IET Nanobiotechnology

Research Article

Nucleic acid detection strategy using gold nanoprobe of two diverse origin

ISSN 1751-8741
Received on 12th October 2018
Revised 23rd May 2019
Accepted on 27th June 2019
E-First on 7th November 2019
doi: 10.1049/iet-nbt.2018.5332
www.ietdl.org

Saranya Vinayagam[1], Paramasivan Rajaiah[2], Amitava Mukerjee[1], Chandrasekaran Natarajan[1] ✉

[1]*Centre for Nanobiotechnology, VIT, Vellore – 632014, Tamil Nadu, India*
[2]*Vector Control Research Centre, Indian Council of Medical Research, Madurai – 625002, Tamil Nadu, India*
✉ *E-mail: nchandrasekaran@vit.ac.in*

Abstract: In recent years, nanoparticles especially with gold and silver nanoparticles based point of care diagnostic methods is being developed for the lethal diseases like dengue. This study focused to work on the dengue virus detection in a simplest method using gold nanoparticles probe (AuNPs) with thiol tagged single strand DNA (ss-DNA). A sensitive, fluorescence-based detection strategy was designed to examine and quantified the hybridisation process and also elucidated the behaviour of AuNPs before and after interaction of biomolecule. The detection process was focused on aggregation of gold nanoprobe in the presence of complementary strand (target region). Hence the percentage of aggregation was measured and as a result, the limit of detection was found to be 10^{-6} dilutions. Current detection method was highly sensitive, easy to perform and the reaction timing is rapid between 5 and 10 min, and it can be observed through naked eye.

1 Introduction

Rapid diagnostic procedures for the detection of acute febrile infection are still requires efficient screening. Dengue is the one of the challenging and deadly viruses for the point of care detection and its control measures. Early detection of dengue virus seeks worldwide attention, as the conventional process are more complicated and it required sensitive probe due to the seasonal variations of the strains. Since it has four serotypes, and the each serotype could cause deadly infection, the urgent need of reliable diagnostic methods using a biosensor needs to be developed. Detection of the disease-causing virus at an earlier stage was the major task of treating such dreadful diseases. Indeed, the problems with the conventional and recent diagnostic methods were their lesser sensitivity and poor selectivity. Hence our paper focused on the enhancement of available techniques; thereby the AuNPs were selected for their uniqueness and physicochemical properties [1]. AuNps facilitate the multi-functionalisation with several organic and biological ligands like protein, DNA, and other antibody for the detection of metals and biological targets [2]. The ligands are considered as deciding factors for the sensitivity and selectivity of the assay. Since, some ligands might end up with non-specific binding to the target biomolecules. For example, the protein and antibody-based ligand could transpire with either false positive or false negative results [3]. The present detection systems were based on the hybridisation of a target sequence to the DNA immobilised AuNPs probe (AuNps-ssDNA). Functionalisation could be achieved by either covalent bonding or electrostatic force. Thiol-based modification was developed by Brust and co-worker as a covalent binding in order to stabilise the nanoparticles and also for further functionalisation [4]. Successively thiol-mediated AuNps were utilised in biosensor application especially for the nucleic-acid-based diagnostics and DNA–AuNps conjugation, it was demonstrated by Mirkin and co-worker [5].

The principle of current detection process involves the sequence-specific hybridisation between the ssDNA functionalised NPs and the targets biomolecules (DNA, RNA) in the biological system [6, 7]. In this approach, two different methods of ssDNA functionalised AuNps probe (nanoprobe) were developed and used for the detection of target dengue virus. Characteristic features and binding mechanisms of two nanoprobes were also examined using various analytical techniques (transmission electron microscope (TEM), FT-Raman). Those nanoprobes were extensively utilised for the colorimetric detection of dengue virus. Before going to the diagnosis, the dengue virus was amplified by PCR, and processed samples were used for the same. Detection knack of both the nanoprobes with target DNA sequence was determined in an efficient manner, and this could elevate the wide range of opportunities for the various molecular diagnostic approaches with the advancement of nanobiotechnology.

2 Materials and methods

2.1 Synthesis of gold nanoparticles

Gold nanoparticles (AuNps) with the diameter of 13 ± 1.5 nm were synthesised using sodium citrate according to a reported method with slight modification [8]. Briefly, 100 ml of 0.01% $HAuCl_4$ in millipore water was brought to boil with stirring, and 3.6 ml of 1% tri-sodium citrate was added to the solution. The colloid solution turned to blue to wine red colour almost after 1 min. The solution was pursued further for 10 and 15 min only with stirring. The solution was allowed to cool to room temperature and stored at 4°C. The AuNPs were concentrated and estimated as 1 nM. The molar extinction coefficient for the nanoparticles calculated to be 3.45×109 L mol^{-1} cm^{-1} [9].

2.2 Bio-conjugation approaches

Two methods were used to prepare gold nanoparticles and single strand DNA (AuNps-ssDNA) conjugation.

2.2.1 Method 1 – nanoprobe 1: AuNps-ssDNA conjugation was prepared according to a reported method with slight modification [10]. Concisely, thiol-tagged ss-DNA was added to a concentrated AuNps in different concentrations (2, 4, 6, 8 and 10 μM) and allowed to stand for 16 h at room temperature. Ageing was performed by bringing the mixture in to 10 mM sodium phosphate buffer (SPB) and 0.1 M NaCl and followed by sonication in the time interval of 1 h. After 48 h of incubation at room temperature, the mixture was centrifuged at 11,000 rpm for 30 min. The pellet was washed 3–4 times in 10 mM SPB with 0.1 M NaCl to remove unbounded DNA; finally the pellet was resuspended in 10 mM SPB with 0.1 M NaCl and stored at 4°C.

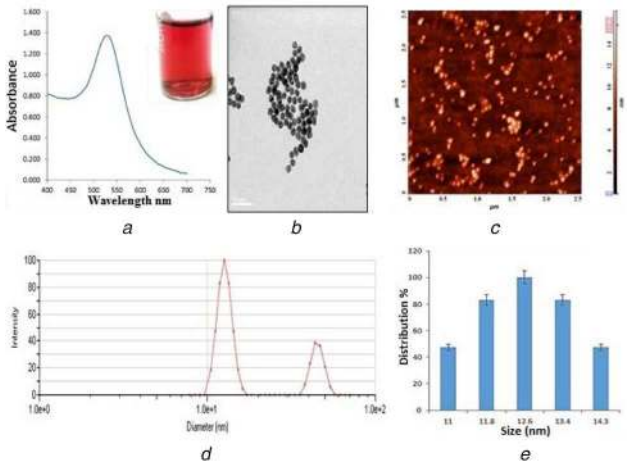

Fig. 1 *Synthesis and characterisation of AuNps*
(a) UV–vis graph of AuNps with image, ***(b)*** TEM image (12 nm), ***(c)*** AFM image (15 nm), ***(d), (e)*** DLS (12 nm)

2.2.2 Method 2 – nanoprobe 2: Thiolated ss-DNAs were conjugated with AuNps according to the previous method with slight modification [1]. Around 100 mM dATPs (deoxyadenosine triphosphate) was added to the concentrated AuNps in the ratio of 600. After 15 min in room temperature, the mixture was brought to 10 mM of SPB with 0.1 MNaCl. Then the different concentrations of thiolated ss-DNA (2, 4, 6, 8 and 10 µM) were added and allowed to stand in the heat for 3 h at 60°C. The solution was then centrifuged at 11,000 rpm for 30 min and washed three times in 10 mM SPB with 0.1M NaCl. The resultant red pellet was re-dispersed in 10 mm SPB with 0.3 M NaCl and stored in 4°C.

2.3 Characterisation of AuNps-ssDNA

AuNps-ssDNA probe was characterised with UV–vis spectroscopy, FT-Raman, and TEM. UV ranges around 200–900 nm wavelength were detected. Nanoprobes were characterised with FT-Raman to find the surface functional groups modifications owing to thiol DNA binding over the AuNps. TEM image was confirming the size and shape stability of AuNps upon addition of 0.1 M NaCl.

2.4 Quantification of thiolssDNA attached to the gold nanoparticles

A number of ssDNA per AuNps were estimated using synergy brand (SYBR) gold nucleic acid dye according to the report [11]. Briefly, to the AuNps-ssDNA probe, 100 mM of dithiothreitol (DTT) were added and kept for overnight. Further, the conjugates with DTT were centrifuged at 11,000 rpm for 10 min. The supernatant was collected and added with 10X SYBR gold dye to make the final concentration of 1X. The resultant was analysed with fluorescence spectroscopy. The excitation wavelength of SYBR was 495 nm and emission wavelength was around 547 nm. The concentration of unknown DNA was determined using standard graph which was plotted with known concentration of DNA. The peak intensity of the solution was recorded and fitted in the formula of a linear graph from the standard plot. The concentration of DNA divided by the concentration of gold nanoparticles in order to quantify the DNA binding number per AuNps.

2.5 Gel electrophoresis

Agarose gel electrophoreses were performed according to the report [12]. Around 2% agarose was dissolved in 0.5× Tris/Borate/EDTA buffer and allowed for solidification. Before loading to the well, 10 µl of AuNps and nanoprobes were mixed with 50% sucrose and subsequently added to their respective lane. The agarose gel was allowed to run for 40 min at 99 V in 0.5% Tris/Borate/EDTA buffer as running buffer. The AuNps-ssDNA yield was calculated based on the intensity of the band in the gel.

2.6 PCR amplification

Dengue virus was procured from CRME, ICMR, Madurai. Further, the RNA was extracted by viral RNA extraction kit and amplified using one step RT-PCR. The sequence of Lanciotti primer was selected for the amplification, and the primer sequence is mentioned as: RT-PCR primer – Dengue forward – TCAATATGCTGAAACGCGCGAGAAACCG – Gene Position – 134-161, Dengue reverse – TTGCACCAACAGTCAATGTCTTCAGGTTC – Gene position – 616-644, Product length – 511 bp. Capture probes – Thiol – TTGCACCAACAGTCAATGTCTTCAGGTTC – Gene position – 616-644, Detection region (Dengue 4) – GAACCTGAAGACATTGACTGTTGGTGCAA – Gene position – 436-466. The amplified product was purified in order to remove the unused enzyme and nucleotides which can interfere with the detection process. The PCR amplified sample was diluted and used for the detection process.

2.7 Colorimetric detection

Nanoprobes were added with 10 µl of the sample and incubated for 5–10 min in the presence 20X saline-sodium citrate (SSC) buffer. Subsequently, centrifuged for 5 min at 10,000 rpm. The results can be observed through naked eyes and also it was evaluated with UV–vis spectroscopy and fluorescence spectroscopy.

3 Results

3.1 Characterisation of AuNps and AuNp-ssDNA conjugates

Characterisation such as UV–vis spectroscopy, dynamic light scattering (DLS), TEM, atomic force microscopy (AFM) and FT-Raman were carried out to the citrate-capped AuNps. The surface plasmon resonance of AuNps was exhibited at 520 nm, and it was represented in Fig. 1*a*. The average size of 12 ± 1 nm was confirmed by TEM, AFM and DLS which was displayed in Figs. 1*B–E,* respectively. The surface functional group and their ligand-binding manner were understood with FT-Raman. Table 1 represented various peak values and their respective functional groups of gold nanoparticles before and after functionalisation with DNA.

3.2 Conjugation of nanoprobes

Fig. 2*a* represents the UV graph of AuNps-ssDNA nanoprobe with five different concentration of ssDNA and compared with AuNps. The conjugates exhibit peak at 260 and 520 nm denoted by ssDNA and AuNps, respectively. The blue shifting of AuNps and the number of ssDNA bounded to the nanoparticles were denoted in Figs. 2*b* and *c*, respectively. The photograph of conjugated AuNps with thiolated, non-thiolated and dATPs and their respective gel electrophoresis was shown in Figs. 2*d* and *e*.

The UV spectroscopy results of nanoprobe 2 was explained in (Figs. 3*a*, *b* and *d*) with the SPR peak at 520 nm which proves that the nanoprobes are stable in salt concentration (0.1 M NaCl) subsequently with the heat treatment at 60°C. The graph also demonstrate the existence of dATPs and NaCl enhances and stabilised the AuNps-ssDNA conjugation. Photography of AuNps with and without ssDNA was showed in (Fig. 3*c*) which explains the aggregation of AuNps without ssDNA with the colour change from red to blue. Quantification of ssDNA per AuNps was plotted against different concentration of DNA (Fig. 3*e*).

Comparative illustration of nanoprobes developed by both methods represented in Figs. 4*a* and *b*. TEM image of nanoprobe of both methods shows a stable, distinct morphology after addition of NaCl was displayed in Figs. 4*c* and *d*

3.3 PCR amplification

Fig. 5*a* illustrates the different diluted RNA expression and the band formed at the 500 bp. Fig. 5*b* represented the image of AuNps-ssDNA probe after addition of sample which has a target sequence.

Table 1 FT Raman spectroscopy of gold nanoparticles and ss DNA conjugated gold nanoparticles

AuNps			AuNps-thiol ss DNA		
Peaks	Functional	Assignment	Peaks	Functional	Assignment
425.85	Cl–C = O in acid chlorides	Cl–C = O in plane deformation	446.28	Cl–C = O in acid chlorides	Cl–C = O in plane deformation
599.02	C–I in iodo compounds	C–I stretch	—	—	—
652.50	C triple bond C–H in alkynes	C triple bond C– H stretch	—	—	—
704.43	C triple bond C– H stretch	C–S stretch strong in Raman	709.55	C–S in sulphides	C–S stretch
—	—	—	786.76	M-di substrate benzene	CH out of plane deformation
923.41	CH = CH_2 in vinyl compounds	CH_2 out of plane wag	—	—	—
1075.89	SO_3 H in sulfonic acids	SO_3 symmetry stretch	1013.03	carbon ring in cyclic compound	ring breathing mode, strong in Raman
2886.03	CH_3 and CH_2 aliphatic compound	CH antisym and sym stretching	2949.80	CH_3 and CH_2 in aliphatic compound	CH antisym and sym stretching
—	—	—	3082.52	CH aromatic and unsaturated hydrocarbon	= C–H stretch

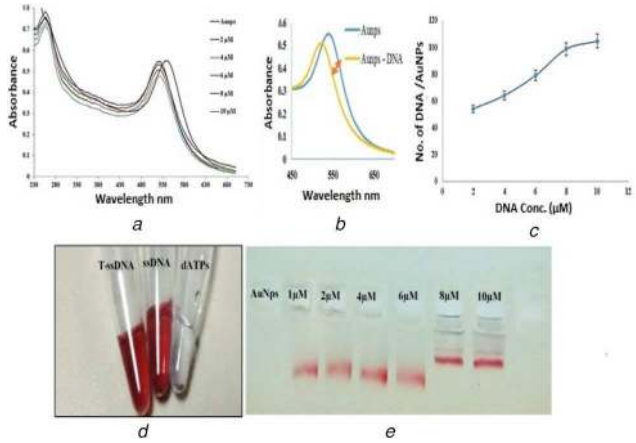

Fig. 2 *Conjugated AuNps-ssDNA 1*

(a) UV–vis spec with different concentrations of DNA, ***(b)*** UV–vis spectroscopy showing blue shifting of peak respective to conjugation, ***(c)*** Surface coverage of ssDNA on AuNps, ***(d)*** Image of AuNps-ssDNA, ***(e)*** Agarose gel electrophoresis of AuNps-ssDNA

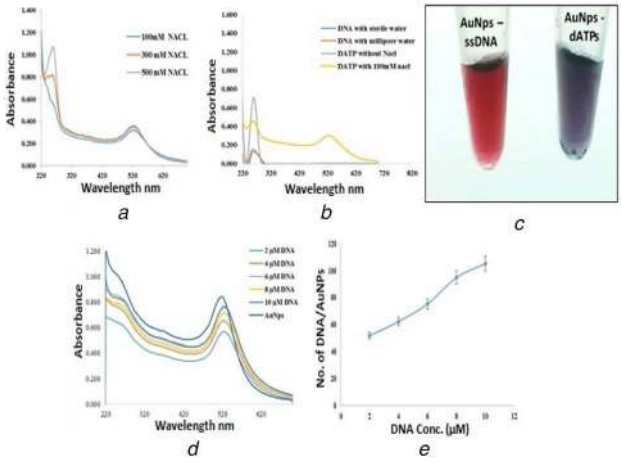

Fig. 3 *Conjugated AuNps-ssDNA 2*

(a) UV spectroscopy with different concentrations of NaCl, ***(b)*** UV spec analysis the binding property of DNA and dATPs, ***(c)*** AuNps-ssDNA and AuNps-dATPs, ***(d)*** UV spec of AuNps-ssDNA with different concentration of DNA, ***(e)*** Surface coverage of ssDNA on AuNps

3.4 Colorimetric detection

Visual discrimination of control (without DNA), complementary sample and non-complementary samples are shown in Fig. 6*a*. Fig. 6*b* illustrates UV absorbance of AuNps after interaction with different concentration of complementary strand.

3.5 Fluorescence detection

The overall fluorescence-based detection of dengue virus with the limit of detection and selective of the nanoprobe was summarised in (Fig. 7). The detection process was further confirmed with fluorescence emission of unbounded DNA.

4 Discussion

Our study demonstrates the preparation and characterisation of two different nanoprobes and further used for the detection of dengue virus. The presence of AuNps was confirmed with UV–vis spectroscopy which shows peak at 520 nm with a sharp and narrow peak which indicates the monodispersed smaller size particles. Before to the conjugation process the AuNps concentrations were determined (0.1 nm) and which was analysed from the combination of inductively coupled plasma-optical emission spectrometry and TEM experiments using formula reported by [9]

$$N = (R\text{cluster}/R\text{atom})3 \tag{1}$$

where N is the number of atom per gold nanoparticles, R cluster obtained with TEM results

$$\text{NNP} = \text{Natom}/N \tag{2}$$

Amount of Au-Nps formed was calculated using NNP and Natom calculated with ICP-OES value

$$\text{Natom} = \text{ICP} - \text{OES}xNA \tag{3}$$

Here the final concentration of gold nanoparticles was calculated with NNP divided by Avogadro's number (NA)

$$\text{CNP} = \text{NNP}/\text{NA} \tag{4}$$

The FT-Raman results show the presence of functional groups such as C = O, C triple bond C–H in alkynes, C = C, C = N stretches, C = O in aldehyde, N = H stretches, NH_3^+ in amino acids was observed in the gold nanoparticles. The ssDNA replaced such group through covalent binding thereby the conjugation was confirmed.

Typically, the binding of a ligand to the nanoparticles surface could be either covalent bonding or non-covalent binding. The majority of conjugation process was based on covalent bonding through thiol group over citrate-capped AuNps and this is considered to be more stable conjugation, as the binding is defined, systematic and very specific to the target. In case of sensor application, the bonding should be more specific in order to get reliable results. Hence, the thiol-tagged ssDNAs were widely used in most of the nucleotide sensors.

Functionalisation can be achieved through various processes. Indeed, the oldest methods of attaining conjugation were through NaCl stabilisation over to the ssDNA addition by gradually

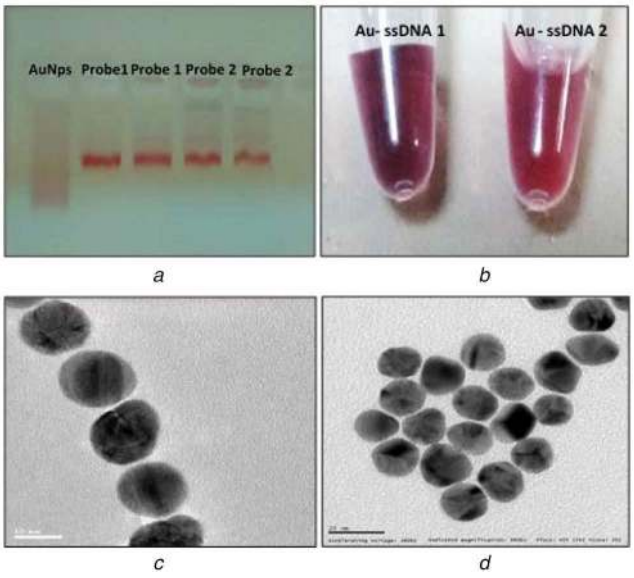

Fig. 4 *Comparative of two methods of conjugation*
(a) Agarose gel electrophoresis of AuNps-ssDNA, ***(b)*** Image of AuNps-ssDNA, ***(c), (d)*** TEM image of AuNps-ssDNA probe 1 and 2, respectively

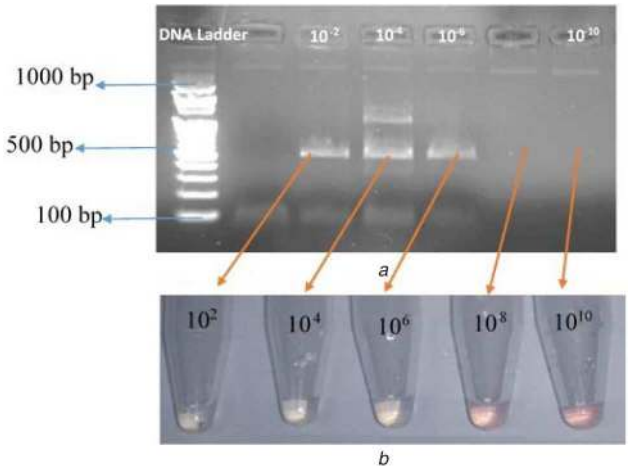

Fig. 5 *PCR amplification*
(a) Agarose gel electrophoresis, dengue virus 4, ***(b)*** Image of colorimetric detection using AuNps-ssDNA probe

increasing the concentration of NaCl, and one of the rapid methods was through pre-stabilising the nanoparticles surface with nucleotides. Pre-stabilisation was supported by deoxy nucleotides tri-phosphate (dNTPs) through non-specific electrostatic adsorption. Under the distinct temperature, these nucleotides were replaced by strong binder like thiol group which was tagged at the 5′ end of the DNA sequence. Among four dNTPs, deoxy adenosine triphosphate (dATPs) demonstrated to have better stabilisation [13]. Hence, for the better understanding of the functionalisation of AuNps with ssDNA, UV spectroscopy, gel electrophoresis and FT-Raman spectroscopy was performed. In case of gel electrophoresis, the band width of the nanoprobe exhibited the quantity of DNA binding to the nanoparticles.

Our UV–vis spectroscopy results point to the blue shifting of AuNps peak confirms the attachment of ssDNA to the AuNps surface through thiol binding. The blue shifting was due to the excitation of properly dispersed particles which observed when the ssDNA covalently bounded to AuNPs [14]. This conjugation efficiency was evaluated with the binding efficiency of both the methods. Here, the pre-stabilisation-based conjugation method was simple and shows similar binding efficiency and the binding of DNA was more stable than the nucleotides. Overall, the binding percentage was calculated through UV–vis, gel electrophoresis. The percentage of DNA binding and stability of the nanoprobes were determined through the mobility of band. The increased binding of ssDNA to AuNps, retarded the band mobility in the gel electrophoresis.

The basic mechanism in pre-stabilisation was the adhered dATPs forms a monolayer over the surface of the AuNps and which conferred the stability even without ssDNA. During the process of heating at 60°C, dATPs on the surface was replaced by the ssDNA. From this result, it is evident that during heating process the AuNps was stable, only when the ssDNA bounded to it. Indeed, the existence of dATPs would be the temporary binding factor and utilised for the stabilisation.

RNA was isolated from dengue serotype 4 virus and diluted and further amplified using one-step RT-PCR. The amplified samples were purified and used for the detection purpose [15]. Various dilution of dengue virus RNA was used to determine the limit of detection of the AuNps-ssDNA probe.

Detection process was based on the aggregation of AuNps-ssDNA probe upon binding to target DNA. The aggregation was attained by binding of two complementary strands together in the presence of 20X SSC buffer which is also known as hybridisation buffer. The result can be viewed through our naked eyes without any analytical instrument. In order to confirm the binding mechanism, UV–vis spectroscopy and fluorescence instrument characterisation were carried out. Target complementary sequence hybridised to the AuNps-ssDNA and formed double-strand helix which brings nanoprobes together and which possibly aggregated the probe. The aggregated gold nanoprobes sedimented at the bottom of the tube during centrifugation. Conversely, the non-complementary strand DNA remains suspended in the solution hence the AuNps being suspended in a solution during centrifugation. The unbound DNA and the un-hybridised probe remains in the suspension which further does not affect stability of AuNPs. Since, the gold nanoprobes were stable in the 20X SSC buffer and it will not affect the conjugation process rather it will enhance the hybridisation process. Even the single mismatch in the base pair sequence could be detected through this simple method of detection. UV spectral results support the premise of detecting the dengue virus through aggregation which can be visual by our eyes with the sensitive of single mismatch sequence.

TThe AuNps aggregation level induced by the concentration of target DNA and the stability of the conjugates owing to the repulsion of ss-DNA. Stability of the nanoprobe is important in the detection process, and that was determined by the percentage of DNA binding to the nanoprobe. During detection process, the obvious difference was observed between the complementary, non-complementary and single base pair mismatch DNA. The binding efficacy of single base pair mismatch to the AuNps-ssDNA was reduced double the time with that of complementary.

The conjugation efficacy of target DNA to the nanoprobes was further confirmed using fluorescence spectroscopy. Since the AuNps has no fluorescence property, our study focused on the DNA. Naturally, DNA has fluorescence property and its fluorescence spectra exhibited at the range between 327 and 370 nm [16]. On the other side, gold nanoparticles metal nanoparticles which is a quencher and that can inhibit the fluorescence emission of the substance was bound to AuNPs. The excitation peak for the DNA was 260 nm, and the emission peak falls at a 332 nm range. DNA in any medium like water and buffer exhibits fluorescence emission at the mentioned range. However in the case of nanoprobes (AuNps-ssDNA), the fluorescence property of bounded DNA was quenched by AuNps. This quenching process is the result of DNA bound to the AuNps. Therefore, only the unbound DNA in the nanoprobe could emit fluorescence peak. The nanoprobe without sample shows no peak as it has no free DNA. On the other hand, the hybridised nanoprobes were unstable and get aggregated. Since the AuNps aggregated, the DNA in the suspension was free and exhibited the fluorescence peak at 532 nm. However, in the presence of non-complimentary DNA, the nanoprobe remains stable, and the unhybridised DNA in the suspension exhibits the corresponding peak. Finally, the colorimetric method of detection was explained by the fluorescence spectroscopy.

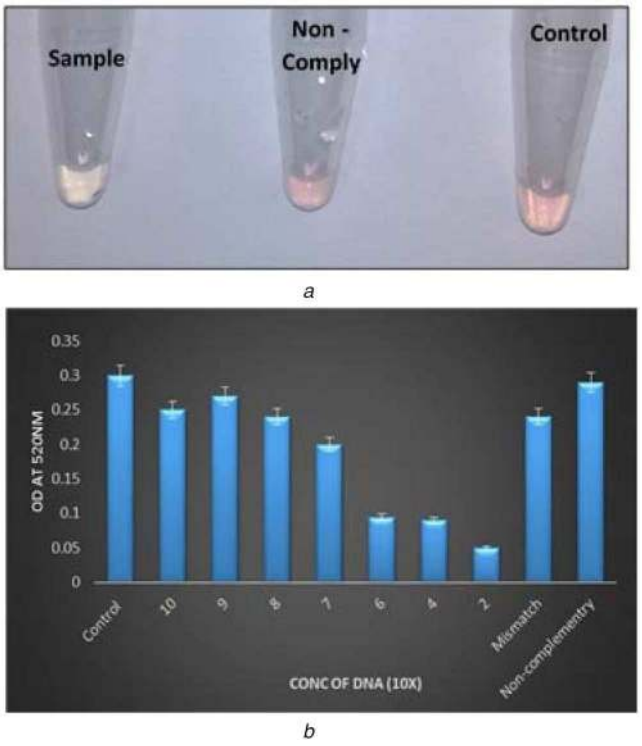

Fig. 6 *Colorimetric detection of dengue 4 virus RNA using AuNps-ssDNA probe*

(a) Photography of AuNps-ssDNA after interacted with sample and non-complementary sample and control, ***(b)*** A graph plotted to determine the LoD

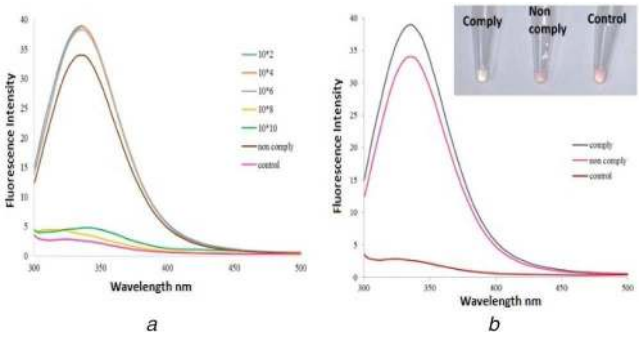

Fig. 7 *Fluorescence spectra of dengue virus detected using AuNps-ssDNA*

(a) Different dilution of sample interacted with AuNps-ssDNA conjugates, ***(b)*** Fluorescence emission of unbounded DNA in the sample during detection process

5 Conclusion

The conjugation efficiency of two different conjugation methods was well understood with the appropriate characterisations. The binding procedure of deoxynucleotide (dATPs) has been defined in both the methods of conjugations and the dATP mediated conjugation was similar to that of NaCl mediated conjugation. We also investigate the extent of binding of thiolated and non-thiolated DNA to the gold nanoparticles. Electrophoresis-based measurements would be the useful components for the better explanation. A simple and sensitive method was developed for the clinical diagnosis of dengue virus. The detection could be performed within 10–15 min with high specificity and sensitivities. The limit of detection was also emphasised with the 10-6 dilutions.

6 Acknowledgments

Authors are thankful to the management of CSIR-SRF (Senior Research Fellow) New Delhi and VIT University, Vellore for providing the facility to carry out this research work

7 References

[1] Zhao, W., Lin, L., Hsing, I.-M.: 'Rapid synthesis of DNA-functionalized gold nanoparticles in salt solution using mononucleotide-mediated conjugation', *Bioconjugate Chem.*, 2009, **20**, pp. 1218–1222

[2] Daniel, M.-C., Astruc, D.: 'Gold nanoparticles: assembly, supramolecular chemistry, quantum size-related properties, and applications toward biology, catalysis, and nanotechnology', *Chem. Rev.*, 2004, **104**, pp. 293–346

[3] Razin, S.: 'DNA probes and PCR in diagnosis of mycoplasma infections', *Mol. Cell. Probes*, 1994, **8**, pp. 497–511

[4] Brust, M., Walker, M.B., Schiffrin, D.*, et al.*: 'Synthesis of thiol-derivatised gold nanoparticles in a two-phase liquid-liquid system', *Chem. Soc. Chem Commun.*, 1994, (7), pp. 801–802,

[5] Mirkin, C.A., Letsinger, R.L., Mucic, R.C.*, et al.*: 'A DNA-based method for rationally assembling nanoparticles into macroscopic materials', *Nature*, 1996, **382**, p. 607

[6] Storhoff, J.J., Elghanian, R., Mucic, R.C.*, et al.*: 'One-pot colorimetric differentiation of polynucleotides with single base imperfections using gold nanoparticle probes', *J. Am. Chem. Soc.*, 1998, **120**, pp. 1959–1964

[7] Xu, X., Han, M.S., Mirkin, C.A.: 'A gold-nanoparticle-based real-time colorimetric screening method for endonuclease activity and inhibition', *Angew. Chem.*, 2007, **119**, pp. 3538–3540

[8] Turkevich, J., Stevenson, P.C., Hillier, J.: 'A study of the nucleation and growth processes in the synthesis of colloidal gold', *Discuss. Faraday Soc.*, 1951, **11**, pp. 55–75

[9] Lewis, D.J., Day, T.M., MacPherson, J.V.*, et al.*: 'Luminescent nanobeads: attachment of surface reactive Eu(III) complexes to gold nanoparticles', *Chem. Commun.*, 2006, **7**, (13), pp. 1433–1435

[10] Zhu, D., Song, P., Shen, J.*, et al.*: 'Polya-mediated DNA assembly on gold nanoparticles for thermodynamically favorable and rapid hybridization analysis', *Anal. Chem.*, 2016, **88**, pp. 4949–4954

[11] Park, S.: 'DNA conjugation to nanoparticles', in Bergese, P., Hamad-Schifferli, K. (Eds.): *Nanomaterial Interfaces in Biology. Methods in Molecular Biology (Methods and Protocols)*, vol 1025. (Humana Press, Totowa, NJ, 2013)

[12] Lu, W., Wang, L., Li, J.*, et al.*: 'Quantitative investigation of the poly-adenine DNA dissociation from the surface of gold nanoparticles', *Sci. Rep.*, 2015, **5** Article ID: 10158

[13] Zhao, W., Lee, T.M., Leung, S.S.*, et al.*: 'Tunable stabilization of gold nanoparticles in aqueous solutions by mononucleotides', *Langmuir*, 2007, **23**, pp. 7143–7147

[14] Palomba, S., Novotny, L., Palmer, R.: 'Blue-shifted plasmon resonance of individual size-selected gold nanoparticles', *Opt. Commun.*, 2008, **281**, pp. 480–483

[15] Lanciotti, R.S., Calisher, C.H., Gubler, D.J.*, et al.*: 'Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction', *J. Clin. Microbiol.*, 1992, **30**, pp. 545–551

[16] Vayá, I., Gustavsson, T., Miannay, F.-A.*, et al.*: 'Fluorescence of natural DNA: from the femtosecond to the nanosecond time scales', *J. Am. Chem. Soc.*, 2010, **132**, pp. 11834–11835