A Graphene Binding-Promoted Fluorescence Enhancement for BSA Recognition

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Materials and general procedure

All the solvents and reagents were of analytic grade and used as received. Water used was ultra filter deionized and purchased from Fisher Scientific. Graphite flake (natural, about 300 mesh, metals basis) was purchased from Alfa Aesar. DNSA and DP were purchased from Acros. UV-Vis spectra were acquired on a Hewlett-Packard 8453 diode-array spectrometer. Fluorescence spectra were obtained on a HORIBA Jobin Yvon NanoLog spectrometer. Dynamic light scattering (DLS) data was obtained on a Malvern Zetasizer Nano S (U.K., ZEN 3600) at 25 °C. AFM images were recorded under ambient conditions using a Park Scientific Autoprobe CP, which is operating in the tapping mode with Micromasch tapping probes with radius of curvature being <4 nm. The tips were brand new. The CCG and "SQ+CCG" solutions were prepared by addition of CCG (8.4 μg/mL) into 3 mL of water and SQ (5 μM) solution, respectively. CD spectra were measured at room temperature and a quartz cell having a path length of 0.1 cm. The concentration of BSA was kept at 2.5×10^{-6} mol·L^{-1}.

Preparation of GO and CCG

Graphene oxide was synthesized from expandable graphitic flake (300 mesh) using a modified Hummer's method.[12] Using mortar and pestle, 1 gram of graphite was ground with 50 grams of NaCl for a period of 10 minutes. NaCl was then dissolved and removed by filtration with water. This caused a small loss in graphite. The ground graphite flakes were then added to 23 ml of H_2SO_4 (98%) and left stirring for 12 hours. Afterwards, while keeping the temperature less than 20ºC, 1 gram of $KMnO_4$ was added. The resulting mixture was stirred at 40 °C for 30 minutes, then stirred at 90 °C for 90 minutes. (When the mixture reaches 70 ºC, temperature

increases rapidly). Next, 46 mL of distilled water were added, and the temperature was increased to 105ºC for 25 min. The reaction was ended by addition of 140 mL distilled water and 10 mL 30% H_2O_2 solution. For purification, the resulting mixture was washed multiple times, first with 5% HCl solution and then with DI water. 200 mL of water were added to the graphite oxide product.

CCG was prepared by using the procedures reported by Shi and co-workers.[4a] The details are described as follows. Fifty milliliters (0.25 mg/mL) of purified GO dispersion was mixed with 14 μL of hydrazine monohydrate and 150 μL of ammonia solution (28 wt% in water). The mixture was stirred at 95°C for 1 h. After reduction, a homogeneous black dispersion with a small amount of black precipitate was obtained. The dispersion was then filtered through glass cotton to remove the precipitate and yield a stable black aqueous dispersion of CCG. The obtained product was characterized by UV-visible spectra (Figure S24).

Structures of DNSA and DP

Dansylproline (DP)   Dansylamide (DNSA)
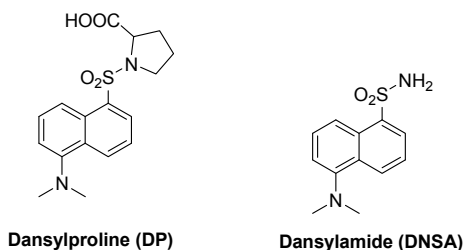
UV and fluorescence experiments

Stock solution (5.0×10^{-4} M) of **SQ** in ethanol, stock solution (0.25 mg/mL) of CCG and 1 mg/mL of BSA in water were prepared. An aliquot (30 μL) of the **SQ** stock solution was added to 3 mL of H_2O in a quartz cuvette. The sample was gently stirred for 5 s before the UV and fluorescence was recorded. For the CCG (0.25 mg/mL based on the initial GO weight content), it was added in 10 μL increments to SQ solution. In all the titration experiments, the total volume was kept not exceed 5% of the original volume. Equivalents of BSA or lysozyme are referenced to squaraine dyes.
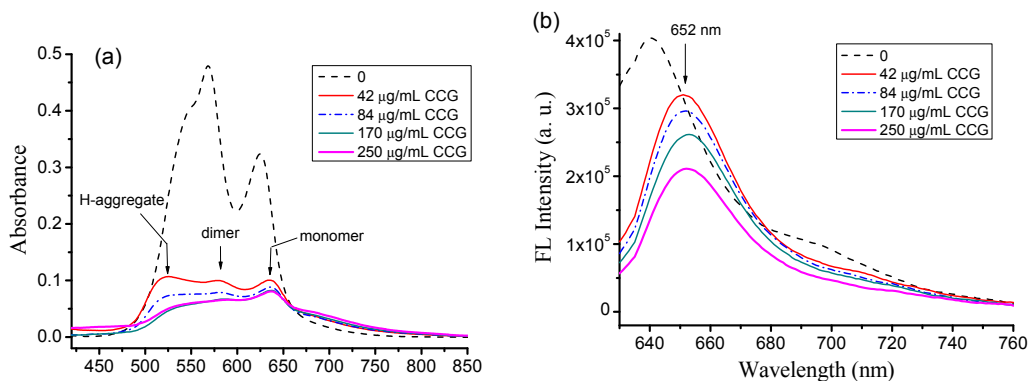
Figure S1. Absorption (a) and fluorescence (b) of **1a** (3 mL, 10 μM) in aqueous solution with different concentration of CCG (0-250 μg/mL).
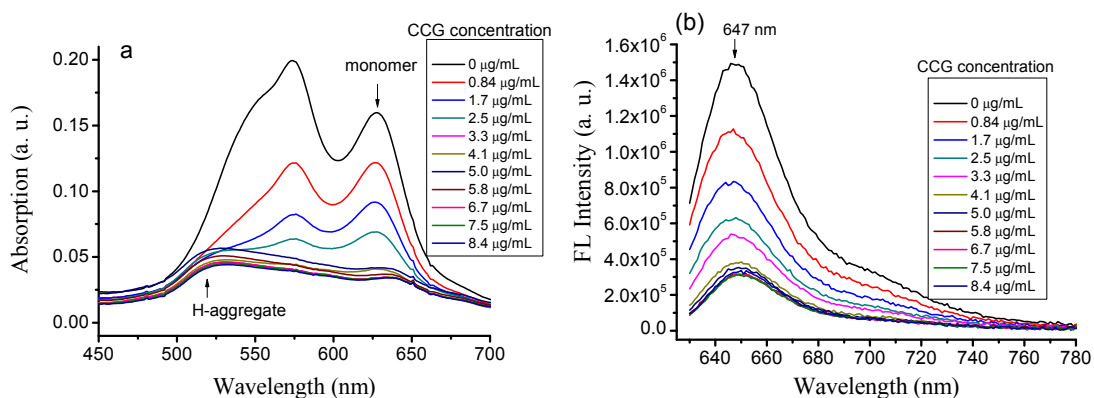

Figure S2. Absorption (a) and fluorescence (b) change of **1a** (5 μM) in aqueous solution with different concentration of CCG (0-8.4 μg/mL).
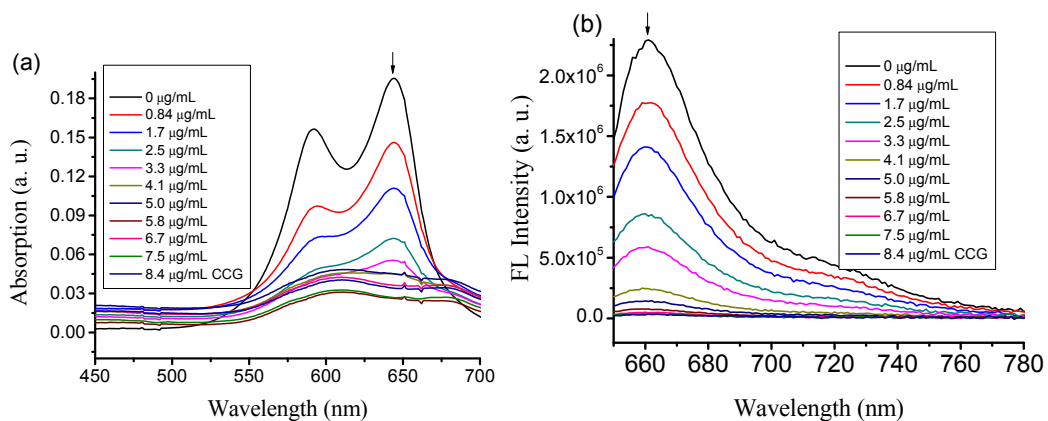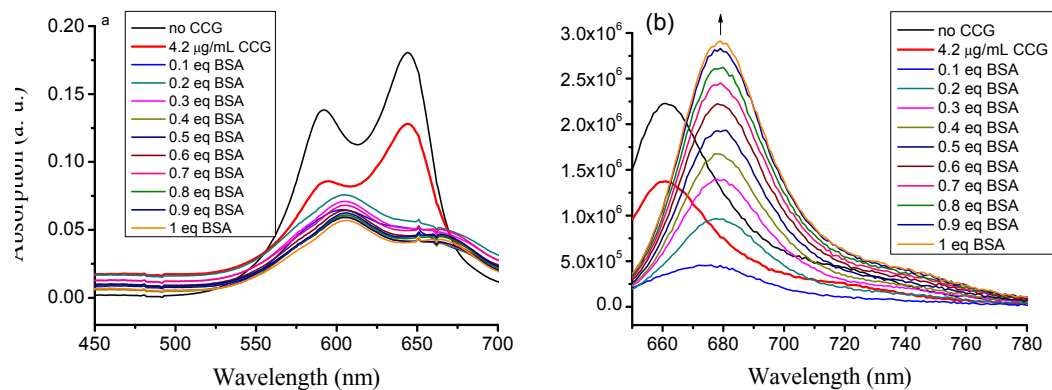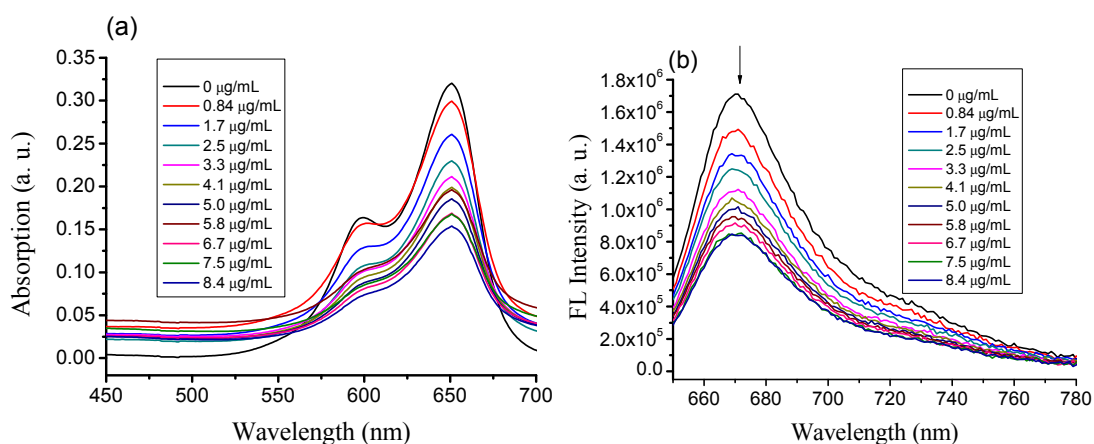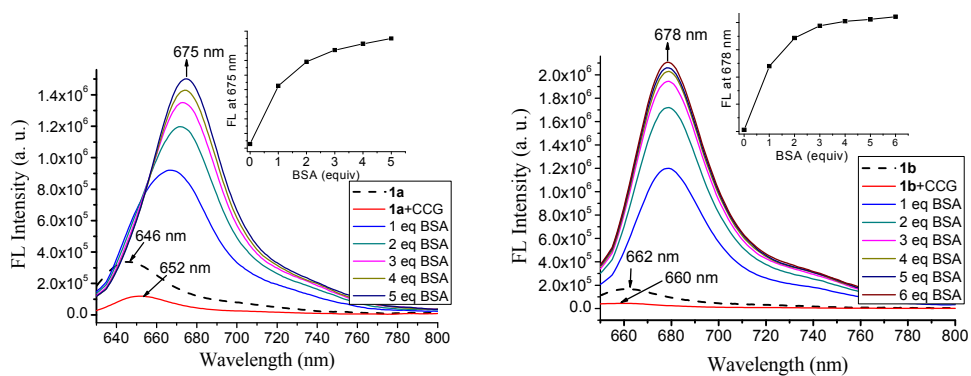

Figure S3. Absorption (a) and fluorescence (b) of **1b** (5 μM) in aqueous solution with different concentration of CCG (0-8.4 μg/mL).
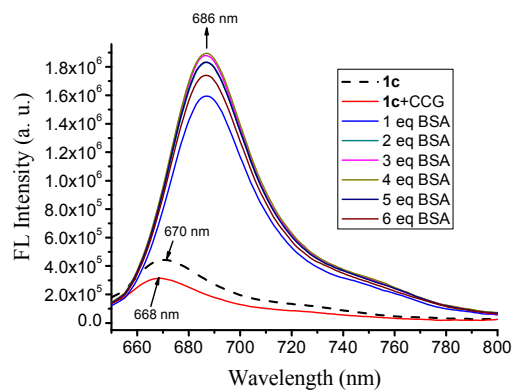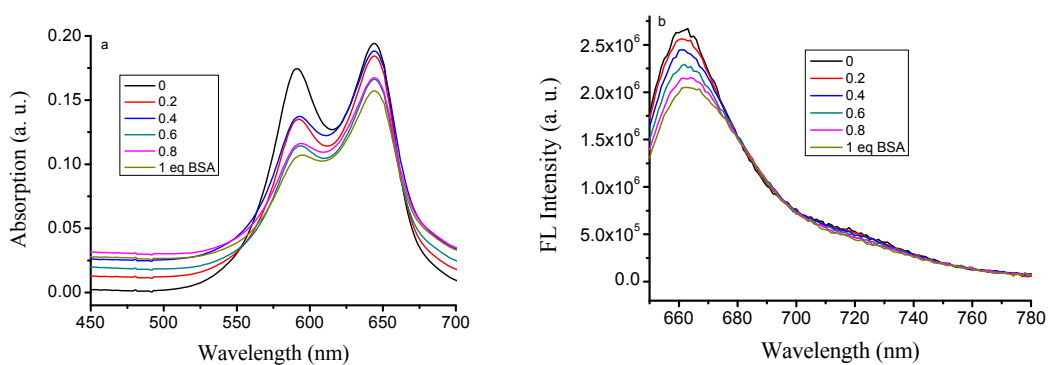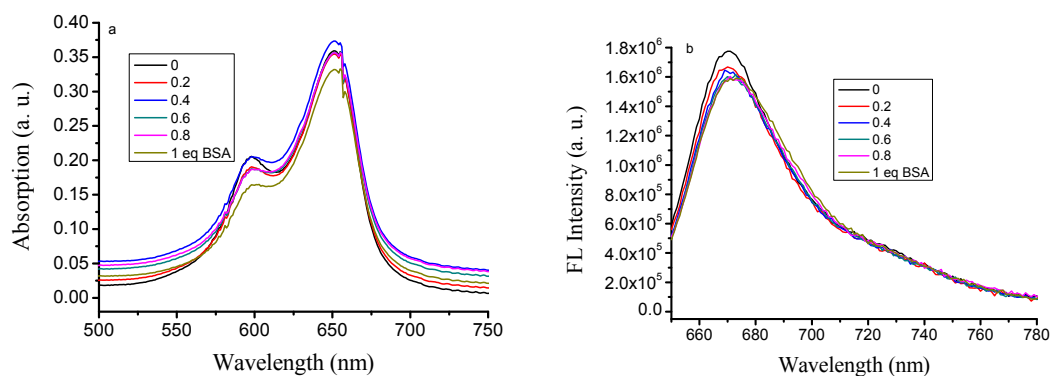
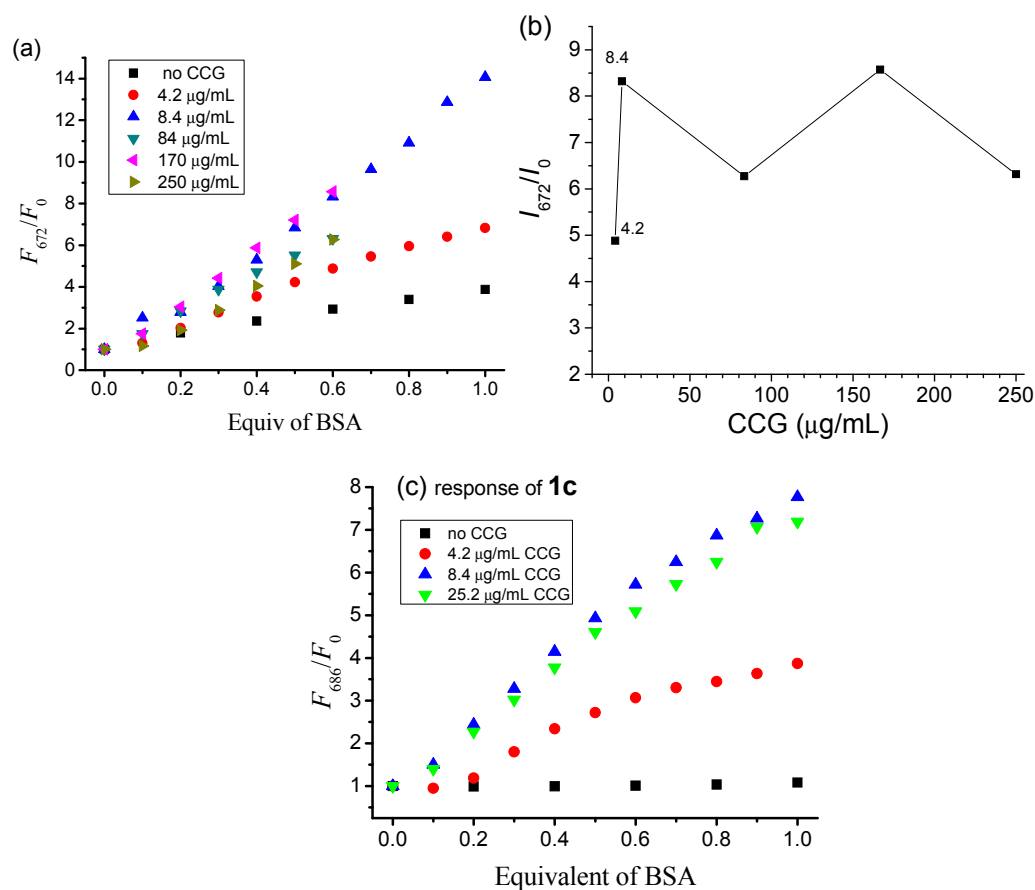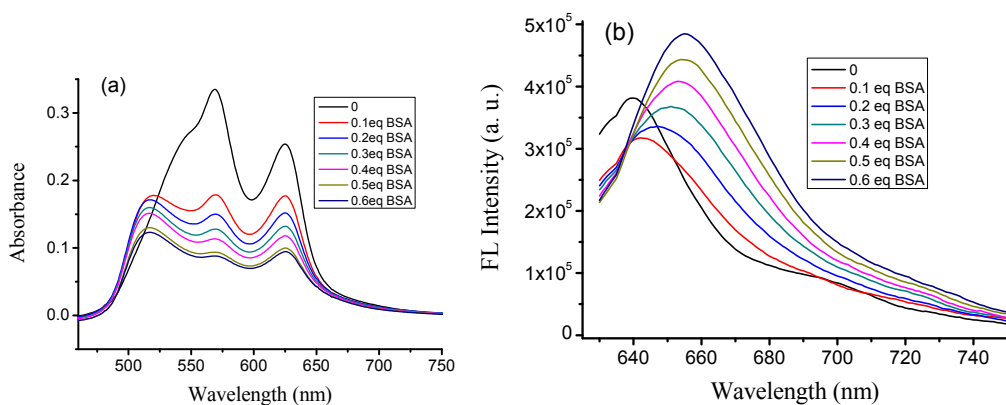Figure S4. Absorption (a) and fluorescence (b) of **1b** (5 μM) in aqueous solution upon addition of BSA in the presence of 4.2 μg/mL CCG.

Figure S5. Absorption (a) and fluorescence (b) of **1c** (5 μM) in aqueous solution with different concentration of CCG (0-8.4 μg/mL).

Figure S6. Fluorescence spectra of **1** (5 μM) in the presence of CCG (8.4 μg/mL) and BSA in water (excitation at 620 nm for **1a** and 640 nm for **1b-1c**).
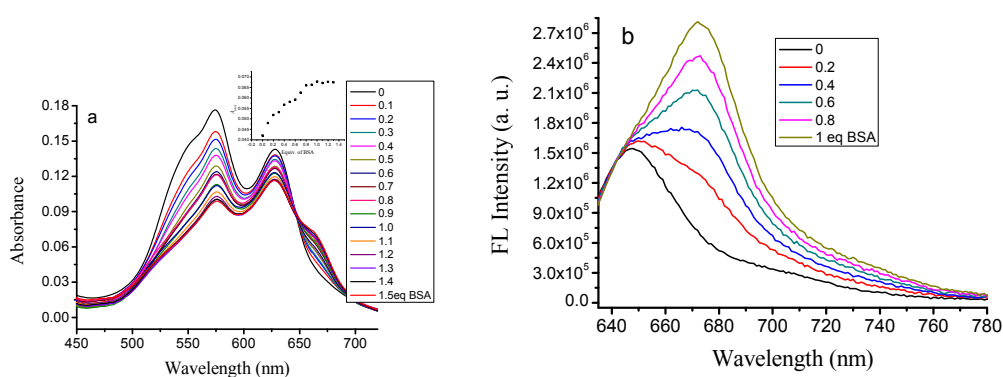
Figure S7. Absorption (a) and fluorescence (b) spectra of **1a** (5 μM) in H₂O upon addition of BSA in the absence of CCG (inset in Figure a: plot of absorbance intensity at 664 nm to equiv of BSA).

Figure S8. Absorption (a) and fluorescence (b) of **1b** (5 μM) in aqueous solution upon addition of BSA in the absence of CCG.

Figure S9. Absorption (a) and fluorescence (b) of **1c** (5 μM) in aqueous solution upon addition of BSA in the absence of CCG.

Figure S10. (a) Fluorescence intensity plot of **1a** (5 μM) at 672 nm in responding to equivalent of BSA in aqueous solution in the presence of various concentration of CCG. (b) Fluorescence response to CCG upon addition of 0.6 equiv of BSA. (c) Fluorescence response of **1c** (5 μM, 3 mL) at the emission peak wavelength 680 nm upon addition of different equivalent of BSA (in reference to SQ dye) in aqueous solution in the presence of various concentration of CCG.

Figure S11. Absorption (a) and fluorescence (b) of **1a** (5 μM) in aqueous solution upon addition of BSA in the presence of 42 μg/mL CCG.
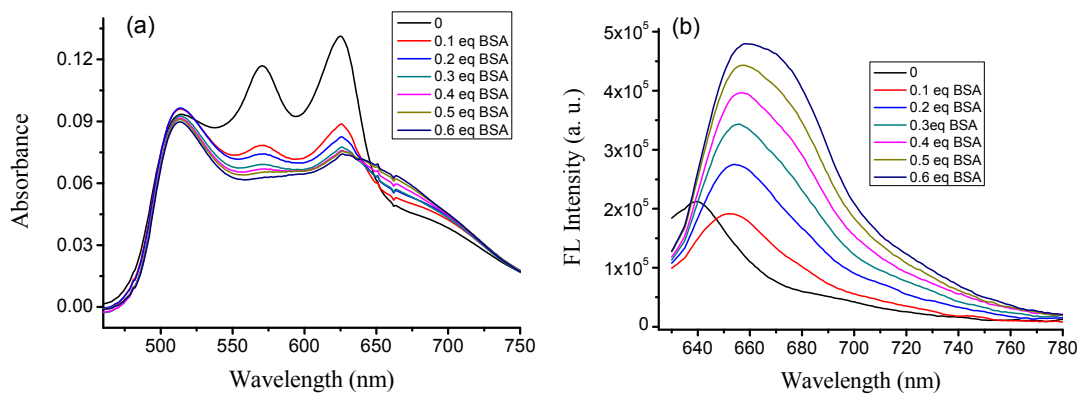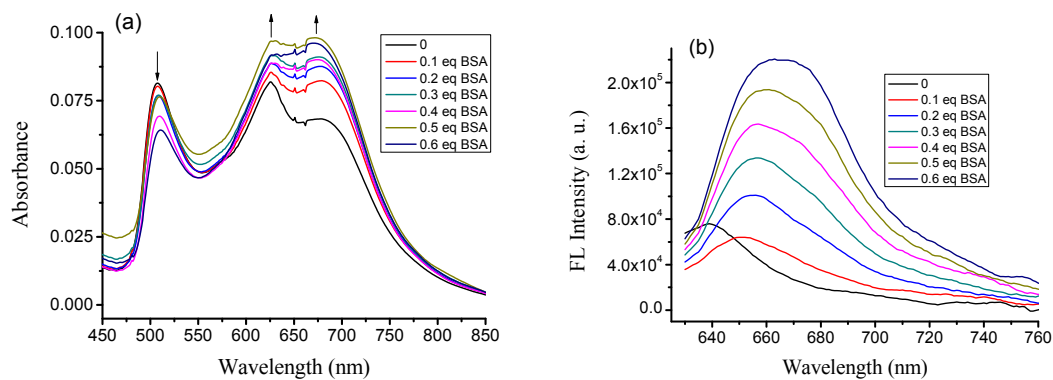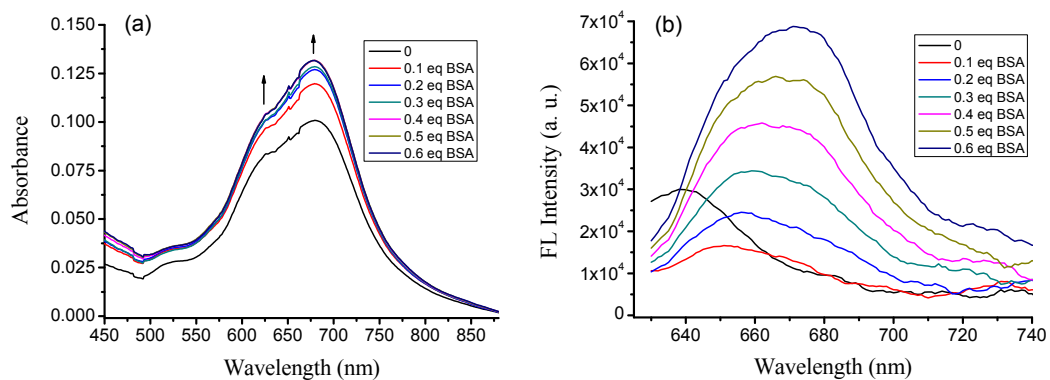

Figure S12. Absorption (a) and fluorescence (b) of **1a** (5 μM) in aqueous solution upon addition of BSA in the presence of 84 μg/mL CCG.

Figure S13. Absorption (a) and fluorescence (b) of **1a** (5 μM) in aqueous solution upon addition of BSA in the presence of 170 μg/mL CCG.

Figure S14. Absorption (a) and fluorescence (b) of **1a** (5 µM) in aqueous solution upon addition of BSA in the presence of 250 µg/mL CCG.
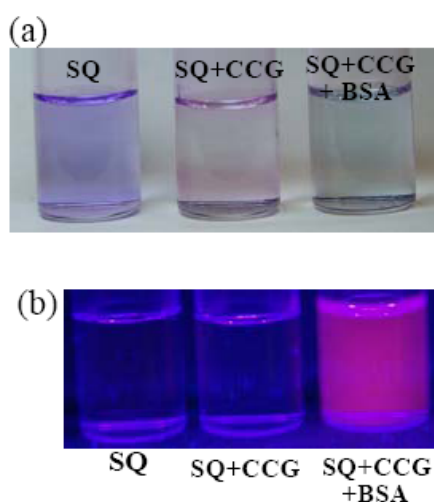

Figure S15. Color changes (a) and fluorescence response (b) of **SQ 1a** (15 µM) in the presence of CCG (0.042 mg/mL) and BSA (0.5 mg) in aqueous solution. In the fluorescence image, the solutions were irradiated with a portable UV-lamp (wavelength: 365 nm).
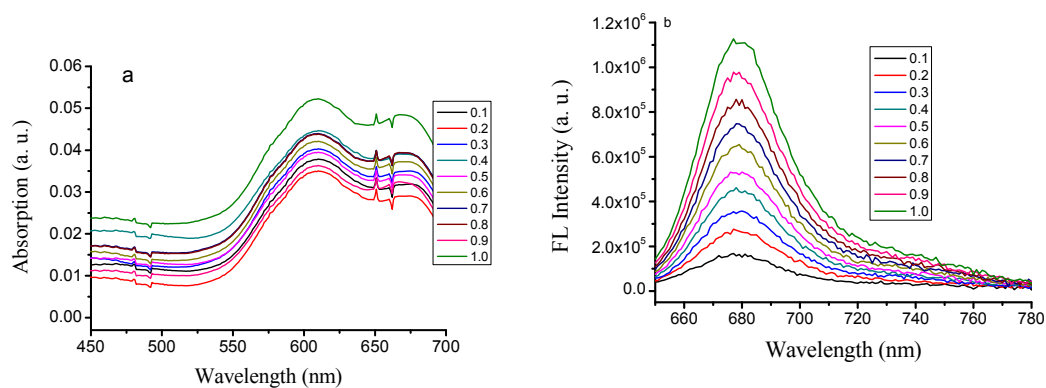

Figure S16. Absorption (a) and fluorescence (b) of **1b** (5 µM) in aqueous solution upon addition of BSA in the presence of 8.4 µg/mL CCG.
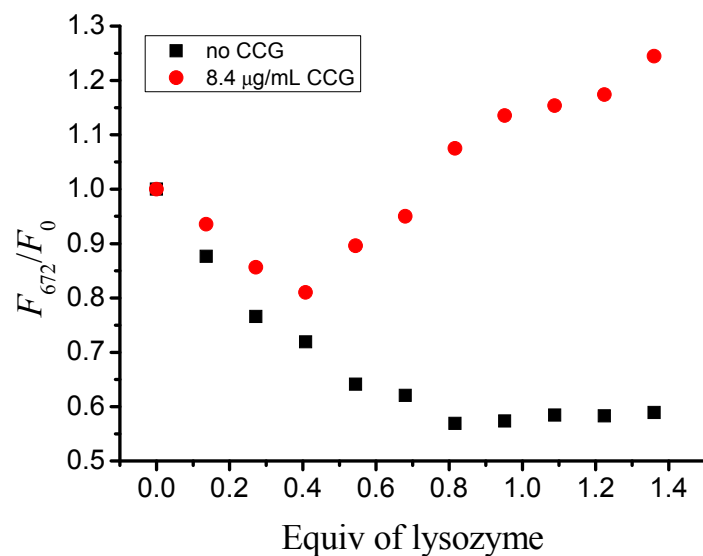
Figure S17. Plot fluorescence intensity at 672 nm of **1a** (5 μM) virsus concentration of lysozyme in aqueous solution in the presence of CCG.
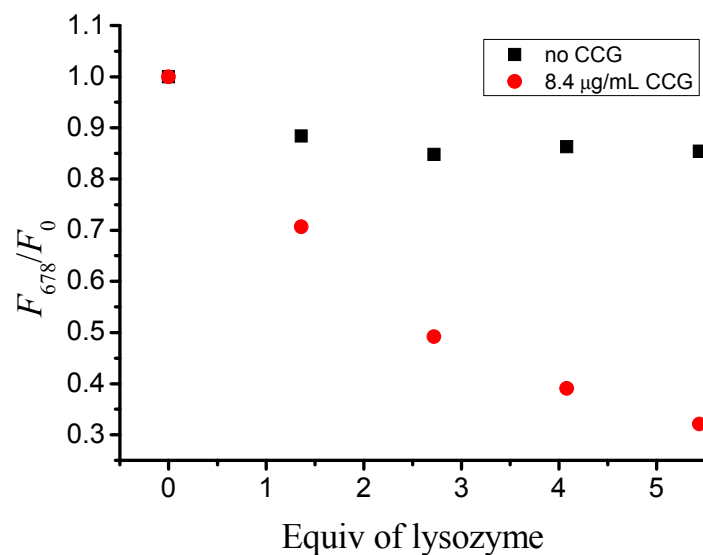

Figure S18. Plot fluorescence intensity at 678 nm of **1b** (5 μM) virsus concentration of lysozyme in aqueous solution in the presence of CCG.
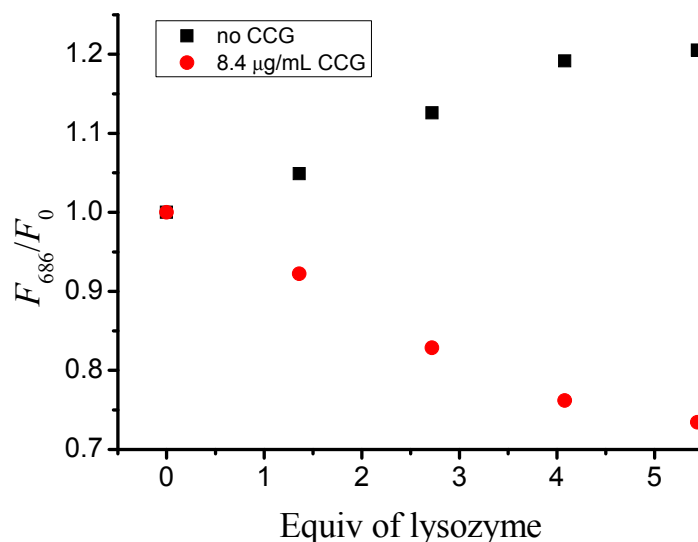
Figure S19. Plot fluorescence intensity at 686 nm of **1c** (5 µM) virsus concentration of lysozyme in aqueous solution in the presence of CCG.
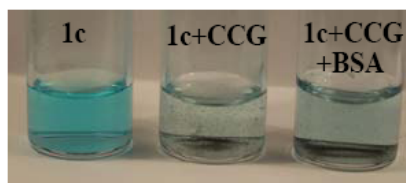

Figure S20. Color changes induced on **SQ 1c** (15 µM) in the presence of CCG (42 µg/mL) and BSA (0.5 mg) in aqueous solution.

Note: The addition of cationic dye and negtively charged graphene could cause the precipitation. This may lower the CCG-SQ concentration, which may cause some difficulty in characterization. It should be noted that the absorption, emission, and light scattering is based on the observation of CCG-SQ in solution. Therefore, the limited precipitation, if it occurrs, will not affect the conclusion, unless the precipitation completely removes the graphene (or SQ dyes) from the solution.
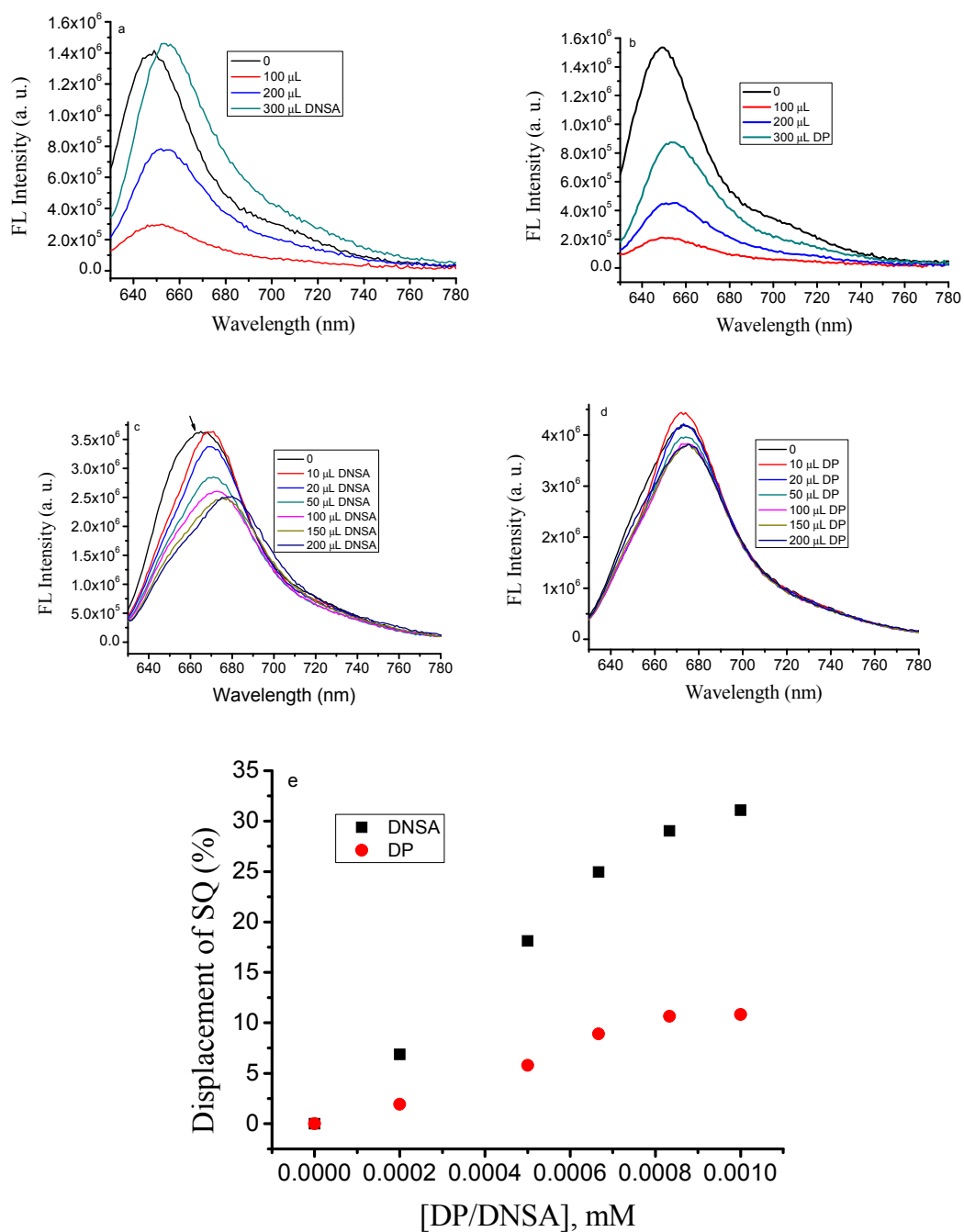
Figure S21. Fluorescence change of **1a** (5 μM) in aqueous solution upon addition of DNSA-DP in the presence of 100 μL CCG (a, b), in the presence of 100 μL CCG and 1 eq BSA (c, d). (e) Displacement of SQ (%) from the SQ/CCG complex by addition of DNSA or DP.

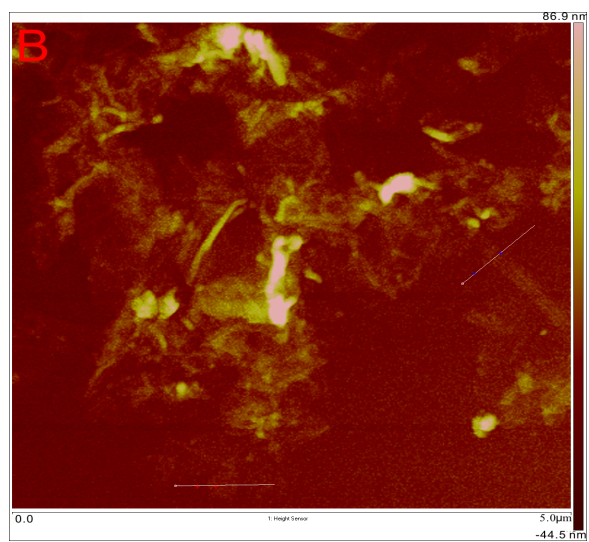The displacement of SQ by DNSA or DP was calculated from the equation:

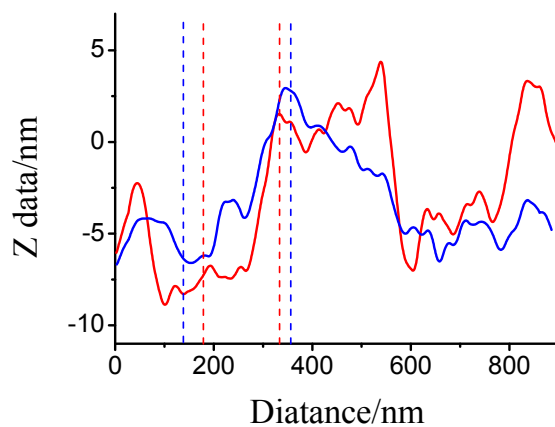$$\text{Displacement of SQ} = (I_s\text{-}I)/I_s \times 100\%$$

Here, I_s is the saturated fluorescence intensity of SQ/CCG in the presence of BSA, I is fluorescence intensity of SQ/CCG/BSA in the presence of different concentration of DNSA or DP.
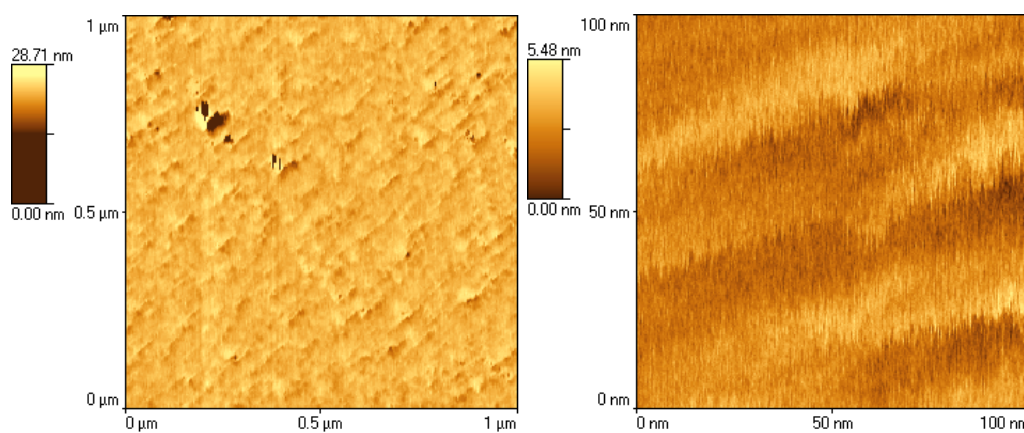
(a)
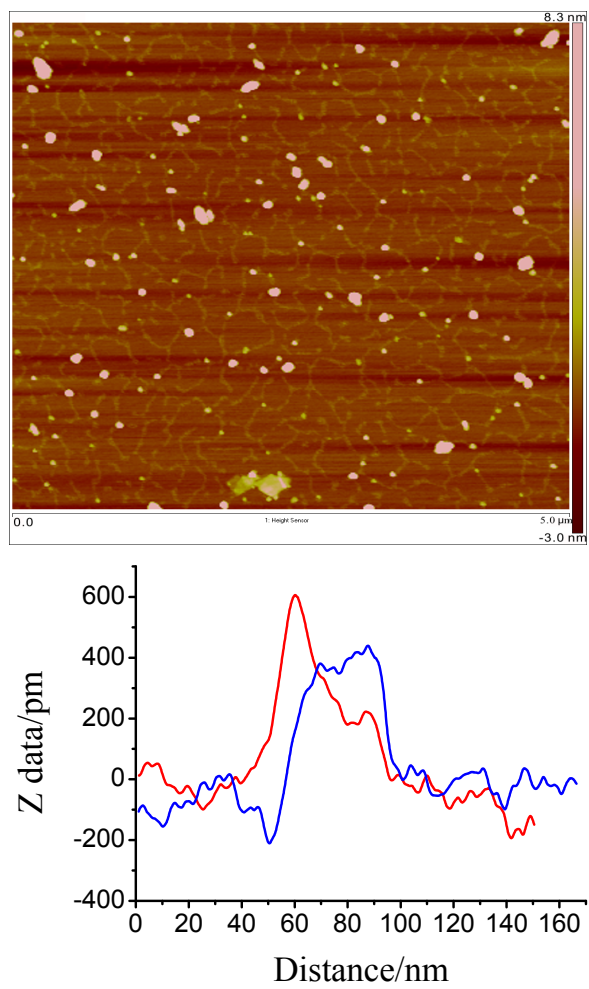
(b)

(c)

Figure S22. AFM spectra of (a) CCG, (b) CCG+SQ, (c) CCG+SQ+BSA. Interaction of BSA with CCG-SQ led to microstructures of smaller sizes.
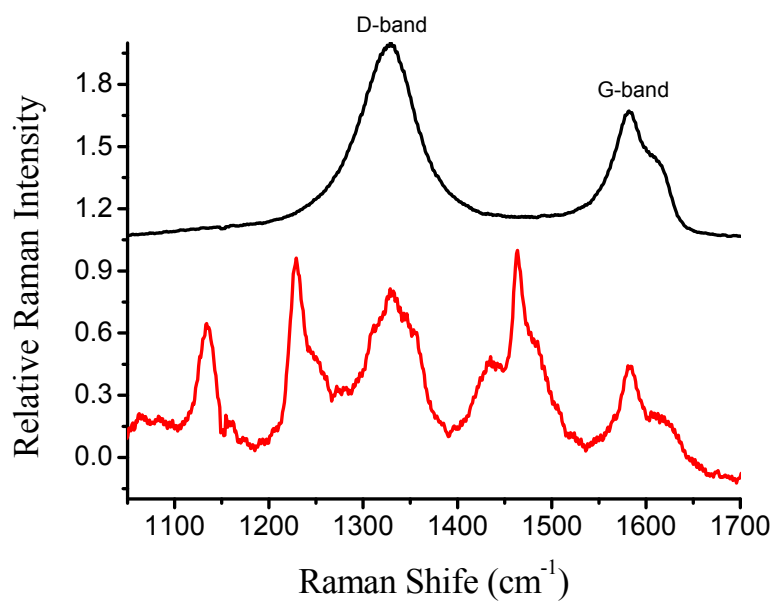

Figure S23. Raman spectra of CCG before (top) and after SQ addition (bottom).

The Raman spectra of CCG showed a disorder-induced D band at 1329 cm^{-1} arisng from the sp^3-hybridized carbon and a tangential stretch G band at 1580 cm^{-1} representing the E$_{2g}$ zone center mode of the crystalline graphite. Compared with CCG, the D and G bands of the SQ/CCG complex were slightly shifted to 1328 and 1583 cm^{-1}, respectively, which could be associated with the noncovalent interactions of SQ with CCG. Moreover, the ratios of the D band and G band of SQ/CCG (1.48) and CCG (1.85) had no apparent difference, indicating that the assembly of CCG with SQ had negligible impact on the electronic band structure of CCG (D. C. Wei, Y. Q. Liu, H. L. Zhang, L. P. Huang, B. Wu, J. Y. Chen, G. Yu, *J. Am. Chem. Soc.* 2009, 131, 11147).
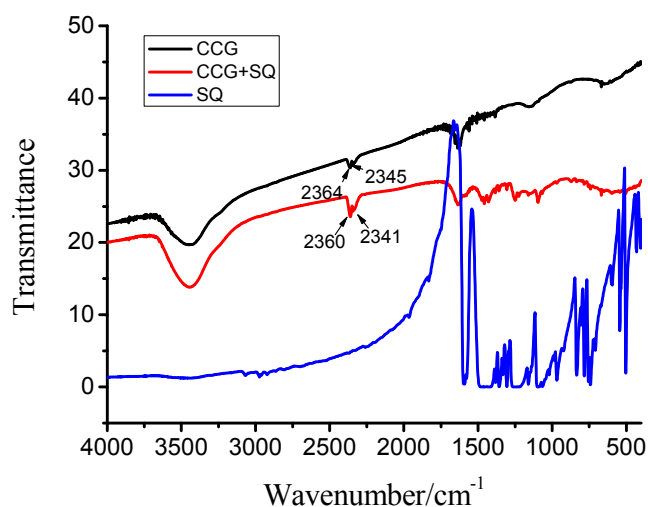

Figure S24. IR spectrum of CCG, CCG+SQ and SQ.

For CCG, peaks at 1718 and 1132 cm^{-1} are attributed to C=O and C-O stretching, respectively. For the CCG/SQ, a new band appeared at 1537 cm^{-1}, which is ascribed to the asymmetric vibration of COO$^-$ moieties, and CCG carboxylic acid moieties were interacted with SQ through ionic interactions (Y. Liu, D. Yu, C. Zeng, Z. Miao and L. Dai, *Langmiur*, 2010, 26, 6158).
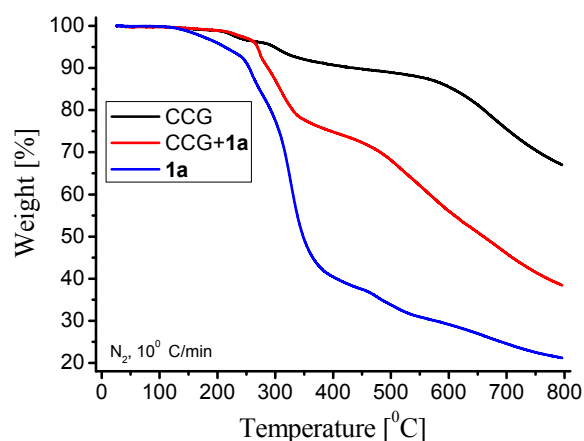
Figure S25. Thermogravimetric analysis (TGA) of **1a**, **1a**+CCG and CCG under a nitrogen flow.

The thermal properties of CCG and SQ-CCG were characterized by TGA (Fig. S25). While pure **SQ 1** started to lose weight at ~150°C, the SQ-CCG complex exhibited the weight loss at a notable higher temperature. The delayed decomposition temperature provided a complementary evidence, supporting the assumption that the **SQ** dye was interacting with CCG via forming complex.
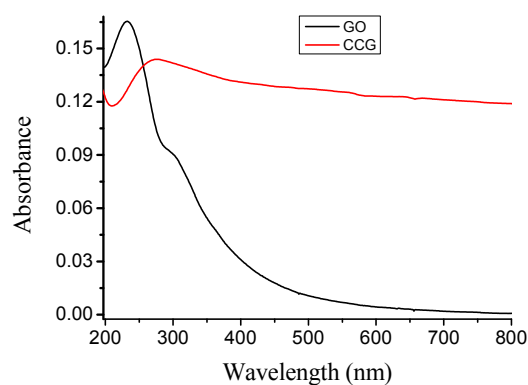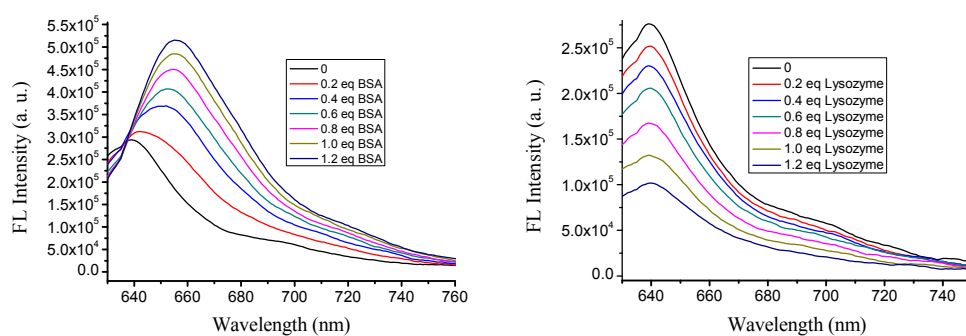

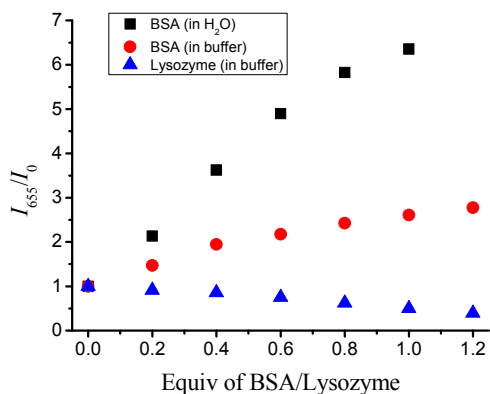Figure S26. Absorption spectra of GO and CCG dispersed in water.

Figure S27. Fluorescence response of **1a** (5 μM) virsus concentration of BSA/lysozyme in aqueous buffer solution (10 mM phosphate, pH 7.2) in the presence of 100 μL of CCG.
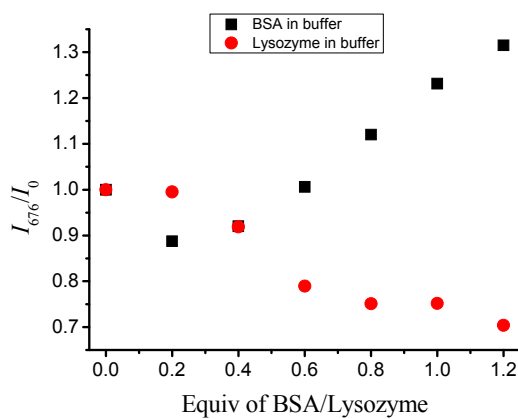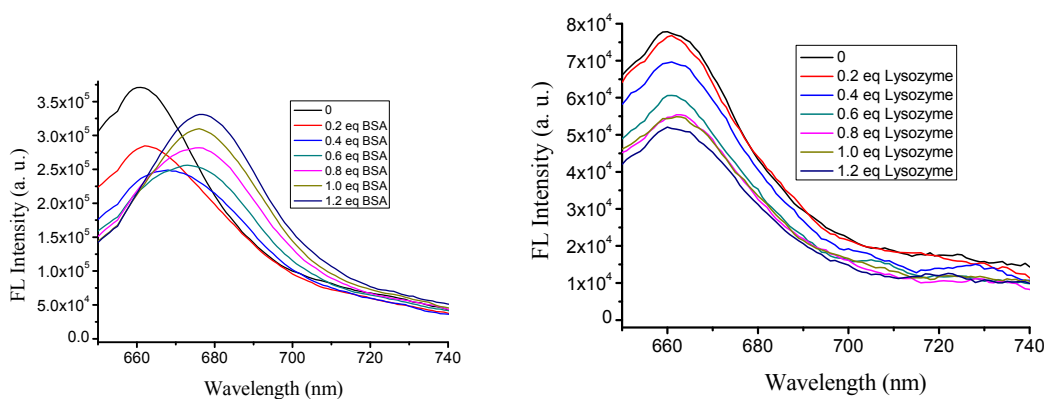

Figure S28. Fluorescence response of **1b** (5 μM) virsus concentration of BSA/lysozyme in aqueous buffer solution (10 mM phosphate, pH 7.2) in the presence of 8.4 μg/mL of CCG.
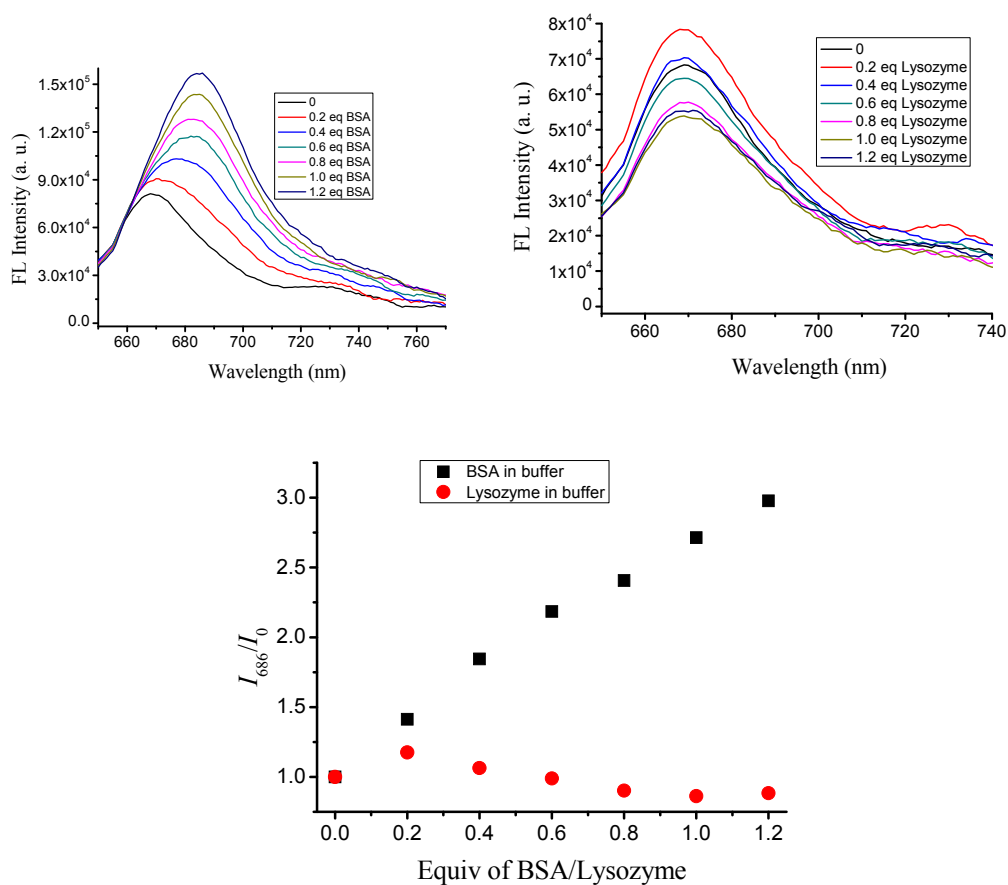
Figure S29. Fluorescence response of **1c** (5 μM) virsus concentration of BSA/lysozyme in aqueous buffer solution (10 mM phosphate, pH 7.2) in the presence of 8.4 μg/mL of CCG.
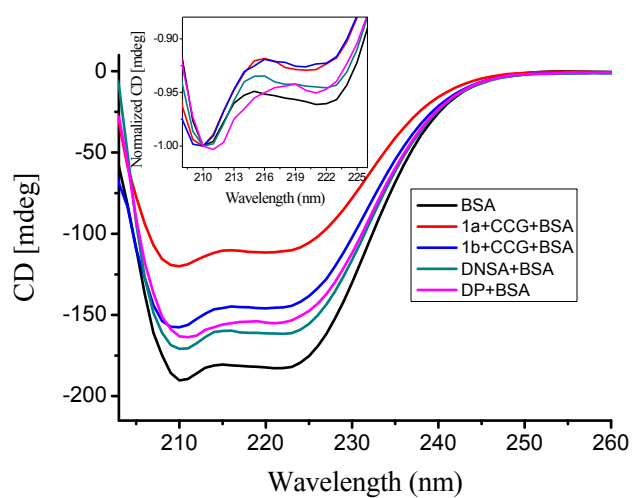

Figure S30. Circular dichroism of BSA (2.5 μM) in the presence of **SQ** (**1a-1c**, 2.5 μM) and CCG (8.4 μg/mL). The concentration for DNSA or DP is 30 μM. Inset: Partial spectra.