Supplementary Material

Construction and comparison of BSA stabilized functionalized-GQDs composite fluorescent probes for selective trypsin detection

Xiao Hu [a], Yan Liu[1*a], Yinhua Jiang [**a], Minjia Meng [a], Zhanchao Liu [b], Liang Ni [a], Weifu Wu [c]

[a] *School of Chemistry and Chemical Engineering, Jiangsu University, Zhenjiang 212013, China.*

[b] *School of Materials Science and Engineering, Jiangsu University of Science and Technology, Zhenjiang 212003, China.*

[c] *School of Environmental and Chemical Engineering, Jiangsu University of Science and Technology, Zhenjiang 212003, China.*

Table of contents:

S1 Chemicals and instruments.

S2 Preparation procedure of GQDs and HGQDs.

S3 Optimization in the preparation of composite nanoprobe

Fig.S1 FT-IR spectrum of GQDs, HGQDs, AGQDs.

Fig.S2 Excitation-dependence fluorescence spectra of three kinds of graphene quantum dots：(a) GQDs, (b) HGQDs, (c) AGQDs.

Fig.S3 Fluorescence spectra of three kind of graphene quantum dots indifferent pH：(a) GQDs, (b) HGQDs, (c) AGQDs.

Fig.S4 Fluorescence spectra intensity changes of three kinds of graphene quantum dots in the presence of different BSA concentration.

Fig.S5 Fluorescence spectra intensity changes of three kinds of composite probes of graphene quantum dots with BSA in different incubation time.

Fig.S6 Fluorescence spectra intensity changes of GQDs/BSA and A GQDs/BSA in different incubation time when detecting trypsin.

* Corresponding author. : Tel. +8613775539306, E-mail address: xiaohu917080@163.com;

* * Corresponding author. : Tel. +8613951408293, E-mail address: 361140113@qq.com.

S1 Chemicals and instruments

Colloidal graphite was purchased from Shanghai Yiji CO. Ltd. Hydrogen peroxide (30%), Potassium permanganate ($KMnO_4$), monobasic sodium phosphate and dibasic sodium phosphate and other chemicals mentioned were purchased from the Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All the chemicals used in this study were analytical grade. Bovine serum albumin (BSA), Trypsin, bromelain, lysozyme, cellulose and pepsin were purchased from Sigma Aldrich (Shanghai, China) and were stored in refrigerator before use. 0.05 M phosphate buffer solution (PBS) was prepared by mixing different ratios of monobasic sodium phosphate and dibasic sodium phosphate, yielding a buffer at pH 6.0, 7.0 and 8.0, which were close to physiological pH range. Doubly distilled water was used for all dilutions.

Fourier transmission infrared spectra (FT-IR, 4000-400 cm^{-1}) in KBr were recorded on a NICOLET NEXUS 4700 FT-IR spectrometer (Nicolet, USA). The transmission electron microscopy (TEM) images were taken with scanning electron microscope (TEM, JEOL, JSM-7001F, Japan). All fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan). Photographs were taken by using a Canon digital camera (IXUS 230 HS, China). Zeta potential was measured with a Zetasizer 3000 (Malvern Instruments, UK). X-ray photoelectron spectroscopy (XPS) analysis was determined on K-Alpha electron spectrometer (Thermo Fisher Scientific, USA).The pH value adjustments were performed using a pHS-3C digital pH meter (LIDA Instrument Factory, Shanghai, China) when investigating effect of pH.

S2 Preparation procedure of GQDs and HGQDs

The graphene quantum dots (GQDs) were prepared from GO. Graphene oxide (GO) was synthesized according to well-known Hummers method from purified colloidal graphite [1]. The graphene quantum dots (GQDs) were prepared from GO. Typically GO (50 mg) was solved in DMF (10 mL) followed by being treated under ultrasonication for 30 min before being transferred to a poly(tetrafluoroethylene) (Teflon)-lined autoclave (50 mL) and heated at 200 ^oC for 5 h. After the reactor cooled down to room temperature naturally, collect the brown transparent suspension contained product and waste the precipitates. The collected suspension was then treated by reduced pressure distillation and the obtained solid was GQDs, which possesses distinguished solvency in many organic solvent and water. GQDs solid was solved in water and purified by going through the

polytetrafluoroethylene (PTFE) microfiltration membrane (220 nm) and dialysis with a Molecular weight cut-off of 3500 Da. The obtained purified GQDs solution was placed in vacuum freeze drier for several days to get purified GQDs solid.

Hydroxylated Graphene quantum dots（HGQDs）used in this study were prepared by GO pyrolysis according to the literature procedure [2]. First of all, 2 g pyrene was added into hot HNO_3 (160 mL) to get trinitropyrene by refluxing and stirring for 12 h at 80 ^oC. The mixture was added into a certain amount of deionized (DI) water slowly after cooled down to room temperature, the diluted solution was then filtered through a 220 nm PTFE microfiltration membrane to remove residual acid and collect the obtained yellow solid, which is 1,3,6-trinitropyrene, drying at 80 ^oC. 50 mg resultant 1,3,6-trinitropyrene was dispersed in 100 mL NaOH aqueous solution with a concentration of 0.2 M before implementing with ultrasonication (500 W) for 2 h. Then, the suspension was transferred to a poly(tetrafluoroethylene) (Teflon)-lined autoclave and heated at 200 ^oC for 10 h and then cooled down to room temperature naturally. The obtained solution was filtered through a 220 nm PTFE microfiltration membrane to gain water soluble H-GQDs, followed by further dialysis with retained molecular weight of 3500 Da and vacuum freeze drying.

S3 Optimization in the preparation of composite nanoprobe

Firstly, the optimized excitation wavelength of GQDs, HGQDs and AGQDs were investigated by fluorescence detection, founding the best excitation were 330 nm, 490 nm and 340 nm respectively as exhibited in Fig.S2. Under the best excitation, the strongest emission of GQDs, HGQDs and AGQDs were 410 nm, 540 nm and 435 nm respectively. Both the optimized excitation and emission were depicted in Fig.2 with inserted fluorescent picture of them from left to right. As we all know that pH of quantum dots solution plays an important role in the fluorescence intensity and stability, the fluorescence intensity of GQDs, HGQDs and AGQDs at pH 6.0, 7.0 and 8.0 were compared. The results were shown in Fig.S3, confirming the fluorescence intensity increased form pH 6.0 to pH 8.0, which can be explained by that their negative zeta potential value make them favorable and stable in slight basic environment, yielding higher intensity and stability. Besides, the zeta potential favor BSA in slight basic environment to assemble with quantum dots to form composite probes.

The BSA concentration plays an important role in the ultimate composite nanoprobe assemble efficiency and thus sensitivity and the accuracy of the different composite nanoprobe detection for trypsin. As we can see from Fig.S4, Fluorescence spectra intensity changes of three kinds of graphene

quantum dots in the presence of different BSA concentration ranging from 0.1 mg/mL to 5.0 mg/mL were recorded, founding that the composite nanoprobe of HGQDs/BSA emission decreased a little with BSA concentration increasing while the other two composite nanoprobe emission increased even though the emission intensity of AGQDs/BSA soared up faster than GQDs/BSA. Considering the further emission intensity change when detecting trypsin, the 1.0 mg/mL of BSA was selected as the optimal concentration for further study to get better sensitivity of the composite nanoprobe. Apart from this, the influence of incubation time after adding BSA solution into initial different quantum dots solution on the intensity of each composite nanoprobe was compared for the reason that the incubation time directly affected the assemble efficiency and the stability of composite nanoprobes. Fig.S5 shows different circumstance of emission intensity of different composite nanoprobe with time prolonged from 15 min to 120 min, suggesting that emission intensity of HGQDs/BSA fell down while emission intensity of both GQDs/BSA and AGQDs/BSA showed upward trend, as well as all composite nanoprobe emission intensity got to a stable level at 120min thus 120 min were chose for optimal incubation time to prepare these three kinds of BSA stabilized composite nanoprobe. It is not hard to observe that the emission intensity growth range of AGQDs/BSA along with increased BSA concentration and prolonged incubation time exceed a lot than that of GQDs/BSA which might be ascribed to the zeta potential value of AGQDs is negative and lower than GQDs.

Reference

[1] Jr W.S.H., Offeman R.E., (1958)Preparation of Graphitic Oxide, J Am Chem Soc 80(6): 1339.

[2] Wang L., Wang Y., Xu T., Liao H., Yao C., Liu Y., Li Z., Chen Z., Pan D., Sun L., (2014) Gram-scale synthesis of single-crystalline graphene quantum dots with superior optical properties, Nat Commun 5:5357.

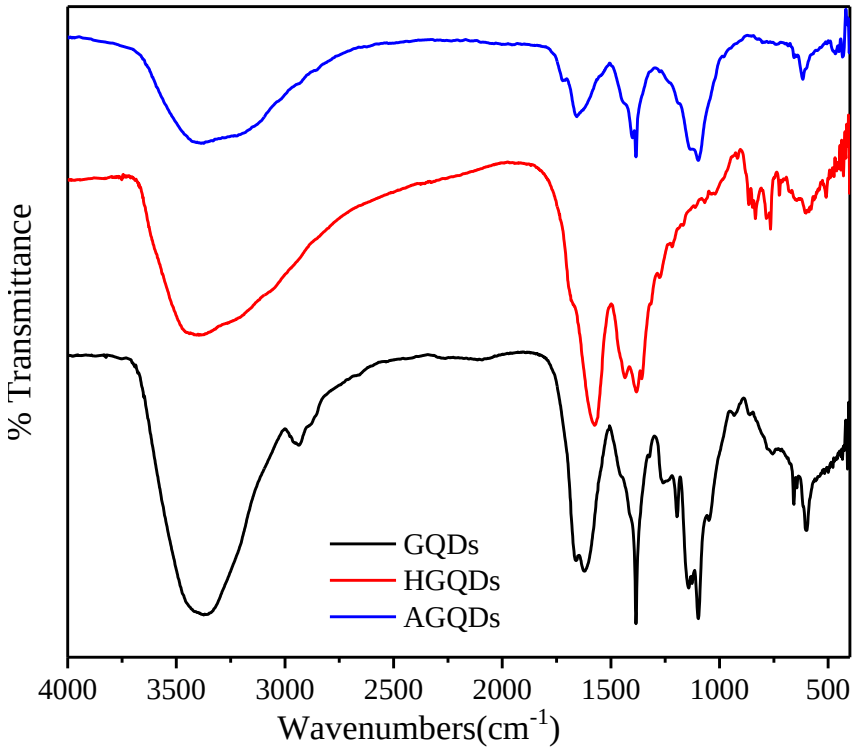

Fig.S1 FT-IR spectrum of GQDs, HGQDs, AGQDs.

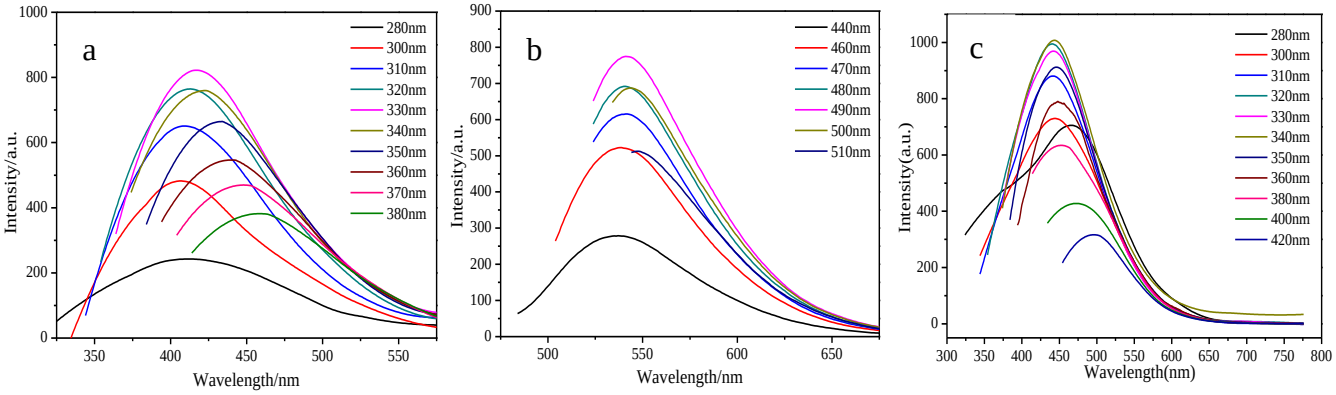

Fig.S2 Excitation-dependence fluorescence spectra of three kinds of graphene quantum dots：(a) GQDs, (b) HGQDs, (c) AGQDs.

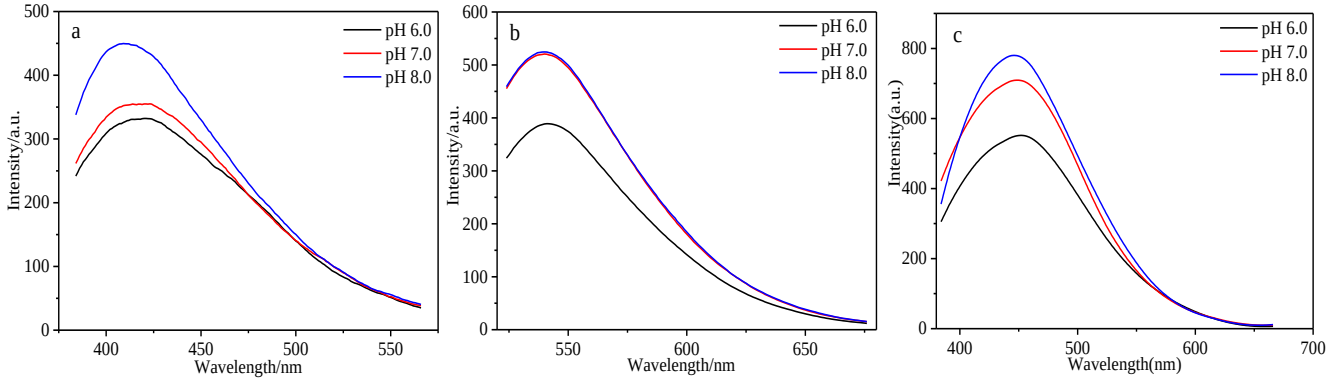

Fig.S3 Fluorescence spectra of three kind of graphene quantum dots indifferent pH：(a) GQDs, (b) HGQDs, (c) AGQDs.

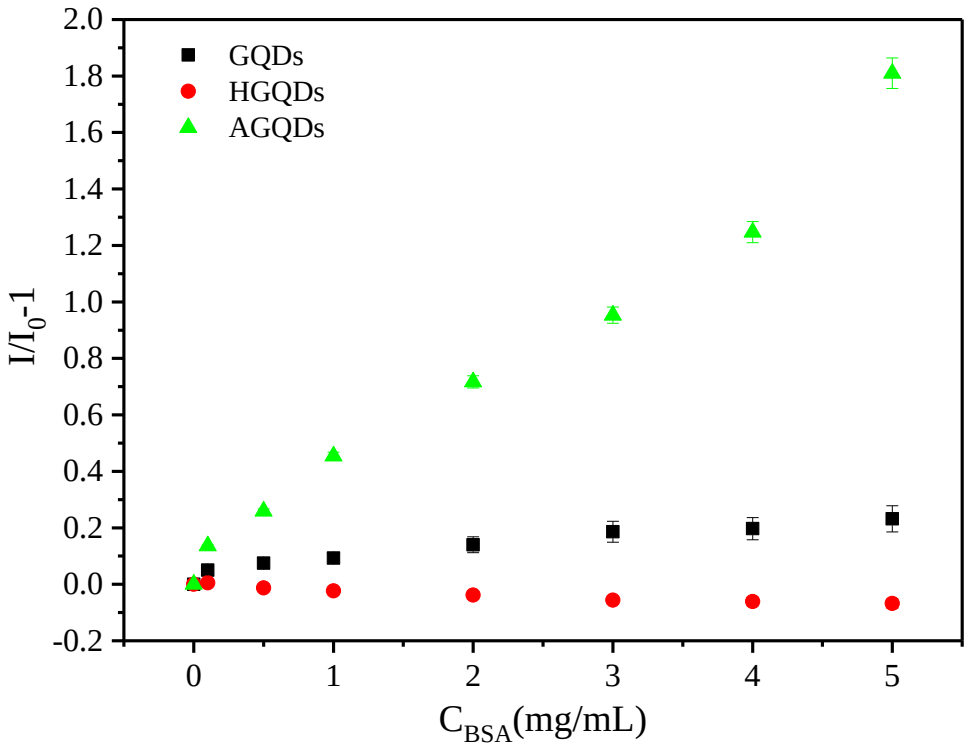

Fig.S4 Fluorescence spectra intensity changes of three kinds of graphene quantum dots in the presence of different BSA concentration.

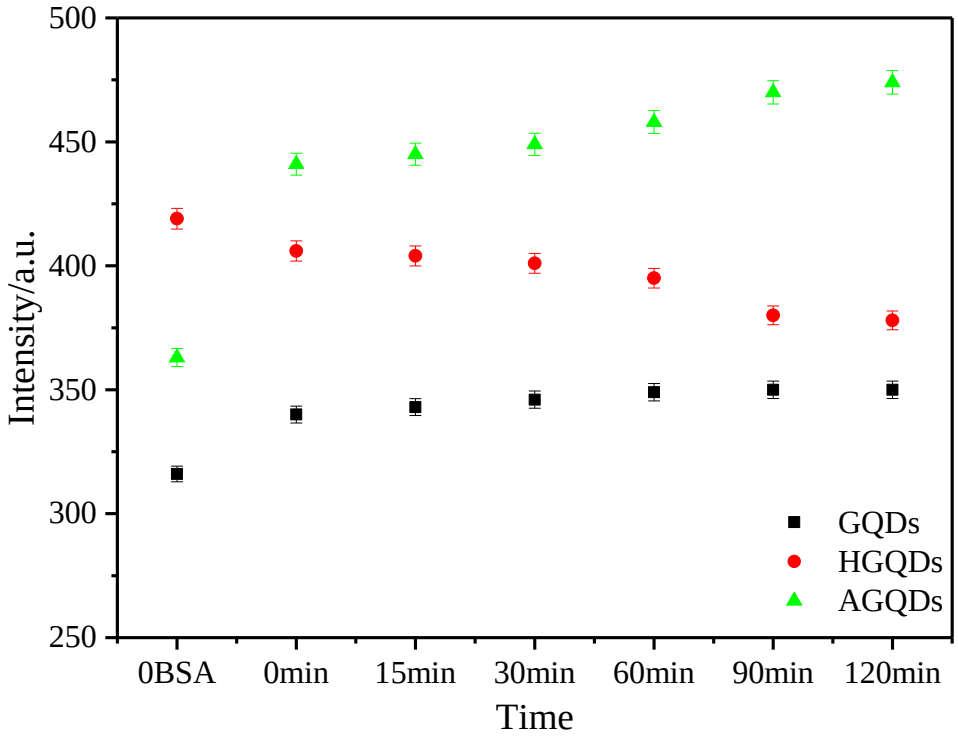

Fig.S5 Fluorescence spectra intensity changes of three kinds of composite probes of graphene quantum dots with BSA in different incubation time.

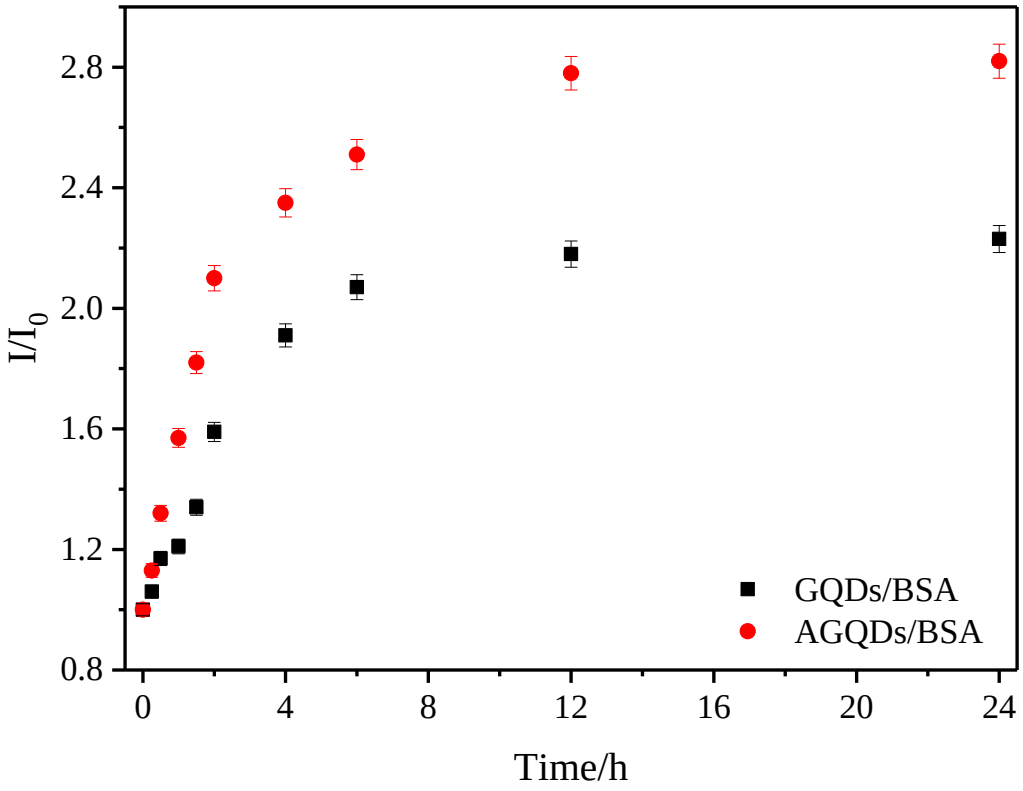

Fig.S6 Fluorescence spectra intensity changes of GQDs/BSA and A GQDs/BSA in different incubation time when detecting trypsin