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DNA Detection Using Plasmonic Enhanced Near-Infrared Photoluminescence of Gallium Arsenide

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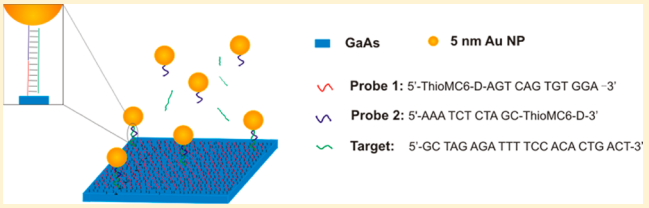
Supporting Information

ABSTRACT: Efficient near-infrared detection of specific DNA with single nucleotide polymorphism selectivity is important for diagnostics and biomedical research. Herein, we report the use of gallium arsenide (GaAs) as a sensing platform for probing DNA immobilization and targeting DNA hybridization, resulting in ∼8-fold enhanced GaAs photoluminescence (PL) at ∼875 nm. The new signal amplification strategy, further coupled with the plasmonic effect of Au nanoparticles, is capable of detecting DNA molecules with a detection limit of 0.8 pM and selectivity against single base mismatches. Such an ultrasensitive near-infrared sensor can find a wide range of biochemical and biomedical applications.



Fast, sensitive, and cost-effective analysis of nucleic acids with single nucleotide polymorphism (SNP) selectivity is of significance due to its broad applications in molecular biology, genetic screening, disease diagnosis, and drug monitoring.[1−4] Because of such importance, a number of optical, acoustic, electrochemical, and electronic approaches have been developed,[3,4] among which hybridization-based optical detection methods are particularly promising due to their versatility and sensitivity.[4] Despite the promises, the applications of optical methods have not been widely adopted, due to several technical limitations, such as the requirement of fluorescence labeling and interference from intrinsic fluorescence from biological tissues or cells.[5] To overcome these limitations, many efforts have been devoted toward developing label-free optical assays in the near-infrared (nIR) window (700 nm to 1300 nm), within which biological samples have minimal absorption.[6]

Semiconducting materials composed of III−V compounds exhibit extraordinary electrical, chemical, and optical properties that can be utilized to develop innovative sensing systems.[7] Gallium arsenide (GaAs), in particular, is an attractive material for electronic and photonic devices because of the high electron mobility and direct band gap structure that result in an intense photoluminescence (PL) signal in the nIR range.[8−10] The intrinsic luminescence of GaAs is remarkably sensitive to the physical and chemical states of its surface, which offers a potential advantage in the development of a new generation of biosensors and biochips.[9,10] This advantage can be further enhanced by implementing optical detection based on GaAs nanostructures, such as nanowires[11] and nanotubes.[12] Therefore, if these materials can be combined with a molecular-recognition element, they will become highly promising candidates in the design of state-of-the-art optical biosensors.[7−10,13]

Herein, we employ GaAs (100) substrates as a sensing platform for label-free nIR optical detection of specific DNA sequences and identification of SNPs down to nanomolar concentrations, based on the GaAs PL intensity difference. Further signal amplification of DNA-recognition events using Au nanoparticles (AuNPs)-induced plasmonic enhancement[14,15] results in an ultrasensitive quantitative method with a detection limit of 0.8 pM.

EXPERIMENTAL SECTION

Chemicals and Materials. The single-crystal n^+-GaAs (100) substrates used in the study were purchased from AXT, Inc. Hydrogen tetrachloroaurate(III) hydrate ($HAuCl_4 \cdot 3H_2O$, 99.999%), L-ascorbic acid (99%), tris(2-carboxyethyl)-phosphine hydrochloride (TCEP), mercaptohexanol (MCH), Na_2HPO_4, NaH_2PO_4, and NaCl were purchased from Sigma-Aldrich. Ultrapure water was obtained through a Nanopure

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Infinity ultrapure water system (Barnstead/Thermolyne Corp, Dubuque, IA) and had an electric resistance >18.3 MΩ. All oligonucleotides used in current study were purchased from Integrated DNA Technologies Inc. (Coralville, IA).

Immobilization of DNA on GaAs Substrates. The procedure to attach DNA onto GaAs was as follows: GaAs substrates were first cleaved into small pieces (1 cm × 1 cm) and then sequentially cleaned by H_2O, ethyl alcohol, acetone, and then H_2O. After that, the GaAs piece was immersed in HCl−H_2O (1:10) for 1 min, then rinsed with double-distilled (DI) H_2O, and dried with N_2. Next, GaAs was incubated with 10 μM DNA probes (FAM-A30-SH, FAM-A30 and A30-SH) for 12 h in PBS solution (pH 6.8) with 500 mM NaCl, then washed by DI H_2O, and blown dry by N_2. The FAM-A30-SH, FAM-A30 were first treated by TCEP before use. To lower nonspecific binding, a backfilling step was necessary. The DNA attached GaAs was immersed in an aqueous solution of MCH (6-mercapto-1-hexanol) with a concentration of 1 mM, for 1 h. Finally, the GaAs samples were measured by fluorescence imaging.

Investigation of Hybridization Activity by DNA Immobilized on GaAs Substrates. As shown in Scheme S1 in Supporting Information, the thiolated-DNA was attached onto GaAs substrates by incubation with 500 mM PBS buffer (pH 6.9) containing 10 μM SH-DNA overnight, then mercaptohexanol (MCH, different concentrations, 0, 0.01, 0.05, and 0.1 mM) for 90 min, and finally thoroughly rinsed by DI H_2O. The DNA hybridization process was accomplished by soaking the DNA attached GaAs in 50 mM Tris-HCl buffer (pH 7.4) and 150 mM NaCl containing 20 μM FAM-cDNA (or FAM-non cDNA) at room temperature for over 90 min. After thoroughly rinsing in 50 mM Tris-HCl buffer (pH 7.4) with 20 M NaCl and drying, the GaAs samples were measured by photoluminescence spectroscopy.

DNA Detection Based on Near-Infrared Photoluminescence of GaAs (100). As illustrated in Scheme S2 in the Supporting Information, we used changes of the nIR bandedge photoluminescence intensities from GaAs as the basis of the conformation change of DNA attached onto GaAs when hybridized with its complementary DNA. The experimental procedure involved DNA immobilization on GaAs, *in situ* DNA hybridization, and PL measurement. Probe DNA attached to a GaAs substrate was placed in a 100 μL volume of target DNA sequences with varying concentrations in hybridization buffer (1× PBS, 0.1% tween 20, pH 7.4). The hybridization was conducted at 37 °C for 2 h. After hybridization the wafers were washed with washing buffer (0.5× PBS, 0.01% tween 20), DI water, and blown dry with N_2. Finally, Renishaw Raman/PL microspectroscopy system was used to collect fluorescence spectra. All sensing measurements were performed under excitation laser of 632 nm, acquisition time of 10 s, and 20× objective lens.

Further Amplification of the nIR Photoluminescence of GaAs by AuNP Plasmonic Effect. The nIR photoluminescence of GaAs was further amplified by the AuNP-plasmonic coupling effect, as shown in Scheme S3 in the Supporting Information. Experimental procedures involved DNA immobilization on the GaAs(100) substrate, target DNA recognition, *in situ* gold plating, and the PL detection. GaAs wafers were placed in 100 μL of target DNA sequences, with varying concentrations, and DNA-Au NPs (10 μM) in hybridization buffer (1× PBS, 0.1% tween 20, pH 7.4). The hybridization was conducted at 37 °C for 2 h. After hybridization, the wafers were washed with washing buffer (0.5× PBS, 0.01% tween 20), then DI H_2O, and blown dry with N_2. Then, the GaAs substrates were immersed in 200 μL of $HAuCl_4$ and AA mixture solution (purchased from Sigma). The attached AuNPs were enlarged by Au metal deposition. Immediately after 2 min of amplification, the reaction was stopped by washing the GaAs substrate with DI H_2O. Finally, a Renishaw Raman/PL microspectroscopy system was used to collect PL spectra. All spectral measurements were performed using an excitation laser of 633 nm, with acquisition time of 5 s, and 20× objective lens.

Instrumentation. Scanning electron microscopy (SEM) images were obtained using a Hitachi S4800 scanning electron microscope. PL spectra of GaAs were collected using a Renishaw microPL/Raman microscope, with the laser-pumping wavelength at 633 nm. Rayleigh line rejection filter for 633 nm was used before collection. The Leica DM2500 M microscope is equipped with objectives of 5×−100×, and a 20× objective was used for this study. A UV coated Deep Depletion CCD array detector (578 × 400 pixels) allows wavelength detection from 200 nm to ∼1050 nm. For PL characterization, the excitation laser used was ∼40 μm in spot size; when a PL intensity comparison of different samples was made, efforts were taken to ensure that multiple spots on the GaAs surface were measured. The error bars correspond to the standard deviation of PL measurements across five repetitive experiments. The excitation power density was in a range of 0.05 to 4.5 kW/cm^2.

RESULTS AND DISCUSSION

The GaAs substrates, which are widely used to manufacture transistors and lasers, are stable under the experimental conditions used and thus are suitable for in vitro diagnostics. A common strategy to modify GaAs surfaces is to use thiol or mercapto-bifunctional molecules to produce an oxide-free surface under mild conditions.[13] Direct immobilization of DNAs onto semiconductor surfaces is an alternative surface passivation method that offers opportunities for facile development of electronic and optoelectronic biosensors.[6,13,15] Our sensing strategy involves immobilization of thiolated probe-DNA (pDNA), subsequent recognition of target complementary DNA (cDNA), and finally signal transduction, all taking place on the GaAs surface (Figure 1A). To adjust the DNA coverage density and improve the DNA hybridization efficiency, 6-mercaptohexanol (MCH) was used before binding to the cDNA. The binding of cDNA transformed the single-stranded structure to duplex, resulting in a different physicochemical adsorption and thus electronic passivation of the GaAs surface, and changed the PL signal.[6,10] To demonstrate the above sensor design, we first immobilized the pDNA labeled with thiol at the 3′-SH end (5′-TTC ACT TCA GTG-ThioMC6-D-3′) on the GaAs surface in its single-stranded form[16] (Figure S1 in the Supporting Information).[10,13] A ∼4-fold increase in the PL peak at ∼875 nm, assigned to the band-to-band emission of GaAs, was observed from the untreated GaAs (black curve) to GaAs-pDNA (red curve, Figure 1B).

Since the abundant negative charge from the DNA backbone depletes electrons from the near surface region of the GaAs, it can effectively increase the band bending and the width of the depletion region, resulting in a decrease in the measured PL intensity. For example, it was reported that the intensity of the GaAs PL peak actually decreased when aptamers were attached,

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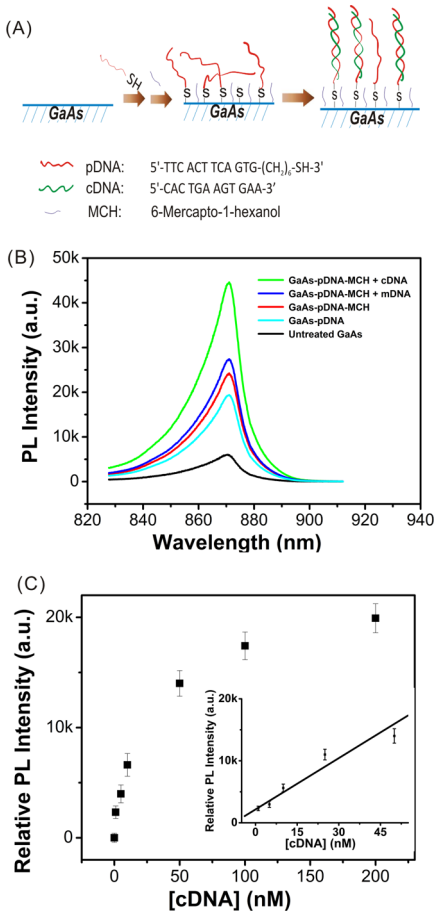

Figure 1. (A) Schematic representation of a GaAs-based near-infrared photoluminescence DNA sensor via DNA conformational changes. A probe DNA (pDNA) with a terminal thiol is immobilized onto oxide free GaAs substrate through self-assembly. In the presence of target complementary DNA (cDNA), duplex DNA is formed, leading DNA to "stand-up" on the GaAs surface, which significantly changes the PL signal. (B) Typical PL response of GaAs-pDNA substrate, with MCH backfilling, hybridized with complementary DNA (cDNA, 100 nM) and single base-mismatched DNA (mDNA, 100 nM), as well as untreated GaAs and GaAs-pDNA without MCH. (C) Relative PL intensities over background of the GaAs-pDNA-MCH-based near-infrared photoluminescence DNA sensor in response to varying concentrations of cDNA. Inset: The linear range of the relative PL intensities under low DNA concentrations. The error bars correspond to the standard deviation of PL measurements across five experiments. Excitation wavelength, 632 nm; acquisition time, 10 s.

and this decrease was attributed to the bending of the energy bands at the GaAs surface due to the adsorption of the negatively charged molecules from the aptamers.[10,17] On the other hand, the modification of GaAs substrates with self-assembled monolayers of basic long-chain alkane thiols resulted in an enhanced luminescence intensity, presumably due to the passivation of midgap surface states through the formation of sulfur-GaAs bonds.[13] In our case, the observed PL enhancement suggests that the passivation effect generated as a result of the thiolated DNA used to tether the molecules to the semiconductor surface dominates any band bending caused by the considerable negative charge of the DNA.

As shown in Figure 1B, the pDNA-GaAs sample with backfilling of MCH showed a slightly higher PL emission, indicating that the addition of MCH further reduces non-radiative surface states of GaAs. Note that direct attachment of MCH to the bare GaAs surfaces where the oxide layer is removed prior to the passivation with the MCH also resulted in enhanced photoluminescence signal (Figure S2 in the Supporting Information). To study the function of MCH, fluorescence microscopy imaging of the dye tagged DNAs (Figure S1 in the Supporting Information) was used. We found that the sample exposed to MCH showed decreased fluorescence, indicating that the MCH spacer can minimize the nonspecific attachment of single-stranded DNA. However, by tuning the ratio of concentrations of SH-DNA and MCH, the hybridization efficiency could be improved as shown in Figure S3 in the Supporting Information. This suggests that the MCH spacer not only adjusted DNA orientation and coverage density on GaAs surface but also prevented nonspecific adsorption of DNA from solution and displaced nonspecifically adsorbed HS−ssDNA.[10]

Interestingly, in the presence of the cDNA (5′-CAC TAA AGT GAA-3′) that is complementary to the pDNA, the peak luminescence intensity increases by ∼8-fold (see Figure 1B, green curve) compared to the untreated GaAs. To eliminate any artifact, a mismatched DNA (mDNA), where an A base of a human aldehyde dehydrogenase 2 gene short fragment was replaced with a G corresponding to a G1459A, SNP was used.[16] Under identical conditions, the mDNA-GaAs resulted in minimal change of the PL peak. Moreover, even by adding substantially higher concentrations of mDNA (1 mM), <10% enhancement in PL intensity was observed in comparison with that of lower concentration (1 nM, see Figure S4 in the Supporting Information). These results demonstrated successful recognition of the target cDNA by the pDNA with excellent selectivity. The enhanced PL intensity (∼8-fold) by adding the target DNA can be attributed to the transformation of flexible single stranded pDNA to a rigid rodlike duplex after hybridization with cDNA.[18] This conformational change should result in the alteration of surface charge distribution, from diffused high density surface charges in the case of ssDNA that often lays flat on the GaAs surface to a much narrower distribution in the rigid duplex that tend to "stand-up" on the surface. As a result, the PL intensity of the GaAs is increased.[10a] In contrast, the lack of change in the PL intensity observed when adding mDNA is consistent with the fact that the SNP discrimination efficacy relies on the melting temperature of probe−target duplexes, i.e., the perfect matched target has a high affinity and the mismatched target has much lower affinity. Therefore the mDNA did not bind to pDNA. Figure 1C shows the increase in PL peak intensity as a function of target DNA concentration ranging from 1 nM to 50 nM. On the basis of the definition that the detection of limit (LOD) is the lowest analyte concentration required to produce a signal at 3 times the standard deviation of the background (3σ, σ was obtained from the measurement of the control experiments), the LOD for this system is determined to be ∼0.2 nM.

While demonstrating the above proof-of-principle experiments is encouraging, the LOD of 0.2 nM is not enough for most DNA detection. The reason is that specific nucleic acids indicating the presence of a disease are often found in only trace amounts,[4] so new technologies with high sensitivity are constantly in demand. Since the presence of a metal surface close to a fluorophore is known to result in the collective electronic oscillations, yielding an enhancement of fluorescence,[14,15,19] we then explored the coupling of surface plasmon effect of gold nanoparticles with PL signal enhancement to

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improve LODs of the system.[19,20] Toward this goal, we designed a sandwiched assay method by assembling AuNPs onto GaAs through DNA hybridization (Figure 2A). The GaAs

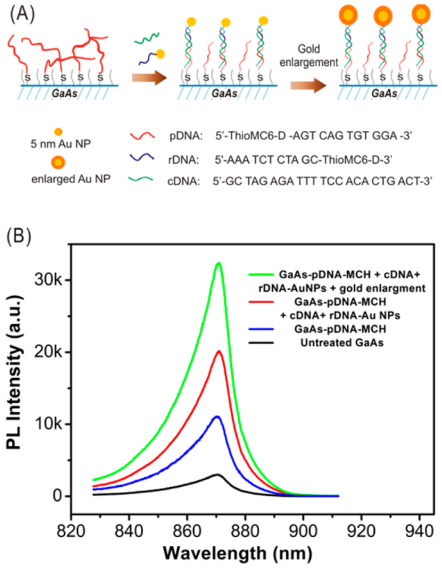

Figure 2. (A) Schematic illustration of the GaAs-based near-infrared photoluminescence DNA sensor with plasmonic enhancement. The capture DNA (pDNA) and report DNA (rDNA) were modified to GaAs and 5 nm AuNPs, respectively. The single-stranded target (cDNA) hybridizes with both the GaAs surface-immobilized pDNA and rDNA on the NPs, thereby attracting 5 nm AuNPs to the GaAs surface. Detection is accomplished using a plasmonic coupling effect of the surface-immobilized AuNP with gold plating, thereby efficiently amplifying PL signal of GaAs. (B) Typical PL response of GaAs-pDNA with MCH backfilling by subsequently introducing with Au NPs and then enhancing by gold enlarging in the presence of 1 nM cDNA as well as untreated GaAs substrate. Excitation laser, 632 nm; acquisition time, 5 s.

surface was modified with the thiolated pDNAs, similar to the previous method shown in Figure 1A. 6-Mercaptohexanol (MCH) was added next to prevent nonspecific adsorption by DNA and AuNPs. A reporter DNA (rDNA) was immobilized onto AuNPs by the salt aging method.[21] If the target cDNA contains a sequence that is complementary to the pDNA at the 3′ end and the rDNA at the 5′ end, the AuNPs will be formed close to the GaAs surface. The AuNP-GaAs surface were subsequently transferred into a solution containing $HAuCl_4$ and L-ascorbic acid (AA) for further gold enlargement.[22] In this way, further signal enhancement was possible.

To validate the sandwich assay method described above, we first immobilized thiolated pDNA (5′-AAA TCT CTA GC-ThioMC6-D-3′) onto the GaAs surface. Similar to the system without AuNPs described earlier, such an immobilization resulted in a ∼3.6-fold increase of PL intensity from untreated GaAs (Figure 2B, black curve) to GaAs-pDNA with MCH backfilling (Figure 2B, blue curve). When the GaAs-pDNA incubated with 1 nM cDNA and 5 nM rDNA-Au NPs, another ∼3-fold increase in PL signal from GaAs was observed (Figure 2B, red curve). However, only minimal PL signal change was found when the GaAs-pDNA incubated with rDNA-AuNPs (Figure S5 in the Supporting Information). These results indicate that the hybridization events among pDNA, cDNA, and rRNA-Au NPs resulted in the enhanced PL intensity of GaAs. After enhancement by a gold enhancer (a mixture of $HAuCl_4$ and ascorbic acid), a highly intense PL peak with a signal gain of ∼10-fold, compared to that without DNA and Au plating, was observed (Figure 2B, green curve). In contrast, no obvious PL enhancement was observed when single- or double-base pair mismatched DNA was added under the same condition, indicating once again the high specificity to DNA sequences. These results are attributed to the plasmon-assisted enhancement of the GaAs photoluminescence, involving electric field induced PL enhancement from metal nanoparticles (Figure S6 in the Supporting Information).

The DNA hybridization and gold enhancement can be directly characterized by scanning electron microscopy (SEM). As shown in Figure S7 in the Supporting Information, a typical AuNP-GaAs structure was obtained by adding complementary target DNA. The NPs were evenly distributed over the whole GaAs surface with a diameter of ∼5 nm. After gold deposition, each of the AuNPs grew, but to different sizes, which may be ascribed to the slightly different levels of oligonucleotide functionalization and, therefore, different activities with respect to their ability to promote the reduction of Au(III) to Au by AA. On the other hand, the presence of noncomplementary DNA resulted in a clean GaAs substrate without any AuNPs being observed, indicating that DNA hybridization was responsible for the attachment of AuNPs on GaAs.

To evaluate the performance of such a sandwich assay system, we investigated the PL response to different concentrations of the target cDNA. As shown in Figure 3A,

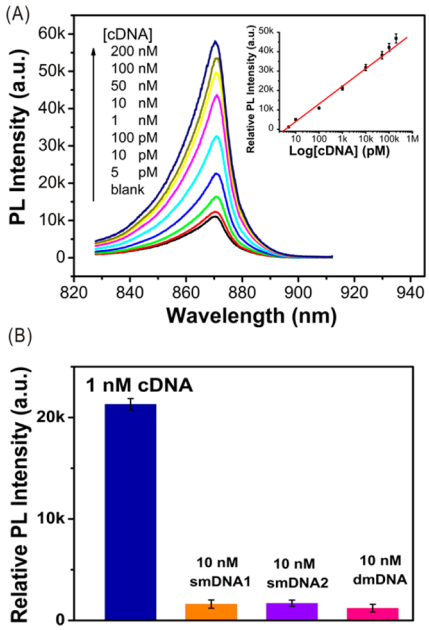

Figure 3. (A) Typical PL spectral responses of the GaAs-based plasmonic enhanced near-infrared photoluminescence DNA sensor to varying concentrations of cDNA from 0 to 200 nM. Inset: A calibration curve demonstrating PL intensity at 875 nm versus target concentration. (B) Typical PL response of GaAs modified capture DNA in the presence of 1 nM target cDNA, 10 nM single-base pair mismatched DNA (smDNA1, 5′-GC AAG AGA TTT TCC ACA CTG ACT-3′; smDNA2, 5′-GC TAG AGA TTT TCC ACA CTG AGT-3′), and double-base pair mismatched DNA (dmDNA, 5′-GC AAG AGA TTT TCC ACA CTG AGT-3′). The error bars correspond to the standard deviation of PL measurements across five repetitive experiments. Excitation laser, 632 nm; acquisition time, 5 s.

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the PL peak intensity gradually increased with increasing concentrations of the target cDNA. A wide dynamic detection range from 1 pM to 10 nM and a LOD (based on the 3σ method) of 0.8 pM were achieved. This LOD is significantly improved over the previously reported systems using GaAs and other hybridization-based DNA sensors (usually nM–pM), as summarized in Table S1 in the Supporting Information. Moreover, three types of mismatched DNA were chosen to examine the selectivity of this method. As shown in Figure 3B, the mismatched DNA targets with 23-base length resulted in a much lower PL intensity under the same condition. These results demonstrate the single-base-mismatched DNA strands can be directly discriminated from the perfectly complementary targets under ambient conditions, indicating the high specificity of the proposed assay. In addition, this nIR optical assay exhibited excellent reproducibility (n = 4, SD = 4.2%) and a good linearity (inset in Figure 3A). Therefore, it is possible to use this system as a robust biosensing platform with high sensitivity and selectivity.

CONCLUSION

In conclusion, we have successfully employed the GaAs surface as a sensing platform for the nIR photoluminescence detection of specific DNA sequences and identification of SNPs with an excellent detection limit of ~0.8 pM. We will build upon the above the proof-of-concept experiments and carry out a more comprehensive study for detection in the real biological sample in the near future. This platform should be readily adaptable to probe photoluminescence enhancement for a range of other nanomaterials. Such systems open a new avenue to integrate biomolecules nIR optical sensors with high-throughput analytical devices, such as microarray and microfluidic chips.

ASSOCIATED CONTENT

Supporting Information

Experimental details and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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