Supplementary Information

On-demand generation of singlet oxygen from a smart graphene-complex for the photodynamic treatment of cancer cells

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Experimental methods

Validation of Fluorescence Response and Singlet Oxygen Generation of Nanocomplex towards cDNA in Solution

Based on Watson-Crick base pairing principle, the selective PDT can also be realized via changing the target molecules to the specific DNA or RNA sequences. To proof this, we also investigated the response of GO/AP-Ce6 towards its cDNA using the similar method except that ATP molecule was replaced by cDNA. Furthermore, the rDNA was used to test the specificity of GO/AP-Ce6 for SOG.

Cell Imaging

The cells were seeded on a quartz-bottom dish at the density of 10000 cells/well, and allowed to attach for 24 h. Afterwards, the cells were washed with PBS and incubated with DCF-DA at 37 °C for 20 min. Then, the cells were divided into six groups: group 1 and 2 with cell only; group 3 incubated with Rusop; group 4 added AP-Ce6; group 5 added GO/AP-Ce6; and group 6 incubated with GO/AP-Ce6+ATP. When incubation for 6 h at 37 °C, group 1 was kept in dark and groups 2-6 were irradiated with light for 20 min. Thereafter, the medium was removed and washed three times with PBS. The treated cells were then stained with Hoechst (10 μg/ml) for 5 min and washed three times with PBS (pH 7.40) again. The fluorescence images were acquired using a fluorescence microscope (Nikon CLIPSE TE2000-S).

Supplementary Figures

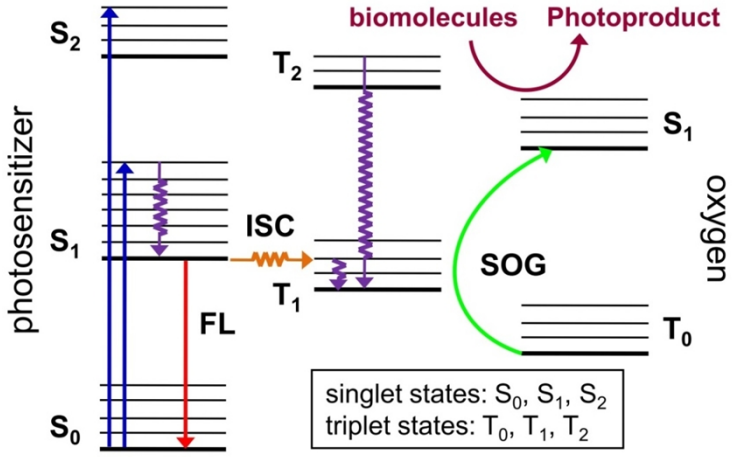

Figure S1. Photophysical mechanism of the fluorescence, SOG and PDT processes. From the energy level diagram, it is found that the photophysical mechanism of SOG is similar to that of fluorescence process; thus, we hypothesize that GO is also capable of quenching SOG.

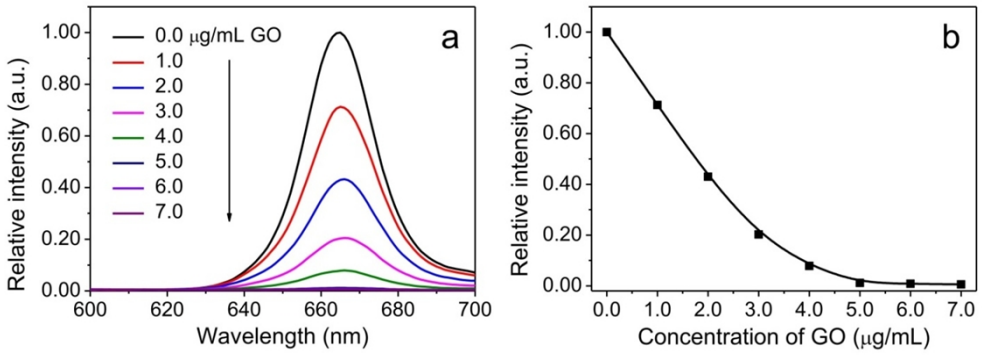

Figure S2. (a) The fluorescence spectra of AP-Ce6 with different concentrations of GO. (b) The AP-Ce6 signal plotted as the function of GO concentration. It is found that the intensity of Ce6 gradually decreased with the increase of GO, indicating that the fluorescence of Ce6 would be quenched by GO.

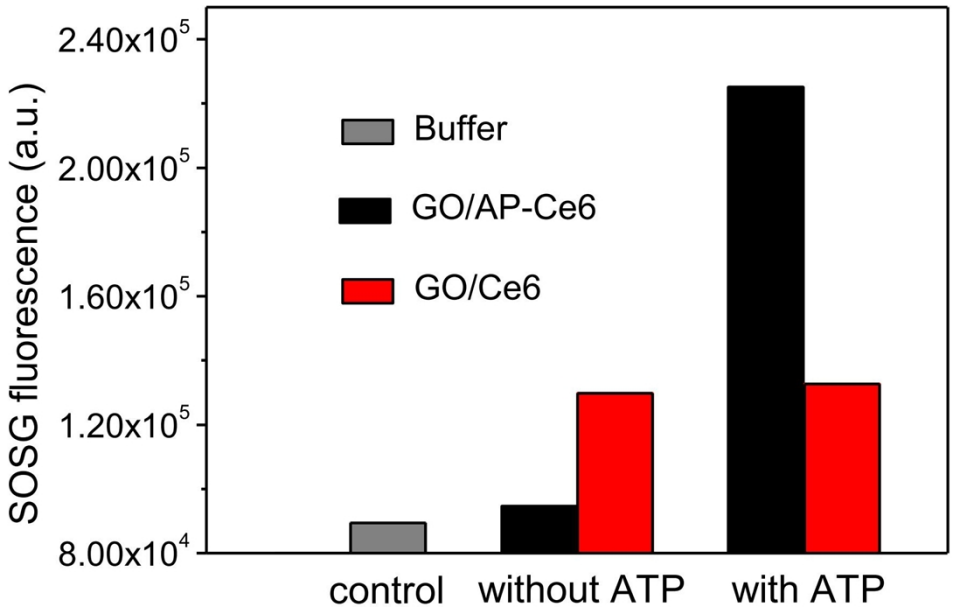

Figure S3. The SOSG signals of GO/AP-Ce6 and GO/Ce6 readout after 10 min of irradiation with excitation at 404 nm. Our result indicates that the quenching effect of SOG from GO/Ce6 complex appears to be less drastic compared to that of GO/AP-Ce6 complex.

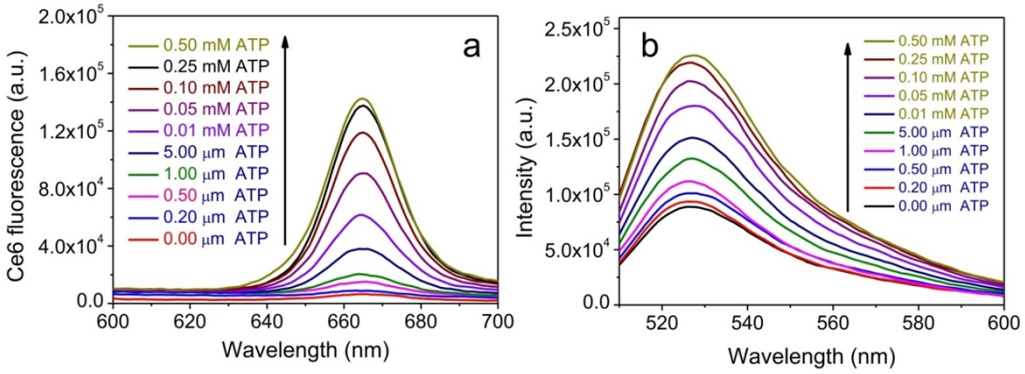

Figure S4. (a) The fluorescence spectra of GO/AP-Ce6 with different concentrations of ATP. (b) The fluorescence spectra of SOSG with different concentrations of ATP. These results indicate that both the fluorescence and SOG of AP-Ce6 are gradually recovered after the introduction of ATP.

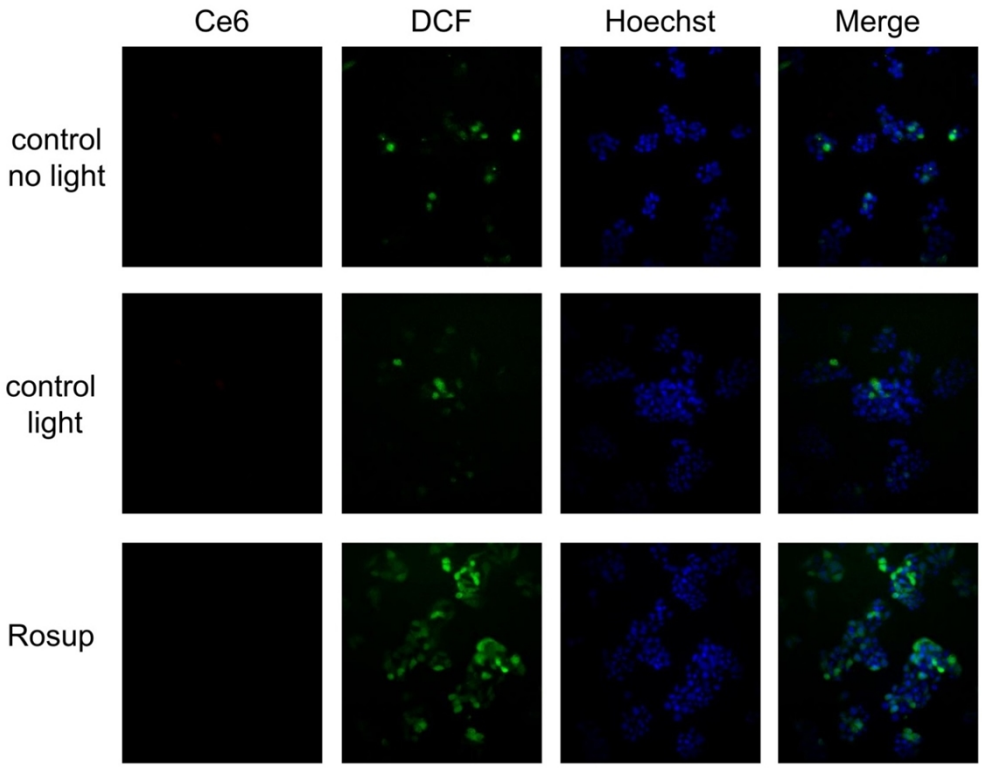

Figure S5. Fluorescence microscopy images of HepG2 cells incubated with Rosup and with or without light, and Ce6 (red), DCF (green) and Hoechst (blue) fluorescence were recorded in the wavelength ranges of 400-500 nm, 500-600 nm and 600-700 nm, respectively. All images were taken under the identical instrumental conditions and presented at the same intensity scale. It is found that almost no increase of fluorescence of DCF is observed in HepG2 cells treated with or without light. However, for the positive control, significant increase of fluorescence of DCF is obtained. These results indicate that $^1O_2$ in the HepG2 cells treated GO/AP-Ce6+ATP is really generated by the AP-Ce6 which is away from the surface of GO.

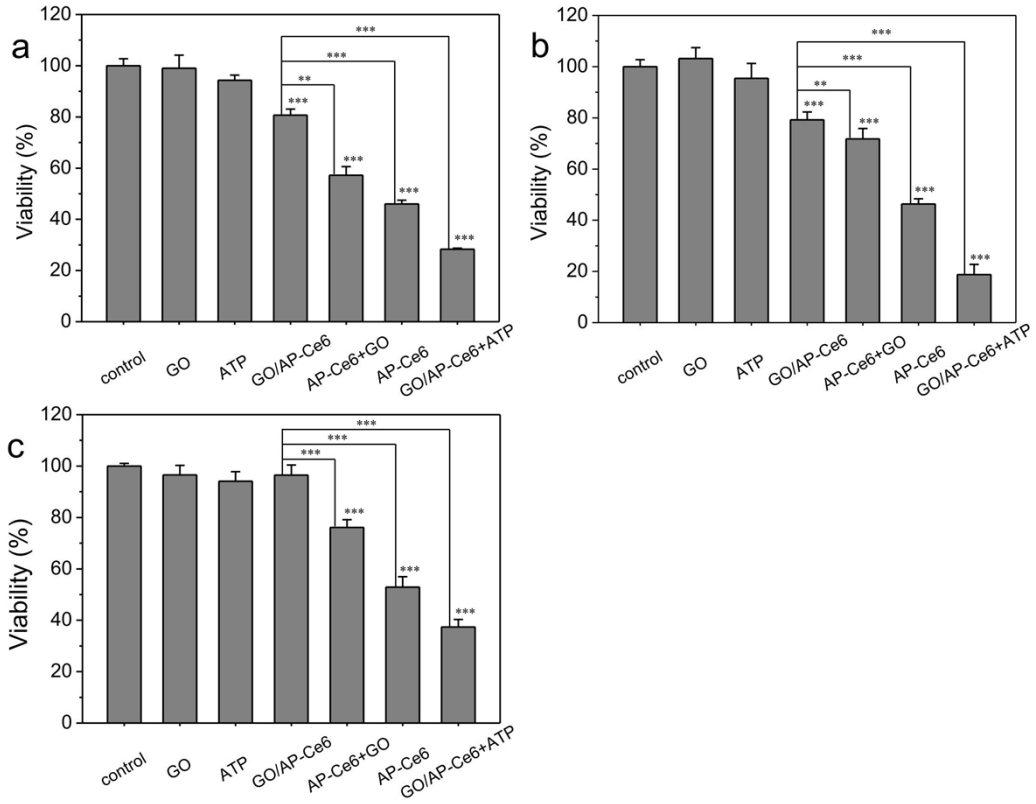

Figure S6. The cellular viability data for MDA (a), HeLa (b) and ECV (c) cells incubated with or without GO, ATP, GO/AP-Ce6, AP-Ce6+GO, AP-Ce6 and GO/AP-Ce6+ATP.These results indicate that GO/AP-Ce6+ATP appear to show similar levels of phototoxicity to MDA, HeLa and ECV cells. (t test, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

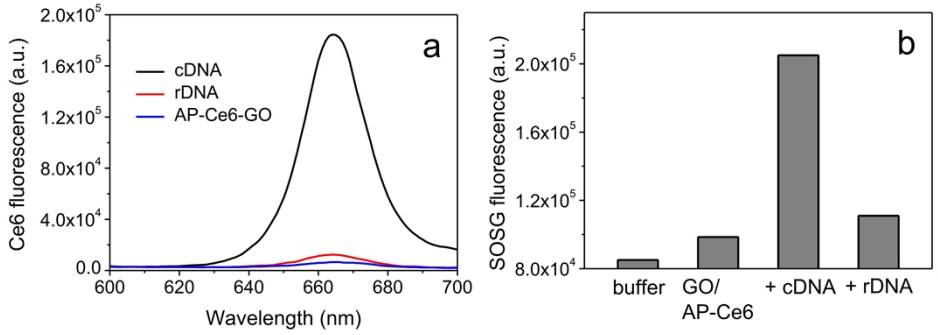

Figure S9. (a) The fluorescence spectra of AP-GO, AP-GO + cDNA and AP-GO + rDNA. The DP-SWNT showed little response to rDNA. (b) The SOSG signals readout after 10 min of irradiation with excitation at 404 nm.

Figure S7. (a) The fluorescence spectra of GO/AP-Ce6, GO/AP-Ce6+cDNA and GO/AP-Ce6+rDNA. The GO/AP-Ce6 showed little response to rDNA. (b) The SOSG signals readout after 10 min of irradiation with excitation at 404 nm. It is found that only cDNA can recover the fluorescence of AP-Ce6 and the GO/AP-Ce6 complex still presents excellent specific response toward cDNA.

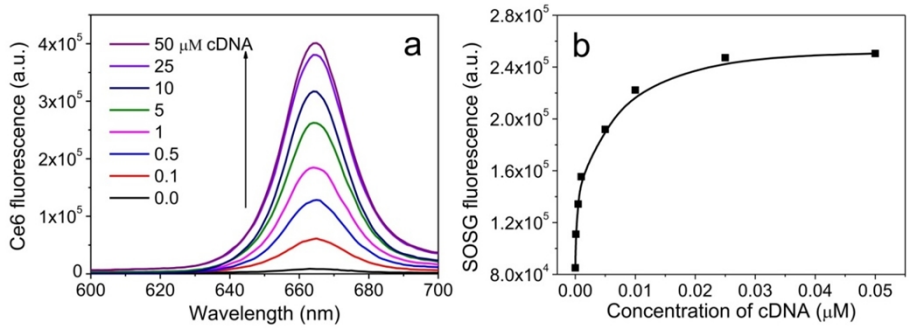

Figure S8. (a) The fluorescence spectra of GO/AP-Ce6 with different concentrations of cDNA. (b) The SOSG signal plotted as the function of cDNA concentration. The purple line indicates the buffer's SOSG signal. It is found that the florescence and SOG of AP-Ce6 could be recover with the increase of cDNA, indicating that a selective PDT agent can be created to trigger SOG with that specific target by simply changing the sequences of the ssDNA.

Table S1. Pearson's correlation and over coefficient of co-localization of Ce6 with DCF.

Sample	Pearson's correlation R_r	Overlap coefficient R
GO/AP-Ce6+ATP	0.732686	0.758301
AP-Ce6	0.853498	0.875324
GO/AP-Ce6	0.768588	0.776337
Control (light)	0.359483	0.521996
Control(no light)	0.021438	0.369955
Rosup	0.033105	0.315502