# Electronic Supplementary Information

# Boronic acid functionalized g-$C_3N_4$ nanosheets for ultrasensitive and selective sensing of glycoprotein in physiological environment

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## Electronic Supplementary Information Included:

$mL^{-1}$. ($\lambda_{ex}$ 370 nm)

**Figure S6.** TEM image of the B-g-CN nanosheets in the presence of 20 μg $mL^{-1}$ IgG.

**Figure S7.** Effect of the B-g-CN nanosheets concentration (0, 10, 50, 100, 200, 500 μg $mL^{-1}$) on the viability of HeLa cells with an incubation time of 24 h.

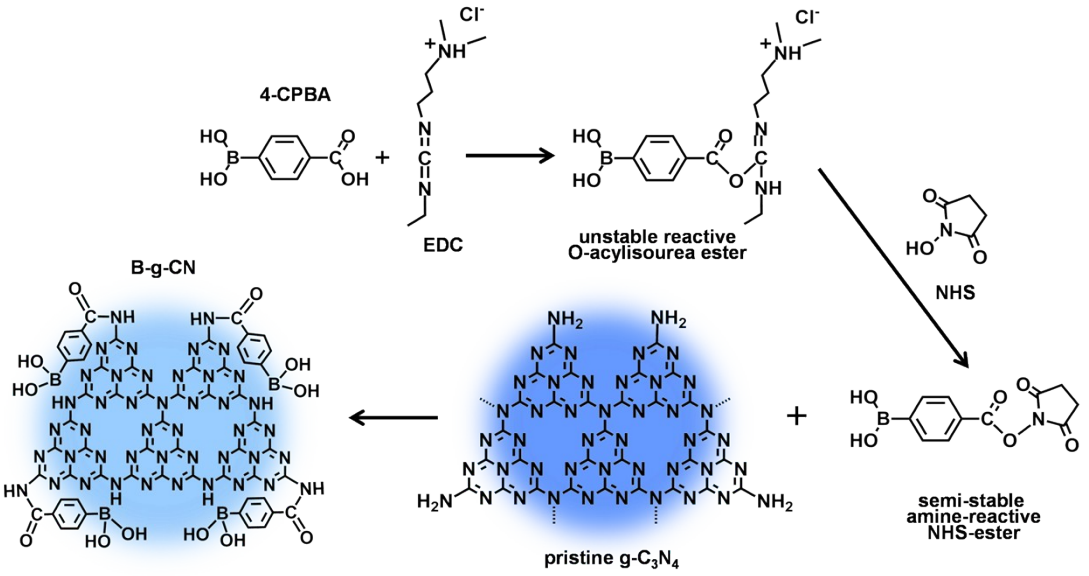
**Figure S8.** The confocal fluorescence images of HeLa cells incubated with B-g-CN nanosheets (10 μg $mL^{-1}$) for 0, 2, 4, 6 and 8 h. Every picture is taken at bright field (top), with excitation/emission at 405/410-490 nm (middle) and overlay of them (bottom). Scale bar: 100 μm.

**Figure S9.** Semiquantitative determination of exogenous IgG (0, 5, 10, 50 μg $mL^{-1}$) in Hela cells according to the relative fluorescence intensity. The calculations are conducted by using the Image J software. (The concentration of B-g-CN nanosheets: 10 μg $mL^{-1}$).

# 1. Experimental detail

## 1.1 Quantification of 4-CPBA content on B-g-CN surface.

As shown in the following reaction scheme, during the EDC/NHS coupling, 4-CPBA covalently binds with -$NH_2$ group on the surface of g-$C_3N_4$ to form -CO-NH-. Therefore, the amount of 4-CPBA can be measured indirectly by quantifying the amount of -$NH_2$ group consumed during the reaction.




**Scheme S1** Preparation scheme for the B-g-CN using 4-CPBA and pristine g-$C_3N_4$.

The -$NH_2$ group was quantified using the ninhydrin coloration method[1] described as follows. In brief, various concentrations (1, 2, 5, 6.25, 12.5 and 25 mM) of n-butylamine standard solutions were prepared for the preparation of calibration curve. 20 μL of n-butylamine standard solutions, 5 μL of phenol/ethanol (w/w=24:76), 100 μL of pyridin and 25 μL of ninhydrin (1 mM, dissolved in ethanol) were mixed and heated at 95 °C for 5 min. Afterwards, the mixture were diluted for 10-fold with 80% ethanol. The resulting colored solution was spectroscopically measured at 570 nm using the UV-vis spectrophotometer. Similarly, 1 mg $mL^{-1}$ g-$C_3N_4$ nanosheets and B-g-CN nanosheets were treated with the same process, deriving an -$NH_2$ concentrations of 5.03 mmol $g^{-1}$ for g-$C_3N_4$ and 2.08 mmol $g^{-1}$ for B-g-CN, respectively. It should be noticed that due to the different composition between B-g-CN and g-$C_3N_4$, the amount of -$NH_2$ consumed during EDC/NHS coupling is not the simple subtraction of

(5.03-2.08) mmol $g^{-1}$. Assuming the reaction ratio of -$NH_2$ was $x$, then for 1 g B-g-CN, the -$NH_2$ concentrations can be written as follows:

$$2.08\ mmol\ g^{-1} = \frac{5.03\ mmol \times (1-x)}{1\ g + 5.03\ \times 10^{-3}\ mol \times x \times (M_{4-CPBA} - M_{H2O})g\ mol^{-1}}$$

$$CPBA\ content = \frac{5.03\ mmol \times x}{1\ g + 5.03\ \times 10^{-3}\ mol \times x \times (M_{4-CPBA} - M_{H2O})g\ mol^{-1}}$$

The amount of 4-CPBA on B-g-CN was calculated to be 1.7 mmol per gram B-g-CN.

**1.2 IgG analysis in human urine samples.**

The urine samples were provided by healthy volunteers and directly diluted for 50-fold with PBS buffer (pH 7.4) and no further pretreatment was involved before applying the sensing procedure. The diluted samples were measured as described in section 2.4 (without the enriching step). For recovery test, 50 μg $mL^{-1}$ of standard IgG was first spiked into the urine sample and then diluted for 50-fold and subjected to the same analysis process mentioned above. The recovery was calculated according to the following equation: Recovery% = ($C_{TF}$ - $C_F$)/$C_S$×100% (TF: Total found; S: Spiked; F: Found).

## 2. Figures

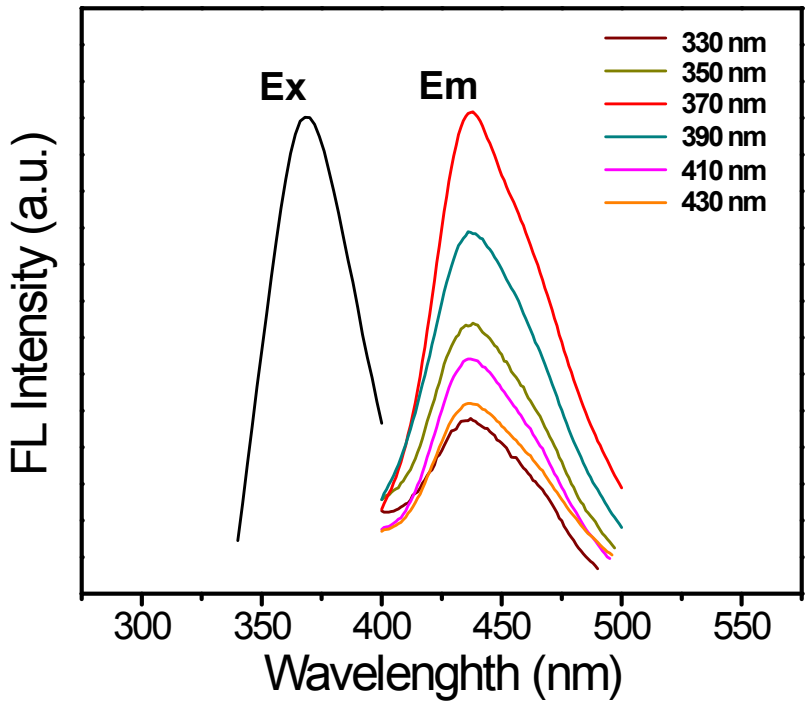



**Figure S1.** The excitation spectra under $\lambda_{em}$ 440 nm and the emission spectra of B-g-CN nanosheets (1.0 mg $mL^{-1}$) at various excitation wavelengths.

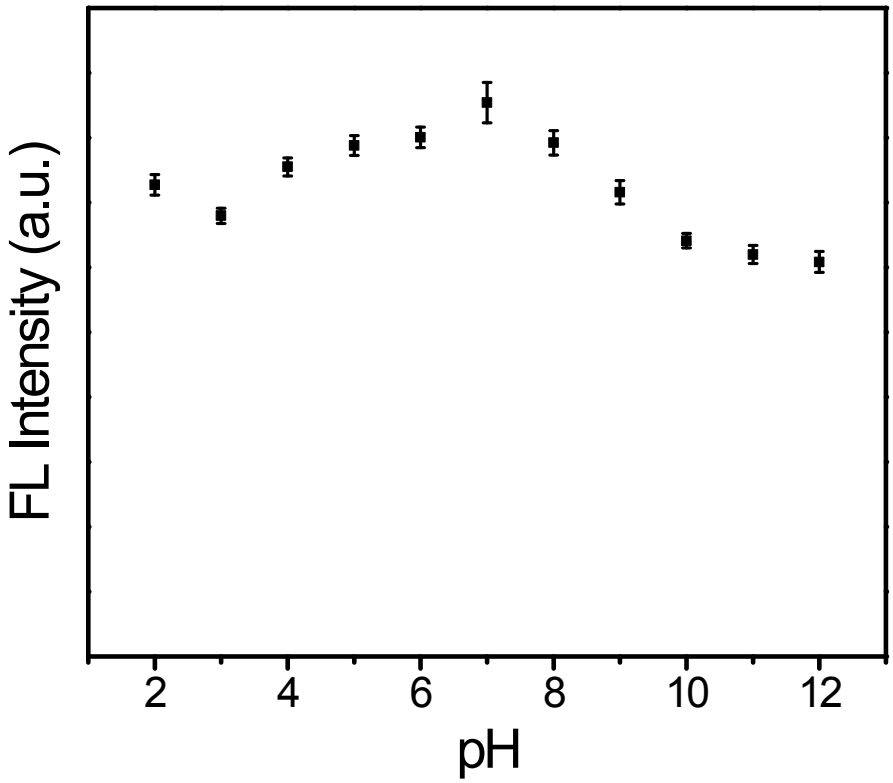



**Figure S2.** The variation of fluorescence intensity of B-g-CN nanosheets (1.0 mg $mL^{-1}$) as a function of pH value under $\lambda_{ex}$ 370 nm.

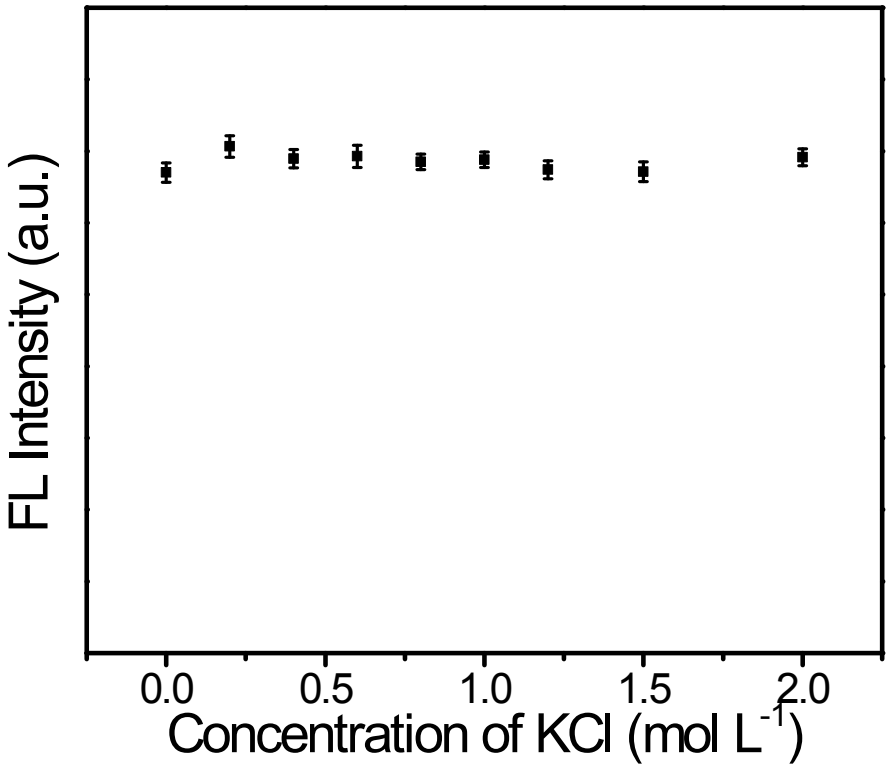



**Figure S3.** The variation of fluorescence intensity of B-g-CN nanosheets (1.0 mg $mL^{-1}$) as a function of ionic strength under $\lambda_{ex}$ 370 nm.

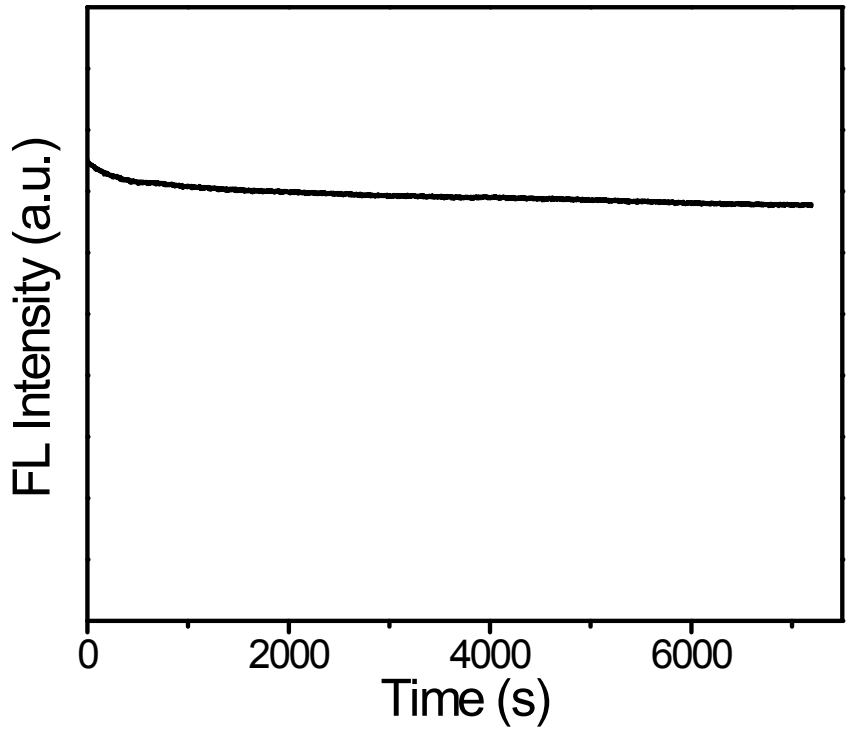



**Figure S4.** The variation of fluorescence intensity of B-g-CN nanosheets (1.0 mg $mL^{-1}$) as a function of irritation time under $\lambda_{ex}$ 370 nm.

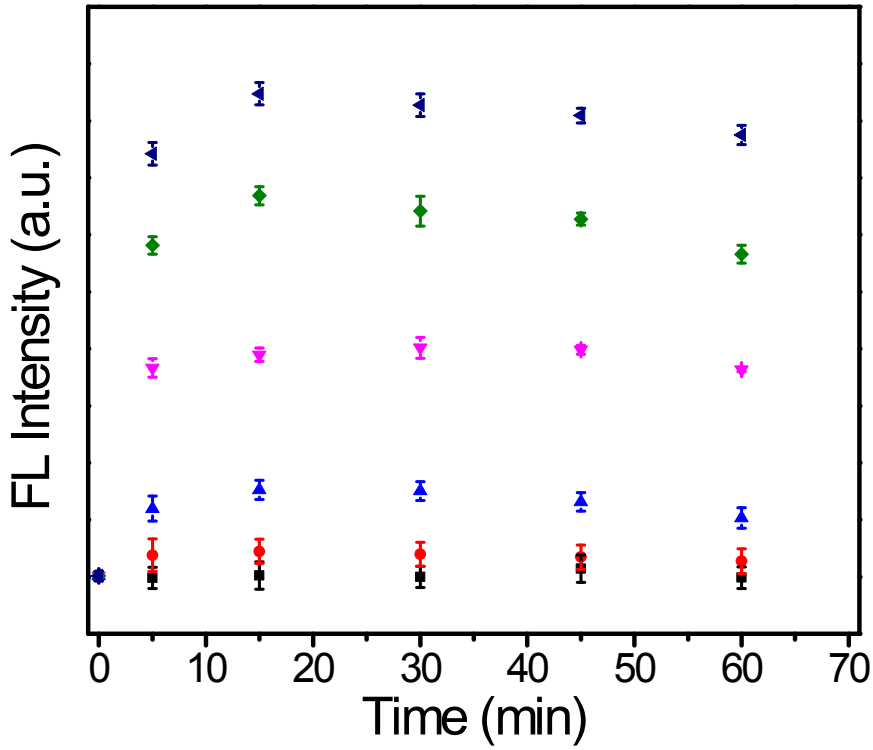



**Figure S5.** Effects of incubation time on the fluorescence intensities of B-g-CN nanosheets (1.0 mg $mL^{-1}$) in the presence of various concentrations of IgG. The concentration of IgG from up to down: 50, 20, 10, 1, 0.5, 0 μg $mL^{-1}$. ($\lambda_{ex}$ 370 nm)

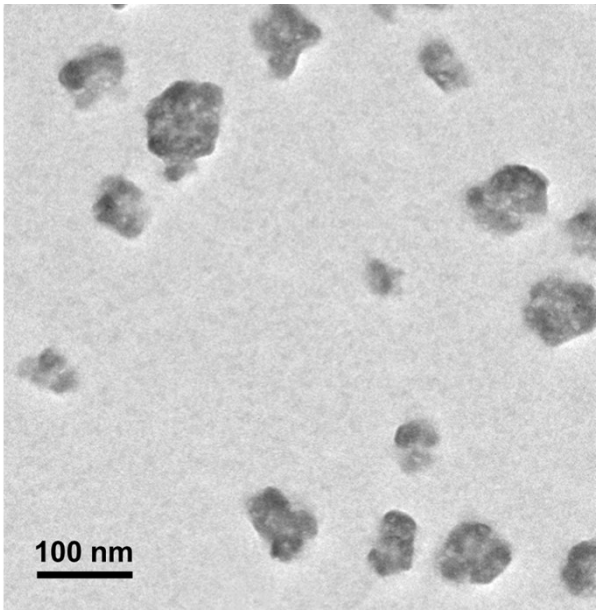



**Figure S6.** TEM image of the B-g-CN nanosheets in the presence of 20 μg $mL^{-1}$ IgG.

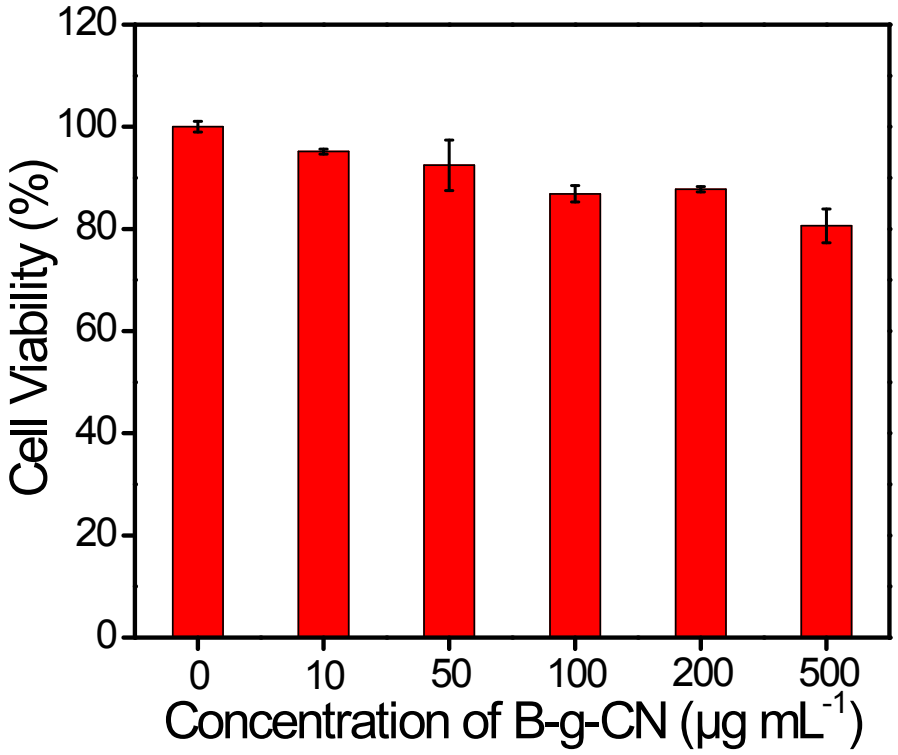



**Figure S7.** Effect of the B-g-CN nanosheets concentration (0, 10, 50, 100, 200, 500 μg $mL^{-1}$) on the viability of HeLa cells with an incubation time of 24 h.

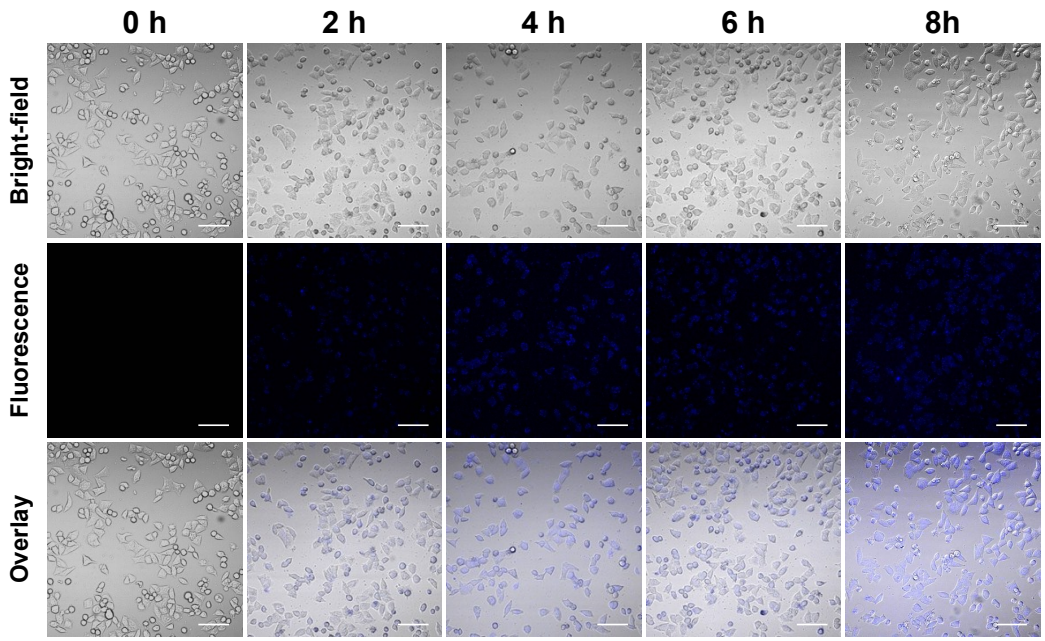
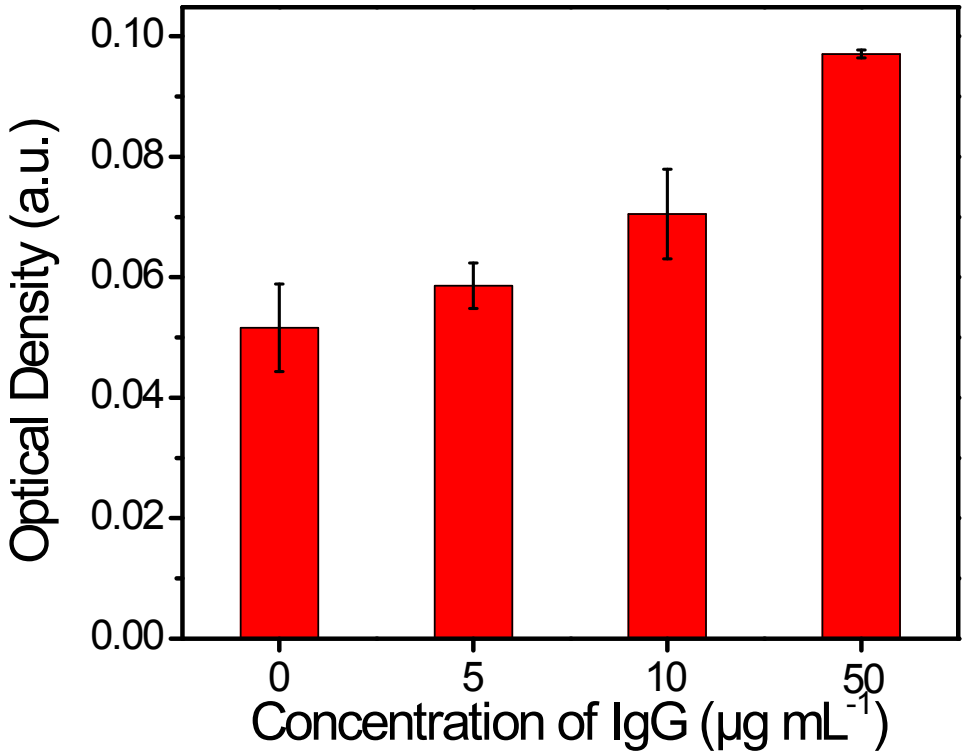



**Figure S8.** The confocal fluorescence images of HeLa cells incubated with B-g-CN nanosheets (10 μg $mL^{-1}$) for 0, 2, 4, 6 and 8h. Every picture is taken at bright field (top), with excitation/emission at 405/410-490 nm (middle) and overlay of them (bottom). Scale bar: 100 μm.

It can be seen that the blue fluorescence in the Hela cells increased as the incubation time increased from 0 to 4 h, and reached a plateau after 4 h. Therefore, 4 h was chosen as the incubation time.

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**Reference**

[1] Mori T, Kubo T and Kaya K, *Colloid Polym. Sci.* 2009, **287**: 513-523.