Electronic Supplementary Information

Fabrication of a Boron Nitride-Gold Nanoclusters Composite and Its Versatile Application for Immunoassay

Guo-Hai Yang,[a] Jian-Jun Shi,[a] Sheng Wang,[a] Wei-Wei Xiong,[a] Li-Ping Jiang,[a] Clemens Burda,[b] and Jun-Jie Zhu*[a]

[a] State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, P. R. China. Fax&Tel: +86 2583597204; E-mail: jjzhu@nju.edu.cn

[b] Center for Chemical Dynamics and Nanomaterials Research, Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106, USA

Materials

BN powder (size 1-2 μm, 99.99%) was purchased from Aladdin Chemistry Co. Ltd. Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was from Shanghai Chemical Reagent Co. (China). Glutathione reduced (GSH), poly(diallyldimethylammonium) (PDDA, 20 wt% in H_2O), 2-(N-morpholino)ethanesulfonic acid (MES), 3-mercaptopropionic acid (MPA), 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS) and bovine serum albumin (BSA) were from Sigma-Aldrich (St. Louis, USA). Human Interleukin-6 (IL-6) (Ag), capture (Ab_1) and signal IL-6 antibody (Ab_2), Carcinoembryonic antigen (CEA), C-reactive protein (CRP), Tumor Necrosis Factor-Alpha (TNF-α) and Human Interleukin-6 ELISA Kit were obtained from Beijing Biosynthesis Biotechnology Co. Ltd. (Beijing, China). The serum samples from leukemia patients were obtained anonymously from Nanjing Gulou Hospital and used as received. When the levels of analyte were over the detection dynamic ranges, serum samples were appropriately diluted with 0.01 M PBS (pH 7.4) prior to the assay. Phosphate buffer saline (PBS, 0.01 M, pH 7.4) contained 136.7 mM NaCl, 2.7 mM KCl, 8.7 mM Na_2HPO_4 and 1.4 mM KH_2PO_4. All other reagents were of analytical grade. All aqueous solutions were prepared using ultra-pure water (Milli-Q, Millipore).

Apparatus

UV-Vis absorption spectra were recorded using a UV-3600 spectrophotometer (Shimadzu, Japan), Fourier transform infrared (FT-IR) spectra were obtained on a Nicolet 6700 spectrophotometer (Nicolet, USA). Zeta potential was measured on a PSA Nano 2590 zeta-potential analyzer (Malvern, UK). Photoluminescence (PL) spectra were obtained on a RF-5301PC spectrophotometer (Shimadzu, Japan). Atomic force microscopy (AFM) image was obtained in tapping mode on an Agilent 5500 (Agilent Technologies, Inc., USA). Scanning electron microscopy (SEM) were recorded with a Hitachi S-4800 scanning electron microscope. High-resolution transmission electron microscopy (HRTEM) images were taken using a JEOL 2010 electron microscope (JEOL Ltd., Japan). MTT assay was recorded at 490 nm using a Bio-Rad 680 microplate reader. Confocal laser scanning microscopy (CLSM) studies

were performed using a Leica TCS SP5 microscope (Germany) with excitation at 405 nm. Fluorescence microscopy images were taken from a Nikon TE2000-U inverted optical microscope. Electrochemical measurements were performed on a CHI 660a workstation (Shanghai Chenhua, China) electrode system comprised of a platinum wire auxiliary, a saturated calomel reference and the modified glass carbon (GCE) working electrode.

Synthesis of PDDA-BN sheets

100 mg of BN powder was dispersed in 50 ml PDDA/water solution (1.5 mg mL^{-1}) by sonication (Sonics VCX-750 ultrasonic processor with flat head tip, 750 W at 30% Amplitude) for 4 h. The dispersion was then allowed for 24 h. Then the dispersion was centrifuged at 2000 rpm for 20 min. After centrifugation, decantation was carried out by pippetting off the top half of the dispersion into a glass pot. Residual PDDA was removed by high speed centrifugation (10000 rpm for 10 min), the complex was washed three times with deionized water to obtained PDDA-BN.

Synthesis of the PDDA-BN/GNCs composite.

GNCs were synthesized according to the literature. In brief, aqueous $HAuCl_4$ solution (0.5 mL, 20 mM, 25 °C) and GSH solution (0.15 mL, 100 mM, 25 °C) were mixed to H_2O (4.35 mL, 25 °C), and the reaction was allowed to proceed under vigorous stirring at 70 °C for 24 h. The obtained GNCs were further purified by ultrafiltration. Then, PDDA-BN was dispersed in 2.0 mL of the above AuNCs solution and sonicated for 30 min. After centrifugation and further washed with distilled water three times, the composite was obtained.

Assembly of Ab_2 onto the PDDA-BN/GNCs composite.

Ab_2 molecules are covalently linked to the PDDA-BN/GNCs composite by a classical strategy, through the interaction between gold and mercapto or primary amine groups of proteins. This process avoided protein crosslinking, and retained their specific immunorecognition ability. For details, Ab_2 (100 μL, 1.0 mg mL^{-1}) was added to a 5.0 mL PDDA-BN/GNCs solution and shaken overnight at 4 °C. The mixture were then centrifuged and washed with distilled water three times. Finally, the bioconjugates were redispersed in 2.0 mL PBST (PBS, 0.05% Tween) that contained 0.1% BSA as

the assay solution and stored at 4 °C.

Preparation of graphene sheets (GS).

GS was prepared from graphite oxide (GO) through a thermal exfoliation method according to previous report.[1] Thermal exfoliation of GO was achieved by placing GO (100 mg) into a quartz tube under argon atmosphere. The quartz tube was flushed with argon for 10 min, and then quickly inserted into a furnace preheated to 1000 °C and held in the furnace for about 1 min.

Preparation of the immunosensor.

Ab_1 was immobilized onto the surfaces of GS through an amidation reaction[1] between the carboxylic acid groups attached to GS and the available amine groups of Ab_1. Typically, into 1.0 mL of GS solution (1.0 mg mL^{-1}), EDC and NHS (100 mM) were added. The mixture was stirred for 4 h and after that, 1.0 mL of Ab_1 solution (100 mg mL^{-1}) was added into the mixture. After another 12 h of reaction, the solution was centrifuged and washed. The resulting GS/Ab_1 conjugates were stored at 4 °C in PBS before use. After washing with PBST, the GS/Ab_1 modified electrode was blocked with blocking solution (PBS containing 0.2% BSA) for 30 min at room temperature and washed with PBST. The electrode was then incubated with several different concentrations (from lower to higher) of Ag (10 μL) at 37 °C for 50 min, followed by washing with PBST to remove the nonspecific adsorption. Next, 10 μL of PDDA-BN/GNCs/Ab_2 was added to the reaction area of the electrode and incubated at 37 °C for another 50 min. To decrease background response, the electrode was washed with double-distilled water to remove nonspecifically bound bioconjugates.

Optical Detection.

Optical immunoassay was performed using gel image systems (Bio-Rad). The relative intensity of each dot was scored using Quantity One (Bio-Rad), a quantitative analysis software program.

Electrochemical Measurements.

Electrochemical detection involved oxidation of the GNCs at 1.4 V for 120 s in 0.1 M HCl, and immediately using a square wave voltammetric (SWV) waveform, from +0.55 to 0 V, with 4-mV potential steps, 25-Hz frequency, and 25-mV amplitude.

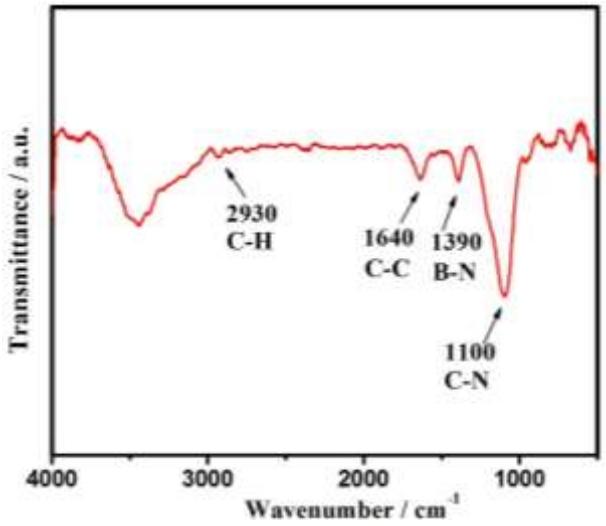

Fig. S1 FT-IR spectra of the as-prepared PDDA-BN sheets.

For the PDDA-BN sheets, the typical IR absorption peaks were at 2930, 1640, 1390 and 1100 cm^{-1}, which attributed to the bond of C-H, C-C, B-N, C-N respectively.

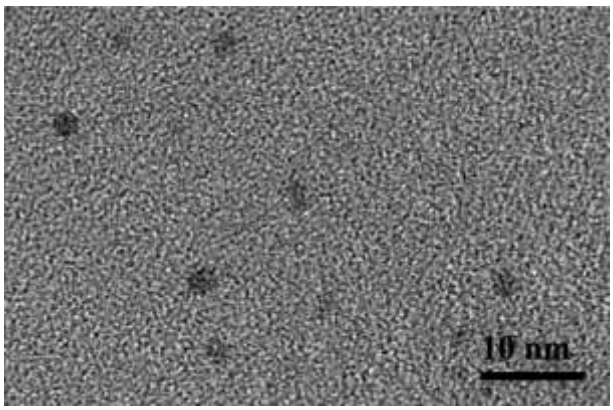

Fig. S2 High-resolution TEM image of Au NCs.

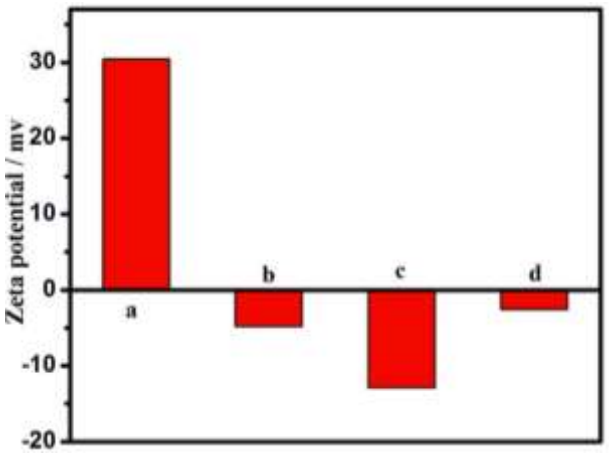

Fig. S3 Zeta potential at the different stages. The bars represent a) PDDA-BN; (b)

GNCs; (c) PDDA-BN/GNCs; (d) PDDA-BN/GNCs-Ab_2 composite.

As shown in Fig. S3, the as-prepared PDDA-BN sheets were positively charged (-30.5 mV) because of the surface coated by PDDA. After the negative GNCs were sonicated with PDDA-BN, the zeta potential turned negative, showing the successful assembling of GNCs. According to Ab_2 probe attaching to the bioconjugate because of the strong interaction between GNCs and mercapto or primary amine groups in biomolecules, the potential was changed again.

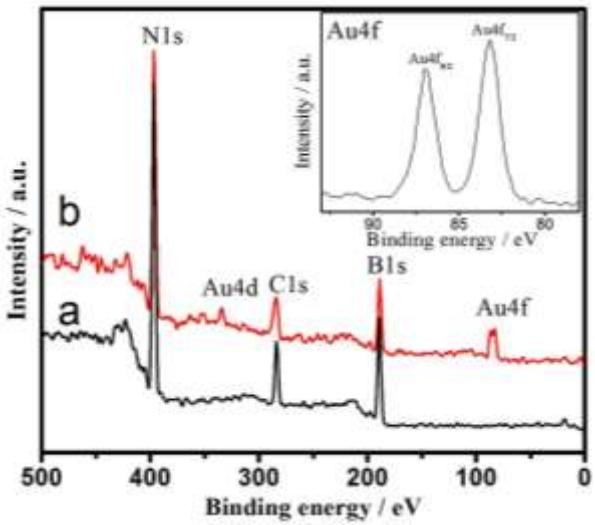

Fig. S4 XPS spectra of (a) PDDA-BN sheets and (b) the PDDA-BN/GNCs composite, inset: high resolution XPS spectra of Au4f orbital of the PDDA-BN/GNCs composite.

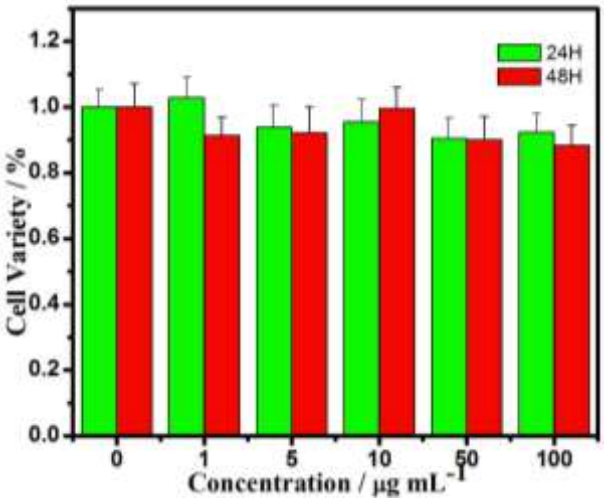

Fig. S5 Cytotoxicity of PDDA-BN/GNCs at different concentrations after incubation with Hell cells for 24 and 48 h, respectively.

The material induced cytotoxic effects on HeLa cells is evaluated by MTT assay, showing the effect of PDDA-BN/GNCs on the HeLa cells viability after 24 and 48h incubation at 37 °C. As can be observed in Fig. S5, the cell viability still keep 91.1 and 87.8% of the original cells after interacting with PDDA-BN/GNCs for 24 and 48 h, respectively, even the concentration of the composite up to 100 μg mL^{-1}.

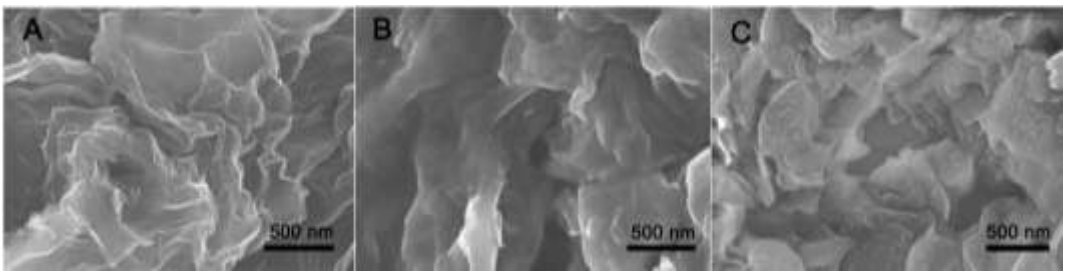

Fig. S6 SEM images of (A) GS modified ITO, (B) IL-6/Ab_1/GS modified ITO and (C) PDDA-BN/GNCs/Ab_2/IL-6/Ab_1/GS modified ITO. The concentration of IL-6: 10 ng mL^{-1}.

As shown in Fig.S6, compared to GS modified ITO (Fig. S6A), the electrode surface of IL-6/Ab_1/GS modified ITO (Fig. S6B) looked rougher in texture due to the attachment of biomolecules. For PDDA-BN/GNCs/Ab_2/IL-6/Ab_1/GS modified ITO, Fig. S6C shows that PDDA-BN/GNCs/Ab_2 biolabels had been immobilized at the electrode surface after immunoreactions. However, there was no PDDA-BN/GNCs/Ab_2 captured at the electrode surface in the absence of IL-6.

Using the sandwich-type immunoreaction, when the concentrations of IL-6 are higher, more signal antibodies (PDDA-BN/GNCs/Ab_2) are captured, and in hence larger signal will be detected. Finally, using optical and electrochemical immunoassay respectively, the fluorescence intensity and SWV responses would increase with the increasing concentrations of IL-6.

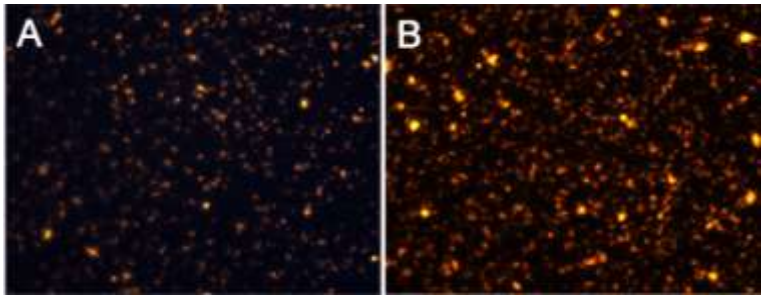

Fig. S7 Fluorescence microscopy image of the immunosensor in the presence of 10 ng mL^{-1} IL-6. (A) With GNCs/Ab_2 bioconjugates and (B) With PDDA-BN/GNCs/Ab_2 bioconjugates.

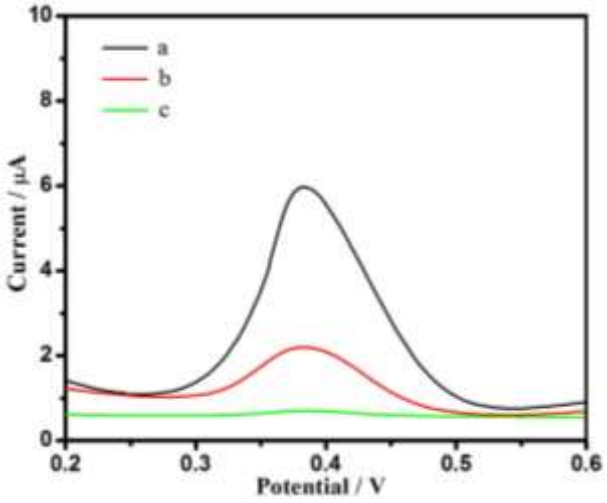

Fig. S8 SWV responses of the immunosensor in the presence of 10 ng mL^{-1} IL-6, with (a) PDDA-BN/GNCs-Ab_2 bioconjugates, (b) GNCs-Ab_2 bioconjugates and (c) Ab_2.

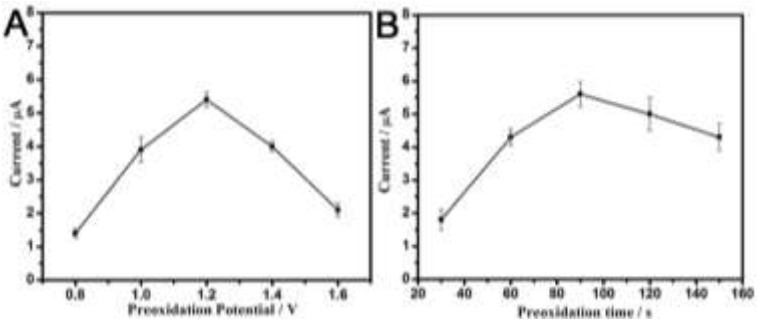

Fig. S9 Effects of (A) preoxidation potential and (B) preoxidation time on SWV response of the immunosensor to IL-6 (10 ng mL^{-1}). The error bars mean the standard deviations from parallel determination of five duplicate immunosensors.

Specificity, reproducibility and stability of the immunoassay

Specificity is an important criterion for any analytical tool. Other proteins such as carcinoembryonic antigen (CEA) and afetoprotein (AFP) were used as the interfere to evaluate the specificity through comparing the electrochemical signals of 1.0 ng mL^{-1} IL-6 solution with a same solution containing additional interferential substance of 100 ng mL^{-1}. The optical responses for CEA and AFP were 7.8 and 8.1% that of IL-6, respectively. The SWV signals for CEA and AFP were 8.0 and 9.1% that of IL-6, respectively. This indicated that the proposed sensor had sufficient selectivity for IL-6 detection, and was capable of distinguishing IL-6 from its analogues in complex samples. The optical assay also showed good reproducibility at one IL-6 level for five replicate measurements with relative standard deviations (RSD) of 5.7%, while 4.8% of that for electrochemical assay. When immunosensors were stored at 4 °C for more than two weeks, the signals remained at about 93.1% for optical response and 92.5% for SWV signal. These results indicated that the proposed immunosensor had acceptable stability.

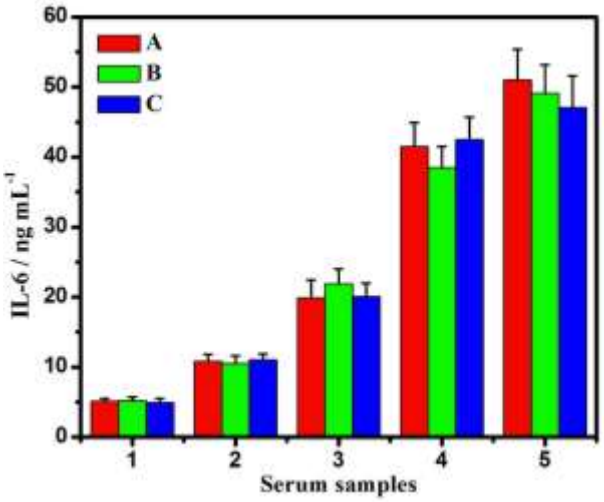

Fig. S10 Comparison of serum IL-6 levels determined by (A) optical assay, (B) ELISA, and (C) electrochemical assay.

The feasibility of the immunoassay in clinical applications was also investigated by analyzing several real samples, and was compared with the ELISA method. As shown in Fig.S10, the relative deviations between the optical immunoassay and the ELISA

method ranged from -9.5 to 7.8%, and the relative deviations between the electrochemical assay and the ELISA method ranged from -7.9 to 8.2%. It obviously indicated that there was no significant difference among the results given by three methods, thus the developed versatile immunoassay might provide an interesting alternative tool for the detection of proteins in clinical laboratories.

References

1. L. Zhao, S. Li, J. He, G. Tian, Q. Wei and H. Li, *Biosens. Bioelectron.* http://dx.doi.org/10.1016/j.bios.2013.05.016.