Electronic Supplementary Information

Loading of an Anti-cancer Drug onto Graphene Oxide and Subsequent Release to DNA/RNA: A Direct Optical Detection

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Note 1

Synthesis of Graphene Oxide (GO)

GO was synthesized using modified Hummers method\(^1\) from graphite flakes. 2 g of graphite flakes was added in 97 wt% H\(_2\)SO\(_4\) (20mL), 1.6 g K\(_2\)S\(_2\)O\(_8\) and 1.6 g P\(_2\)O\(_5\) mixture. The mixture was kept at 80 °C for 8 hrs, cooled at room temperature, diluted with 0.5 L of de-ionized water and left for overnight. Then the mixture was filtered, washed several times with de-ionized water for removal of residual acids, and dried in vacuum oven overnight at 60 °C. Finally, we obtained 1.3 g of pre-oxidized graphite. The pre-oxidized graphite was further subjected to oxidation process for synthesizing GO. 1 g of pre-oxidized graphite powder and sodium nitrate (NaNO\(_3\); 0.5 g, 0.5 eq.) mixture was taken in a flask kept in ice water bath. Then, concentrated H\(_2\)SO\(_4\) (97 wt-%, 40 ml) was added with stirring. After that potassium permanganate (KMnO\(_4\); 3 g, 2 eq.) added very slowly over ~1 hr, continue the stirring for 2 hrs in ice water bath. After removing from the ice water bath, the stirring was continued vigorously for 2 days at room temperature and after that aqueous H\(_2\)SO\(_4\) (5 wt%, 100 ml) was added very slowly up to ~1 hr with constant stirring. The temperature was automatically raised up to 60 °C, and then the final temperature of the reaction mixture was kept above 90 °C by supplying external heat. The resultant mixture was further stirred for 2 hrs at 98 °C, and the temperature was then reduced to 60 °C. Then H\(_2\)O\(_2\) aqueous solution (30 wt-%, 10 ml) was added and the mixture was stirred for 2 hrs at room temperature. The product was repeatedly washed with aqueous HCl solution (10 wt-%) and then rinsed with de-ionized water repeatedly, up to neutral pH obtained. Then mixture was washed with ethanol solution for three times and finally oxidized compound was dried at ambient conditions overnight.
FE-SEM Images and Raman Spectra

Figure S1. FE-SEM images of (a) ellipticine (7 μM) with HSA (100 μM), (b) ellipticine (7 μM) with DNA (100 μM), (c) ellipticine (7 μM) with TAR RNA (10 μM), (d) Phosphate buffer (PBS, pH 7); and (e) Raman Spectra of graphite; (f) Powder X-ray diffraction of graphite.
Table S1: Intensity ratio of D, G bands and the crystalline size (L_c) of the graphene domains.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ID/ IG</th>
<th>Lc = 4.4 (ID/IG)^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graphite</td>
<td>0.34</td>
<td>12.81</td>
</tr>
<tr>
<td>GO</td>
<td>1.10</td>
<td>3.97</td>
</tr>
<tr>
<td>GO + DNA</td>
<td>1.24</td>
<td>3.53</td>
</tr>
<tr>
<td>GO + RNA</td>
<td>1.15</td>
<td>3.80</td>
</tr>
<tr>
<td>GO + HSA</td>
<td>1.14</td>
<td>3.86</td>
</tr>
<tr>
<td>GO + HSA + DNA</td>
<td>1.17</td>
<td>3.75</td>
</tr>
</tbody>
</table>

Absorption Spectra

**Figure S2:** Absorption spectra of (a) ellipticine in aqueous PBS with increasing concentration GO (0-3.8 µg/ml), (b) ellipticine-GO (E-GO) complex with increasing concentration of DNA (0-100 µM).
Figure S3: Fluorescence excitation spectra of ellipticine in aqueous PBS with increasing concentration of GO ($\lambda_{em}$= 450 nm).

Figure S4. Fluorescence profiles ($\lambda_{ex}$=355 nm) of E-GO in aqueous PBS with increasing concentration of Glucose (0-110 mM).
Note 2

**Loading Capacity of Drug On GO:** The drug (ellipticine) loading on GO was monitored in phosphate buffer saline (PBS, pH ~7) at room temperature. As ellipticine (E) is sparingly soluble in water, hence, a small amount of concentrated DMSO stock solution of E was added into aqueous PBS buffer solution and strongly sonicated to make system homogeneous. After each addition of GO, the solution is strongly sonicated for 10-20 minutes. The loading capacity of drug was calculated by monitoring excitation spectra of the drug, rather than the conventional absorption spectra, as fluorescence excitation spectra is more sensitive than absorption spectra particularly when the concentration of the drug is in the range of nano-molar to micro-molar. The drug loading capacity was determined by monitoring its peak intensity at 300 nm (the peak at 300 nm arises due to the protonated form of E in PBS) (Figure S5) with the help of following equation:

$$W_{\text{loading capacity}} = 100 - \left\{ \frac{\text{ellipticine intensity in presence of GO}}{\text{ellipticine intensity without GO}} \right\} \times 100$$

where, $W_{\text{loading capacity}}$ stands for loading capacity of E, and we found ~76% drug (from its initial concentration) was loaded on GO surface. The high loading capacity of E indicates that drug binds to both sides of single layer GO sheet predominantly by π-π stacking interactions.
**Figure S5:** Fluorescence excitation spectra of ellipticine in presence of GO ($\lambda_{em}$ = 520 nm).

**Unloading Capacity of Drug from GO:** The progressive addition of DNA/RNA into ellipticine-GO (E-GO) composite, drug releasing from GO takes place, and it is evident from Figure 3 that after coming out from GO surface the drug binds to DNA/RNA in its protonated form. The releasing capacity of the drug from GO was calculated monitoring the intensity at 315 nm (as a new peak starts appearing at ~315 nm after addition of DNA, RNA)\(^2\) in the excitation spectra (Figure S6) with the help of following equation,

$$W_{\text{unloading capacity}} = 100 - \left( \frac{\text{ellipticine final intensity in presence of GO}}{\text{E-GO intensity in presence of DNA or RNA}} \right) \times 100$$

where, $W_{\text{unloading capacity}}$ stands for releasing capacity of drug, and finally we found ~ 60% of drug was released from GO.
**Figure S6**: Fluorescence excitation spectra of ellipticine-GO (E-GO) system in presence of (a) DNA and (b) RNA ($\lambda_{em} = 520$ nm).
Note 3

Circular Dichroism (CD) Measurements

Figure S7: CD spectra of HSA in presence of E-GO solution.

CD spectra of HSA were monitored by the progressive increase concentration of the E-GO in the medium. HSA shows two distinct characteristic peaks at 208 nm and 222 nm, which appears from α-helices of the protein as HSA contains 65% of α-helix in its structure. GO itself doesn’t show any characteristic signal in CD. By the progressively increasing concentration of GO in the fixed concentration of HSA, a prominent decrease in CD signal at both of the dips is observed. The decreasing CD signal is surely an outcome of modulation in the secondary structure of protein in presence of GO. This ascribed to protein molecule adsorption over the GO surface by hydrophobic, π-π stacking interaction between aromatic moieties of protein and π-conjugate domains of GO. Moreover electrostatic, H-bonding interactions also play crucial role in protein-GO complexation process. 
**Note 4**

**Fluorescence Lifetime Measurements:**

Fluorescence lifetime is an excellent technique to explore the excited state milieu around the fluorophore and is highly sensitive to the excited-state interaction between the probe and host.\(^5\) Therefore, fluorescence lifetime measurements of ellipticine (E) are performed in GO, GO-HSA, GO-DNA/RNA, and GO-HSA-DNA systems. We have monitored the decay profiles both at 450 nm as well as at 520 nm in order to probe the neutral (E\(_N\)) as well as protonated form (E\(_H^+\)) of the drug. Instead of emphasizing individual components, we have considered average lifetime to provide insight into the binding behavior of E in the above mentioned systems. The decay characteristics of neutral drug molecules (monitored at 450 nm) in presence of GO, GO-HSA, GO-DNA/RNA, and GO-HSA-DNA systems are displayed in Figure S8a, and corresponding fitting results are given in Table S2a. Neutral ellipticine (E\(_N\)) in buffer solution gives an average fluorescence lifetime of \(~710\) ps, which is enhanced to 2 ns in presence of GO. The increased life-time may be attributed to \(\pi-\pi\) and hydrophobic interactions between neutral form of the drug and GO basal planes. When E-GO complex is titrated by bio-macromolecules like serum albumin (HSA), DNA and RNA, then significant changes are observed in the decay profiles of E (Figure S8a). In presence of HSA, fluorescence lifetime of E-GO increases from 2 ns to 6.2 ns, which indicates that the drug in E-GO complex are further getting stabilized when protein adsorbs on the GO surface, which is consistent with the intensity hike at 450 nm in presence of protein. In case of DNA/RNA, no such significant changes are observed inferring that neutral ellipticine (E\(_N\)) molecules in the GO surface are not interacting with DNA/RNA at all, which is also consistent with steady state observation, where we have seen the intensity at \(~450\) nm remains almost same in presence of DNA/RNA.
Table S2: Fluorescence decay fitting parameters of ellipticine in presence of GO and E-Go complex in presence of various bio-macromolecules, decays collected at 450 nm and (b) at 520 nm.

(a)

<table>
<thead>
<tr>
<th>Sample</th>
<th>(\tau_1) (ns)</th>
<th>(\tau_2) (ns)</th>
<th>(\tau_3) (ns)</th>
<th>R_1</th>
<th>R_2</th>
<th>R_3</th>
<th>(\langle\tau\rangle^#) (ns)</th>
<th>(\chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ellip in PBS</td>
<td>0.22</td>
<td>1.64</td>
<td>8.92</td>
<td>0.82</td>
<td>0.14</td>
<td>0.03</td>
<td>0.71</td>
<td>1.25</td>
</tr>
<tr>
<td>Ellip + GO (3.8 μg/ml)</td>
<td>0.15</td>
<td>1.52</td>
<td>8.77</td>
<td>0.6</td>
<td>0.22</td>
<td>0.18</td>
<td>2.05</td>
<td>1.06</td>
</tr>
<tr>
<td>(E-GO)</td>
<td>0.12</td>
<td>1.57</td>
<td>9.42</td>
<td>0.54</td>
<td>0.22</td>
<td>0.24</td>
<td>2.53</td>
<td>1.06</td>
</tr>
<tr>
<td>E-GO + DNA (100 μM)</td>
<td>0.18</td>
<td>1.97</td>
<td>9.5</td>
<td>0.53</td>
<td>0.28</td>
<td>0.2</td>
<td>2.53</td>
<td>1.09</td>
</tr>
<tr>
<td>E-GO + RNA (10 μM)</td>
<td>0.49</td>
<td>4.57</td>
<td>20.3</td>
<td>0.47</td>
<td>0.3</td>
<td>0.23</td>
<td>6.2</td>
<td>1.08</td>
</tr>
<tr>
<td>E-GO + HSA (100 μM)</td>
<td>0.24</td>
<td>2.62</td>
<td>12.9</td>
<td>0.62</td>
<td>0.27</td>
<td>0.11</td>
<td>2.26</td>
<td>1.14</td>
</tr>
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</table>

(b)

<table>
<thead>
<tr>
<th>Sample</th>
<th>(\tau_1) (ns)</th>
<th>(\tau_2) (ns)</th>
<th>(\tau_3) (ns)</th>
<th>R_1</th>
<th>R_2</th>
<th>R_3</th>
<th>(\langle\tau\rangle^#) (ns)</th>
<th>(\chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ellip in PBS</td>
<td>-</td>
<td>1.95</td>
<td>6.46</td>
<td>-</td>
<td>0.87</td>
<td>0.13</td>
<td>2.52</td>
<td>1.08</td>
</tr>
<tr>
<td>Ellip + GO (3.8 μg/ml)</td>
<td>0.22</td>
<td>1.89</td>
<td>9.12</td>
<td>0.51</td>
<td>0.37</td>
<td>0.12</td>
<td>1.88</td>
<td>1.04</td>
</tr>
<tr>
<td>(E-GO)</td>
<td>0.37</td>
<td>3.63</td>
<td>15.3</td>
<td>0.36</td>
<td>0.2</td>
<td>0.44</td>
<td>7.6</td>
<td>1.01</td>
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<tr>
<td>E-GO + DNA (100 μM)</td>
<td>0.31</td>
<td>3</td>
<td>15</td>
<td>0.3</td>
<td>0.46</td>
<td>0.25</td>
<td>7.71</td>
<td>0.99</td>
</tr>
<tr>
<td>E-GO + RNA (10 μM)</td>
<td>0.39</td>
<td>3.32</td>
<td>16.7</td>
<td>0.57</td>
<td>0.31</td>
<td>0.12</td>
<td>3.23</td>
<td>1.14</td>
</tr>
<tr>
<td>E-GO + HSA (100 μM)</td>
<td>0.26</td>
<td>2.76</td>
<td>13.3</td>
<td>0.57</td>
<td>0.27</td>
<td>0.17</td>
<td>3.08</td>
<td>1.12</td>
</tr>
<tr>
<td>EGH + DNA (100 μM)</td>
<td>0.26</td>
<td>2.76</td>
<td>13.3</td>
<td>0.57</td>
<td>0.27</td>
<td>0.17</td>
<td>3.08</td>
<td>1.12</td>
</tr>
</tbody>
</table>

\(\langle\tau\rangle = \tau_1R_1 + \tau_2R_2 + \tau_3R_3\); whereas \(\chi^2\) is the measure of goodness of the fit. \(\chi^2\) close to 1 is considered as good fit.

To verify the drug release behavior inside the cell, we have gradually added DNA to the ellipticine-GO-HSA (EGH) complex. The average lifetime of EGH complex drastically drops from 6.2 ns to 2.26 ns in presence of DNA, inferring that \(E_N\) form of drug is destabilized when DNA/RNA interacts with EGH complex. This is because in presence of DNA/RNA, \(E_{H^+}\) form of drug preferably interacts, and therefore, the equilibrium \(E_N \rightleftharpoons E_{H^+}\) is shifted towards the latter.
side. So, the lifetime results also supports our claim based on the emission spectra that drug molecules are released from EGH complex in presence of DNA/RNA and binds to DNA/RNA.

We have also probed the interaction behavior between E-GO and glucose, and we found that glucose is not perturbing the decay profile of GO-bound E, suggesting that glucose is not at all interacting with drug, which corroborates well with our steady state observation where we found that glucose does not alter the emission profile of GO-bound E. Fluorescence lifetime profiles of protonated ellipticine (E$_H^+$) collected at 520 nm in presence of GO, GO-DNA, GO-HSA, GO-HSA-DNA, GO-RNA and GO-glucose are shown in Figure S8b, and the results are compiled in Table S2b. E$_H^+$ in aqueous buffer (pH 7) exhibits an average lifetime of 2.52 ns. In the presence of GO the average lifetime slightly decreases compared to E$_H^+$ in aqueous buffer, and it is attributed to the quenching effect by GO. However, when DNA/RNA is added to the GO-bound E, there is huge increment of average lifetime of E$_H^+$, inferring that ellipticine (E) is getting stabilized through interaction (probably through intercalation binding mode) with DNA/RNA in its protonated form.

**Figure S8**: Fluorescence decay profiles of ellipticine in presence of presence of GO and E-GO complex in presence of various bio-macromolecules, decays at (a) 450 nm and (b) 520 nm.
The average lifetime of E$_H^+$ bound to E-GO-HSA complex increases when it was being titrated by DNA, and this suggests that E$_H^+$ is generating and getting stabilized in presence of DNA. This corroborates well with steady state results where we have observed that the peak at 520 starts appearing when E-GO-HSA complex is being titrated by DNA. Therefore, both steady state and time-resolved experiments confirm that DNA has higher binding affinity compared to E-GO as well as E-GO-HSA complex, which has important consequence in the context of drug release inside the cell. In presence of glucose there is no significant change in the average lifetime of E$_H^+$, indicating that E$_H^+$ in presence of glucose neither interacts nor release from surface of GO, and this observation is quite consistent with steady state emission results, where we have seen that emission spectra of E is unperturbed in presence of glucose.
Note 5

**Time-Resolved Fluorescence Anisotropy:** To obtain further information about the microenvironments of the drug in presence of GO, GO-DNA, GO-RNA, GO-HSA-DNA, and GO-glucose systems, we have collected time-resolved anisotropy decays, which directly reflect the restriction over rotational motion imposed by the surrounding environment. The typical anisotropy decays are shown in Figure S9, and corresponding data are given Table S3. The average rotational correlation time of GO-bound E monitored at 450 nm is 110 ps. When GO-bound E is exposed to DNA/RNA, severe restriction of rotational motion of \(E_H^+\) takes place (Figure S9), inferring that the drug molecules are interacting to DNA/RNA probably through intercalation binding mode. Interestingly, the decay profile consists of ‘dip-rise’ feature in case of E-GO-DNA composite system, and the decay exhibits some residual anisotropy, which does not fit within our experimental time window. This kind of not-so-common time-resolved anisotropy arises due to the presence of multiple species, each characterized by its own lifetime and anisotropy decay.\(^6\)\(^7\) However, for the sake of relevance of the present work, we mainly focus on normal anisotropy decay features.

**Table S3:** Time-resolved fluorescence anisotropy fitting parameters of ellipticine in presence of GO and various bio-macromolecules containing GO in aqueous buffer solution.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(\tau_{r1}) (ns)</th>
<th>(\tau_{r2}) (ns)</th>
<th>(\tau_{r3}) (ns)</th>
<th>(f_1)</th>
<th>(f_2)</th>
<th>(f_3)</th>
<th>(r_0)</th>
<th>(\langle \tau_r \rangle)(^#) (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ellip + GO (3.8 μg/ml)</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>0.37</td>
<td>-</td>
<td>-</td>
<td>0.37</td>
<td>0.15</td>
</tr>
<tr>
<td>E-GO + RNA (10 μM)</td>
<td>0.08</td>
<td>8.08</td>
<td>-</td>
<td>0.19</td>
<td>0.15</td>
<td>-</td>
<td>0.34</td>
<td>3.7</td>
</tr>
<tr>
<td>Ellip + HSA (100 μM)</td>
<td>0.10</td>
<td>0.88</td>
<td>20.0</td>
<td>0.19</td>
<td>0.02</td>
<td>0.09</td>
<td>0.33</td>
<td>6.12</td>
</tr>
<tr>
<td>E-GO + HSA (100 μM)</td>
<td>0.082</td>
<td>0.86</td>
<td>32.0</td>
<td>0.19</td>
<td>0.09</td>
<td>0.10</td>
<td>0.38</td>
<td>9.03</td>
</tr>
<tr>
<td>EGH + DNA (100 μM)</td>
<td>0.12</td>
<td>2.92</td>
<td>35.0</td>
<td>0.14</td>
<td>0.06</td>
<td>0.08</td>
<td>0.39</td>
<td>11.03</td>
</tr>
</tbody>
</table>

\(^\#\langle \tau_r \rangle = (\tau_1 f_1 + \tau_2 f_2 + \tau_3 f_3) / (f_1 + f_2 + f_3)\)
Further, we performed anisotropy measurements for ellipticine and E-GO in presence of serum protein (Figure S9) and the results are summarized in Table S3. Compared to ellipticine-HSA, E-GO-HSA system exhibits significantly slower rotational correlation time of ellipticine (Table S3b), it attributes to the more confined environment of ellipticine molecules in E-GO-HSA composite than E-HSA system. This observation infers that protein adsorption takes place on the GO surface probably through hydrophobic, and π-π stacking interactions with electron conjugated sub-domains, hydrogen bonding interactions (between the oxygen functional groups of GO and nitrogen and oxygen containing functional groups of protein) as well as electrostatic interactions between protein surface charges and oxygen functional groups over GO.8-12

Figure S9: Time-Resolved fluorescence anisotropy decay profiles of (a) E-GO (4 μg/ml), E-GO-DNA (100 μM) and E-GO-RNA (10 μM), (b) Ellip-HSA (100 μM), E-GO-HSA (100 μM) and EGH-DNA (100 μM).

In order to probe drug releasing behavior from E-GO-HSA (EGH) in presence of DNA, anisotropy measurement was monitored (at 520 nm) in presence of DNA to the above mentioned system. It is clearly shown from the results (Table S3 and Figure S9) that rotational motion of the
drug is getting more restricted in EGH-DNA system. This observation leads us to conclude that DNA extracts the drug from EGH system by the formation of ternary complex with protein absorbed on GO surface, thereby; drug is experiencing slightly restricted motion compared to EGH system. When GO-bound E is exposed to glucose, the anisotropy decay profile remains same, indicating glucose is not interacting with E-GO, which is in agreement with steady state and lifetime results.

**Note 6**

**Binding Constants:** The binding parameters of ellipticine (E) in presence of GO-HSA, GO-DNA, GO-RNA and GO-HSA-DNA were estimated from modified Scatchard plot,\textsuperscript{13} which is described as follows

\[
\frac{[M]_{\text{total}}}{f} = \frac{1}{NK_f(1-f)} + \frac{[L]_{\text{total}}}{N}
\]  

Where, \([M]_{\text{total}}\) is the final concentration of the macromolecule, \([L]_{\text{total}}\) is the total concentration of the drug, “N” is the number of sites in macromolecule and “f” represents the fraction of ligand bound to macromolecule. The value of “f” can be evaluated from the following equation

\[
f = \frac{I_{\text{obs}} - I_L}{I_{\text{max}} - I_L}
\]

where \(I_{\text{obs}}, I_L, \) and \(I_{\text{max}}\) represents observed fluorescence intensity of the free drug and maximum intensity after saturation of all binding sites, respectively. A plot of \([M]_{\text{total}}/f\) vs. \(1/(1-f)\) produces a straight line and one can calculate binding constant (\(K_f\)) from the slope. The Scatchard plot for neutral ellipticine (\(E_N\)) towards HSA was obtained by monitoring the peak intensity at 450 nm with the progressive addition of HSA into the E-GO complex (Figure S10a), and the binding
constant of $E_N$ towards HSA is determined to be $K_f = (1.44\pm0.15) \times 10^5 \text{ M}^{-1}$. The high binding constant along with the favorable free energy change ($\Delta G^0 = -29.43\pm2.96 \text{ kJ mol}^{-1}$) indicates that HSA interacts with GO surface. It is evident from steady state spectra that when DNA/RNA interacts with E-GO complex, then protonated form ($E_H^+$) of the drug binds with those polynucleotides. The binding affinity of $E_H^+$ with DNA in presence of GO is estimated to be $K_f = (6.18\pm0.6) \times 10^5 \text{ M}^{-1}$ from Scatchard plot (Figure S10b). The high binding constant and the favorable free energy change calculated ($\Delta G^0 = -33.03\pm3.3 \text{ kJ mol}^{-1}$) from the binding constant indicates that the E is released from GO surface and intercalates with DNA as protonated form of the drug. Very similar results are observed in presence of TAR-RNA (Figure S10c), where the association constant is estimated to be $K_f = (1.014\pm0.1) \times 10^5 \text{ M}^{-1}$ which suggests the binding process is highly energy favored ($\Delta G^0 = -28.56\pm2.84 \text{ kJ mol}^{-1}$) one. Drug release from EGO-HSA complex in presence of DNA and its binding affinity to DNA can be quantitatively determined from the binding constant estimated from the Scatchard plot (Figure S10d), and the estimated association constant is $K_f = (2.21\pm0.22) \times 10^5 \text{ M}^{-1}$. The high association constant and the favorable free energy ($\Delta G^0 = -30.5\pm3.0 \text{ kJ mol}^{-1}$) change indicates that even though protein was giving further protection to drug on the surface of GO, drug releasing takes place even in presence of DNA, because of the higher binding affinity of protonated ellipticine ($E_H^+$) to DNA compared to protein.
Figure S10: Scatchard binding plots constructed from emission intensities of ellipticine in presence of (a) GO-DNA, (b) GO-RNA, (c) GO-HSA and (d) GO-HSA-DNA.
Note 7

**Steady State Anisotropy**: Fluorescence anisotropy is a good tool to predict the milieu around the drug molecule in complex macromolecule systems.\(^5\) Therefore, to understand the environment around the drug, steady state fluorescence anisotropy was monitored for E-GO complex in the presence of HSA, DNA, and HSA-DNA (Figure S11). In the presence of serum protein, a higher value of anisotropy of the drug monitored at 450 nm indicates that neutral forms of the drug are experiencing restricted environment probably due to adsorption of HSA on GO surface.\(^{11,12}\) Like HSA, we have also observed the increment of drug anisotropy (monitored at 520 nm) in the presence of DNA/RNA (Figure S11), and it is attributed to the interaction between GO-bound drug with the above-mentioned biomolecules. Moreover, anisotropy value of the same system is hiked when it is monitored at 520 nm (Figure S11), inferring that neutral drug molecules those are coming out from GO-HSA surface interacts with DNA in its protonated form. However, when GO-bound E is exposed to glucose, anisotropy almost remains unchanged, inferring that there is no interaction going on between glucose and GO-bound E.

![Graph](image)

**Figure S11**: Steady state anisotropy of ellipticine in presence of (a) GO-HSA, GO-DNA, and GO-HSA-DNA.
Figure S12: Pictures of (a) ellipticine-GO (E-GO) in aqueous PBS, (b) E-GO-HSA (EGH) in aqueous PBS (c) E-GO-DNA in aqueous PBS (d) EGH-DNA in aqueous PBS at different time intervals.

Notably, the drug release in-vitro takes place within 72 hours.\textsuperscript{14-16} Therefore, we have monitored the precipitation of E-GO, E-GO-DNA, E-GO-HSA and E-GO-HSA-DNA solution for 72 hours. Interestingly, all the solutions remains well dispersed even for 72 hours (Figure S12), which
suggest that additional functionalization of GO, which has been routinely used in various drug delivery experiments, is not necessary.

REFERENCES