SUPPLEMENTARY INFORMATION

1. Flow Cytometry and Uptake of Doxorubicin in MCF-7 Cells

MCF-7 Cells were plated at a density of 400,000 cells/well in a 24 well cell culture plate. After 24 hours, cells were incubated for 12 hours with 0, 100 nM, 400 nM, 600 nM DOX, as well as 1 nM DNA-AuNP and 1 nM DOX-DNA-AuNP. Cells were washed 3 times with PBS, and designated DOX-DNA-AuNP samples were then irradiated with 10 Gy. The cells were then trypsinized and placed in round bottom test tubes. Flow cytometry was used to measure the fluorescence from at least 10,000 cells using an upgraded BD FACScan – five-color Cytek (Cytek Development, Fremont, CA). Data was analyzed using FlowJo v7.6.5, Ashland, OR. Results of the flow cytometry data of DOX uptake is shown in Figure SI-1.

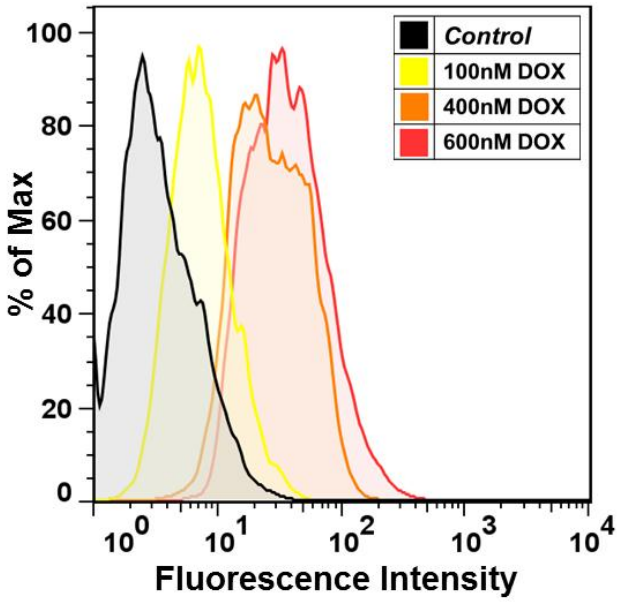

Figure SI-1. Flow cytometry data of cellular uptake of free DOX at three different incubation concentrations of 100, 400, and 600 nM.

2. AuNPs

Gold chloride ($HAuCl_4$) and sodium citrate were purchased from Sigma-Aldrich. Dimethylsulfoxide (DMSO) was obtained from Fluka. Modified oligonucleotides were purchased from Sigma-Genosys and IDT. Water was purified with a Millipore Biocel system (18.2 MΩ). X-ray irradiation was performed in an HP Faxitron Model 4385SA (3.3 Gy/min at 100 kVp). Fluorescence measurements were made, using a Jobin-Yvon Horiba Fluoromax-P fluorometer. Transmission Electron Microscopy (TEM) was performed using a JEOL 1230 at 120 KeV.

3. DNA-Coated Gold Nanoparticles (DNA-AuNP)

16 nm gold nanoparticles (AuNPs) were synthesized using a modified method from Turkevich.[1] Briefly, 5.25 mL of a 1% (w/v) sodium citrate solution were injected to a solution of 15 mg $HAuCl_4$ in 150 mL H_2O which had been brought to reflux, under stirring. The solution was stirred at reflux for an additional 15 minutes, and then allowed to cool to room temperature. TEM image of these AuNPs is given in Figure SI-2. The AuNPs were functionalized with

Carboxyfluorescein (6-FAM) labeled thiol-DNA (6-FAM-5'-$(TTAGGG)_n$-3'-C3-SH) of various lengths (n=1, 2, 4) using a modified method from Dhar et al.[2] Briefly, 50 µL of 100 µM 6-FAM-DNA was added to 5 mL of 1 nM AuNPs and allowed to incubate for 10 minutes before the addition of 50 µL of a 1% (w/v) Tween 20 solution. After an additional 10 minutes, 50 µL of 1 M phosphate buffer (PB), and 5 mL of 2M NaCl (in 10 mM PB) were added and the solution was allowed to incubate overnight. The free DNA was removed from solution by centrifuging five times (13,000 rpm, 15 min) and redispersed in PBS (100mM NaCl, 10 mM PB, pH=7.4).

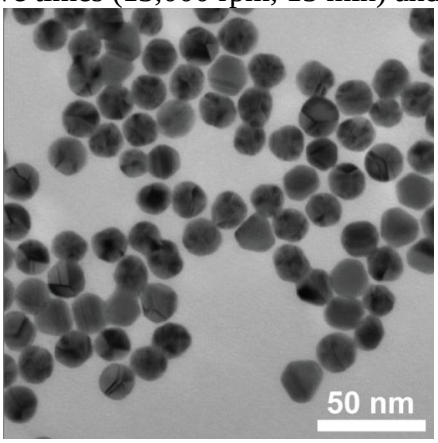

Figure SI-2. TEM image of synthesized AuNPs. The average size is 15.9 ± 1.2 nm.

4. Doxorubicin Conjugation to DNA-AuNPs

Using a method developed by Wang et al.,[3] all solutions were prepared in anhydrous dimethylformamide (DMF). To 431 µL of 2 mM doxorubicin purchased from LC Laboratories, 37.4 µL of 0.0717 M triethylamine (TEA) and 20 µL of 0.0472 M succinic anhydride were added and reacted for 3 hours. Solutions of 1-Ethyl-3-[3- dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were prepared by dissolving 33.0 mg of EDC and 19.8 mg NHS in 1 mL volumes of DMF with vortexing and sonication. To the succinic-doxorubicin solution, 20 µL each of the EDC and NHS solutions were added. All subsequent reactions were performed in PBS (100mM NaCl, 10mM PB, pH =7.4). To 2.50 mL of amine-terminated DNA-AuNPs (NH_2-C_6-5'-TTAGGGTTAGGG-3'-C_3-SH-AuNP), 75 µL of 2 mM polyethylene glycol (mPEG-SH, MW 2000) were added. The solution was briefly vortexed to mix, and allowed to react for 10 minutes before 132 µL of the activated doxorubicin was added. The solution reacted overnight, and the DOX-DNA-AuNPs were then washed five times by centrifuging (13,000 rpm, 15 min) with PBS (100 mM NaCl, 10 mM PB, pH=7.4). The resuspended solution was subsequently cleaned by centrifuging twice in a 10K Millipore filter (7,000 rpm, 10 min) and the solution in the filter was recovered. Figure SI-3 shows the absorption spectra of DOX, AuNPs, and 6-FAM.

5. X-ray Radiation with DMSO

X-ray radiation studies were performed, using DMSO as a hydroxyl radical scavenger at concentrations from 0-100 mM. In a 24-well plate, 400 µL of purified 6-FAM-DNA-AuNPs were mixed with 400 µL of 2X DMSO solution, or PBS and the samples were irradiated with X-rays. After radiation, the AuNPs were removed from solution by centrifugation (13,000 rpm, 15 min) and supernatant fluorescence was measured (Excitation at 490 nm and Emission at 514 nm). Non-radiated controls were identically prepared and measured.

6. X-ray Radiation with Quenching DNA

The 6-FAM-DNA-SH compound has one 6-FAM attached to the end of a thiolated DNA strand, and is available commercially. The average size of AuNPs was measured, using TEM counting over 200 nanoparticles (15.9±1.2 nm, Figure SI-2). The low fluorescence yield of DOX (4%), coupled with incomplete quenching (0.3%) when bound to the surface of AuNPs, made spectroscopic measurements difficult.[3] Such an interference is much less a problem in the 6-FAM case due to the high fluorescence yield of 6-FAM (>80%) and more complete quenching when 6-FAM is attached to AuNPs (see Figure SI-3). The cleavage via X-ray irradiation was studied with optical fluorescence spectroscopy.

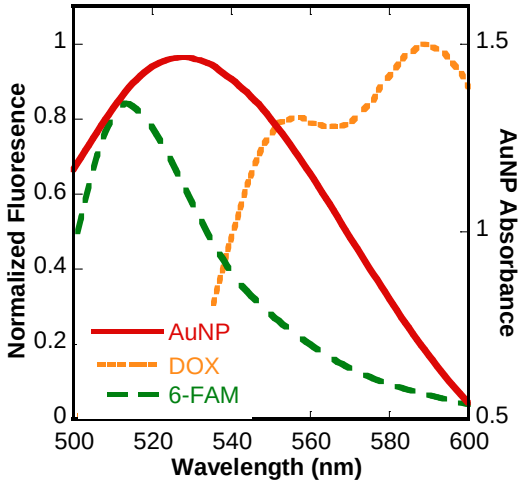

Figure SI-3. Measured optical absorption spectra of AuNPs, DOX, and 6-FAM. The spectral overlap between 6-FAM and AuNPs is larger than that between DOX and AuNPs, causing more quenching between 6-FAM and AuNPs.

Black Hole Quencher®-1 DNA, coupled to 6-FAM, was purchased from IDT (6-FAM-5'-TTAGGGTTAGGG-3'- BHQ-1®). It was irradiated with DMSO at concentrations from 0-100 mM in 24 well plates, and the fluorescence from cleaved DNA was measured. The results of irradiation are shown in Figure SI-4. The fluorescence signal and, hence cleavage was found to be approximately linearly dependent of the length of DNA and the dose of X-rays; both are shown in Figure SI-4. The nonlinearity in the dose dependence results was caused by the destruction of 6-FAM by X-ray radiation. Only single data points were obtained in two DNA chain length study, due to limited sample sizes for those two types of DNA strands. X-ray radiation of cleaved 6-FAM were incubated with MCF-7 cells for 4 hours.

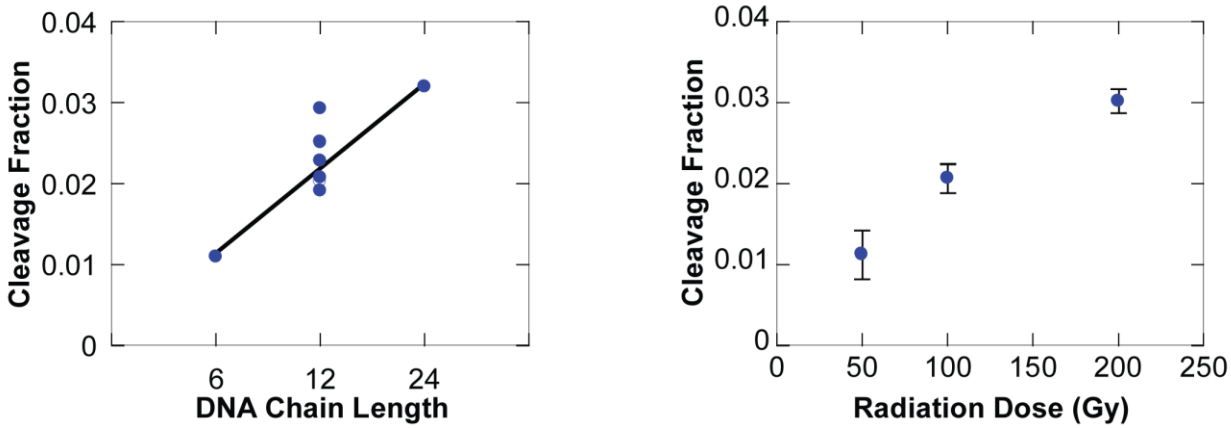

Figure SI-4.Fluorimetry measurements of 6-FAM after X-ray radiation. The left panel shows the cleavage as a function of radiation dose. The right panel shows the DNA length dependency.

Optical fluorescence microscopy (Leica DFC310 FX) was used to image the cells. Figure SI-5 shows the fluorescent and bright field images of the samples. Optical microscopy was also used to verify that 6-FAM molecules were cleaved with X-rays. Figure SI-5 in Supporting Information shows images of MCF-7 cells, incubated with X-ray irradiated 6-FAM-DNA-AuNPs. Due to the cleavage of DNA by X-ray radiation, 6-FAM was cleaved off the surface of AuNPs and they caused cells to be fluorescent, as shown in Figure SI-5. It is worth noting that each cleaved 6-FAM molecule may carry a short segment of DNA with it.

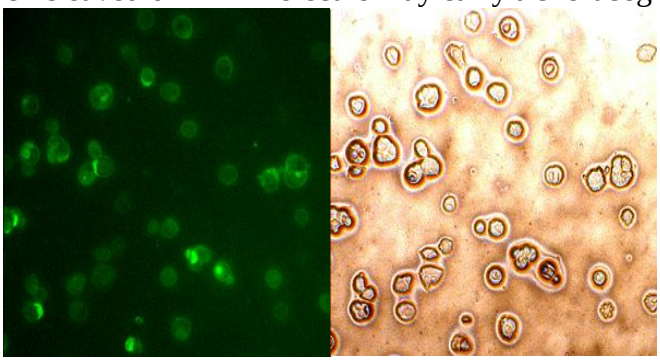

Figure SI-5. Optical microscopy imaging of MCF-7 breast cancer cells incubated with X-ray irradiated 6-FAM-DNA-AuNPs. The left panel shows the fluorescence from the 6-FAM cleaved off 6-FAM-DNA-AuNPs, and the right panel shows the bright field image of the same region.

7. Clonogenic Assay

MCF-7 cells were plated at a rate of 50,000 cells/ml in a 24-well cell culture plate. They were then incubated with DOX, DOX-DNA, and DOX-DNA-AuNPs for 12 hours, and then irritated with 0, 2, 5, and 10 Gy X-rays. The cells were then counted, using a hemocytometer, and replated in a 6 well plate at 1000 cell per well for the 0 and 2 Gy dose; 3000 cells for the 5 Gy; and 9000 for the 10 Gy. The plates were incubated for 14 days, and then fixed with gluteraldhyde and stained with crystal violet. Then the colonies with 50 or more cells were then counted.

8. Statistics:

Table 1: All uptake experiments were performed in triplicate
Figure 1. 10,000 cells were counted for each data set.
Figure 2 and Table 2: All clonogenic assay experiments were performed in triplicate $P < 0.05$
Figure 3. All cleavage experiments were performed in triplicate.

Figure SI-1. A. All data points were collected in triplicate. B. Multiple data points were collected for 12-mer DNA, but only single data points were collected for 6- and 24-mer DNA with a linearity R^2=0.93.
Figure SI-2. Image.
Figure SI-3. Spectral measurements, STD < 2%.
Figure SI-4. Data points are given as obtained.
Figure SI-5. Image (No statistics)

1. Turkevich, J.;Stevenson, P.C.;Hillier, J., *Discussions of the Faraday Society* **1951**, (11), 55-&.
2. Dhar, S.;Daniel, W.L.;Giljohann, D.A.;Mirkin, C.A.;Lippard, S.J., *Journal of the American Chemical Society* **2009,** 131, (41), 14652-+.
3. Wang, F.;Wang, Y.C.;Dou, S.;Xiong, M.H.;Sun, T.M.;Wang, J., *Acs Nano* **2011,** 5, (5), 3679-3692.