Electronic Supplementary Material (ESI) for Chemical Communications
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Electronic supplementary information

Fluorescent detection of cholesterol using β-cyclodextrin functionalized graphene

Experimental details

Materials and Reagents. Graphite powder (< 20 μm), β-cyclodextrin, cholesterol, ammonia solution (25%), hydrazine monohydrate (98%), rhodamine 6G (R6G), rhodamine B, fluoresecein and dialysis membrane (MWCO 12000) were purchased from Sigma Aldrich and used as received. Fetal bovine serum was purchased from Invitrogen.

Synthesis of β-Cyclodextrin Functionalized Graphene (β-CD-G). β-CD-G was synthesized following the method reported earlier.[27] Aqueous solution of GO (5 mg/mL) was prepared by modified Hummer's method.[25] In a separate vial 200 mg of β-cyclodextrin was dissolved in 5 mL water and mixed with 100 μL NH_4OH and 200 μL GO solution. The whole solution was continuously stirred for one hour, followed by addition of 10 μL hydrazine monohydrate solution (0.04 M). Next, the solution was heated to 80-90° C for one hour with constant stirring. The stable black solution was then dialysed against distilled water with cellulose membrane (MWCO 12,000) for overnight. Finally, the solution was diluted with tris(hydroxymethyl)aminomethane (Tris) buffer of pH 7.4 and used as stock solution. Polystyrene sulfonate functionalized graphene was synthesized using the reported procedure.[31]

Fluorescence Detection of Cholesterol. Aqueous solution of R6G (0.1 mM) and ethanolic solution of cholesterol (10 mM) were prepared separately and diluted if necessary. A 0.5 mL stock solution of β-CD-G was taken in a vial and mixed with 150 μL of R6G (10^{-4}M) solution and diluted to 2 mL by adding distilled water. Next, 180 μL of this solution was taken in each

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well of microplate and each well was mixed with 20 μL cholesterol solution of different concentration. Fluorescence of each well was measured after 30 minutes using microplate reader, under the 480 nm excitation. Control experiments were performed using graphene oxide solution or polystyrene sulfonate functionalized graphene solution using same procedure as described above.

Cholesterol Detection in Serum. Cholesterol detection in serum was performed using fetal bovine serum. The proteins and other substances were removed from serum according to reported method,[1] before they were used. Typically, 200 μL of serum was mixed with 1.8 mL ethanol and after 10 minutes it was centrifuged at 4000 rpm. The supernatant serum was collected and mixed with known amount of cholesterol. Next, this solution was used to detect cholesterol according to the procedure described above.

Instrumentation: Emission spectra and cholesterol complexation assay were performed using the Synergy TMMX Multimode Microplate Reader. Absorbance spectra of different solutions were measured using Shimadzu UV-2550 UV visible spectrometer. FTIR study was performed using NICOLET 6700 FT-IR spectroscopy after making pellet with solid KBr. Diluted sample solutions were deposited on mica disk and then AFM was measured using VEECO DICP II autoprobe (model AP 0100). Agiltron R3000 Raman spectrometer was used to obtain Raman spectra with 785 nm excitation laser. Transmission electron microscopic (TEM) image of graphene was captured with FEI Tecnai G2 F20 microscope by putting a drop of sample solution on carbon coated copper grid.

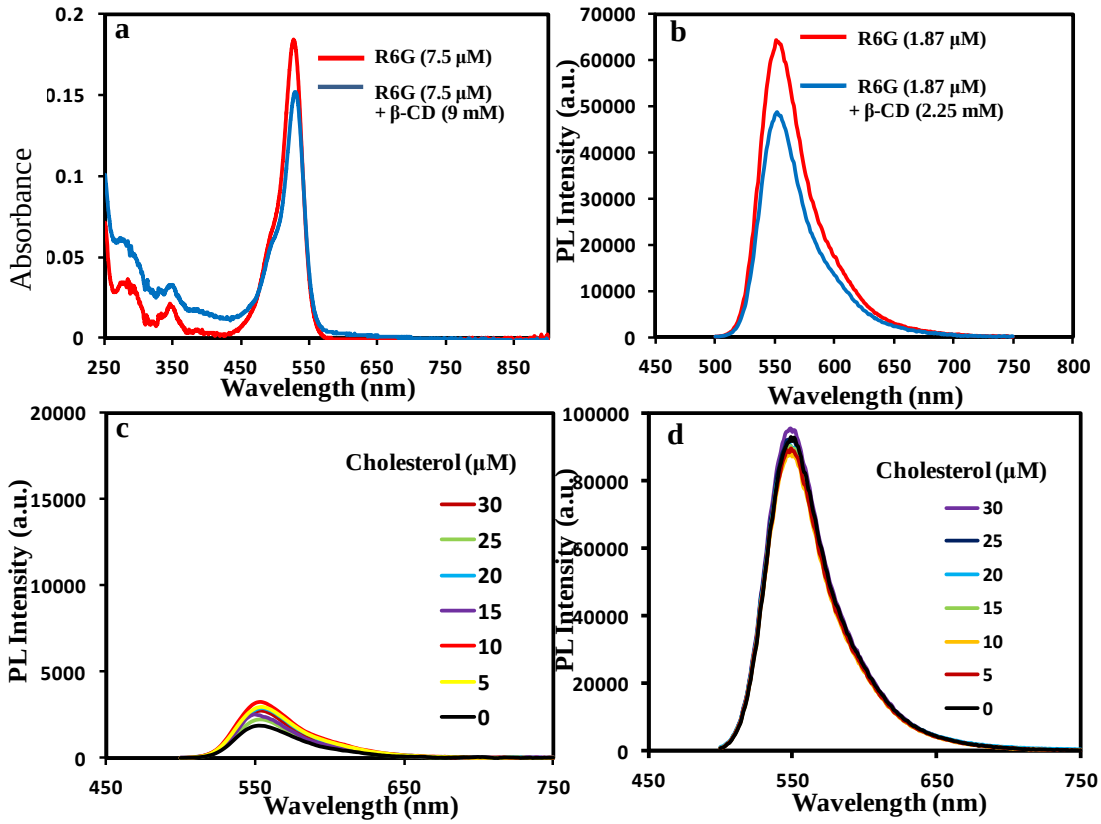

Figure S1. Control experiments for R6G interaction with graphene and β-CD. a) UV-visible and b) fluorescence spectra of free R6G (red) and in presence of β-CD (blue). c) Effect of adding cholesterol into a solution mixture of polystyrene sulfonate capped graphene and R6G, showing very small change of fluorescence d) Effect of adding cholesterol into a solution mixture of graphene oxide and R6G, showing a negligible change of fluorescence.

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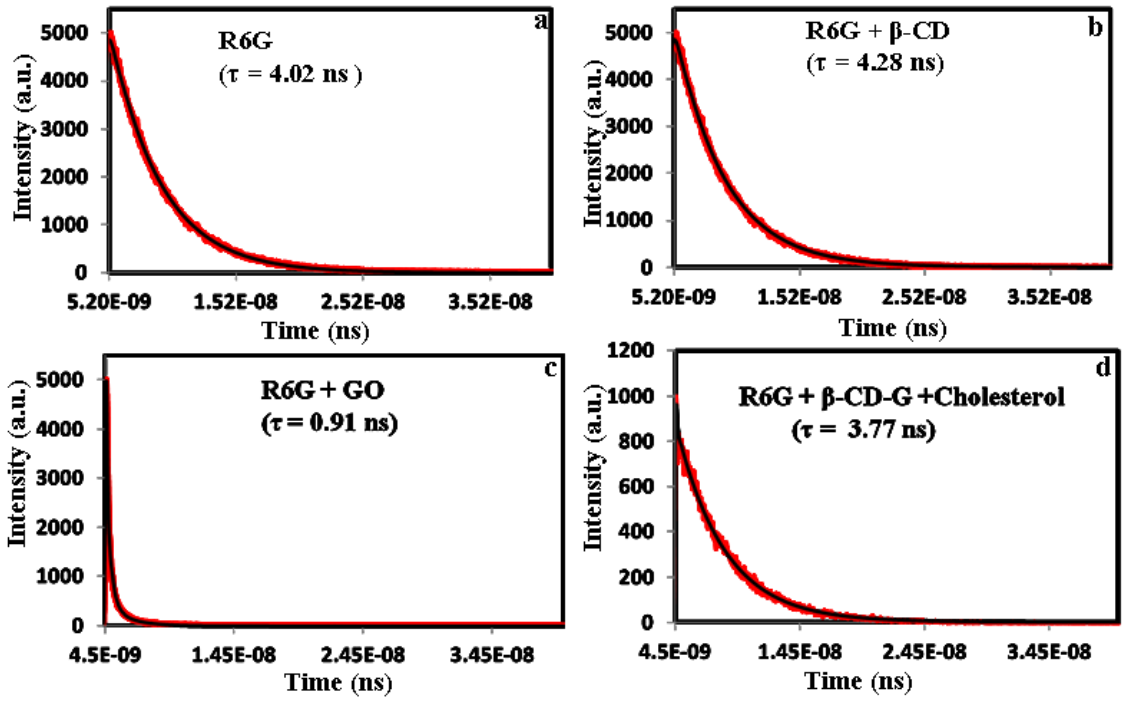

Figure S2. Fluorescence lifetime decay curve of R6G (red) along with fitting curve (black) in water (a), in presence of β-CD (b), in presence of GO (c) and in presence of cholesterol and β-CD-G.(d) Lifetime of R6G in presence of β-CD-G is not measured low signal (due to strong quenching effect) and sub nanosecond timescale which is beyond our experimental facility.

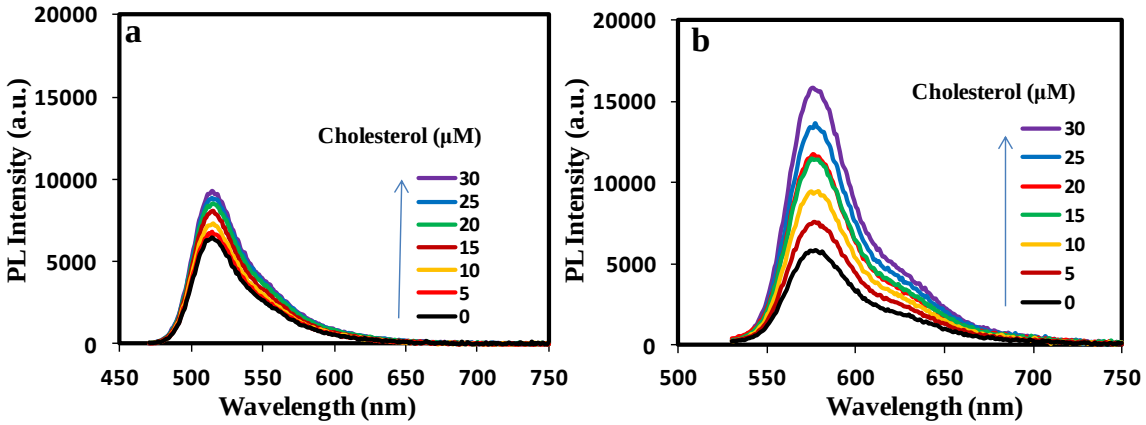

Figure S3. Cholesterol induced 'turn on' fluorescence using (a) fluorescein and (b) rhodamone B. Different amount of cholesterol is added into a solution of β-CD-G-fluorescein or β-CD-G-rhodamine B. Note the fluorescence responses are poor compared to R6G based approach as shown in Figure 3.

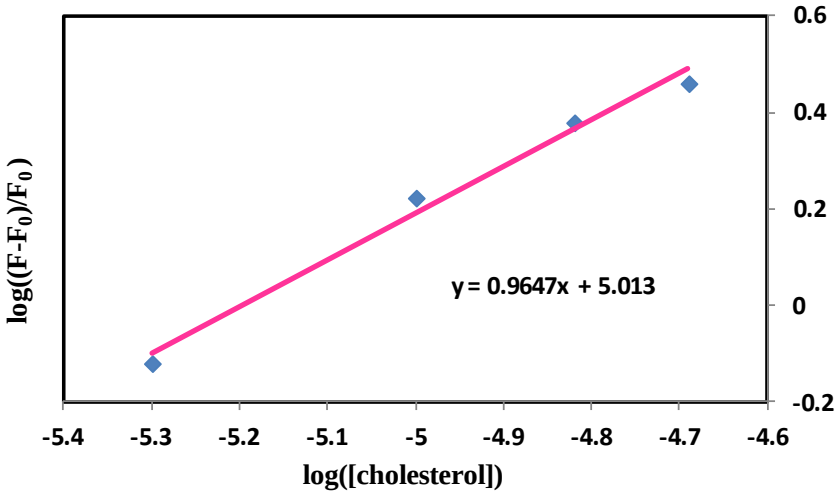

Figure S4. Determination of binding constant between cholesterol and β-CD-G by plotting log $((F-F_0)/F_0)$ vs log[cholesterol] using the equation log $((F-F_0)/F_0)$ = n log([cholesterol]/ K_d) taken from the reference[2] where F and F_0 are fluorescence intensity after and before addition of cholesterol, and K_d = dissociation constant. The binding constant (K_b) between cholesterol and β-CD-G = $1/K_d$ = 1.55 M^{-1} where n = 0.964.

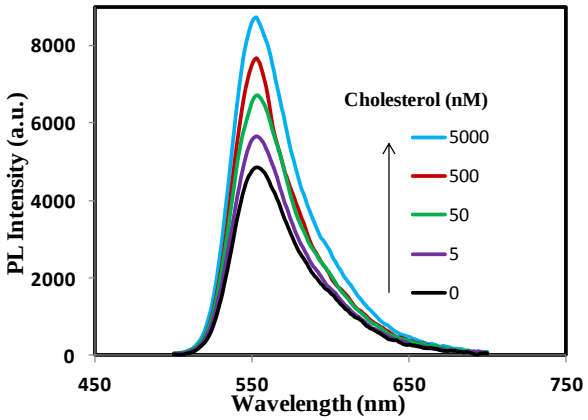

Figure S5. Low concentration detection of cholesterol using solution of β-CD-G-R6G via cholesterol induced 'turn on' fluorescence. The cholesterol can be detected upto 5 nM concentration.

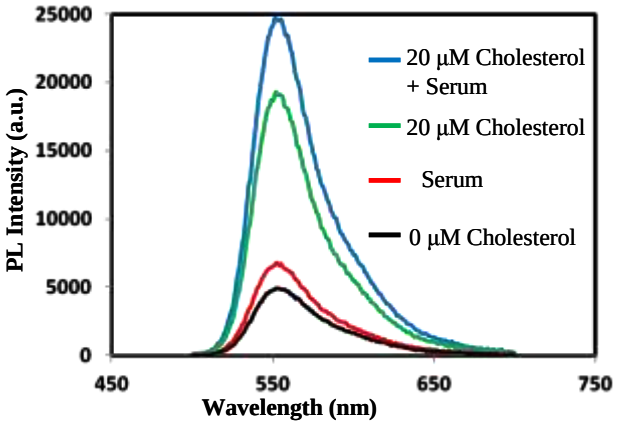

Figure S6. Comparative result of cholesterol induced 'turn on' fluorescence in presence and absence of serum. Here β-CD-G-R6G has been used for cholesterol detection. Note the comparable fluorescence response for same amount of cholesterol.

References

1. N. Zhang, Y. Liu, L. Tong, K. Xu, L. Zhuo, B. Tang, *Analyst*, **2008**, 133, 1176.

2. N. Wangoo, C. R. Suri, G. Shekhawat, *Appl. Phys. Lett*. 2008, **92**, 133104.