Plasmonic Response of DNA-Assembled Gold Nanorods:

Effect of DNA Linker Length, Temperature and

Linker/Nanoparticles Ratio

Stephanie Vial^{a, I, *}, Dmytro Nykypanchuk^b, Francis Leonard Deepak^a, Marta Prado^a, and Oleg Gang^b

^a International Iberian Nanotechnology Laboratory, Avenida Mestre Jose Veiga s/n, 4715-330, Braga, Portugal. Emails: leonard.francis@inl.int; marta.prado@inl.int

^b Center for Functional Nanomaterials, Brookhaven National Laboratory, Upton, NY 11973, United States. Emails: dmytro@bnl.gov; ogang@bnl.gov

*Corresponding Author (Email: stephanie.vial@dep.uminho.pt)

^I 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, 4806-909 Taipas, Guimarães, Portugal and ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

Abstract

Optical properties of gold nanorod (AuNR) particles self-assembled with DNA are systematically investigated. The particles assembly is driven by specific base-pair recognition between single strand (ss) DNA linker and DNA anchored to AuNRs, and it results in the distance- and morphology-dependent plasmonic coupling of AuNRs. The longitudinal plasmon band is distinctly affected by tuning the length of DNA linker, the temperature and linker/AuNRs. We observed that the increase of temperature enhances the interparticle interactions and leads to clear distinguishable plasmonic signals between linker lengths up to 100 bases. Both absorbance decrease and shift of the longitudinal plasmon allow for use of AuNR for the DNA sensing applications.

Keywords: plasmon coupling, gold nanorod, DNA, self-assembly

1. INTRODUCTION

The assembly of gold nanoparticles (AuNPs) and the control of their hierarchical organization in one, two, and three dimensions are attracting great current interest for applications such as optoelectronic, sensing and drug delivery.^{1,2} AuNPs present Localized Surface Plasmon Resonance, a collective oscillation of the conduction electron at the metallic surface stimulated by incident light, a property that is greatly affected by the collective interactions of the nanoparticles. For individual gold spheres, typically, the excitation of the plasmon occurs in the visible region and is easily detected with UV-visible spectroscopy or even by eye. A small change in the size, shape, local environment, surface nature, interparticle distance and degree of aggregation of gold nanoparticles leads to a change in their optical properties. The use of gold nanorods (AuNRs) as an alternative to spherical nanoparticles was studied and showed interesting features.^{3,4} AuNRs present two surface plasmon resonances (SPR) – longitudinal and transverse.⁵ The longitudinal plasmon band (LPB) maximum is, typically, in the near-infrared region and its position is highly sensitive to the local environment making them more versatile as a platform for biodetection.⁶ Also, nanorods, unlike isotropic spheres, can assemble end-to-end or side-by-side providing two distinguishable signals depending on the interaction pathway, potentially leading to a multilevel detection. For end-to-end interactions one expects red shift of LPB while side-by-side assembly affects both plasmon bands and leads to a blue shift in LPB and the red shift of the transverse one, as shown by both discrete dipole simulations⁷ and experimental studies.⁸⁻⁹

Different AuNPs arrangements from multimers¹⁰⁻¹³ to 3D well-ordered nanostructures^{14, 15} were built up thanks to the possibility of using the programmability of

DNA base pairing as outstanding strategy for controlled attachment of nanoparticles. In terms of plasmon effect,¹⁶ tuning the length and the sequence of DNA and thus the distance between particles and the nanoparticles orientation is attractive in order to modulate the optical properties of nanoparticles-based systems. Therefore, promising nanoparticle-based DNA sensors showing high sensitivity and selectivity were widely developed.¹⁷⁻²¹

The use of DNA as a template might provide a variety of rods orientation in the assembled state leading to new and more interesting plasmonic responses. For example, assembly of nanorods in well-defined 2D²² and 1D²³ architectures has been demonstrated. Moreover, the use of triangular DNA origami structures²⁴ showed the ability to direct the organization of dimers with control of the distance and angle between rods, which results theoretically and experimentally to diverse plasmonic signatures.

The length, the flexibility of the DNA linker, the temperature and the ionic strength has shown^{13,16,21,25-27} to be very important in defining the performance of spherical nanoparticles-based DNA system as manifested via SPR coupling. However, to date, no specific investigation regarding how these factors may affect the plasmon coupling in AuNR system with DNA mediated interactions has been reported. In order to establish an efficient and reliable method to control the interparticle interactions, the parameters affecting the surface plasmon resonances must be carefully evaluated and characterized.

In the present study, the plasmon coupling arising from the self-assembly of AuNRs driven by hybridization between ss-DNA linker and DNA-functionalized to AuNRs was systematically investigated. DNA linker length, the temperature and DNA linker/AuNRs

ratio were studied and discussed in order to determine their effects on the optical properties. Upon adding the linker, a side-by-side AuNRs assembly is immediately induced as noticed by a blue shift of LPB.²³ This trend is observed in our system using flexible DNA linker and elevated temperature of assembly. On the other hand, *Parab et al*²⁸ have reported a red shift of LPB using a more rigid system designed for the detection of 24-mer ss-DNA at room temperature. We also find that temperature significantly affects the interactions between nanorods, which leads to increase in plasmon coupling strength at higher temperatures. The findings might be relevant for a number of biosensing applications, and the results are discussed in the context. We report that whereas, previous studies^{28,29} follow the drop of absorbance to detect shorter DNA, LPB blue shift can be used for DNA detection, quantification and length discrimination at least up to 100 bases when the particles get organized in a side-by-side fashion.

2. MATERIALS AND METHODS

2.1 Chemicals and Materials

Gold (III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), silver nitrate (AgNO_3), ascorbic acid 99%, sodium borohydride (NaBH_4), cetyltrimethylammomium bromide (CTAB) and phosphate buffer solution 1 M were purchased from Sigma-Aldrich. All the oligonucleotides were purchased from Sigma Life Science. GE Healthcare NAP-5 Columns (Sephadex G-25 DNA grade) were purchased from VWR.

2.2 Nanorods synthesis

The gold nanorods were synthesized by the seed-mediated method.³⁰ First, a gold seed solution was prepared by the borohydride reduction of 0.25 mM HAuCl₄ in an aqueous 0.1 M Cetyltrimethylammonium bromide (CTAB) solution. Subsequently, the seed solution was added to a 10 ml growth solution containing 0.1 M CTAB, 0.5 mM HAuCl₄, 0.7 mM ascorbic acid and 0.06 mM silver nitrate. The solution was aged for 24 h to ensure the complete formation of AuNRs. This protocol gives CTAB-stabilized gold nanorods, 40 nm $\pm$ 3 nm long, with an average diameter around 12 nm $\pm$ 1.7 nm (Figures S1a and S1b). The concentration of gold nanorods was determined at the LPB peak maximum using molar extinction coefficient $\epsilon = 4.10^9 \text{ M}^{-1} \text{ cm}^{-1}$.³¹

2.3 Nanoparticle modification with DNA

Single-stranded thiolated-DNAs were immobilized on the surface of the nanorods through S-Au bonds following the procedure that we have developed previously.²³ First, DNA for particle functionalization was reduced by adding dithiolthreitol (DTT) solution and purified with a sephadex column (NAP-5, GE Healthcare) by elution with 10 mM phosphate buffer (pH = 7.4). From the gold nanorods stock solution, two different batches of particles were, separately, functionalized with Sequence-A DNA and Sequence-B DNA (see Table S1 for DNA sequences). Before DNA functionalization, the gold nanorods were centrifuged twice and supernatant was exchanged with purified water in order to remove the excess of CTAB. Typically, an excess of 3000 purified DNA per nanorods was added to 1 ml of a purified solution of AuNRs ($\sim 2 \text{ nM}$), and the mixture incubated for at least 12 h at room temperature. The salt aging process was carried out in the presence of 1% w/v sodium

dodecylsulfate. The mixture was brought to 10 mM concentration of phosphate buffer (pH = 7.4), and left for 1 h. Afterwards, the salt concentration was increased stepwise in order to reach a final concentration of 0.5 M of NaCl, and the samples incubated overnight. The solution was freed of excess DNA by three consecutive centrifugations and supernatant exchange of phosphate buffer 10 mM, pH 7.4 and 0.5 M of NaCl. The average number of thiolated DNA strands attached to AuNRs, about 500-600 DNA per AuNRs, was determined from changes in the DNA concentration before and after rods functionalization, as measured by UV-visible spectroscopy.³²

The DNA-AuNRs were stable for at least 2 weeks at this salt concentration. The final concentration of AuNRs was adjusted to 0.35 nM.

2.4 DNA-AuNRs assembly at 25 °C

Particle assembly was carried out at 25 °C by combining equimolar amounts ([AuNRs] = ~ 0.35 nM) of sequence-A and sequence-B DNA-capped gold particles with the corresponding DNA linkers (see Table S1 for DNA linker sequences) in 100 μ l solution of 10 mM phosphate buffer, 0.5 M NaCl, pH = 7.4. The UV-visible spectra of the assembled AuNRs were monitored from 1000 to 450 nm using a Shimadzu UV-2550 spectrometer (wavelength accuracy is $\pm$ 0.3 nm and the photometric accuracy is $\pm$ 0.004 absorbance) with temperature controlled cell holder.

For ease of data comparison, we have normalized the absorption of all spectra by the maximum of the longitudinal resonance of the AuNRs sample before linker addition. The shift of the LPB ($\Delta\lambda$) and the absorbance drop at the maximum position of the DNA-capped

AuNRs located at 770 nm versus time of incubation were plotted in order to follow in detail the LPB responses arising from AuNRs self-assembly.

2.5 Thermal profile procedure

For temperature dependent studies we have used the following procedures. Heating process: The corresponding linker at linker to AuNRs ratio 100 was incubated in an equimolar mixture of sequence-A and sequence-B DNA-capped gold AuNRs at 25 °C for 30 minutes to initiate gold nanorods assembly. Then, the different samples were heated to a corresponding temperature and UV-visible spectra were taken after 5 min incubation at the required temperature.

Annealing process: After 15 min at 70°C, each sample was cooled down to 60 °C. UV-visible measurement was performed after 10 min incubation time. All absorption spectra were normalized at the longitudinal plasmon band maximum.

2.6 DNA-AuNRs assembly at 60 °C

Particle assembly was carried out at 60 °C by combining equimolar amounts ($[AuNRs] = \sim 0.35 \text{ nM}$) of sequence-A and sequence-B DNA-capped gold particles with the corresponding DNA linkers in 100 μl solution of 10 mM phosphate buffer, 0.5 M NaCl, pH = 7.4. For the Linker/AuNRs (R) ratio studies, the same procedure was used for L33 at different concentrations in order to obtain $R = 2.5, 10, 25, 50, 100$.

The UV-visible spectra of the assembled AuNRs were monitored from 1000 to 450 nm using a Shimadzu UV-2550 spectrometer with temperature controlled cell holder. For ease of data comparison, we have normalized the absorption of all spectra by the maximum of

the longitudinal resonance of the AuNRs sample before linker addition. The shift of the LPB ($\Delta\lambda$) and the absorbance drop at the maximum position of the DNA-capped AuNRs located at 770 nm versus time of incubation were plotted in order to follow in detail the LPB responses arising from AuNRs self-assembly.

2.7 Characterization

Transmission Electron Microscopy (TEM/HRTEM) of the AuNRs was carried out at 200 kV with 80-200 kV probe corrected Titan ChemiSTEM microscope. For the TEM analysis, the Au nanorods were prepared by adding a drop of the solution onto a lacey carbon Cu grid (300 mesh, Ted Pella) and allowing it to dry. Images were acquired with GATAN ULTRASCAN 1000 P camera controlled with a Digital Micrograph software integrated in the microscope's user interface.

3. RESULTS AND DISCUSSION

In this work, the particle assembly is induced by DNA hybridization. The design as shown in Figure 1 allows an external ss-DNA to link the AuNRs through base pair recognition with complementary DNA strands attached to the NPs. The DNA sequences are shown in Table S1. Well-dispersed CTAB-gold nanorods were prepared and present an average length of $40\text{ nm} \pm 3\text{ nm}$, a diameter of $12\text{ nm} \pm 1.7\text{ nm}$ with an aspect ratio of approximately 3.3, as characterized by UV-vis spectroscopy and TEM imaging (Figures S1a and S1b). Two sets of AuNRs were separately functionalized with two non-complementary 20 and 23-base ss-DNAs, named A and B (Figure 1 and Table S1). The ss-DNA were composed of two parts: the recognition part consisting of 15 and 18 bases for A

and B, respectively, and a 5 bases poly(T) spacer between the AuNRs surface and the recognition part. The UV-vis spectra before and after the DNA functionalization, shown in Figure S1c, confirmed the presence of DNA on the AuNRs surface with the appearance of a new peak at ca. 260 nm.

DNA-AuNRs assembly

The particle assembly was driven by Watson-Crick hybridization of 15 bp AA' and 18 bp BB' between the recognition parts of the DNA attached to AuNRs and the corresponding ends of the A'B'-ss-DNA linker (Figure 1). Three flexible ss-DNA linkers of 33, 52, and 100 bases denoted L33, L52, and L100, respectively, were designed for this study. For each of them, the recognition parts were conserved, only the spacer between ends was increased by adding poly dT sequence of 0, 19 and 67 bases, respectively (Figure 1).

Initially, to confirm that the gold nanorods self-assembly was induced by the presence of the linker, the absorbance of equimolar mixture of sequence-A and sequence-B DNA-capped gold nanorods without the linker (Figure 2) was measured. The spectra did not change for at least 30 min at 25 °C, showing that the particles were stable and no aggregation occurred. Whereas, the addition of a linker at linker/AuNRs ratio = 100 after 30 min at 25 °C led to a significant change of spectral shape suggesting particle aggregation (Figure 2).

Many parameters govern the efficiency of DNA hybridization, such as buffer pH and ionic strength, DNA sequences, the hybridization temperature and the DNA concentration. Keeping the buffer conditions (pH and ionic strength) constant, the effect of linker length,

the temperature and linker/AuNRs ratio were studied in order to investigate their influence on the nanoparticles interactions and plasmon coupling.

The effect of linker length at 25 °C

Figure 2 shows that the linker length has a dramatic effect on the spectral signature and the linker induced assembly of nanorods. For all systems, the longitudinal plasmon band is blue shifted and the shift is accompanied by a decrease in absorbance intensity and peak widening, whereas the transverse band showed a slight red shift of 4 nm for L33 and 2 nm for L52 and L100. This behavior suggests a side-by-side assembly of gold nanorods.^{7, 23} As we have recently reported,²³ the rods are organized in 1D ladderlike ribbons structure favored by a side-by-side alignment when using flexible DNA linker. The side-by-side rod arrangement leads to a blue shift of the LPB due to the repulsive dipole-dipole interactions, which results in the band shift to a higher energy relatively to isolated rods.⁷ The DNA mediated assembly is often a hierarchical process that involves bond and structure relaxation at different time and length scales. Thus it is important to understand the time evolution of the assembled structures. Figure 3 presents changes of absorbance at 770 nm and the variation of the LPB position $\Delta\lambda$ ($\Delta\lambda = \lambda_t - \lambda_{t=0}$) *versus* the time of incubation (t). The largest drop in absorbance occurs within the first 10 minutes upon linker addition. Then, a plateau is reached for L52 and L100 linkers, whereas for L33 the absorbance continues to decrease slightly which may relate to the onset of precipitation of AuNRs from the solution for the shortest linker. Moreover, the LPB underwent a maximum blue shift of 2, 8, and 6 nm just after 5 min of incubation for L33, L52, and L100, respectively.

In general, one expects that shorter linkers should bring nanoparticles closer thus facilitating the stronger plasmon coupling and leading to a larger shift. From a universal scaling behavior,³³ the plasmon coupling is interparticle distance dependent – shorter distance between particles leading to the larger plasmon shift. As we have shown previously²³, the interparticle distance is increased by using longer DNA linker leading to a decrease of the plasmon coupling, as evident from the diminution of the longitudinal plasmon band variation. However, here, the observed dependence of LPB at 25 °C on linker lengths is somewhat counterintuitive – shortest linker, L33, showing the smallest shift in LPB; and then, for L52 and L100, the system recovers the expected behavior – larger plasmon shift for the shorter linker (Figure 3). For anisotropic object such as a rod, as we have discussed above, the plasmon shift direction and magnitude are governed by both interparticle distance and the assembly orientation (side-by-side, random or end-to-end). Since only side-by-side orientation leads to a blue shift of LPB, plasmon coupling of shortest linker, L33, should be the largest. However, the observed LPB blue shift is the least pronounced that might be induced by a larger orientational disorder compared to longer linkers in side-by-side assembly, which counterbalances the expected strong coupling in the presence of L33. This behavior can be explained by a reduced flexibility of nanostructures assembled with short linkers.^{23,27} Since the rod concentration in the assembly solution is low, one does not expect the prealignment of nanorods due to entropic effects and upon initial contact of nanorods during the assembly, their mutual orientation should be close to random. If, then, the connection between nanorods is established with a short rigid linker, such as L33, the initial randomness in the orientation should be preserved as nanorods would have fewer degrees of freedom to adjust mutual orientation in comparison with larger more flexible linkers. Also, long flexible linkers have

shown to favor the formation of a crystalline structure that in the case of gold nanorods brings the particles to be aligned in side-by-side orientations.²³

In term of sensing, considering the linker as the target, the results indicate that the detection scheme is sensitive to the ss-DNA targets up to 100 bases long, in contrast to previous AuNRs systems studies where much shorter targets were used, and as well suggest size discrimination,^{28,29} thus, potentially, widening the applicability of our system for DNA detection or biodetection.

However, the diverse optical signal observed with the different linker lengths due to the structural differences at the assembly are not beneficial for size discrimination. To address this issue, we conducted temperature dependent studies

Effect of temperature

DNA assembled nanostructures often undergo structural changes with temperature^{14,16,25,26} and may require thermal annealing to transition from metastable to ground state. Thus we investigated how plasmonic response of AuNRs systems changes with the temperature upon heating from 25 °C to assembly melting temperature (Figure 4) and during annealing – cooling from above melting temperature to 60 °C. UV-Vis results are presented in Figures S2 and S3 for heating and Figure 5 for annealing. It was found that thermal treatment of the sample has a significant effect on the plasmonic response of the system and the response changes both qualitatively and quantitatively in heating and annealing regimes.

Plasmonic response, both absorbance decrease and LPB shift (Figure 4), of the samples during the heating process is more pronounced than at 25 °C, but the trend is qualitatively similar. The more obvious absorption decrease and, more importantly,

absorption peak widening, indicate faster assembly at elevated temperatures (Figure S2). Similarly to 25 °C assembly, in the L33 system, the LPB does not shift significantly while the absorbance decreases dramatically with the temperature increase. The L52 and L100 show a blue shift however the effect is much more dramatic – reaching 34 and 20 nm, respectively, at 60 °C - when contrasted with 8 and 6 nm shifts at 25 °C. The increased blue shift for L52 and L100 linkers indicates that temperature improves side-by-side AuNRs orientation and leads to the larger size of the ordered aggregates. This behavior persists until the systems reach assembly melting temperatures (T_m) at 66, 64, and 62 °C for L33, L52, and L100 respectively, and then it starts to recover the plasmon signature of individual nanorods (Figure S3). For all system, the T_m is determined following the absorbance at 770 nm (Figure 4). It can be noticed that, similarly to spherical NPs^{17, 34} assembled with DNA, gold nanorod assemblies exhibit sharp melting profiles caused by collective nature of DNA mediated interactions between nanorods, confirming that DNA hybridization governs the nanoparticles interactions. Moreover, as it was observed by *Smith et al*³⁴, the T_m is linker spacer-dependent since a decrease of 4 °C is observed when the spacer increases from 0 to 67 poly(T) sequences.

However, the T_m can also be determined following the shift of the LPB (Figure 4) as we can see for the system L52 and L100 i.e. when clear blue shift of the LPB is observed during the assembly of AuNRs.

Upon cooling from above melting temperature (70 °C) to 60 °C (Figure 5), each system showed a remarkable blue shift of the longitudinal plasmon band of 42, 16 and 10 nm for L33, L52 and L100, respectively, and a slight red shift of the transverse one. It should be noted that upon this annealing step the system recovered the expected behavior of larger LPB shift with shorter linker length. In addition, the strength of the coupling – blue

shift of LPB - is stronger than that at 25 °C suggesting a rearrangement of the DNA connections and better side-by-side orientation of the nanorods for linkers of all lengths. In-situ structural studies¹⁴ showed that heating to T_m and annealing samples of nanoparticles pre-assembled at room temperature can lead to well-ordered structure due to local re-arrangement of NPs; as, typically, DNA assembled nanoparticles are arrested in meta-stable state if assembled far below the melting temperature of the connecting DNA duplex, and only upon annealing close to the melting temperature they may reach the equilibrium structure. Based on the results obtained for spherical AuNPs system, we can hypothesize that gold nanorods systems are similar and thus, at temperature close to T_m , DNA linkers and, as the result, AuNRs re-organize to form close-to equilibrium structure which for nanorods correspond to side-by-side arrangement²³ thus leading to a more pronounced blue shift of the LPB as compared to assembly at room temperature.

The qualitative change in the LPB shift with the temperature and the significant enhancement of the observed signal shows that the thermal history is of a paramount importance in order to increase the interactions between plasmonic nanorods or other anisotropic nanoparticles. Since the thermal history significantly affects the LPB response of the nanorod assembly and the high temperature annealing improves the strength of the LPB shift, the assembly process and the system spectral response at 60 °C in more detail were investigated (Figure 6). To confirm that working at 60 °C has no effect on the LPB change, the absorbance of equimolar mixture of sequence-A and sequence-B DNA-capped gold nanorods without the linker (Figure S4) was measured. The spectra did not change and no blue shift of LPB was observed for at least 30 min at 60 °C, showing that the particles were stable and no aggregation occurred. During the incubation at 60 °C, we observed broadening, blue shift and absorbance decrease of the LPB for all systems (Figure

6) and the effect was more pronounced than that at 25 °C for each system, as we can see in Fig S5. In contrast to 25 °C assembly, gold nanorods interact side-by-side independently of the linker length, as revealed by a blue shift of LPB. While the linker length defines the magnitude of the blue shift there was no obvious difference observed for the absorbance drop (Fig 6b).

Immediately upon mixing, particles assemble side-by-side and within 5-10 min a maximum LPB blue shift of 36 nm, 24 nm and 14 nm for the corresponding L33, L52 and L100 are observed, and then they remain constant as indicated by a plateau (Figure 6c). These results are in agreement with our structural study²³ of the analogous systems that shows 1D ladderlike ribbon structure in the initial assembly stage due side-by-side interactions. The increasing length of the linker, i.e. the increased gap between particles, decreases the strength of the plasmon coupling, resulting in diminished LPB shift. Interestingly, the absorbance decrease with time is continuous and, unlike LPB shift, it does not reach a plateau at early stages of assembly (Figure 6b). This indicates that LPB shift emerges from a local orientational order of nanorods and not from the total size of the aggregate. Then, for longer time, larger aggregates might start to form, as suggested by a slight red shift and a continuous decrease in absorbance. Based on our previous observations, the later stages of assembly should be attributed to ribbon-ribbon interactions.

It is evident that the effect of temperature is crucial in order to increase the strength of the plasmon coupling, and thus accelerate the detection method and has to be taken in consideration for future use of DNA assembled nanorods in biosensing applications. First, the absorbance decrease and the shift of LPB are clearly enhanced compare to 25 °C. Second, the sensitivity toward linker length is more pronounced at 60 °C and allows clear

discrimination between the linkers. We believe the reason for this behavior can be due to intra- and inter-strand interactions between DNAs on the same particles as well as secondary structure formation for the linker strand. While it is possible to design DNA based model system where intra-strand interactions (formation of hairpins) are minimal, however these interactions are unavoidable in the real life applications thus, more closely mimicked by our study.

The effect of linker to AuNR ratio

For DNA/nanoparticle based assembly the relevant parameter to modify the interparticle interactions is the linker to particle ratio. As we have shown, the shortest linker, L33, at L33/NRs=100 provides the largest LPB shift at 60 °C and allows a clear spectral change. Therefore, we investigated how linker/AuNRs ratio (R) with R= 0, 2.5, 10, 25, 50, and 100 may affect the plasmon coupling. Following the procedure for the study at 60 °C as described in the previous section, the plasmonic response of the system within 30 min time frame after the addition of the linker strand was investigated. As R increased, the plasmon coupling strength increases as shown in Fig 7a and indicated by increase of blue shift of the LPB. The absorbance magnitude at 770 nm (Figure 7b) and the variation of the longitudinal plasmon band position ($\Delta\lambda = \lambda_{(t)} - \lambda_{(t=0)}$) (Figure 7c) were plotted as a function of the time of incubation (t). As we have noted above, the sharp changes in absorbance and $\Delta\lambda$ are observed within the first 10 min with little change afterwards. Therefore, these parameters were used at 10 min assembly time to follow the system response at different R (Fig 8). Interestingly, both, absorbance and $\Delta\lambda$, change proportionally with R, for R = 0 through 50, then, a plateau was reached at higher R. While reason for the linear dependence

of LPB shift on R is not clear, but, qualitatively, the dependence can likely be caused by two factors. First, at small R the connection between AuNRs is softer thus causing a weaker side-by-side alignment of the nanorods in equilibrium state. Second, due to entropic effects, the interparticle distance decreases with the larger number of linkers in nanoparticle-DNA assembly thus leading to the greater plasmon coupling and larger shifts.³⁵

Whereas previous studies^{28,29} use the difference in absorbance in order to quantify the DNA target, here the linear dependence of $\Delta\lambda$ vs R for linker/NRs ratio between 2.5 and 50, proved that the absorbance as the shift $\Delta\lambda$ can both detect and quantify the target concentration. This observation is especially valuable since the optical response based on LPB shift with the target concentration is not a kinetic effect, which is highly dependent on experimental conditions, but a manifestation of polymeric nature of DNA molecules and equilibrium state nanorod connectivity.

4. CONCLUSIONS

In summary, we have reported the optical responses arising from the self-assembly of gold nanorods driven by linker-induced hybridization and the effect of different factors such as linker length, temperature, or linker to AuNR ratio. Our previous study showed a side-by-side morphology dominates assembly of gold nanorods. In the present work, we explored how plasmon coupling depends on DNA linker length and the linker/NRs ratio. For sensing applications, this design of self-assembly using gold nanorods allows detection of ss-DNA targets up to 100 bases in contrast to previous AuNRs system studies where shorter DNA targets (less than 33 bases) were used. Moreover, the detailed temperature

studies showed that temperature significantly affect the detection efficiency. At optimized conditions sensitive detection and quantification of the target DNA following both the absorbance intensity and the shift of the longitudinal plasmon were observed. The presented optical studies of nanorods might be also relevant for therapeutic and energy transfer applications.

ACKNOWLEDGMENT

The authors thank Dr. Jhon Gonzalez for the discussion, his technical help and his support during the manuscript process. SV acknowledges financial support from “Self assembled nanostructures and functionalization protocols for biomedical, food quality, and environment control” *integrated programme (SAESCTN-PIIC&DT/1/2011), financed by the Norte Regional Operational programme (ON.2 – O Novo Norte), under the National Strategic Referenced Framework Programme (QREN), through the European Regional Devevelopment Fund (FEDER).*” Research carried out at the Center for Functional Nanomaterials, Brookhaven National Laboratory, was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, under Contract No. DE-AC02-98CH10886.

REFERENCES

- (1) Barrow, S. J., Funston, A. M., Wei, X., Mulvaney, P. DNA-directed Self-assembly and Optical Properties of Discrete 1D, 2D and 3D Plasmonic Structures, *Nano Today* **2013**, 8, 138-167.
- (2) Gao, Y., Tang, Z. Design and Application of Inorganic Nanoparticle Superstructures: Current Status and Future Challenge, *Small* **2011**, 7, 2133-2146.
- (3) Chen, H., Shao, L., Li, Q., Wang, J. Gold Nanorods and their Plasmonic Properties, *Chem. Soc. Rev.* **2013**, 42, 2679–2724.
- (4) Huang, X., Neretina, S., El-Sayed, M. A. Gold Nanorods: From Synthesis and Properties to Biological and Biomedical Applications, *Adv. Mater.* **2009**, 21, 4880–4910.
- (5) Pérez-Juste, J., Pastoriza-Santos, I., Liz-Marzán, L. M., Mulvaney, P. Gold Nanorods: Synthesis, Characterization and Applications, *Coordin. Chem. Rev.* **2005**, 249, 1870–1901.
- (6) Liz-Marzán, L. M., Pérez-Juste, J., Pastoriza-Santos, I. Plasmonics of Gold Nanorods. Considerations for Biosensing, Nanomaterials for Applications in Medicine and Biology (Giersing, M., Khomutov, G, B., Eds), Springer, Netherlands **2008**, 103–111.
- (7) Jain, P. K., Eustis, S., El-Sayed, M. A. Plasmon Coupling in Nanorod Assemblies: Optical Absorption, Discrete Dipole Approximation Simulation, and Exciton-Coupling Model, *J. Phys. Chem. B* **2006**, 110, 18243–18253.
- (8) Wang, L., Zhu, Y., Xu, L., Chen, W., Kuang, H., Liu, L., Agarwal, A., Xu, C., Kotov, N. A. Side-by-Side and End-to-End Gold Nanorod Assemblies for Environmental Toxin Sensing, *Angew. Chem. Int. Edit* **2010**, 49, 5472–5475.
- (9) Wang, Y., Li, Y. F., Wang, J., Sang, Y., Huang, C. Z. End-to-End Assembly of Gold Nanorods by means of Oligonucleotide-Mercury (II) Molecular Recognition, *Chem. Commun.* **2010**, 46, 1332–1334.
- (10) Wang, J., Zhang, P., Li, C. M., Li, Y. F., Huang, C. Z. A Highly Selective and Colorimetric Assay of Lysine by Molecular-driven Gold Nanorods Assembly, *Biosens. Bioelectron.* **2012**, 34, 197–201.
- (11) Mastroianni, A. J., Claridge, S. A., Alivisatos, P. A. Pyramidal and Chiral Groupings of Gold Nanocrystals Assembled Using DNA Scaffolds, *J. Am. Chem. Soc.* **2009**, 131, 8455-8459
- (12) Busson, M. P., Rolly, B., Stout, B., Bonod, N., Larquet, E., Polman, A., Bidault, S. Optical and Topological Characterization of Gold Nanoparticle Dimers Linked by a Single DNA Double Strand, *Nano Lett.* **2011**, 11, 5060-5065

- (13) Chi, C., Maye, M. M., Stadler, A. L., Van der Lelie, D., Gang, O. Sensing Nucleic Acids with Dimer Nanoclusters, *Adv. Funct. Mater.* **2011**, *21*, 1051–1057.
- (14) Nykypanchuk, D., Maye, M. M., Van Der Lelie, D., Gang, O. DNA-guided Crystallization of Colloidal Nanoparticles, *Nature* **2008**, *451*, 549–552.
- (15) Park, S. Y., Lytton-Jean, A. K. R., Lee, B., Weigand, S., Schatz, G. C., Mirkin, C. A. DNA-Programmable Nanoparticle Crystallization, *Nature* **2008**, *451*, 553–556.
- (16) Storhoff, J. J., Lazarides, A. A., Mucic, R. C., Mirkin, C. A., Letsinger, R. L., Schatz, G. C. What Controls the Optical Properties of DNA-Linked Gold Nanoparticle Assemblies?, *J. Am. Chem. Soc.* **2000**, *122*, 4640–4650.
- (17) Nam, J.-M., Stoeva, S. I., Mirkin, C. A. Bio-Bar-Code-Based DNA Detection with PCR-like Sensitivity, *J. Am. Chem. Soc.* **2004**, *126*, 5932–5933.
- (18) Storhoff, J. J., Lucas, A. D., Garimella, V., Bao, Y. P., Müller, U. R. Homogeneous Detection of Unamplified Genomic DNA Sequences Based on Colorimetric Scatter of Gold Nanoparticle Probes, *Nat. Biotechnol.* **2004**, *22*, 883–887.
- (19) Sato, K., Hosokawa, K., Maeda, M. Rapid Aggregation of Gold Nanoparticles Induced by Non-Cross-Linking DNA Hybridization, *J. Am. Chem. Soc.* **2003**, *125*, 8102–8103.
- (20) Zanolli, L. M., D’Agata, R., Spoto, G. Functionalized Gold Nanoparticles for Ultrasensitive DNA Detection, *Anal. Bioanal. Chem.* **2012**, *402*, 1759–1771.
- (21) Stadler, A., Chi, C., Van Der Lelie, D., Gang, O. DNA-incorporating Nanomaterials in Biotechnological Applications, *Nanomedicine (Lond)* **2010**, *5*, 319–334.
- (22) Jones, M. R., Macfarlane, R. J., Lee, B., Zhang, J., Young, K. L., Senesi, A. J., Mirkin, C. A. DNA-Nanoparticle Superlattices Formed from Anisotropic Building Blocks, *Nat. Mater.* **2010**, *9*, 913–917.
- (23) Vial, S., Nykypanchuk, D., Yager, K. G., Tkachenko, A. V., Gang, O. Linear Mesostructures in DNA-Nanorod Self-Assembly, *ACS Nano* **2013**, *7*, 5437–5445.
- (24) Pal, S., Deng, Z., Wang, H., Zou, S., Liu, Y., Yan, H. DNA Directed Self-Assembly of Anisotropic Plasmonic Nanostructures, *J. Am. Chem. Soc.* **2011**, *133*, 17606–17609.
- (25) Jin, R., Wu, G., Li, Z.; Mirkin, C. A., Schatz, G. C. What Controls the Melting Properties of DNA-Linked Gold Nanoparticle Assemblies?, *J. Am. Chem. Soc.* **2003**, *125*, 1643–1654.
- (26) Xiong, H. M., Van Der Lelie, D., Gang, O. DNA Linker-Mediated Crystallization of Nanocolloids, *J. Am. Chem. Soc.* **2008**, *130*, 2442–2443.

- (27) Xiong, H., Van Der Lelie, D., Gang, O. Phase Behavior of Nanoparticles Assembled by DNA Linkers, *Phys. Rev. Lett.* **2009**, *102*, 015504.
- (28) Parab, H. J., Jung, C., Lee, J.-H., Park, H. G. A Gold Nanorod-based Optical DNA Biosensor for the Diagnosis of Pathogens, *Biosens. Bioelectron.* **2010**, *26*, 667–673.
- (29) Ma, Z., Tian, L., Wang, T., Wang, C. Optical DNA Detection Based on Gold Nanorods Aggregation, *Anal. Chim. Acta* **2010**, *673*, 179–184.
- (30) Nikoobakht, B., El-Sayed, M. A. Preparation and Growth Mechanism of Gold Nanorods (NRs) Using Seed-Mediated Growth Method, *Chem. Mater.* **2003**, *15*, 1957–1962.
- (31) Orendorff, C. J., Murphy, C. J. Quantitation of Metal Content in the Silver-Assisted Growth of Gold Nanorods, *J. Phys. Chem. B* **2006**, *110*, 3990–3994.
- (32) Maye, M. M., Nykypanchuk, D., Van Der Lelie, D., Gang, O. A Simple Method for Kinetic Control of DNA-Induced Nanoparticle Assembly, *J. Am. Chem. Soc.* **2006**, *128*, 14020–14021
- (33) Jain, P. K., Huang, W., El-Sayed, M. A. On the Universal Scaling Behavior of the Distance Decay of Plasmon Coupling in Metal Nanoparticle Pairs: A Plasmon Ruler Equation, *Nano Lett.* **2007**, *7*, 2080–2088.
- (34) Smith, B. D., Dave, N., Huang, P. J. J., Liu, J. Assembly of DNA-Functionalized Gold Nanoparticles with Gaps and Overhangs in Linker DNA, *J. Phys. Chem. C* **2011**, *115*, 7851–7857
- (35) Chi, C., Vargas-Lara, F., Tkachenko, A. V., Starr, F. W., Gang, O. Internal Structure of Nanoparticle Dimers Linked by DNA, *ACS Nano* **2012**, *6*, 6793 - 6802.

FIGURE CAPTIONS

Figure 1. Schematic of linker induced gold nanorods assembly via DNA hybridization (the interparticle linkage, for clarity, is shown, not to scale).

Figure 2. UV-vis spectra of a mixture of A and B-capped AuNRs incubated for 30 min at 25 °C without linker (spectrum taken every 10 min) and in presence of L33, L52, and L100 with linker/AuNRs = 100 (spectrum taken every 5 min). (Buffer conditions: phosphate buffer 10 mM, pH 7.4 and 0.5 M of NaCl)

Figure 3. Comparison of (left) the evolution of the absorbance at 770 nm and (right) the variation of LPB position of each system versus time.

Figure 4. Comparison of (left) the evolution of the absorbance at 770 nm and (right) the variation of LPB position of each system versus temperature

Figure 5. Normalized UV-vis spectra at 70°C and after cooling to 60 °C for each sample

Figure 6. a) UV-vis spectra of L33, L52 and L100 after 30 min incubation at 60 °C (Spectrum taken every 5 min); b) evolution of the absorbance with time c) LPB shift ($\Delta\lambda = \lambda_{(t)} - \lambda_{(t=0)}$) with time. (Buffer conditions: phosphate buffer 10 mM, pH 7.4 and 0.5 M of NaCl)

Figure 7. a) Normalized UV-vis spectra of different L33/AuNRs ratios (R) taken after 30 min of incubation at 60 °C and evolution of the absorbance (b) and LPB shift (c) versus time between R= 2.5 to 100. (Buffer conditions: phosphate buffer 10 mM, pH 7.4 and 0.5 M of NaCl). Lines in panel (c) represent exponential fits.

Figure 8. a) Absorbance at 770 nm and b) shift of the LPB vs R. Lines represent linear fits.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8