Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supplementary Information

Graphene-sandwiched DNA nano-system: Regulation of intercalated doxorubicin for controlled cellular localization

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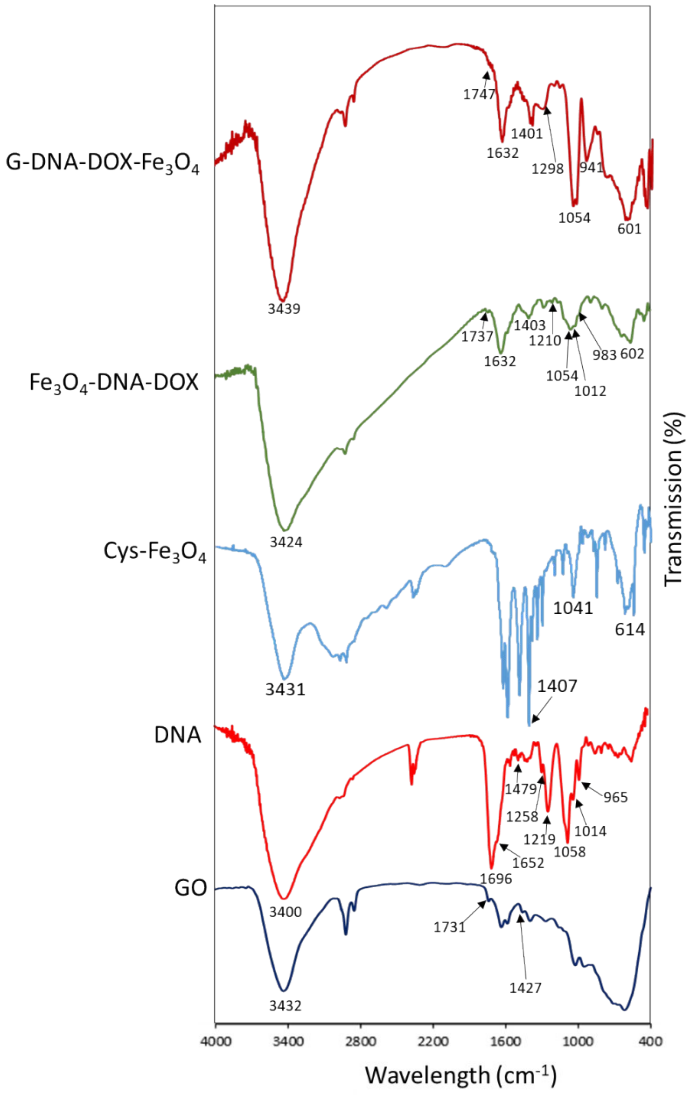

Figure S1. FTIR spectra. FTIR spectra of G-COOH, DNA, Cys-Fe_3O_4, G-DNA-DOX-Fe_3O_4 and Fe_3O_4-DNA-DOX.

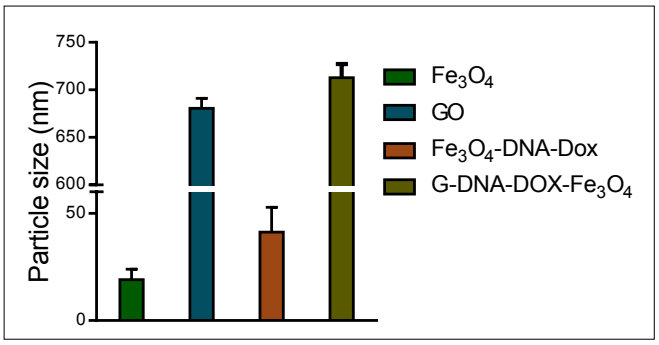

Figure S2. DLS particle size analysis. DLS particle size analysis of Fe_3O_4, GO, G-DNA-DOX-Fe_3O_4 and Fe_3O_4-DNA-DOX.

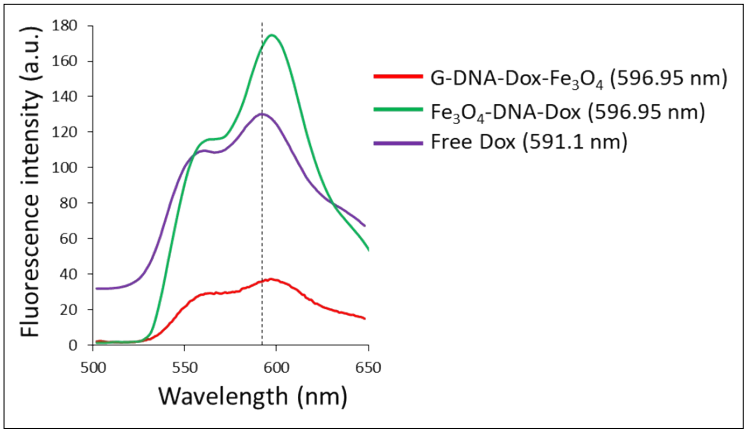

Figure S3. Fluorescence emission spectra of DOX nanosystems. The λ_{em} of free DOX, G-DNA-DOX-Fe_3O_4 and Fe_3O_4-DNA-DOX excited at 480 nm was at 591.10, 596.95, and 596.95 nm respectively. The dashed line indicates a wavelength of 591.10 nm.

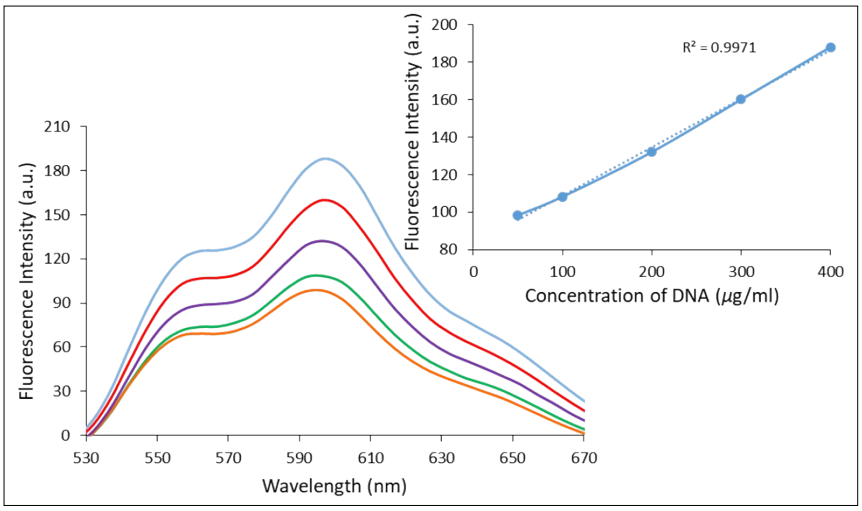

Figure S4. DNA-DOX calibration curve. Fluorescence spectra of intercalated DNA-DOX (10:1) was obtained upon excitation at 480 nm and displayed maximum emission at 596.95 nm (λ_{em}). Calibration curve was obtained in the concentration range 50 μg/mL to 400 μg/mL of DNA in the ratio of 10:1 (DNA:DOX).

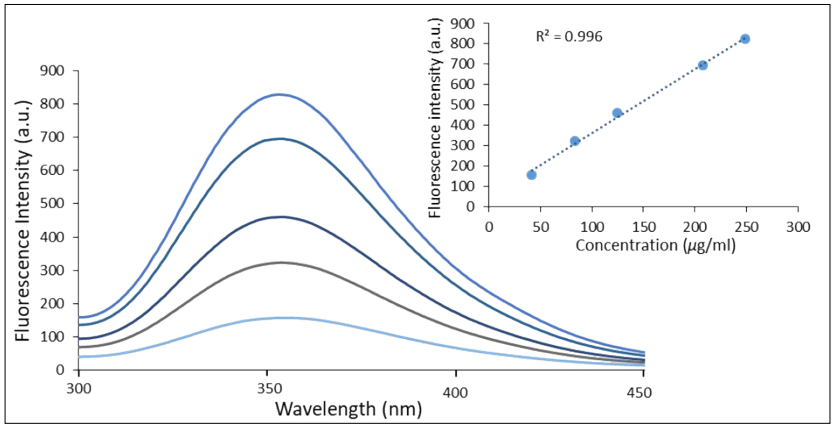

Figure S5. BSA calibration curve. Fluorescence spectra of BSA was obtained upon excitation at 280 nm and displayed maximum emission at 347.07 nm (λ_{em}). Calibration curve was obtained in the concentration range 40 μg/mL to 250 μg/mL.

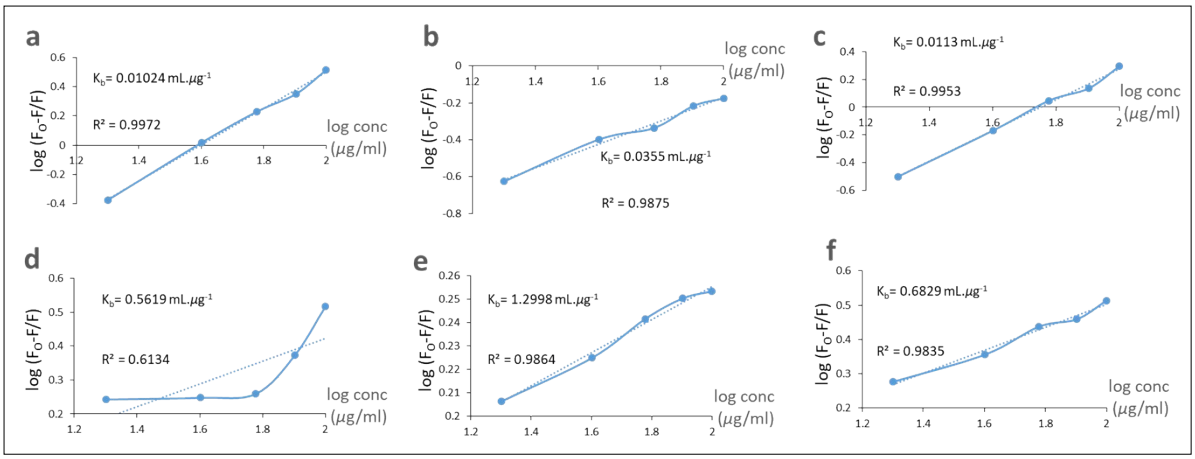

Figure S6. The plot of log (F_o/F) as a function of log (drug concentration) for BSA-ligand systems a) DNA, b) DOX, c) GO, d) Fe_3O_4, e) Fe_3O_4-DNA-DOX and f) G-DNA-DOX-Fe_3O_4. F_o is the initial fluorescence intensity of pure BSA and F is the fluorescence intensity in the presence of ligand (quenching agent). The binding constant (K_b) is obtained from the intercept of the plot.

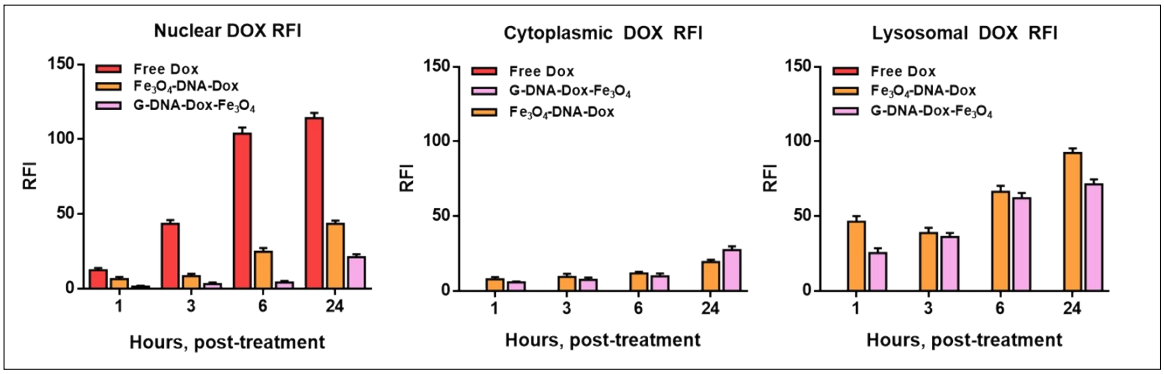

Fig. S7. Measured Relative Fluorescent Intensity (RFI) corresponding to DOX content in the nucleus, cytoplasm and lysosome were noted at 1, 3, 6 and 24 h.

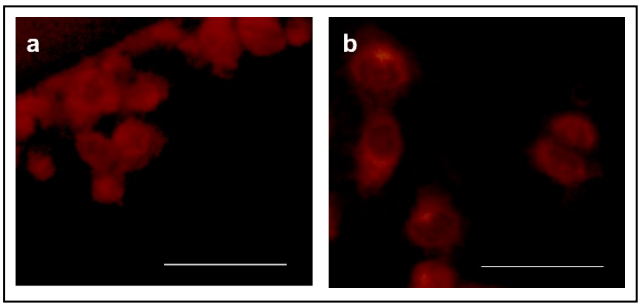

Figure S8. Fluorescence images of HeLa cells treated with a) Fe_3O_4-DNA-DOX and b) G-DNA-DOX-Fe_3O_4, and imaged after 48 h. Scale bar is 50 μm.

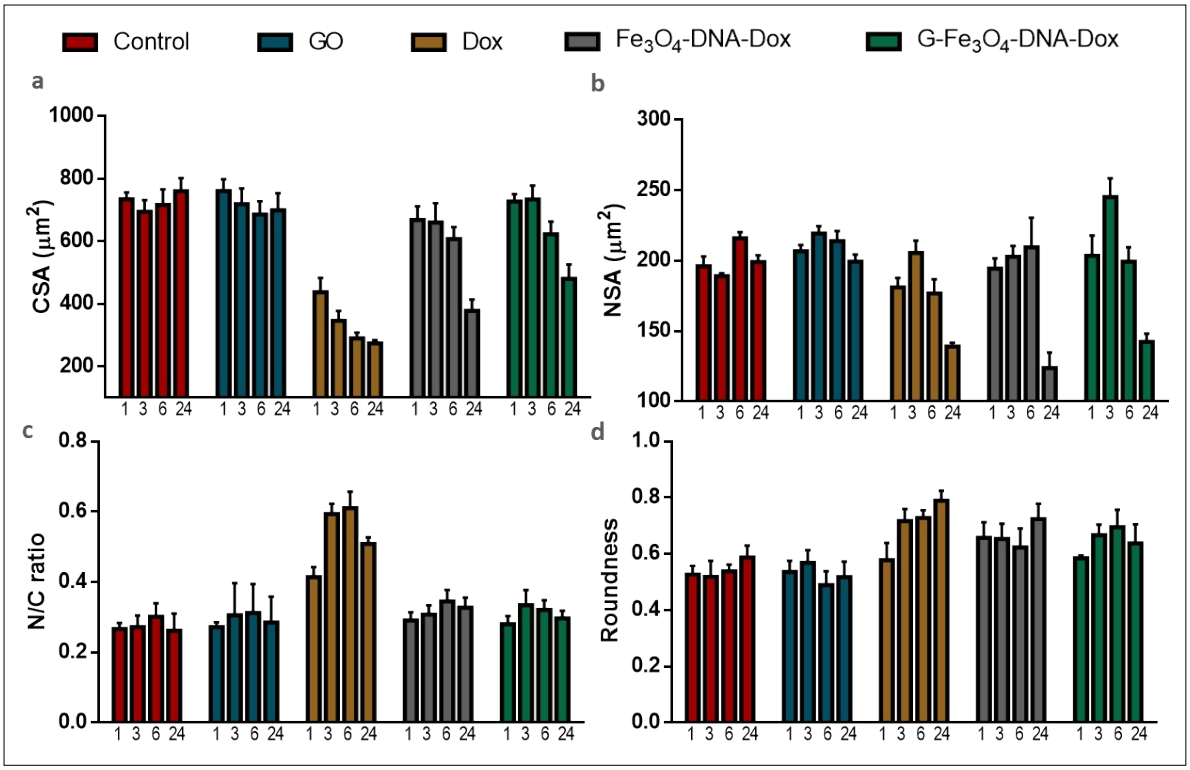

Figure S9. Morphological parameters studied on HeLa cells. NSA, CSA, N/C ratio and roundness of HeLa cells were measured upon exposure to GO, Dox, Fe_3O_4-DNA-DOX and G-DNA-DOX-Fe_3O_4 at 1, 3, 6 and 24 h.

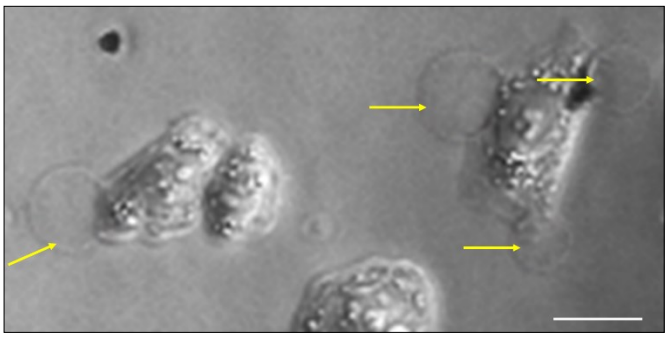

Figure S10. Cell membrane blebbing demonstrated by HeLa cells exposed to G-DNA-DOX-Fe_3O_4. Yellow arrows indicate blebs on the cell membrane. Scale bar is 20 μm.

Table S1. Significance Challenge in DOX delivery systems across cellular compartment and temporal domains (Nuclear/Cytoplasmic/Lysosomal; 1-24h).

Comparators			
Nanosystems/ drug	**Cell compartment**	**Time points**	**p value**
Free Dox	nucleus	1 and 24 h	< 0.0001
Fe_3O_4-DNA-Dox	nucleus	1 and 24 h	< 0.0001
G-DNA-Dox-Fe_3O_4	nucleus	1 and 24 h	< 0.0001
Free Dox vs Fe_3O_4-DNA-Dox	nucleus	24 h	< 0.0001
Free Dox vs Fe_3O_4-DNA-Dox	nucleus	1 h	0.0086
Free Dox vs Fe_3O_4-DNA-Dox	nucleus	3 h	< 0.0001
Free Dox vs Fe_3O_4-DNA-Dox	nucleus	6 h	< 0.0001
Free Dox vs G-DNA-Dox-Fe_3O_4	nucleus	3 h	< 0.0001
Free Dox vs G-DNA-Dox-Fe_3O_4	nucleus	6 h	< 0.0001
Fe_3O_4-DNA-Dox	nucleus and cytoplasm	24 h	< 0.0001
Fe_3O_4-DNA-Dox	nucleus and lysosome	24 h	< 0.0001
Fe_3O_4-DNA-Dox	nucleus and lysosome	1 h	0.3453
G-DNA-Dox-Fe_3O_4	nucleus and cytoplasm	24 h	0.027
G-DNA-Dox-Fe_3O_4	nucleus and lysosome	24 h	< 0.0001
G-DNA-Dox-Fe_3O_4	nucleus and lysosome	1 h	0.0002
Fe_3O_4-DNA-Dox	cytoplasm and lysosome	1 h	< 0.0001
Fe_3O_4-DNA-Dox	cytoplasm and lysosome	24 h	< 0.0001
G-DNA-Dox-Fe_3O_4	cytoplasm and lysosome	1 h	0.0005
G-DNA-Dox-Fe_3O_4	cytoplasm and lysosome	24 h	< 0.0001

Table S2. Comparison of DOX loading on various nanosystems.

Nanosystems	**DOX loading**
G-Dox	25 μg/mg
G-Cys-Fe_3O_4-Dox	15 μg/mg
G-DNA-DOX-Fe_3O_4	18 μg/mg
Fe_3O_4-DNA-DOX	25 μg/mg