Electronic Supplementary Information (ESI)

Fluorescence detection of cholesterol using
a nitrogen-doping graphene quantum dots/chromium
picolinate complex-based sensor

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**Fig. S1** Fluorescence responses of N-GQDs (0.05 g L\(^{-1}\)) at various pH conditions.

![Fluorescence responses of N-GQDs](image)

**Fig. S2** Grafting capacity of Cys in N-GQDs aqueous solution (0.05 g L\(^{-1}\)) at (a) various pH conditions with \(n_{\text{NHS}}:n_{\text{EDC}}=1:2\) (molar ratio); and (b) different molar ratio of NHS to EDC with pH=5.0.

![Grafting capacity of Cys in N-GQDs](image)

**Date notes:**
To evaluate Cys grafting capacity, the grafted Cys concentration in modified N-GQDs aqueous solution (0.05 g L\(^{-1}\)) is measured using the calibration curve of Cys standard aqueous solution by monitoring the absorbance at 230 nm using an UV-Vis spectrophotometer. The grafting capacity is calculated as follows:

\[
\text{Grafting capacity (mg/g)} = \frac{\text{Amount of grafted Cys (mg)}}{\text{Amount of N-GQDs(g)}}
\]
**Fig. S3** AFM image and the height profile along the line in the topographic image of CrPic.

![AFM Image and Height Profile](image)

**Date notes:**
According to the statistical analyses, the average topographic height of CrPic is 0.64 nm.

**Fig. S4** AFM image and the height profile along the line (a and b) in the topographic image of N-GQDs/CrPic.

![AFM Image and Height Profile](image)

**Date notes:**
According to the statistical analyses, the average topographic height of CrPic is 1.91 nm.
**Fig. S5** Electron energy loss: (a) C, N and O element mapping images of N-GQDs; and (b) C, N, O, S and Cr element mapping images of N-GQDs/CrPic.

![Electron energy loss images](image1)

**Date note:**
Scanning Electron Microscopy (SEM) images and Energy Dispersive spectroscopy (EDS) are carried out on a Hitachi S-4800 scanning electron microscope equipped with a Noran energy dispersive spectrometer. These images are acquired by visualizing the inelastically scattered electrons in the energy loss windows for elements with different color areas.

**Fig. S6** Absorbance responses of CrPic aqueous solution at various concentrations; the error bar represented the standard deviation of three parallel tests.

![Absorbance response](image2)

**Date notes:**
To evaluate CrPic loading capacity, the grafted CrPic concentration in N-GQDs/CrPic aqueous solution (0.06 g L\(^{-1}\)) is measured using the calibration curve of CrPic standard aqueous solution by monitoring the absorbance at 265 nm using an UV-Vis spectrophotometer. The loading capacity is calculated as follows:

\[
\text{Loading capacity (mg/g)} = \frac{\text{Amount of grafted CrPic (mg)}}{\text{Amount of N-GQDs/CrPic (g)}}
\]
**Fig. S7** Relative PL intensities of N-GQDs ($F_B/F_A$, where $F_B$ and $F_A$ indicate PL intensities at 360 nm excitation before and after modifying, respectively) under different ions and biomolecules aqueous solutions at the same final concentration of 240 μM.

**Fig. S8** Fluorescence of N-GQDs/CrPic as a function of time after adding cholesterol (240 μM).

**Fig. S9** The plot of the $F/F_0$ (where $F_0$ and $F$ indicate fluorescence intensity of the N-GQDs/CrPic complex before and after addition of cholesterol (240 μM), respectively) as a function of pH.
**Fig. S10** Molecular structures of (a) cholesterol, (b) testosterone, (c) estrone, (d) progesterone and (e) glucocorticoid.

**Fig. S11** Fluorescence intensity of the N-GQDs/CrPic complex after addition of different biomolecules.
In this work, the Gaussian 09 suite of programs is used through, and the B3LYP density functional method is employed to carry out all the computations. The 6-31G(d) basis set is used for the computations of C,H,O and N atom. Vibrational frequency analyses at the same level of theory are performed on all optimized structures to characterize stationary points as local minima.

**Table S1** Quantum chemical parameters of cholesterol (Cho), testosterone (Tes) and estrone (Est).

<table>
<thead>
<tr>
<th>Molecules</th>
<th>$E_{\text{HOMO}}$ (eV)</th>
<th>$E_{\text{LUMO}}$ (eV)</th>
<th>$\Delta E$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cho</td>
<td>-5.87</td>
<td>-1.14</td>
<td>4.73</td>
</tr>
<tr>
<td>Tes</td>
<td>-5.94</td>
<td>0.97</td>
<td>6.91</td>
</tr>
<tr>
<td>Est</td>
<td>-5.91</td>
<td>0.46</td>
<td>6.37</td>
</tr>
</tbody>
</table>

**Fig. S12** Optimized structures and molecular orbital densities of cholesterol (a and b), testosterone (c and d) and estrone (e and f). Left: HOMO, right: LUMO.
**Fig. S13** Bright-field microphotographs of HeLa cells incubated for 24 h under various concentrations of N-GQDs/CrPic (a-f: 0.00, 0.04, 0.08, 0.16, 0.32 and 0.64 g L\(^{-1}\)).

**Date notes:**
Cell image is recorded by using an inverted microscope (SANYO, Japan).