nanotechnology.

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References

- Haes AJ, Haynes CL, McFarland AD et al (2005) Plasmonic materials for surface-enhanced sensing and spectroscopy. *MRS Bull* 30:368–375
- Azizi A, Ranjbar B, Khajeh K et al (2011) Effects of trehalose and sorbitol on the activity and structure of *Pseudomonas cepacia* lipase: spectroscopic insight. *Int J Biol Macromol* 49:652–656
- Murray W, Barnes W (2007) Plasmonic materials. *Adv Math* 19: 3771–3782
- Li H, Carter JD, LaBean TH (2009) Nanofabrication by DNA self-assembly. *Mater Today* 12:24–32
- Kuzyk A, Schreiber R, Fan Z et al (2012) DNA-based self-assembly of chiral plasmonic nanostructures with tailored optical response. *Nature* 483:311–314
- Yang D, Campolongo MJ, Nhi Tran TN et al (2010) Novel DNA materials and their applications. *Nanomed-Nanotechnol* 2:648–669
- Mirkin CA, Letsinger RL, Mucic RC et al (1996) A DNA-based method for rationally assembling into macroscopic materials. *Lett Nature* 382:607–609
- Tan SJ, Campolongo MJ, Luo D et al (2011) Building plasmonic nanostructures with DNA. *Nature Nanotechnol* 6:268–276
- Park SY, Gibbs-Davis JM, Nguyen ST et al (2007) Sharp melting in DNA-linked nanostructure systems: thermodynamic models of DNA-linked polymers. *J Phys Chem B* 111:8785–8791
- Storhoff JJ, Lazarides MRC et al (2000) What controls the optical properties of DNA-linked gold nanoparticle assemblies? *J Am Chem Soc* 122:4640–4650
- Jin R, Wu G, Li Z et al (2003) What controls the melting properties of DNA-linked gold nanoparticle assemblies? *J Am Chem Soc* 125: 1643–1654
- Ranjbar B, Gill P (2009) Circular dichroism techniques: biomolecular and nanostructural analyses—a review. *Chem Biol Drug Des* 74:101–120
- Fan Z, Govorov AO (2010) Plasmonic circular dichroism of chiral metal nanoparticle assemblies. *Nano Lett* 10:2580–2587
- Li Z, Zhu Z, Liu W et al (2012) Reversible plasmonic circular dichroism of Au nanorod and DNA assemblies. *J Am Chem Soc* 134:3322–3325
- George J, Thomas KG (2010) Surface plasmon coupled circular dichroism of Au nanoparticles on peptide nanotubes. *J Am Chem Soc* 132:2502–2503
- Gérard VA, Gun'ko YK, Defrancq E et al (2011) Plasmon-induced CD response of oligonucleotide-conjugated metal nanoparticles. *Chem Comm* 47:7383–7385
- Anker JN, Hall WP, Lyandres O et al (2008) Biosensing plasmonic nanosensors. *Nat Math* 7:442–453