Electronic Supplementary Material (ESI) for Analyst.
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Support Information Materials

Ru(II) polypyridyl complexes incorporated and folate-conjugated vehicle for cancer cell imaging and photoinduced inactivation

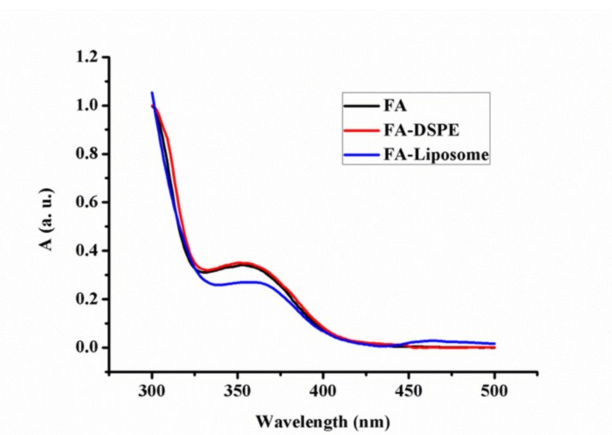

Figure S1. UV-Vis absorption spectra of free FA, FA-PEG2000-DSPE and FA-liposomes.

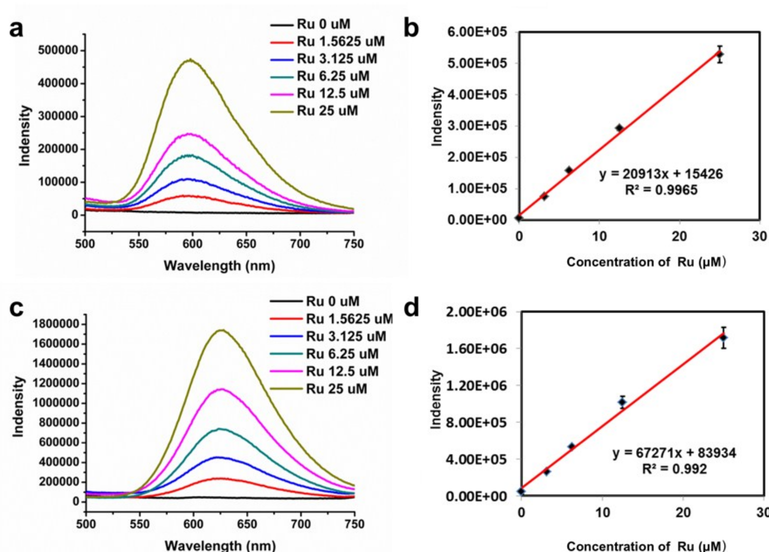

Figure S2. Luminescence emission spectra of (a) $[Ru(phen)_2(dppz)]^{2+}$ in 0.01 M PBS and (b) $[Ru(phen)_2(dppz)]^{2+}$ in 1% Triton X-100 (pH 7.4, λ_{ex} = 405 nm). From up to down, the concentrations of $[Ru(phen)_2(dppz)]^{2+}$ were 25, 12.5, 6.25, 3.125, 1.5625, 0 μM, respectively. Standard curve of $[Ru(phen)_2(dppz)]^{2+}$ linear relationship between the relative luminescence intensity enhancement and the concentrations of $[Ru(phen)_2(dppz)]^{2+}$ in 0.01 M PBS (c) or in 1% Triton X-100 (d). Error bars were based on standard deviations of triplicated samples. It could be noticed that the emission maximum of $[Ru(phen)_2(dppz)]^{2+}$ shifted to 625 nm when treated with Triton X-100, the addition of the neutral surfactant to complex resulted in a shift in their emission maxima, which may be due to the microencapsulation of the ruthenium(II) complexes into the micellar medium, resulting in a change in the microenvironment experienced by the ruthenium(II) complexes. [1-2]

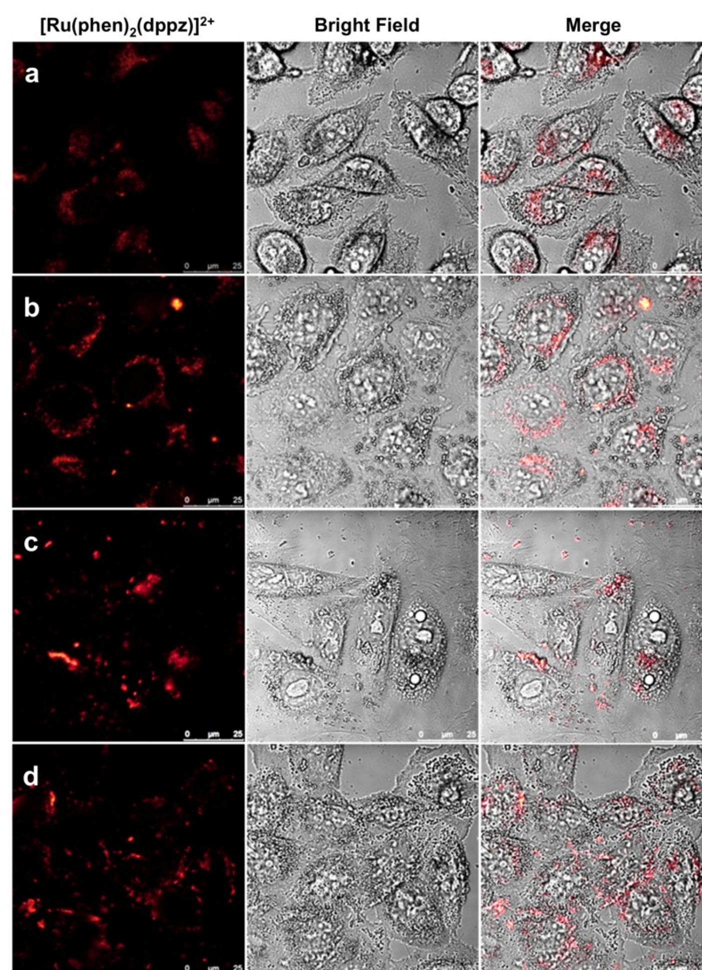

Figure S3. Confocal laser scanning microcopy images of $[Ru(phen)_2(dppz)]^{2+}$ in FR^+ HeLa (a), HeLa (b), A549 (c) and MCF-7 (d) cells. The cells were incubated with 100 μM $[Ru(phen)_2(dppz)]^{2+}$ for 24 h, and then free $[Ru(phen)_2(dppz)]^{2+}$ was washed away three times with 0.01 M PBS before imaging. The $[Ru(phen)_2(dppz)]^{2+}$ were excited using a 488 nm laser, emitting 600-650 nm luminescence (scale bar: 25μm).

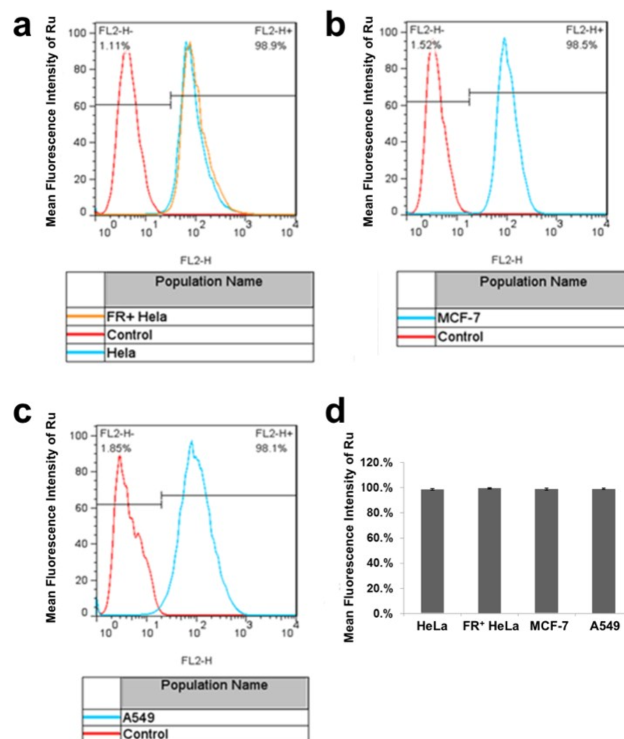

Figure S4. Flow cytometry profiles of luminescence from $[Ru(phen)_2(dppz)]^{2+}$ that uptaken in HeLa (a), MCF-7 (b) and A549 (c) cells. The cells were incubated with 100 μM $[Ru(phen)_2(dppz)]^{2+}$ for 24 h before flow cytometry assay. (d) Percentage of luminescence cells calculated from a, b, c. The data from three independent experiments were normalized as a percentage of the control.

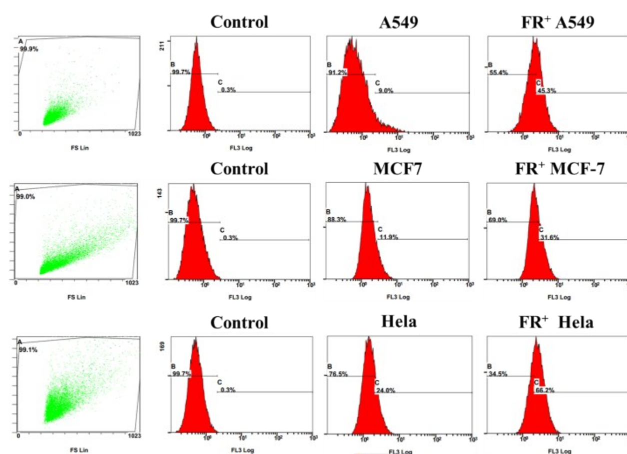

Figure S5. Flow cytometry profiles of luminescence from f-LP-Ru in HeLa, MCF-7 and A549 cells. The cells were incubated with 1mM f-LP-Ru for 24 h before flow cytometry assay.

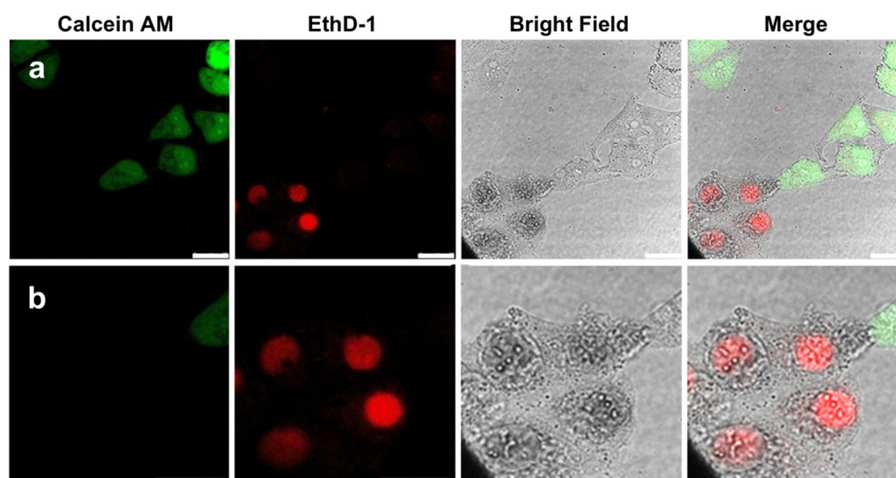

Figure S6. The LIVE/DEAD Viability/Cytotoxicity Assay of HeLa treated with $[Ru(phen)_2(dppz)]^{2+}$. (a) After incubation with $[Ru(phen)_2(dppz)]^{2+}$ for 24 h, cells were rinsed with PBS for three times and irradiated with the laser. The cells were then stained by a mixture of calcein-AM and EthD-1 solution for 20 min. The dead cells were examined under a confocal laser scanning microscope. Calcein-AM was excited using a 488 nm laser, emitting 500-520 nm fluorescence, EthD-1 was excited using a 543 nm laser, emitting 650-670 nm fluorescence. (b) Magnification view of (a).

References

1 E. Rajkumar, P. M. Mareeswaran and S. Rajagopal. *Photochem. Photobiol. Sci.* 2014, **13,** 1261-1269.

2 R. Sangiliapillai, R. Arumugam, R. Eswaranc and Rajagopal Seenivasanb. *Luminescence* 2015, DOI: 10.1002/bio.2917