Graphene Oxide Biosensors

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# Photoluminescent Graphene Oxide Ink to Print Sensors onto Microporous Membranes for Versatile Visualization Bioassays**

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The existence and quantities of biomarkers, including peptides, proteins, and DNA, are often the significant signs of certain disease states and physiological processes.[1] The rapid and low-cost method for the identifications and detections of these biomolecules is of crucial importance for disease diagnosis and medical research, which is difficultly achieved by the well-established instrumental techniques owing to expensive equipment and complex sample pretreatments.[2] Recently, great efforts have been devoted to the development of simple nanosensors by the employments of superior physical properties and chemosensory flexibility of nanomaterials. In particular, the optical nanosensors based on the unique fluorescence emission and optical absorption of nanomaterials are widely explored for the detections of biological species.[3–7] For example, fluorescent polymer–nanoparticle complexes identify proteins by fluorescence;[1] gold nanorods or nanoparticles detect peptides[4] and DNA sequences[5] by color changes; hybrid quantum dots visualize biomolecules in cells[6] and explosive particulates at surfaces by imaging.[7] These colorimetric responses can easily be observed by either the naked eye or simple measurements. Among them, colorimetric test papers having the advantage of low cost, easy operation, portable feasibility, and quantitative analysis have been considered as the most desirable approach for chemical or biological assays.[8] However, most of optical nanomaterials are not suitable to make paper sensors owing to the loss of optical/sensitive activeness at the dry and aggregating states, the difficulty of fabrication on solid substrates, and the limit of chemosensory mechanism. To date, the paper sensor, in particular, for the detection of divergent biological species, is rather few.

Herein, we report photoluminescent graphene oxide (GO) paper-like sensors for the ultrasensitive visual bioassays of peptide, protein, and DNA. GO nanosheets are a two dimensional oxidized derivative of graphene, which can be easily exfoliated from graphite in high yield under simple oxidizing conditions. Owing to the huge surface area and a thickness of several nanometers,[9] the ultrathin flexible nanosheets can easily be assembled into the form of paper sheets[10a] or directly fabricated on solid substrates.[10b] Furthermore, GO bears numerous phenol hydroxy and epoxy groups at the basal plane and carboxylic groups at the lateral edge,[9] thus providing the direct interaction with biological species and the flexibility of chemical modification.[11] In principle, these features make GO nanosheets an excellent candidate as basic materials for paper chemosensors. However, common GO nanosheets possess a finite electronic bandgap and are usually used as a highly efficient fluorescence quencher in many reported biosensors.[12] The lack of signal output capability greatly limit application in colorimetric paper sensors. On the other hand, unmodified GO nanosheets can emit a weak fluorescence at about 550 nm.[13] In recent work, highly photoluminescent GO nanosheets were successfully synthesized through the amine reaction and ring-opening amination at the surface of GO nanosheets.[14] The emissive peak at 440 nm exhibits a large blue-shift relative to that of pure GO. Generally, the weak emissions of GO are attributed to a large number of conjugated aromatic $sp^2$ clusters at GO nanosheets. When the epoxy rings at GO nanosheets are opened by the present amination reaction, the degree of $sp^2$ conjugation will largely be reduced, making the emissive peak exhibit a large blue shift.

Herein we propose a fluorescence "off-to-on" mechanism of GO nanosheets for assays of different biological species by the employment of silver nanoparticles (AgNPs) functionalized with ligands, antibodies, and oligonucleotides. Furthermore, the sensors are formed by jet-printing GO ink onto a piece of commercial microporous membrane for the ultrasensitive visual detection of peptide, protein, and DNA in a very simple fashion.

Scheme 1 illustrates the fluorescence off-to-on mechanism of GO nanosheets by various functionalized AgNPs for the detections of different biomolecules. GO was chemically treated with *n*-butylamine (NHBu), and the resultant nanosheets (GO-NHBu) emitted a bright blue fluorescence at 440 nm with the excitation of 350 nm light.[14] The emission exhibits an excellent fluorescence stability under different pH values and in the presence of organic/biological species. Here, the zeta potential of the used GO-NHBu aqueous solution is about −21.6 mV.[14] Futhermore, the GO-NHBu nanosheets are several hundred nanometers in dimension at the basal surface and about 2 nm in thickness (Supporting Information, Figure S1 a), and possess a huge surface that bears hydrophobic aromatic rings at their basal plane and a great number of hydrophilic end groups, including amine, hydroxy, and alkylamide, thus exhibiting various interactions with biological molecules, such as hydrogen bonds and π–π conjuga-

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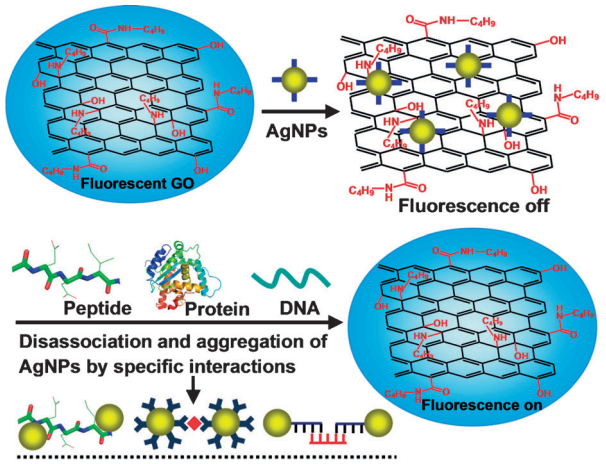


***Scheme 1.*** Fluorescence "off-to-on" mechanism of GO-NHBu nanosheets for biological assays. The photoluminescent GO nanosheets adsorb functionalized AgNPs, leading to the quenching of GO fluorescence. The addition of target species leads to the disassociation and aggregation of AgNPs, and the fluorescence of GO is thus recovered.

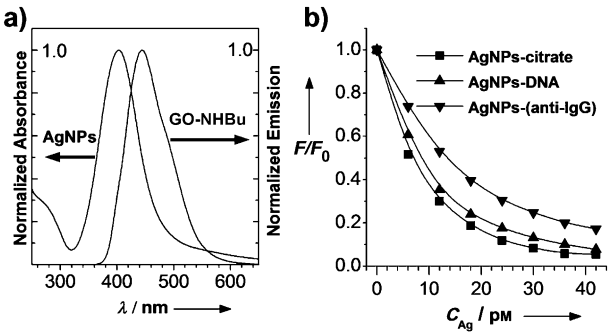


***Figure 1.*** Fluorescence quenching of GO-NHBu with AgNPs. a) Absorption spectrum of AgNPs and emission spectrum of aqueous GO-NHBu solution under excitation at 350 nm. b) Plots of fluorescence quenching efficiencies ($F/F_0$) versus the concentrations of citrate-stabilized, oligonucleotide-conjugated, and anti-IgG-modified AgNPs, respectively.

tion.[11] To devise the GO-based sensors, we synthesized a series of functionalized AgNPs (30 nm) with different recognition elements, including ligand, antibody, and oligonucleotide (see the Experimental Section in the Supporting Information). Upon addition into the aqueous GO-NHBu solution, these AgNPs can thus adsorb onto the surface of GO-NHBu nanosheets by nonspecific surface interactions, forming GO-NHBu/AgNPs complexes that are still highly dispersive in water owing to the huge surface area of GO-NHBu and very small amount of AgNPs. Simultaneously, the fluorescence of GO-NHBu is strongly quenched by the AgNPs through resonance energy transfer or charge-transfer process (see below). When the corresponding analyte is added into the system, specific donor–acceptor interactions occur between analytes and AgNPs, for example, antigen–antibody reaction and DNA strand hybridization, which will lead to the disassociation of AgNPs from the GO-NHBu nanosheets and their aggregation. The fluorescence of GO-NHBu nanosheets is immediately recovered, thus providing the rapid identification and quantification of analytes by the fluorescence.

When GO-NHBu and citrate-stabilized AgNPs are mixed in aqueous solution, the AgNPs obviously adsorb onto the surface of GO-NHBu nanosheets and do not aggregate, as revealed by TEM observation (Supporting Information, Figure S1 b), which is similarly observed with oligonucleotide-conjugated and anti-IgG-modified AgNPs, respectively. The attaching behavior clearly confirms that the surface of GO-NHBu nanosheets involves homogeneous interactions with the functionalized AgNPs. On the other hand, the 30 nm AgNPs exhibit a strong absorption centered at 405 nm, and have a partial spectral overlap with the GO-NHBu emission centered at 440 nm (Figure 1 a). The spectral relationship implies that the homogeneous adsorption of AgNPs at the surface of GO-NHBu may lead to highly efficient quenching of GO-NHBu fluorescence by resonance energy transfer or charge transfer. With the increase of three kinds of AgNPs in the aqueous GO-NHBu, we can simultaneously monitor the gradual quenching of GO-NHBu fluorescence, and the bright blue fluorescence of GO-NHBu under UV lamp completely disappears with AgNP concentrations of up to 60 pM (Supporting Information, Figure S2). The fluorescence intensity $F/F_0$ rapidly decreases with the amount of three AgNPs in the 1.2 $\mu g\,mL^{-1}$ aqueous GO-NHBu solution (Figure 1 b). However, these AgNPs exhibit the similarly high efficiencies of quenching fluorescence at only picomole levels. The disassociation of trace AgNPs from GO-NHBu nanosheets will provide an ultrasensitive/rapid bioassay method by the recovery of fluorescence of GO-NHBu.

In general, metal nanoparticles are usually an efficient quencher of many emitters, such as dyes and fluorescent polymers, by the strong charge transfer.[15] However, the highly efficient quenching by AgNPs should originate from the resonance energy transfer from GO-NHBu to AgNPs owing to spectral overlapping (Figure 1 a). Various evidence has enforced the belief that 1) the fluorescence of GO-NHBu is not quenched by other metal nanoparticles, including often used gold nanoparticles; and 2) the emissive peak of GO-NHBu gradually shifts to a longer wavelength from 440 nm to 485 nm while becoming weaker (Supporting Information, Figure S2) because the absorption of AgNPs only overlaps with the short-wavelength part of GO-NHBu emission. More experimental evidence is detailed in the Supporting Information, Figure S3.

Herein, we validate the above sensory mechanism and its sensitivity for the detection of three representative biomolecules, namely biothiols (glutathione and cysteine), immunoglobin (IgG), and a DNA sequence (Figure 2). The detection of glutathione (GSH) with citrate-stabilized AgNPs is shown in Figure 2 a. Citrate-stabilized AgNPs are negatively charged at the surface. However, the isoelectric points of GSH and cysteine are 5.92 and 5.02, respectively, and thus they are the forms of positively charged ions in the citrate buffer (pH 3.0). The electrostatic interaction will damage the negatively charged repulsion among silver colloids,[16] and immediately lead to the disassociation and aggregation of AgNPs (Sup-

  

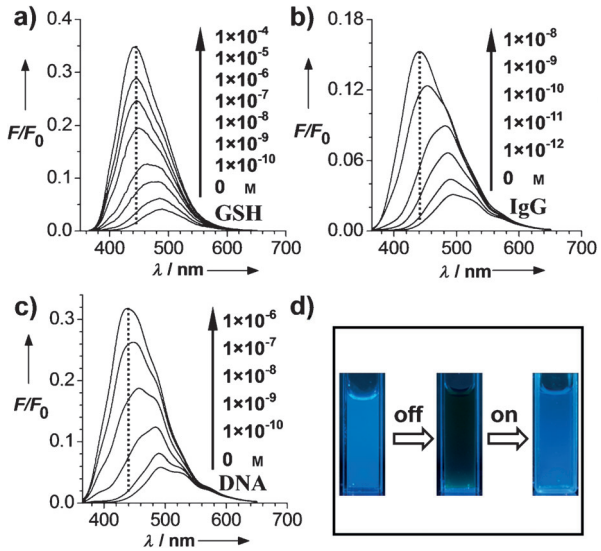


***Figure 2.*** Switching-on of GO-NHBu fluorescence by analytes in solution. a)–c) Evolution of fluorescence spectra of GO-NHBu/AgNPs complex with the addition of a) glutathione, b) IgG, and c) target DNA. d) Photographs of the aqueous GO-NHBu solution in the fluorescence off-to-on process under a 365 nm UV lamp. The fluorescence of GO-NHBu is first quenched with 60 pM AgNPs. $F_0$ represents the original intensity before the addition of AgNPs.

porting Information, Figure S4). As expected, fluorescence of GO-NHBu is gradually enhanced with the addition of GSH into the solution containing GO-NHBu/AgNPs complex (Figure 2a). Similar results are also obtained for cysteine (Supporting Information, Figure S5). Meanwhile, the fluorescence peak gradually shifts from 485 nm back to original 440 nm with the recovery of fluorescence intensity, which further confirms the quenching mechanism of resonance energy transfer. Furthermore, the fluorescence enhancement is proportional to the logarithm of glutathione and cysteine concentrations, and the limit of sensitivity for glutathione and cysteine is about 20 pM (Supporting Information, Figure S6), which exceeds the currently reported methods.[3] On the other hand, the fluorescence is highly selective and exhibits an excellent anti-interference with metal ions and other α-amino acids, and the method is also applicable in the determination of total biothiols in blood plasma after the pre-reductive reactions (Supporting Information, Figure S7–S9 and Table S1).

Figure 2b shows an experimental example for the detection of protein, namely human immunoglobin (IgG). The fluorescence of GO-NHBu nanosheets is first quenched by the addition of AgNPs-(anti-IgG). Subsequently, the introduction of IgG will exfoliate AgNPs-(anti-IgG) from the GO-NHBu nanosheets and lead to the aggregation of the AgNPs by the widely known antigen–antibody reaction (Scheme 1). Therefore, the fluorescence of GO-NHBu is gradually turned on with the increase in the amount of IgG in solution (Figure 2b). Similarly, the fluorescence peak shifts from 485 nm back to 440 nm with the recovery of fluorescence intensity, which is again consistent with our expectations. The fluorescence enhancement $F/F_0$ also exhibits an excellent linear correlation with the logarithm of IgG concentrations, with a limit of detection as low as 0.1 pM (Supporting Information, Figure S10). Furthermore, the high specificity of this method is also demonstrated by the observations of fluorescence enhancement with the additions of other serum proteins: transferrin, serum albumin, and thrombin (Supporting Information, Figure S11).

Meanwhile, we have demonstrated the detection of target DNA sequences in GO-NHBu solution (Figure 2c). Two sets of non-complementary oligonucleotide segments (oligonu-1: 5′AGTCAGGTGCAC-$(CH_2)_6$-SH3′, and oligonu-2: 5′GGACACCTCTTC-$(CH_2)_6$-SH3′) are separately conjugated to AgNPs (see the Experimental Section in the Supporting Information). The target DNA (5′GAAGAGGTGTCCGTGCACCTGACT3′) contains the two sequences pairing with oligonu-1 and oligonu-2, respectively. The AgNPs-(oligonu-1) and AgNPs-(oligonu-2) are mixed in an equal amount, and then added into the phosphate buffer solution of GO-NHBu containing 20 mM NaCl, leading to the fluorescence quenching of GO-NHBu. The addition of target DNA will cause the aggregation of AgNPs by the hybridization of the target DNA with oligonu-1 and oligonu-2. The fluorescence is immediately turned on with the increase of target DNA (Figure 2c). Even at 0.1 nM target DNA, the fluorescence intensity is still enhanced by a factor of about 41.5%. The limit of detection reaches 10 pM, and more results have also suggested a high selectivity for the assay of DNA sequences (Supporting Information, Figure S12, S13).

Fluorescent GO-NHBu consists of two-dimensional ultrathin nanosheets (ca. 2 nm thickness), and thus provides feasibility for printing the sensors on the paper-like materials (Figure 3a). The fabrication necessarily satisfies two basic requirements: 1) the substrate materials do not have any intrinsic fluorescence under UV lamp; and 2) the GO-NHBu nanosheets can firmly stick to the substrate through jet-printing and should not exfoliate or disassociate upon

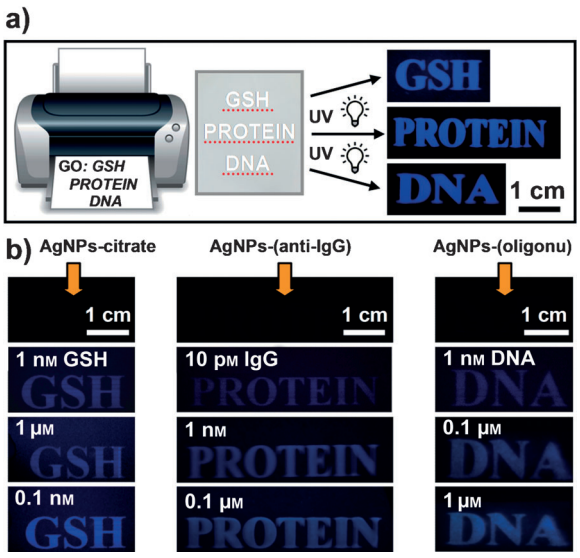


***Figure 3.*** Visual biosensors. a) The inkjet printing of GO-NHBu into "words" on microporous polyvinylidene fluoride membrane. b) Visual detections of GSH, IgG, and DNA under a UV lamp.

contacting the aqueous solution of analytes. In the case of normal printing papers, the strong background fluorescence under UV lamp seriously interferes the colorimetric observations. In particular, the printed GO-NHBu nanosheets automatically exfoliate from the normal papers or common filter paper upon contacting aqueous solution, which is due to the obvious swell and dilation of these highly hydrophilic papers (Supporting Information, Figure S14). However, the commercial polyvinylidene fluoride (PVDF) microporous membrane has a highly compact and stable structure (Supporting Information, Figure S15) and a large number of micrometer-scaled (0.22 μm) pores, which do not only overcome the drawbacks of swell and dilation of normal papers in water, but also significantly increase the fastening of printed GO-NHBu nanosheets on this membrane. Although small degree of diffusion from the printed spots on the PVDF membrane may occur, it is very negligible and does not affect the use in detection. (Supporting Information, Figure S16).

Aqueous GO-NHBu nanosheets (2 mL, 0.9 mg mL$^{-1}$) acting as colorless "imaging ink" were first injected into a vacant cartridge of commercial inkjet printer. The desired word or image is printed onto a piece of PVDF microporous membrane by a common printer connected with a computer. The invisible words GSH, PROTEIN, and DNA appear in bright blue under a UV lamp (the right images of Figure 3a). Surprisingly, the edges of the words are very sharp and their brightnesses of fluorescence are completely uniform. Moreover, the brightness of the words is tunable by the concentration of GO-NHBu. These results indicate that the method is highly accurate, reliable, and applicable in the fabrication of sensors on the PVDF membrane. In principle, the printed GO-NHBu nanosheets may fold on the surface, creating pockets that could trap Ag nanoparticles to prevent their disassociation, and this effect might reduce the sensitivity. However, the sizes of used nanosheets are only 2–3 hundred nanometers, the aqueous solution is at a very dilute concentration, and the inkjet printing avoids laser heat. These greatly reduce the possibility of nanosheet curling.

We repeated the above detections of GSH, IgG, and DNA on the test paper by the fluorescence off-to-on operation (Figure 3b). First, 10 μL of AgNPs-citrate, AgNPs-(anti-IgG), and AgNPs-(oligonu) (0.1 nM) was dropped onto the test paper, and these AgNPs adsorbed onto the words GSH, PROTEIN, and DNA, accompanied by the evaporation of solution. Owing to the energy transfer between the GO-NHBu and AgNPs, these bright blue words on the test paper disappeared five minutes later under the UV lamp. Subsequently, the analyte solutions of GSH, IgG, and DNA were added onto the corresponding test papers. The AgNPs disassociated from these words and aggregated owing to the donor–acceptor interactions mentioned above. Thus, the GSH, PROTEIN, and DNA words gradually appeared with the increase of analyte amounts under UV lamp. When 1 nM of GSH was added, the fluorescent GSH can still clearly be discerned under UV lamp. Meanwhile, IgG and DNA were clearly visualized with the limits of 10 pM and 1 nM, respectively, under the currently unoptimized conditions. Established routine techniques for the detection of DNA are performed by a reaction of DNA amplification and a spectroscopic assay using fluorescence or chemiluminescence spectrometers. However, the present work shows that the paper-like biosensors can directly, rapidly, and sensitively detect DNA, which does not need the complicated amplification reaction and spectroscopic equipment. Thus, the method exhibits the advantages of low cost, easy operation, and portability. Although the detection limits do not exceed some reported methods, such as chemiluminescence, the sensitivity can meet the requirements of biological detection and medical diagnosis.

Even if there are some successful techniques that use the release of quenched fluorophores from GO as a readout method,[12] most of the sensors based on the organic dyes usually have the drawback of either fluorescence self-quenching or photobleaching at solid state. Therefore, they are not suitable to construct a paper-like biosensor.

In summary, the reported graphene oxide nanosheets emit a highly stable fluorescence in soluble and dry states and can adsorb various functionalized silver nanoparticles by non-specific interactions at surface. These make the ultrathin nanosheets an excellent chemosensory material for direct use in solution detection and easy fabrication of sensors on microporous membranes. Very few silver nanoparticles can quench the fluorescence of graphene oxide by resonance energy transfer and then are disassociated from the nanosheets by the analytes through the specific interactions in which the fluorescence on provides an ultrasensitive biological detection. In particular, the ink of graphene oxide can easily be printed into the highly uniform word or image for the fabrication of test paper. The portable sensors for the visual detections of peptide, protein, and DNA are rapid, simple, efficient, and inexpensive. If a library of silver nanoparticles with various recognition elements is available, a nearly universal bioassay will be achieved.

## Experimental Section

The photoluminescent GO nanosheets were synthesized as reported previously.[14] AgNPs were prepared by the reduction of $AgNO_3$ with trisodium citrate in water. The preparations of anti-IgG and oligonucleotide conjugated AgNPs are detailed in the Supporting Information.

Printing of photoluminescent graphene oxide sensors: A common cartridge of a commercial inkjet printer was washed with deionized water until the ink powder was cleared away completely. Then, an aqueous solution of GO-NHBu nanosheets (2 mL, 0.9 mg mL$^{-1}$) as ink was injected into the vacant cartridge by a syringe. Subsequently, GO-NHBu can be printed into different "words" on a piece of polyvinylidene fluoride (PVDF) microporous membrane by an inkjet printer connected with a computer. After the treatment by drying at room temperature, photoluminescent graphene oxide sensors were obtained.

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