Supporting information

Prolonged Fluorescence Lifetime of Carbon Quantum Dots by Combining with Hydroxyapatite Nanorods for Bio-applications

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S1. The morphology of water soluble CQDs and pure HAp nanorods

To compare the difference of free CQDs, CQD-HAp hybrid nanorods and pure HAp nanorods, these three kinds of materials were prepared and characterized (Figure S1). The water soluble CQDs were prepared according a common method. In brief, 0.8 g gelatin was added to 40 mL water under stirring. After total dissolution, the mixture was transferred into 50 mL Teflon liner heated at 200℃ for 3 h. The resulting light yellow solution was centrifuged at 12000 rpm for 30 min to remove weight precipitate and agglomerated particles and then yielded a light brown aqueous solution of CQDs. Figure S1a shows the size of water soluble CQDs. The size of CQDs is about 3-4 nm, and consistent with the previous result. Figure S1b shows the HAp without any organic agent. The prepared HAp have the rod-like morphology, but the uniformity is low compared with CQD-HAp hybrid nanorods. The reason of morphology change of HAp added oleic acid and octadecylamine or not bases on the fact that oleic acid and octadecylamine play an important part in morphology control.

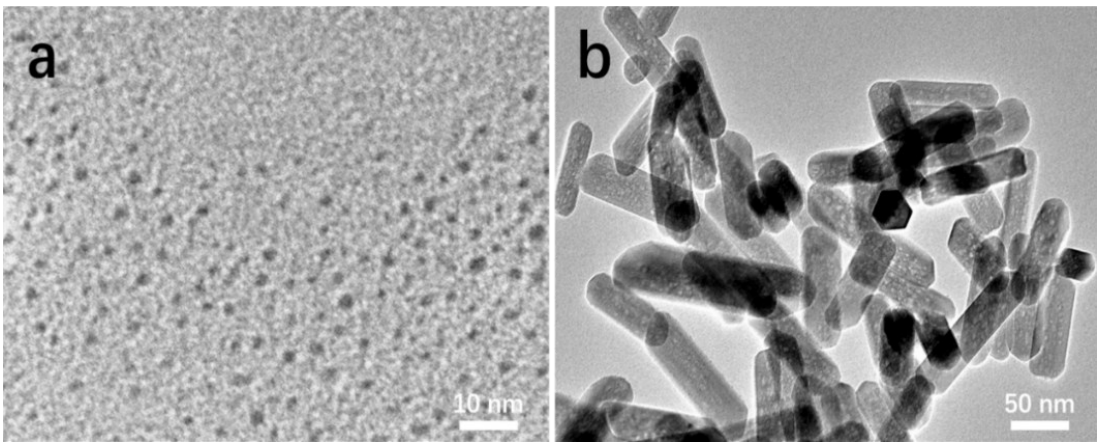

Figure S1. TEM images of prepared CQDs (a) and HAp without any organic reagent (b).

S2. Dispersity of CQD-HAp hybrid nanorods in water.

To check the polarity of CQD-HAp hybrid nanorods, CQD-HAp hybrid nanorods were dispersed in water and water-cyclohexane mixture system. As Figure S2a shown,

CQD-HAp hybrid nanorods were dispersed well in water. In the water-cyclohexane mixture system, CQD-HAp hybrid nanorods stably exist in water not in cyclohexane (Figure S2b). All the results show that CQD-HAp hybrid nanorods have good hydrophilic, and it means that CQD-HAp hybrid nanorods dispersed well in buffer solution following more easily endocytosed by cells.

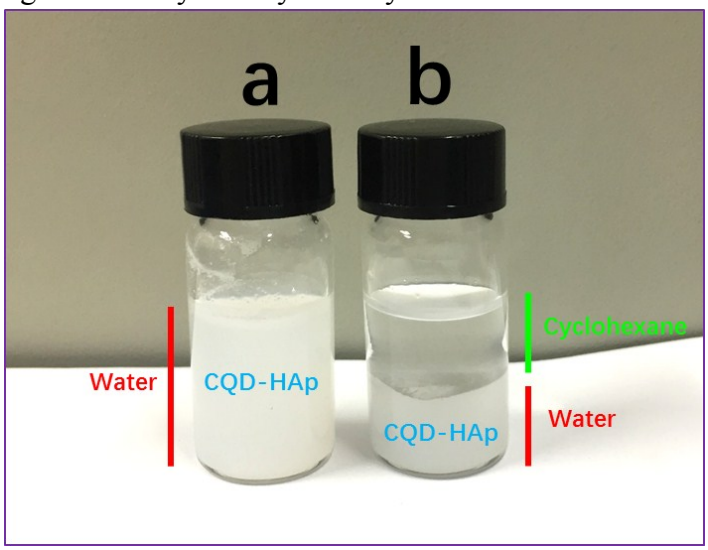

Figure S2. Dispersity of CQD-HAp hybrid nanorods in different solvent. a, in water; b in water-cyclohexane mixture.

S3. Emission spectra at the maximum of emission at 310 nm excitation and rhodamine B-HAp composite nanorods fluorescent stability.

The emission spectra at the maximum emission of CQD and CQD-HAp hybrid nanorods at 310 nm excitation are shown in Figure S3a. CQD-HAp hybrid nanorods possess two more emission peaks than CQD. While the characteristic peak of CQD at 380 nm still exist in the CQD-HAp hybrid nanorods.

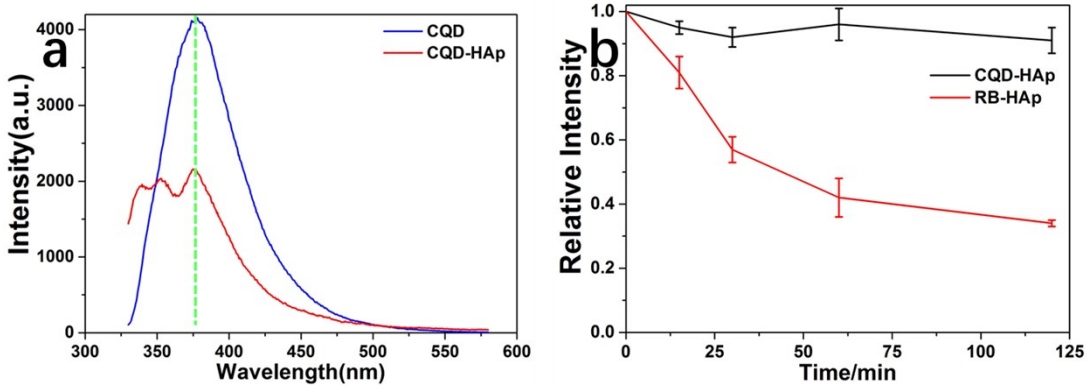

Figure S3. The emission spectra of CQDs and CQD-HAp hybrid nanorods at the maximum of emission at 310 nm excitation (a) and fluorescent intensity stability of CQD-HAp hybrid nanorods compared with rhodamine B loaded on the pure HAp nanorods (b).

To compare the fluorescent intensity stability of CQD-HAp hybrid nanorods, rhodamine B was incorporated in the nanorods as the reference dye. Firstly,

rhodamine B was mixed with pure HAp nanorods, and the mixture was shaken for 12 h to form rhodamine B-HAp (RB-HAp) composite nanorods. Then RB-HAp composite nanorods were collected by configuration and washed by water. RB-HAp composite nanorods were set into six groups in 100 μg/ml concentration. Fluorescent intensity of RB-HAp composite nanorods left were measured by collection through configuration. As Figure S3b shown, CQD-HAp hybrid nanorods have high fluorescent stability than rhodamine B-HAp composite nanorods, whereas the fluorescent intensity of RB-HAp composite nanorods decreases with the time. Therefore, CQD-HAp nanorods have good fluorescent stability for long-time cell imaging.

S4. Absorption, emission and quantum yield in PBS

Absorption spectra of CQD-HAp hybrid nanorods and pure HAp nanorods dispersed in physiological media-PBS (phosphate buffer solution) are shown below. As we can see, the spectra of CQD-HAp hybrid nanorods in PBS (Figure S4a) have some signal distortion because of the muddy station when HAp nanorods and CQD-HAp hybrid nanorods were dispersed in PBS. However, it still shows that there is obviously increased absorption from 450 to 200 nm which can be attributed to the CQD.

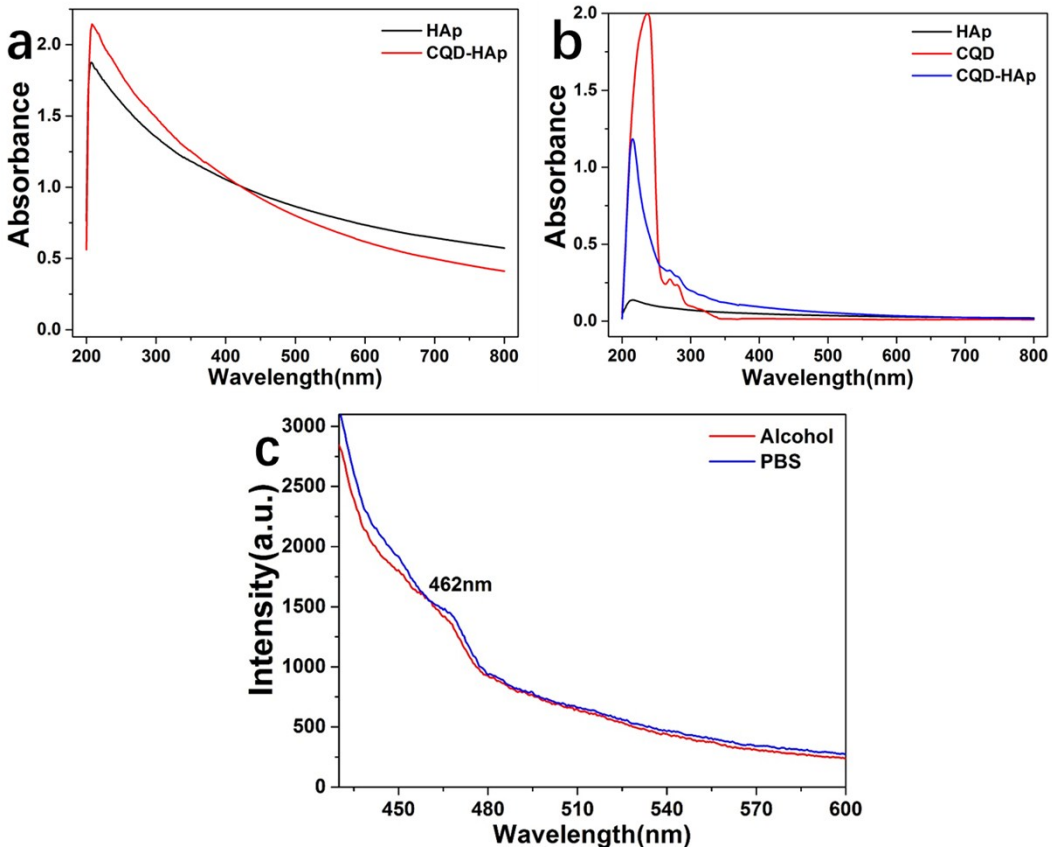

Figure S4. The absorption and emission spectra of CQD-HAp hybrid nanorods. a, absorption spectrum; b, emission spectra in different medium at 405 nm excitation.

To more clearly and accurately obtain the absorption spectra of CQD-HAp hybrid nanorods, cyclohexane was chosen to get the more uniform system (Figure S4b). It is

clear that HAp nanorods almost have no absorption whereas CQD-HAp hybrid nanorods have strongly absorption as similar as pure CQD. And CQD-HAp hybrid nanorods spectra have a blue shift in certain content which is consistent with the result of emission spectra.

Emission spectra of CQD-HAp hybrid nanorods in PBS and alcohol are shown in Figure S4c. As we can see, there is not obviously change in fluorescent spectra intensity and shape, which means CQD-HAp hybrid nanorods still possess good fluorescence property in PBS.

Further, the quantum yield of CQD-HAp hybrid nanorods in PBS were obtained and is 19.21%. After connection with HAp nanorods, CQD-HAp hybrid nanorods possess higher quantum yield than pure CQD (14.77%). In conclusion, CQD-HAp hybrid nanorods have shown great potentials to be used in cell imaging, drug delivery and other applications.

S5. PI staining to further confirm cell viability

PI has the ability to quickly discriminate live cells from dead cells because it only penetrates into the nucleus of dead cells. PI will show a bright red color excited at 534 nm when localized in the nucleus. Firstly, Hela cells were cultured in FluoroDish™ and incubated for 24 h. Then, the medium was changed by new H-DMEM medium with 5 μM PI concentration. After incubating for 25 min, cells were washed for three times with PBS and examined by fluorescence microscope in the bright field at the 510-550 nm wavelength. The result is shown in Figure S5. It clearly indicates that there were only few dead cell after cultured for 3 d even at 800 μg/ml. The results are consistent with the conclusion of CCK-8 results.

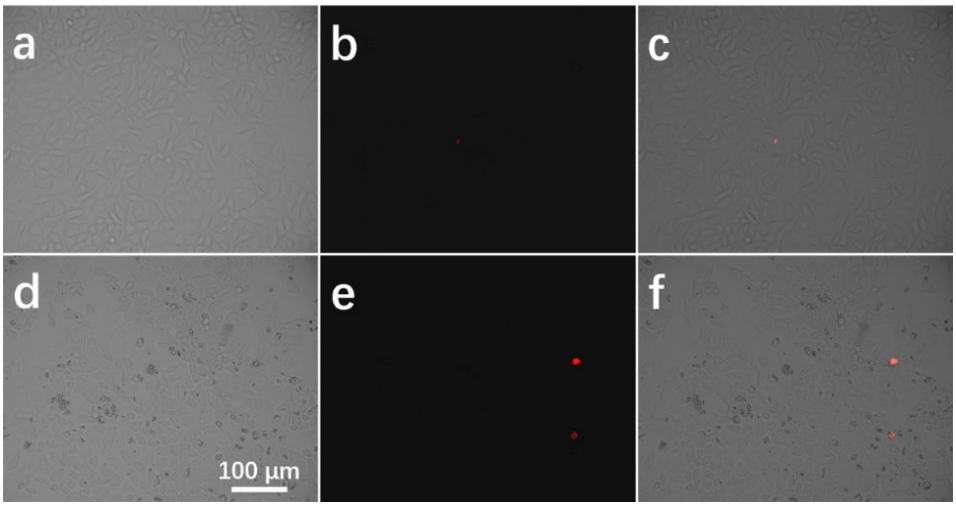

Figure S5. PI staining results for Hela cell at free and 800 μg/ml CQD-HAp hybrid nanorods concentration cultured for 3 d. a-c, free CQD-HAp hybrid nanorods; d-f, at 800 μg/ml concentration. a, d, at bright field; d, e. at 510-550 nm wavelength excitation; c, f, merge of a, b and d, e respectively. The bright red dot shows the location of dead Hela cells.

S6. The measurement of standard curve of Dox at different concentration

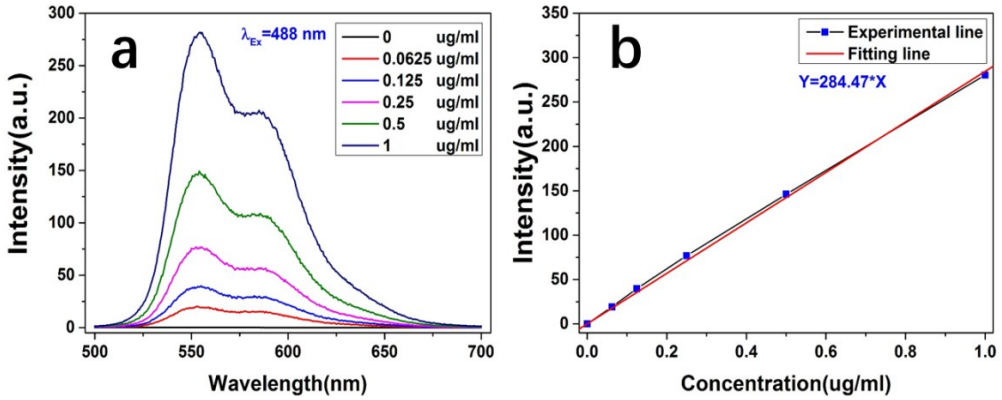

Figure S6. The relationship between fluorescence intensity and content of Dox. a, the fluorescence intensity of Dox at different concentration; b, the fitting line according a.

At the different free Dox concentration, the intensity at 550 nm under excited at 488 nm wavelength light is different (Figure S6a). According the concentration and the intensity at 550 nm, the standard curve of the relationship between fluorescence intensity and Dox concentration is calculated (Figure S6b). At the concentration range from 0-1 μg/ml, the linearity degree is very high. Therefore, it is proper to measure the content of Dox loaded on Dox-CQD-HAp hybrid nanorods surface according the fitting line.

S7. Accuracy testing of Dox concentration

For one aspect, CQD prepared in this work almost have no absorption in the 500-700 nm wavelength range (Figure S7a). Therefore, CQD-HAp hybrid nanorods should have no obvious influence to the concentration measurement in theoretically.

Further, we preformed experiment to check whether CQD-HAp hybrid nanorods can quench a fluorescence yield of Dox. Simple mathematic addition process was used to compare the total fluorescent intensity of Dox before loading and the sum of Dox-CQD-HAp hybrid nanorods fluorescent intensity and left Dox fluorescent intensity in solution. As Figure S7b shown, the total fluorescent intensity (2661) of Dox before loading approximates to the sum (2700) of Dox-CQD-HAp hybrid nanorods fluorescent intensity (2430) and left Dox fluorescent intensity in solution (270). Excluding the little intensity of CQD-HAp hybrid nanorods at 488nm (actually, we have excluded the little influence of CQD-HAp nanorods in calculation the concentration of Dox in CQD-HAp hybrid nanorods), the value of the total fluorescent intensity of Dox before loading and the sum of Dox-CQD-HAp hybrid nanorods fluorescent intensity and left Dox fluorescent intensity in solution should be almost equal. In conclusion, CQD-HAp hybrid nanorods almost have no interference to measure the concentration of Dox loaded on Dox-CQD-HAp hybrid nanorods. Therefore, the Dox concentration measurement has relatively high accuracy and reliability.

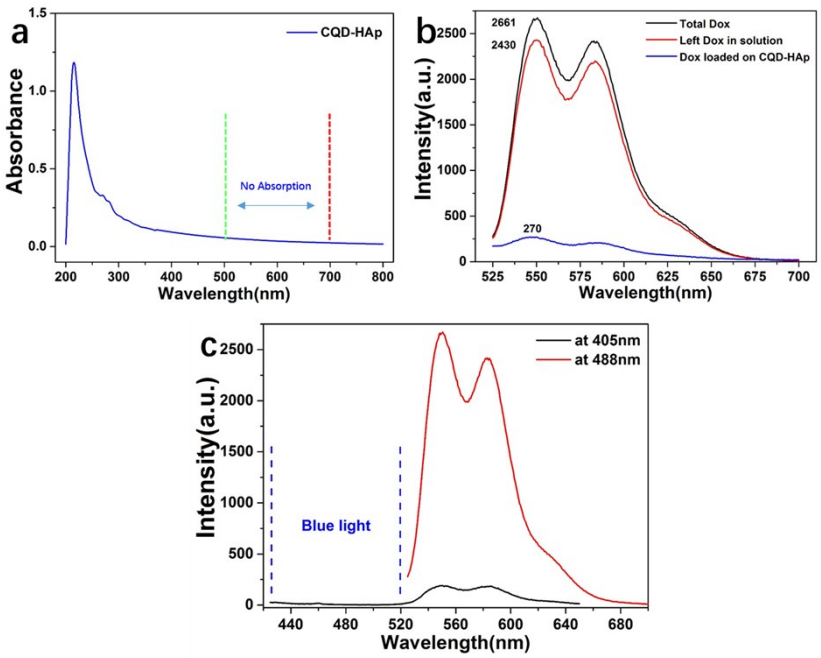

Figure S7. The absorption spectrum of CQD-HAp hybrid nanorods (a); the respective fluorescent intensity of total Dox, Left Dox in solution and Dox loaded on CQD-HAp hybrid nanorods (b); emission spectra of Dox at different excitation. Dashed lines show the emission range of light of CQD-HAp nanorods excited by 405 nm.

We also check the Dox fluorescence spectra at 405 nm (Figure S7c). As we can see, there is no emission in blue light range. When Dox-CQD-HAp hybrid nanorods were used as drug delivery, the place of blue light only shows the CQD-HAp hybrid nanorods without influence of Dox fluorescence. Therefore, CQD-HAp hybrid nanorods have good potential to function as cell imaging nano-materials and drug delivery system in bio-field.

S8. The location of CQD-HAp hybrid nanorods after endocytosed by Hela cells

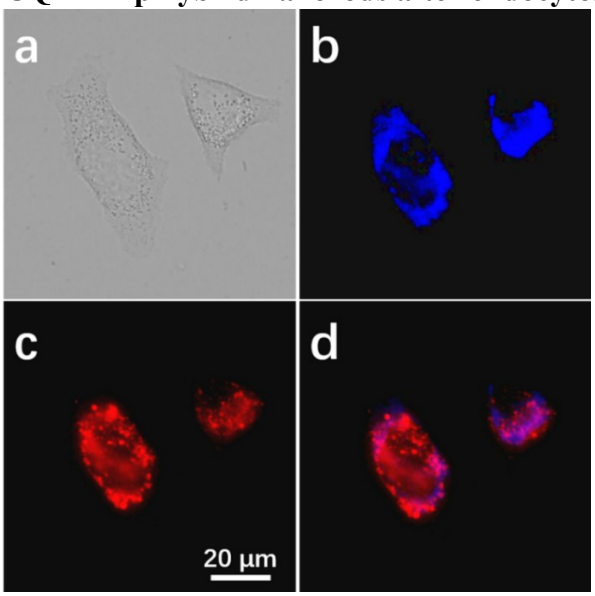

Figure S8. Lyso-Tracker Red imaging to confirm the location of Dox released from

Dox- CQD-HAp hybrid nanorods after cultured for 12 h at 300 μg/ml concentration. a, under bright field; b, CQD-HAp fluorescence under 330-385 nm wavelength excitation; c, Lyso-Tracker Red fluorescence under 510-550 nm wavelength excitation; d, merge of b, c.

Lyso-Tracker Red is a kind of dye to connect with lysosomes selectively. Hela cells were cultured at FluoroDish™ firstly, and incubated for 24 h. Then, the medium was changed by new H-DMEM medium with 50 nM Lyso-Tracker Red concentration. After incubating for 25 min, cells were washed for three times with PBS and examined by fluorescence microscope in the bright field at different wavelength. Figure S8a shows the morphology of Hela cells under bright field. The location of CQD-HAp nanorods and lysosomes is presented in Figure S8b and c. Figure S8d is the merge of b and c. It is clear that the location of CQD-HAp hybrid nanorods (blue light) and lysosome (red) almost coincide with each other. Therefore, we can conclude that the location of CQD-HAp hybrid nanorods locates in lysosomes. The morphology of Hela cells has some change because dimethylsulfoxide which Lyso-Tracker Red dissolved in has toxicity to cells.

S9. The release rate of Dox from Dox-CQD-HAp hybrid nanorods at different pH

The pH in lysosomes is higher (about 5) than cytoplasm (about 7.4). Therefore, the release rate of Dox from Dox-CQD-HAp hybrid nanorods at different pH was measured at PBS to simulate cells' environment.

In pH=7.4 PBS, the release rate is high before 6 h. However, the rate becomes very slow after 12 h, and the Dox release content only reaches about 30 % even after 72 h. In pH=5.0 PBS, the release rate is very high until 24 h, and the Dox release content reaches about 65 % after 24 h. The release rate of Dox from Dox-CQD-HAp hybrid nanorods in pH=5.0 PBS is higher than the one in pH=7.4 PBS. Further, the release rate curve in pH=5.0 PBS is consistent with lethality curve. Therefore, the result provides more evidence to confirm the location of Dox from Dox-CQD-HAp hybrid nanorods located in lysosome, and consistent with the result of Lyso-Tracker Red imaging.

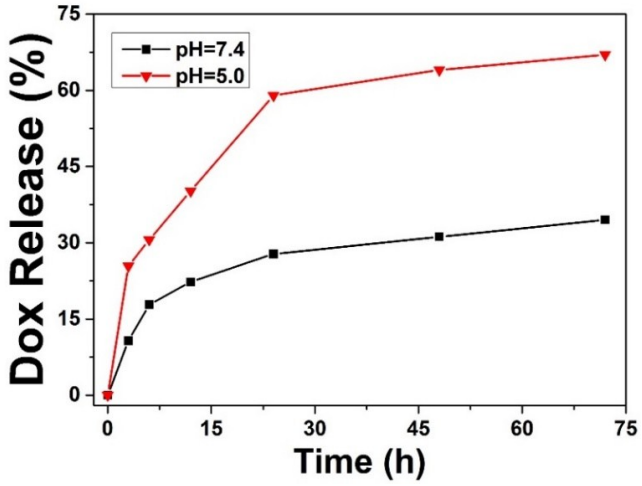

Figure S9. The rate of Dox released from Dox- CQD-HAp hybrid nanorods at

different pH value in PBS (original PBS, pH=7.4; PBS added HCl, pH=5.0).

S10. Endocytosed content of Dox-CQD-HAp hybrid nanorods in per Hela or PC-3 cell.

We measured the content of Dox-CQD-HAp hybrid nanorods endocytosed by PC-3 cells and Hela cells. PC-3 cells and Hela cells were cultured in 3 confocal dish respectively with the same cells' number per dish. After culture for 16 h (cell spreading well but no proliferation), 1 ml new medium with Dox-CQD-HAp hybrid nanorods at 300 μg/ml concentration were added in the two groups. After culture for another 6 h, the Dox-CQD-HAp hybrid nanorods left in the medium were collected carefully and dispersed in same volume alcohol, and measured by fluorescence spectrophotometer to check the content of left Dox.

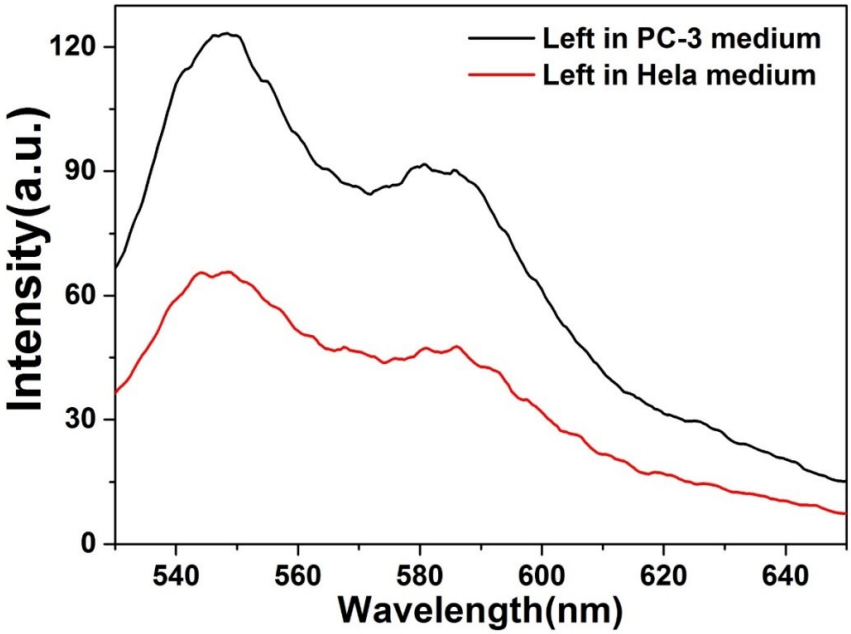

Figure S10. The emission spectra of Dox-CQD-HAp hybrid nanorods left in medium in Hela cells and PC-3 cells respectively.

To decrease the measurement error, three parallels was averaged. Result is shown in Figure S10. It is clear that there are more Dox-CQD-HAp hybrid nanorods left in PC-3 cells' medium than in Hela cells' medium, which means that there are more Dox-CQD-HAp hybrid nanorods endocytosed by Hela cells than PC-3 cells in per cell.

Dox can be easily released from Dox-CQD-HAp hybrid nanorods in pH=5 (Figure S9). As we all known, pH in cancer cells is lower than normal cells, and cytoplasm is of acidity. Therefore, more Dox can be released from Dox-CQD-HAp hybrid nanorods in per Hela cell in certain time. Therefore, Dox-CQD-HAp hybrid nanorods have higher lethality to Hela cells.

S11. The process of pure Dox into Hela cells

To compare the process difference between pure Dox and Dox released from Dox-CQD-HAp hybrid nanorods, the process of pure Dox into Hela cells was measured by

fluorescence microscope.

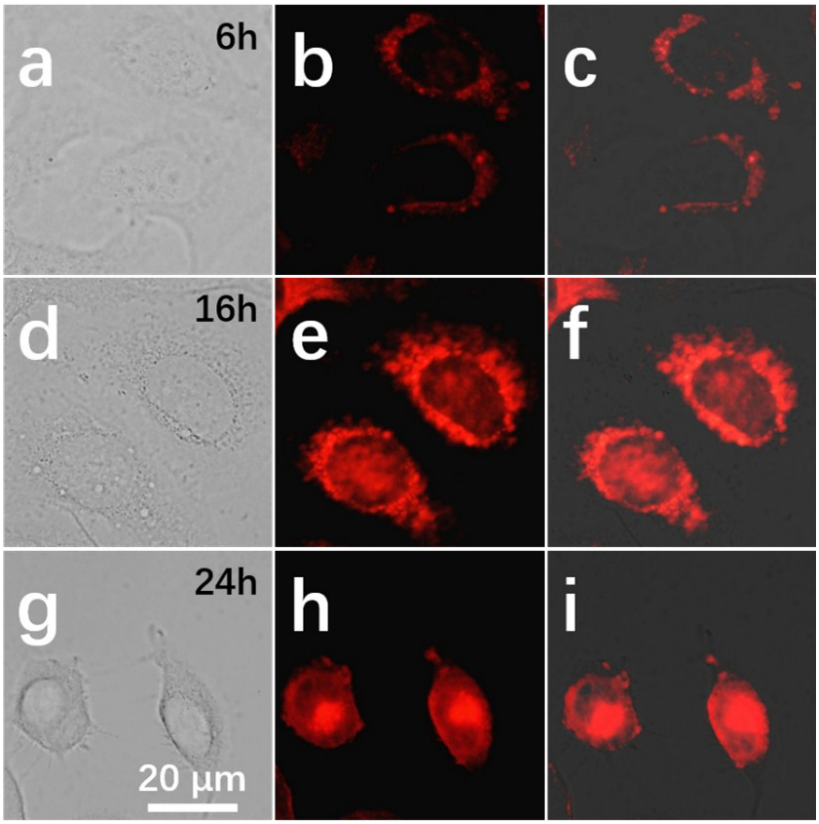

Figure S11. The process of free Dox endocytosed into cytoplasm and nucleus at 1 μg/ml concentration. a-c, after 6 h; d-f, after 16 h; g-i, after 24 h; a, d and g, under bright field; b, e and h, Dox fluorescence under 510-550 nm wavelength excitation; c, f and i, merge of a, d, g and b, e, h respectively.

The result is shown in Figure S11. Little Dox was endocytosed by Hela cells after 6 h at 1 μg/ml concentration. After 16 h, there was only some Dox into nucleus, a lot of Dox remained in cytoplasm. After 24 h, there was much Dox into nucleus. The process of free Dox into nucleus is consistent with the lethality curve of free Dox. It is clear that free Dox into Hela nucleus is slower than Dox released from Dox-CQD-HAp hybrid nanorods, and the result support the conclusion that Dox-CQD-HAp hybrid nanorods have higher lethality than free Dox.