

Gold nanoparticles-assisted plasmonic enhancement for DNA detection on the graphene-based portable SPR sensor

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Abstract

The impact of different gold nanoparticles (GNPs) structures on the plasmonic enhancement for DNA detection is investigated on a few-layer graphene (FLG) surface plasmon resonance (SPR) sensor. Two distinct structures of gold nanourchins (GNu) and gold nanorods (GNr) were used to bind the uniquely designed single-stranded probe DNA (ssDNA) of *Mycobacterium tuberculosis* complex (MTBC) DNA. The two types of GNPs-ssDNA mixture were adsorbed onto the FLG-coated SPR sensor through the π - π stacking force between the ssDNA and the graphene layer. In the presence of the complementary single-stranded DNA (cssDNA), the hybridization process took place and gradually removed the probes from the graphene surface. From SPR sensor preparation, the annealing process of the Au layer of the SPR sensor effectively enhanced the FLG coverage leading to a higher load of the probe DNA onto the sensing interface. The FLG was shown effective in providing a larger surface area for biomolecular capture due to its roughness. Carried out in the DNA hybridization study with SPR sensor, GNu, with its rough and spikey structures, significantly reinforced the overall DNA hybridization signal than the GNr with smooth superfices, especially in capturing the probe DNA. The DNA hybridization detection assisted by GNu reached the femtomolar range limit of detection (LoD). An optical simulation validated the extreme plasmonic field enhancement at the tip of the GNu spicules. The overall integrated approach of graphene-based SPR sensor and GNu-assisted DNA detection provided the proof-of-concept for the possibility for Tuberculosis disease screening using a low-cost and portable system potentially applied in a remote or the third world countries.

Keywords:

DNA biosensor, *Mycobacterium tuberculosis*, few-layer graphene, surface plasmon resonance, gold nanourchins, gold nanorods

1. Introduction

The world-shattering Coronavirus disease (COVID-19) pandemic has been an important reminder for the urging needs on the rapid screening and diagnostic methods of respiratory and airborne pathogens infection [1–3]. Another major threat from the respiratory disease class is Tuberculosis (TB) infection, which typically spreads in numbers of poor and developing countries [4]. The shot-up of the morbidity and mortality rate of TB was predicted to increase significantly as the consequence of the reallocation of resources for the COVID-19 pandemic worldwide[5,6]. The *M. tuberculosis* complex (MTBC) bacterium is highly noticed as the pathogenic species causing the tuberculosis disease. It has been declared to bring out deadly effects that have infected almost a quarter of the world population, dominantly in the low-middle income countries in Africa, Asia, South America, and Eastern Europe[3,4]. Unfortunately, the human-to-human airborne transmission of this bacterium makes it hard to be tested in a rapid diagnostic or screening protocol. One of the well-known DNA sequence biomarkers of the MTBC is the fragment of IS6110, which is stable for decades from the mutation genetic part, and extremely specific to the MTBC species. This fragment exists in several copies in different locations of the MTBC genomic profile. This versatility has made this sequence a favorable biomarker for the PCR and sequence-based screening diagnostics.

The polymerase chain reaction (PCR), as the golden standard for quantification of the pathogen through specific nucleic acid amplification, is still regarded indirect, laborious, and expensive. These drawbacks have been the main challenges for the middle-low income countries to perform the routine and extensive PCR tests in society[1,7]. Additionally, the typical bench-top PCR system is far from portability means to be applied for TB cases, which most likely occur in

remote and harsh sites. Biosensor technology has been long spotlighted to provide an alternative to the time-consuming standard diagnostic methods, and its growth has been momentarily accelerated by the current pandemic situation toward the realization of the next-generation diagnostics methods. Among myriads of disease biomarkers, the preference of the nucleic acid biomarker from the antigen is highly advantageous because its base-pairing hybridization is highly specific and robust [8] and easy to be functionalized with nanoparticles and other surfaces [9]. On the other hand, the detection of serology biomarkers requires complicated purification-separation protocols due to the complex protein matrices in blood or body liquid[10,11].

In the application of DNA on a biosensor, the uniform surface coverage by probe DNA molecules is essential to achieve the overall inherent biorecognition signal after hybridization with the target. The interfacial engineering for biomolecular captures, such as via cross-linking agent [12], electrostatic attraction [13], nanoparticles (NPs)-assisted capture [14], and many other methods have been crucially developed to achieve the best sensing device performance. Gowtham et al. reported that the mechanism of nucleobases physical absorption onto graphene and carbon nanotubes. Adenine (A), Thymine (T), Cytosine (C), and Guanine (G) interaction between the graphene layer were dominated by the molecular polarizability, which played a vital role in an attractive dispersion force between these materials[15]. The orientation of the binding was reported almost parallel to the graphene layer with a separation distance around 3.5 Å, and it indicated the interaction characteristic for the π - π stacking mechanism[15,16]. The aptamer binding on graphene was also studied for the α -thrombin monitoring application. The aptamer probes were functionalized by the amine or sulfide group and adsorbed on the graphene surface by the π - π stacking force mechanism. Subsequently, the presence of the α -thrombins attracted the labeled-aptamer from the graphene surface due to a massive covalent bond between the amino or sulfide

group to the α -thrombin[17]. In addition, the nanomolar and sub-nanomolar range detection of DNA hybridization were reported as well by the use of graphene for surface-assisted adsorption of the DNA strand[18,19].

The utilization of solution-based graphene. Such as graphene oxide (GO) for capturing the DNA strands via the π - π interaction was highly regarded as a simple and low-cost approach to initialize the biomolecular recognition on the sensor's interface [20–22]. A significant challenge in working with solution-based graphene is transferring onto another solid substrate, such as the gold (Au) layer, typically used in the surface plasmon resonance (SPR) sensing setup. Song et al. recommended the improvement of the Au {111} crystallization on the surface to enhance the adsorption energy of the graphene onto the gold layer [23]. Another method emphasized the high possibility for colloidal graphene onto the Au SPR chip through metal-carbon bonding with a considerably high uniform coverage rate [20]. The application of deformed multi-layer graphene for effective biomolecular capture was also studied by Hwang et al., who successfully created electronic hotspots from the capture of DNA on the crumpled graphene layer [24].

Among the metallic nanoparticle clusters, AuNPs have gained significant attention to be combined with DNA in numbers of sensing platforms, such as in electrochemical, transistor-based, and optical sensors due to their high bio-affinity, chemical and physical stability, surface area to volume ratio, and excellent optical features [25–31]. Specifically, the plasmonic enhancement from AuNPs has been applied in a variety of optical sensing setups, such as in SPR, photoluminescence, and surface-enhanced Raman spectroscopy (SERS) biosensors showing ultrasensitive DNA or aptamer detection[20,32–35]. The plasmonic coupling is predominantly defined by nature, size, shape, structure, composition, and aspect ratio of the NPs and the environment dielectric constant and inter-spatial arrangement within the NPs on a solid surface

[36]. Some studies reported the remarkable electric field enhancement of branched AuNPs with protruding spikes and sharp tips useful in biosensing configuration [37,38].

In this article, we presented the proof-of-concept of the MTBC screening method, using FLG on an SPR sensing system. The biorecognition signal from DNA was assisted by the tagging of probe DNA with gold nanoparticles (GNPs) with two distinct structures, gold nanourchins (GNu) or gold nanorods (GNr), to be adsorbed onto the FLG layer via the π - π stacking. The hybridization event with the target DNA was checked through the release of the gold nanostructures-tagged probe from the sensing interface. The plasmonic activity from both gold nanostructures was compared, and it was denoted that the GNu provided the most significant enhancement with an impressive limit of detection (LoD). The proposed work combined both the ease of solution-based graphene integration onto the portable SPR chip and the facile detection methods for nucleic acid-based Tuberculosis detection. Among experimental using clinical samples of the infectious diseases, that the future outlook will be performed in the high biosafety level laboratory, the screening and detection using biosensor technology can be a potential approach. Moreover, the modular biosensing technology with the feature of components disposability can be an advantage to avoid contamination during the measurements.

2. Material and method

The primary platform of the portable SPR biosensor utilizing organic light source in this experiment was described in our previous reports and depicted in Fig. 1[39]. Modular based instruments and its portability offer an advantage for the real detection scheme of MTBC, which

commonly occurs in the remote area or third world countries. The disposability of the modular component is beneficial for the handling of the toxicity of the samples.

2.1. Material and Instruments

Graphene powder and its dispersion solution were purchased from Angstrom Materials Inc. (Ohio, USA). The G_{Nu} with 70 nm size and G_{Nr} with the aspect ratio of 2.5 were bought from NanoSeedz (Hongkong, P.R.C). DNA sequences were purchased from Purigo Biotechnology Co. (Taipei, Taiwan). The DNA probe and target sequences were derived from the MTBC DNA fragment IS6110. The single-stranded (ssDNA) probe sequence used in this study was SH-CGTGCGGCTA TTACGAGGAC TCCACGCTGG (30 mers). The sequence of the complementary ssDNA (cssDNA) target was CCGAT AATGCTCCTG AGGTGCGACC (25 mers). In comparison, the sequence of mismatched single-stranded DNA (mssDNA) utilized in the specificity test was ACAGC ATTGCGCGTT CAGACACCGC (25 mers). The target DNA strand had been designed with shorter sequences (5-mers shorter) to accommodate the spacing activity in the probe DNA. The phosphate buffer saline (PBS) tablet from Sigma Aldrich (Missouri, US) was employed to produce the PBS buffer solution. NaCl, K₂HPO₄, and KH₂PO₄ for salt buffer solution were purchased from Sigma Aldrich (Missouri, US). The RAMaker system from Protrustech was applied to perform Raman spectroscopy. Co, Ltd (Tainan City, Taiwan) with charge-coupled device (CCD) camera system and coupled with Olympus microscope body. The collimation of light excitation was performed by 100 times objective lens (NA 0.5). The laser power source was 100 mW, the exposure time was 2 seconds, and the accumulation number is 3. The electron microscopy image was obtained from the field-emission scanning electron microscope (FESEM) with the instrument series of JSM-7500F (JEOL co., Tokyo, Japan), transmission electron

microscopes (TEM) with the instrument type of JEOL JEM-1230 and JEOL JEM-2100 PLUS (JEOL co., Tokyo, Japan). The Finite-difference time-domain (FDTD) simulation was performed by Lumerical (Pensylvania, US).

Figure 1 is preferred in this location

2.2. Sensing chip preparation

A gold sensing chip was prepared and produced based on the protocol explained in our previous reports [40]. Next, the chip was annealed in the baking chamber at 300 °C for 30 min with a very slow ramp-up of the temperature, followed by an immediate quenching process by exposing it to the N₂ gas at 20 °C. The quenching ramp-down temperature should be carefully handled to avoid substrate crack. The annealing-quenching step are critical for improving Au {111} crystallization[23,41]. The dispersant solution of graphene was diluted in the Deionized (DI) water to reach a 1% concentration. To prepare the FLG solution, the graphene powder was then dispersed in 1% of the dispersant solution until reaching the concentration of 0.1 mg/mL before the ultrasonic treatment for 20 mins. Subsequently, the FLG solution was dropped on the sensing surface and baked at 80 °C (the boiling temperature point of the solution was avoided) until the dryness of the sensing layer was obtained.

2.3. The DNA immobilization and detection

In this study, the ssDNA probe was tagged with the G_{Nu} before the adsorption onto the sensing membrane. First, a 70 nM G_{Nu} solution was centrifuged and suspended in the PBS solution with a rotation speed of 700 G for 30 minutes. The G_{Nu} solution was then mixed with the ssDNA in PBS until the final concentration of 100 nM ssDNA was reached. This mixed solution

was kept at room temperature for 20-24 hours to allow the effective covalent binding between GNu and ssDNA probe. The target DNA was prepared through a serial dilution using the salt buffer to complete a series of concentrations from 100 fM to 100 nM. The salt buffer for target dilution was prepared by mixing 1 M K_2HPO_4 and 1 M KH_2PO_4 in a 1 M NaCl solution. For GNR-assisted DNA detection, similar stages were applied by replacing the GNu with GNR solution.

3. Results and discussion

3.1. Few-layer graphene (FLG) deposition on Au SPR chip

The deposition of graphene was notably pivotal in interfacing DNA oligonucleotides onto the SPR sensing system. We observed that the drop-casting deposition of the FLG solution onto the Au sensing chip of the portable SPR sensor had favorably resulted in graphene with a few-layer structure. In Fig S1a, the optical microscopy figure showed a considerably uniform coverage of FLG on the Au layer, indicated by the homogenous transparent color spread onto the surface with tiny clumped particles over it. The surface morphologies visualized by FESEM in Fig S2 demonstrates the FLG on top of the Au chip, indicated by some visible creases on the surface with contrast seen to the Au layer without graphene on top. The FLG structure is typically found in the drop-casting method as a result of the graphene film wrinkling due to the surface tension of water when the evaporation takes place during drying protocols [42]. It is noteworthy that the wrinkled structure of graphene is ascribed to the defects and holes in the lattice of tetrahedral sp^3 hybridized carbon atoms providing extra bonds that finally form the atomically rough graphene sheets as compared to pristine graphene[43,44]. Raman detection of the FLG film in Fig S2 presents a substantial uniformity of graphene coverage by considerably similar spectral features taken at three different sites. The graphene fingerprint peaks, such as a visible 2D ($\sim 2680\text{ cm}^{-1}$), considerably

sharp G ($\sim 1571\text{ cm}^{-1}$), and an intense D ($\sim 1340\text{ cm}^{-1}$) peaks ascribing to the phonons coupling with two opposite wave vectors, sp^2 carbon and sp^3 carbon, respectively[45], were observed. The lesser intensity of 2D than the G peak indicates the few-layer graphene formation [46,47], and the highly pronounced D peak (defects), as well as small 2D peak, is attributed to the defects due to the removal of oxygen in the basal plane [45,48]. The rough graphene sheet would be beneficial in trapping the probe due to the high surface area in the next stage of surface functionalization. This FLG solution drop-casting method holds a potency for a straightforward and brief process for graphene-based SPR biosensor development.

Figure 2 is preferred in this location

3.2. Absorption profiles of GNu and GNR.

The architectural building block of the gold nanostructures greatly determines its optical properties. The transmission electron micrograph (TEM) in Fig 2 inset shows that the GNu consists of poly-branch tips with an approximate tip-to-tip diameter of around 70-80 nm. The absorption spectra of GNu and GNR in the PBS medium are demonstrated in Fig. 2. The GNu absorption appears optimally at around 601 nm of wavelength. In the presence of ssDNA on the GNu following up the preparation of the probe, the absorption peak was shifted to ~ 619 nm of wavelength. This finding is in good accordance with Rotz et al., 2015 who reported a similar range of wavelength shifts of gold nanostars spectrum after DNA conjugation with good maintenance of colloidal stability[49]. In contrast, as seen in the TEM image, in Fig 2 inset, the dimension of GNR has a length of ca. 40 nm and thickness of rod of about 10 nm. The GNR absorption showed two major peaks, with a maximum peak at 724 nm of wavelength and the minimum one at 522 nm. These peaks emerged as the consequence of dual orientations of the GNR, which has longitudinal and transverse peaks [50,51]. In the presence of ssDNA, the minor and major peaks were blue-

shifted to 531 nm and 734 nm of wavelength, respectively, indicating a successful formation of ssDNA and GNR nanoprobe complex

3.3. Annealing-quenching protocol of Au sensing chip and GNPs effects on the SPR signal enhancement

The annealing and quenching step of the Au SPR sensing chip before graphene solution deposition was an attempt to enhance Au {111} orientation on the surface[41]. Higher temperature annealing was suggested to obtain highly ordered Au {111}. However, considering that the melting point of the BK7 substrate is around 350 °C, the annealing point in our experiment was set up to 300 °C. Moreover, the quenching step with the exposure of the substrate to the cold N₂ gas was conducted to avoid substrate crack, which easily occurs in the quenching techniques with cool liquid immersion. Song et al. reported that Au {111} film exhibited better binding absorption for graphene solution [23]. For the comparison purpose, we measured the probe immobilization on the FLG-covered Au SPR chip in the presence and absence of the annealing-quenching protocol, as displayed in Fig 3a. The strong absorption and homogenous coverage of the FLG film onto the Au SPR sensing chip were assisted by the annealing-quenching protocols, which in comparison to the non-treated Au chip, dramatically amplified the SPR signal after the immobilization of GNP-DNA matrix via the π - π stacking force between graphene and ssDNA. The π - π stacking force exists in the mechanism of ssDNA immobilization since both graphene and ssDNA shared identical characteristics as the electron-rich materials [16,23]. It is also essential to note the topological impact of the uniform FLG coverage on the Au sensing chip for probe capture. Pertaining to the convection-diffusion-reaction during the FLG drop-casting on the Au chip, the convection-triggered evaporation of liquid and surface roughness of the graphene layer could

enable molecular absorption facilitating the DNA transfer to the graphene interface, which in turn cut off the diffusion time and yield high-sensitivity detection[24].

On the other hand, in the Au SPR chip without the annealing-quenching step, the probe immobilization stage yielded a negligible SPR signal. This is indicative of the poor FLG sheet coverage onto the Au chip. The FLG degradation was plausibly caused by buffer streaming in the reaction chamber creating the lack of Au {111} orientation with higher energy absorption of the graphene layer. This outcome is in a linear agreement with the report of Song et al. [23]. The morphology of Au film in the presence and absence of the annealing is depicted in Fig. S3.

Figure 3 is preferred in this location

3.4. Probe immobilization

The gold nanorods (GNr) with the length of ~40-60 nm and aspect ratio 2.5 were applied to compare the SPR signal enhancement in the GNu-assisted probe immobilization stage. The SPR signal levels are presented in Fig. 3(b). The GNu-ssDNA performs three times higher SPR signal enhancement than the GNr-ssDNA probe's signal. This phenomenon is closely linked with the plasmonic absorption behavior of GNu and GNr, which likely occur in different wavelength regions. The plasmonic absorption of GNu is observed to be in the range of 600-620 nm, which is correlated to the intensity peak of the OLED light source in this experiment. Contrarily, the GNr maximum plasmonic absorption was observed in the range of 720-750 nm wavelength, where the intensity of the OLED light source had dropped below the full-width half maximum (FWHM) [39]. As displayed in Fig 3b, The GNu in this study had successfully provided around three times higher enhancement in the SPR signal than the GNr.

3.5 Detection of cssDNA target

The probe DNA immobilization is pivotal in the overall study. In GNR-assisted DNA detection (Fig S4a and Fig 3b), the SPR signal from the GNR was indicative of the probe DNA immobilization. However, compared to the GNR-assisted one shown in Fig 3a and 3b, the GNR signal is extremely low since the saturation level was achieved very rapidly within the low range level of SPR signal (Fig S4a). In the detection of a series of cssDNA concentrations, the SPR sensor detected the degradation of the signal level after the washing step by PBS solution in both GNR and GNR assisted-DNA detection (Fig S4b and Fig 4a), respectively. It is also notable that in both approaches, the SPR signal dramatically leaped as the cssDNA target was injected into the reaction chamber due to the immediate change in the refractive index value resulted from the salt buffer from the target dilution. The DNA binding affinity is greatly affected by the ionic strength[52], and salt mainly shows interactions with the negatively charged phosphate molecules[53]. The contrast was seen in the SPR signals after PBS wash in GNR-assisted DNA detection, which shows more pronounced signal reduction trends as higher cssDNA concentration was injected into the sensing chamber (Fig 4a). The drop of SPR signal below the reference signal level (negative Δ SPR signal) implies the release of ssDNA probes from the graphene surface by stronger hydrogen bond force during DNA hybridization [54] than the π - π stacking force between graphene and probe DNA [55–57]. The removal of the probe is related to the different binding energy where the DNA hybridization binding energy was estimated at around 65.7 kcal/mol (or equivalent to 2.85 eV)[35], while the π - π stacking force between ssDNA to the graphene surface is only approximately 0.49 to 0.61 eV[7,36]. The removal of the ssDNA and the GNPs away from the sensing area had resulted in a significant drop in the plasmonic field represented by a negative Δ SPR signal. This, by nature, structure, and morphology of the GNPs, was much stronger in the

GNu than GNr in providing the plasmonic effects. This phenomenon leads to the susceptible sensing method with the negative signals.

Figure 4 is preferred in this location

Fig 4b demonstrates the calibration plots from the level signal after PBS washing protocols in the real-time dynamic measurement from GNu and GNr-assisted DNA detection (Fig 4a and Fig S4b). The GNu-assisted DNA detection yields a trendline with a correlation coefficient of 99% and an estimated limit of detection (LOD) based on the International Union of Pure and Applied Chemistry (IUPAC) definition using confidence level 3. It means the LOD was calculated by the estimation of the concentration (the x-axis), when the signal level is reached 3 times of standard deviation of reference measurement, in correlation to the dose-response standard curve. The SD of the reference measurement was 0.23, consequently, the calculated LOD of ~24.5 fM cssDNA was achieved, significantly lower than that estimated in GNr SPR signal shown by 8.2 pM of cssDNA.

Figure 5 is preferred in this location

The calibration plot from the specificity test performed using non-complementary mssDNA target hybridization with the GNu-cssDNA probe was displayed in Fig 4b. In this test, the hydrogen bond of the DNA hybridization was not supposed to exist, and hence, the mssDNA strands were accumulated in the graphene layer due to the π - π stacking force. This mechanism produced a slight shifting of the delta SPR signals to the positive signal[58], which opposed the results from the specific binding of hybridized DNA. The combined calibration plots clearly distinguished the GNu paramount characteristics in providing SPR signal enhancement for both

highly sensitive and specific detection of DNA hybridization. The plasmonic field enhancement characteristics of GNu and GNr simulated in FDTD is presented in Fig. 5. The GNu showed prominent hotspots in every tip of its spicules, while the GNr hotspots were likely distributed in the edge of its diameter. The robust plasmonic profile of the GNu in the simulation affirms the findings on its UV-VIS absorption behavior shown in Fig 2. It is important to note two crucial contributions of the GNu in DNA detection using our constructed SPR sensor. One, the surface roughness effects on the GNu facilitates higher probe DNA binding sites proven by the larger shift in the UV-VIS absorption outcomes than that in the GNr bonded-probe DNA. Two, the sharp spicules and rough morphology of the GNu exhibits a drastic improvement of the SPR signals than the smooth-surfaced GNr shown in the FDTD simulation.

Table 1 is preferred in this location

Compared with the reported studies in related fields in Table 1, it is indicated that our modular and portable sensor using a graphene-based SPR sensor combined with GNu-assisted DNA detection shows excellent advantages in terms of simplicity, portability, specificity. The detection limit in our proposed study is highly comparable to the commercial SPR, laboratory SPR as well as the SPR imaging system, which indicates a promising technique for DNA detection in a low concentration.

4. Conclusion

The proof-of-concept of the DNA hybridization of MTBC, assisted by the plasmonic field from GNu has been presented in a portable graphene-based SPR sensor. The drop-casting of a few-layer graphene solution on the Au chip of the SPR sensor has paved the way for simple, time-

saving, and low-cost sensor production. The high accuracy of the detection reflected from the low detection limit and high specificity in DNA detection performed in this study offer new insights for early screening methods of bacterial infection, such as Tuberculosis infection in a rapid a low-cost manners with portable sensors possibly applied in a remote area or the third world countries as well as applicable for the use in the pandemic scenes.

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Figures and captions

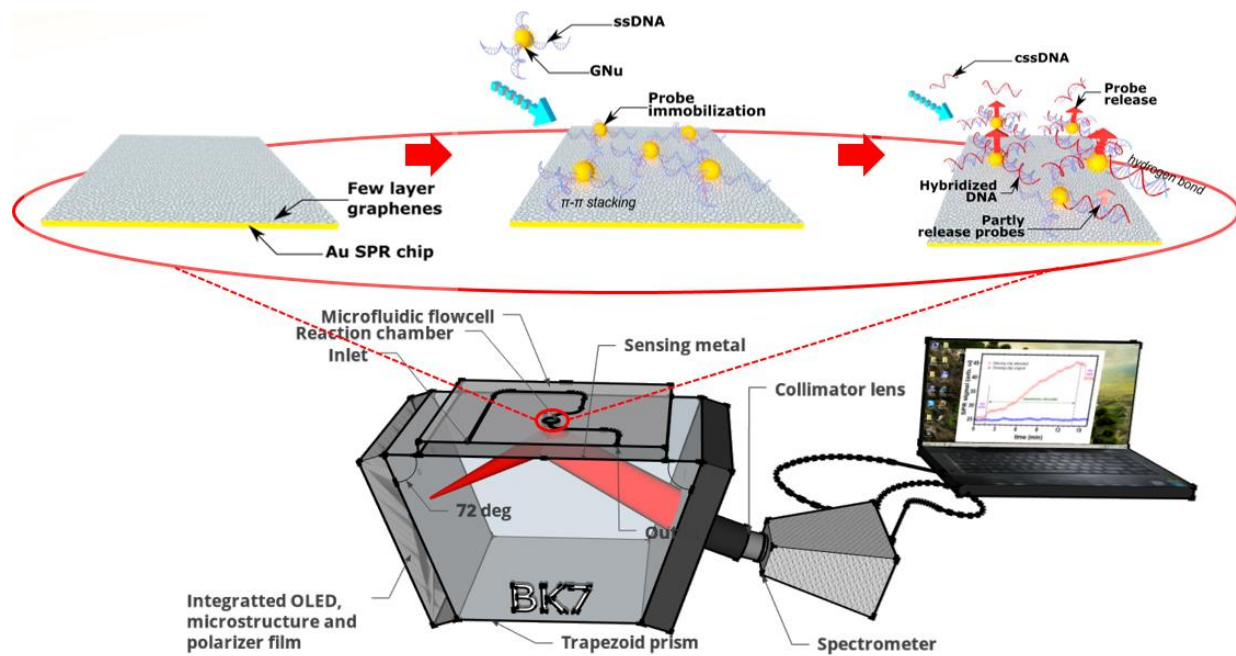


Fig. 1. Experimental configuration of DNA hybridization on a graphene-based SPR sensor.

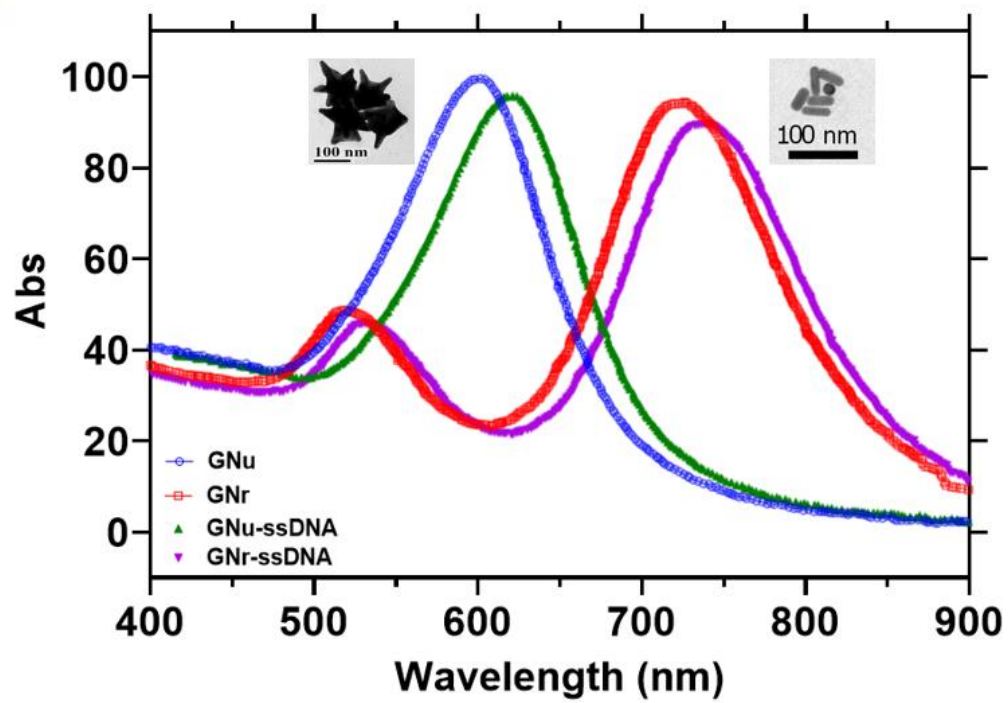
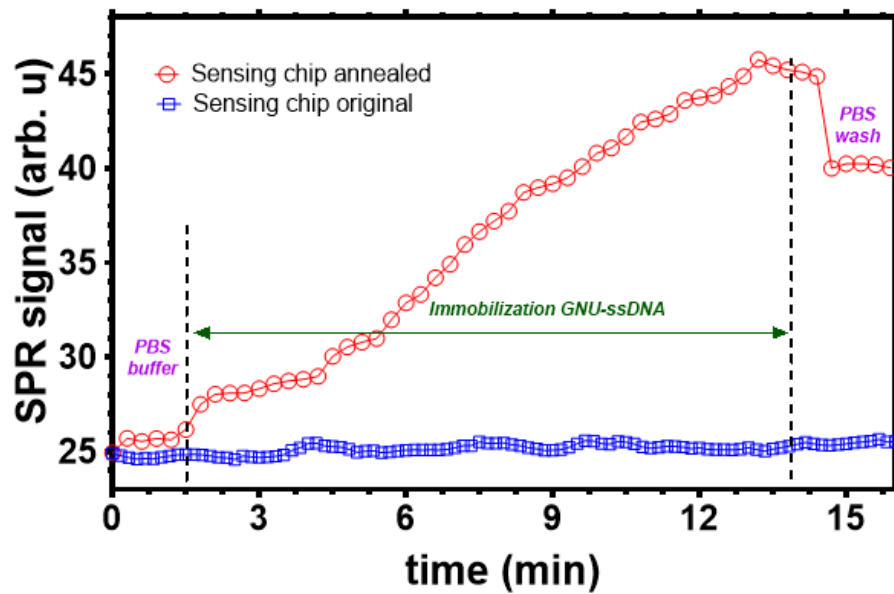
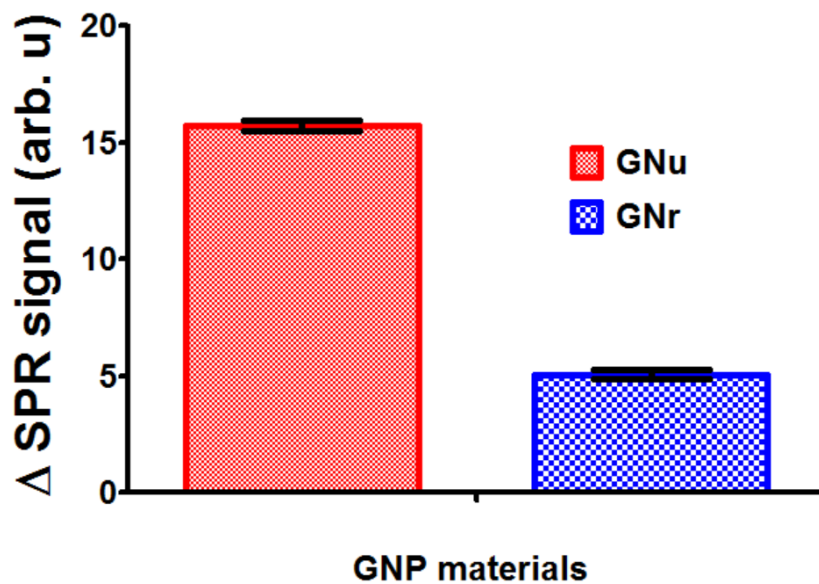


Fig. 2 UV/VIS absorption spectra of GNu and GNr in the absence and presence of ssDNA probe.



(a)



(b)

Fig. 3. (a) The real-time measured SPR signal comparison of GNU-probe immobilization on the FLG layers deposited on Au chip with and without annealing treatment, (b). The comparison of SPR signal level from GNU-ssDNA and GNR-ssDNA probe immobilization on the FLG layer deposited on an annealed Au SPR chip

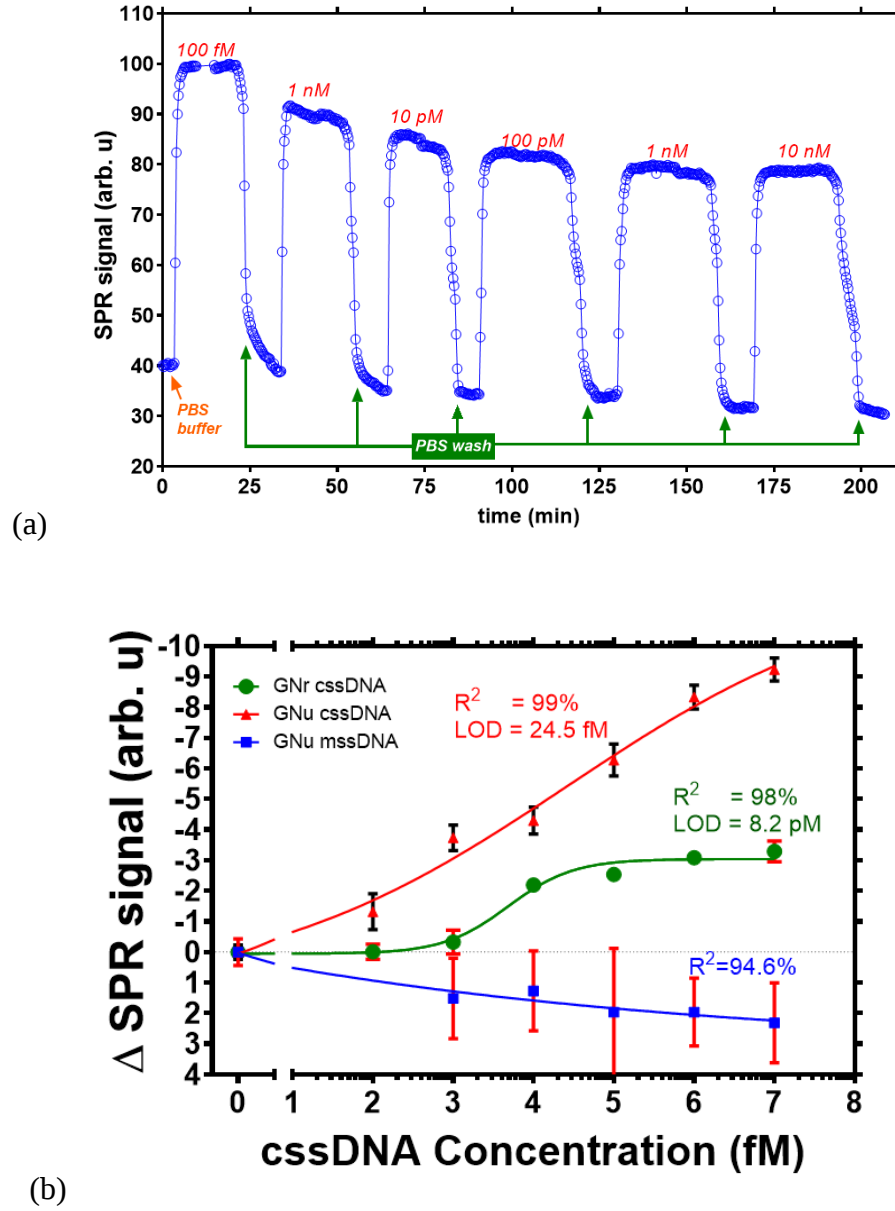


Fig. 4. (a) Real-time SPR sensor measurement of the series concentrations of cssDNA target hybridization with GNU-ssDNA probe, (b) The calibration plots showing the cssDNA target hybridization with GNU-ssDNA and GNr-ssDNA probes, and the specificity test from the hybridization of non-complementary mssDNA and GNU-ssDNA probe.

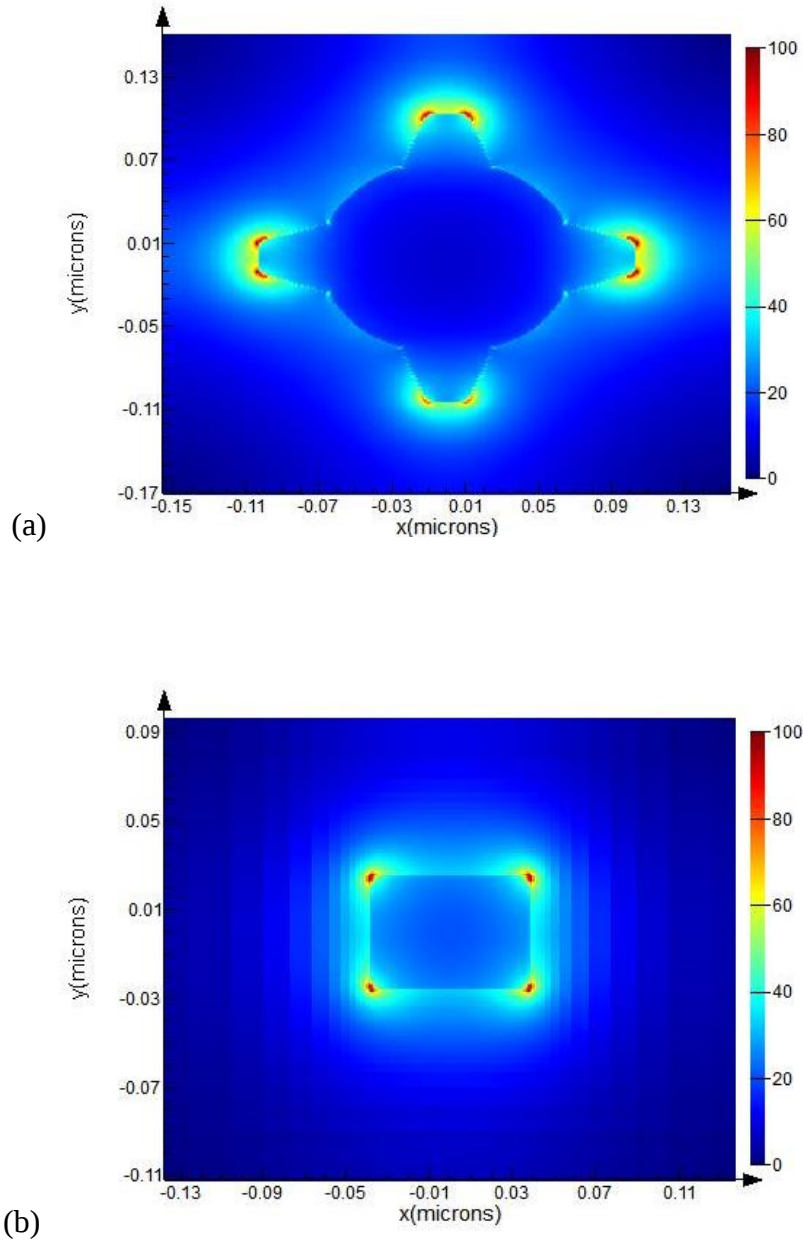


Fig. 5. The FDTD. simulation of plasmonic distribution on GNu and GNr.

Table and caption

Table 1. Performance comparisons of our work with other SPR-based DNA detection studies

No	Sensing platform	Remarkable Technique	Detection Target	LOD.	Ref.
1.	Fiber optic SPR	SAM-PEG surface chemistry binds the streptavidin for signal enhancement	DNA-protein	2 nM	[59]
2.	SPR.	DNA-templated polyaniline deposition	DNA-PNA	5 nM	[60]
3.	SPR imaging	Signal enhancement by colloidal Au NP	DNA hybridization	10 pM	[61]
4.	SPR imaging	Signal enhancement by Au NP	MicroRNAs	10 fM	[62]
5.	Laboratory SPR	GO, signal enhancement by AuNP	DNA hybridization	10 fM	[20]
6.	Commercial SPR Sensia	Graphene, signal enhancement by Au nanostar	DNA Hybridization	0.5 fM	[32]
7.	Localized SPR.	Au nanoisland, Signal enhancement by Au nanostar	DNA hybridization	0.2–40 nM	[63]
8.	Localized SPR.	Doxorubicin-modified gold nanoparticles for signal enhancement	DNA hybridization	600 pM	[64]
9.	Commercial SPR Biacore X	Signal enhancement by streptavidin	MicroRNA	17 pM	[65]
10.	Commercial SPR Biacore X	Signal enhancement by super-sandwich assembly and streptavidin	MicroRNA	9 pM	[66]
11.	Laboratory SPR.	Surface-anchored rolling circle and AuNP amplification	Tuberculosis DNA.	5 pM	[67]
12.	Modular and portable SPR.	Few graphene layers, signal enhancement by Au nano urchin	Tuberculosis DNA	24.5 fM	This work

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