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A Mitochondria-targeted and NO-based Anticancer Nanosystem with Enhanced Photo- controllability and Low Dark-toxicity

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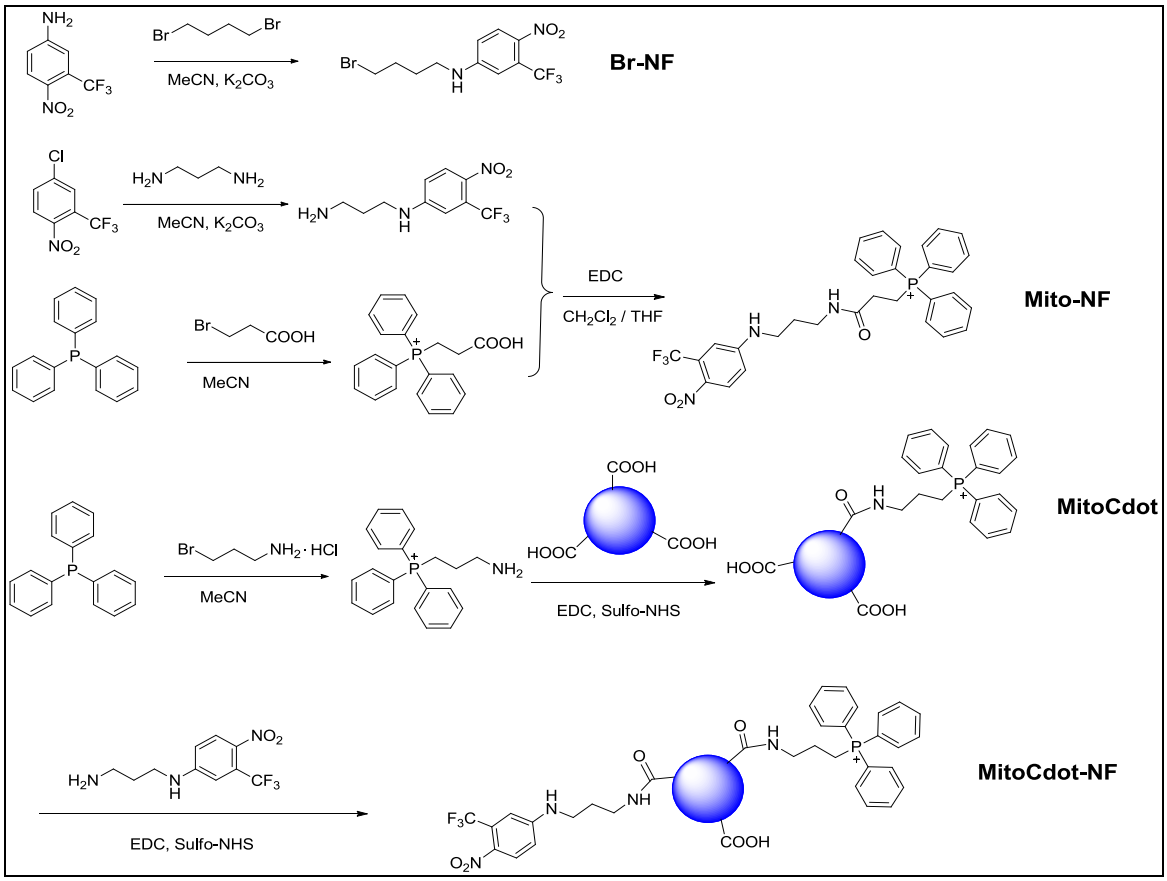
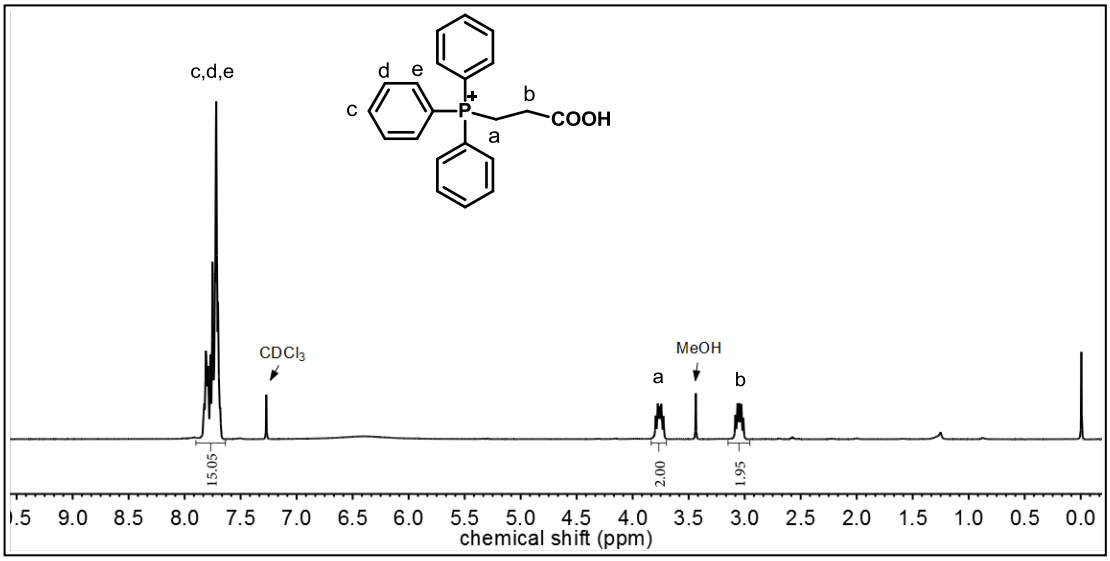
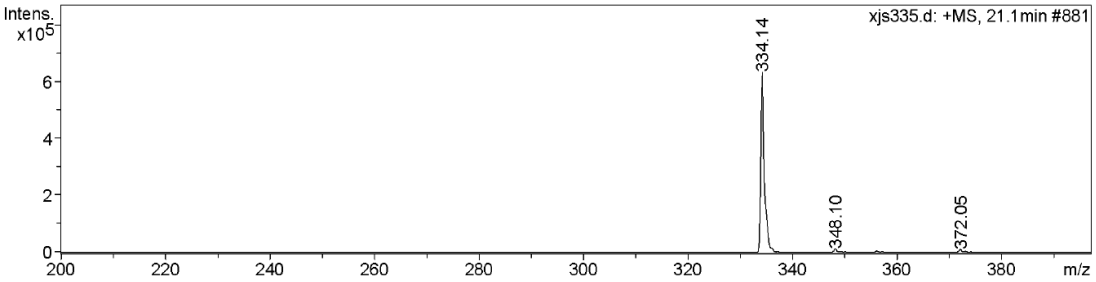


Scheme S1. Synthesis route for the targeted NO-releasing nanosystem. The numbers for the functional groups on the C-dot do not reflect their actual numbers.

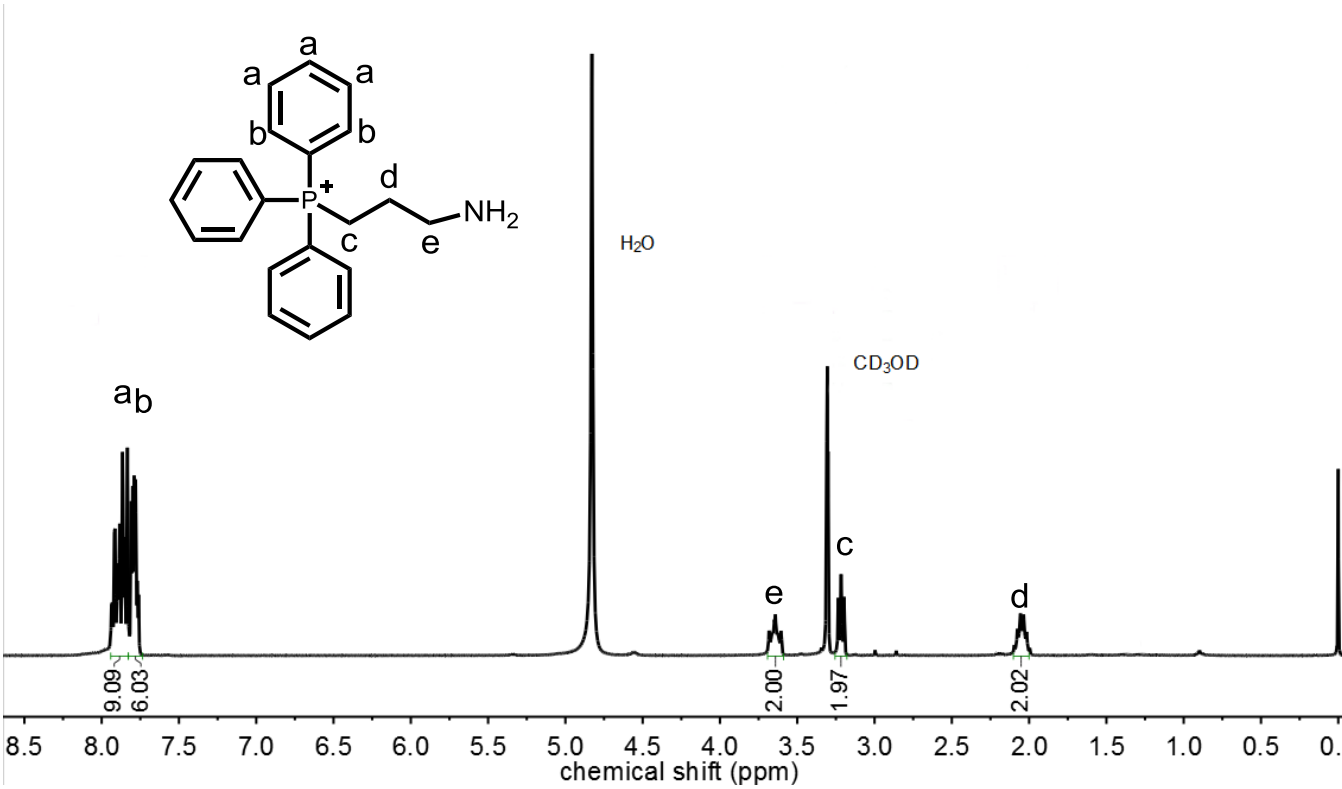
For the synthesis of the C-dots, citric acid was used as carbon source and 1,2-ethylenediamine (EDA) as the surface passivation agent.





#	m/z	I	I %
1	334.14	631907	100.0
2	337.15	1300	0.2
3	348.10	11026	1.7
4	349.09	1821	0.3
5	350.08	742	0.1
6	356.06	4688	0.7
7	357.12	1033	0.2
8	372.05	9635	1.5
9	372.99	2681	0.4
10	373.98	996	0.2

Figure S1. ^{1}H NMR and mass spectra for the TPP-COOH.



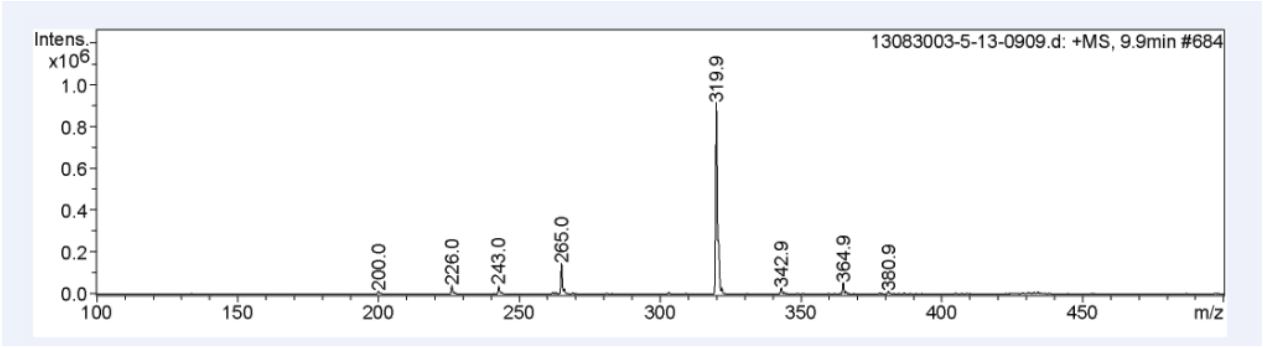


Figure S2. ^{1}H NMR and mass spectra for the TPP-NH_2.

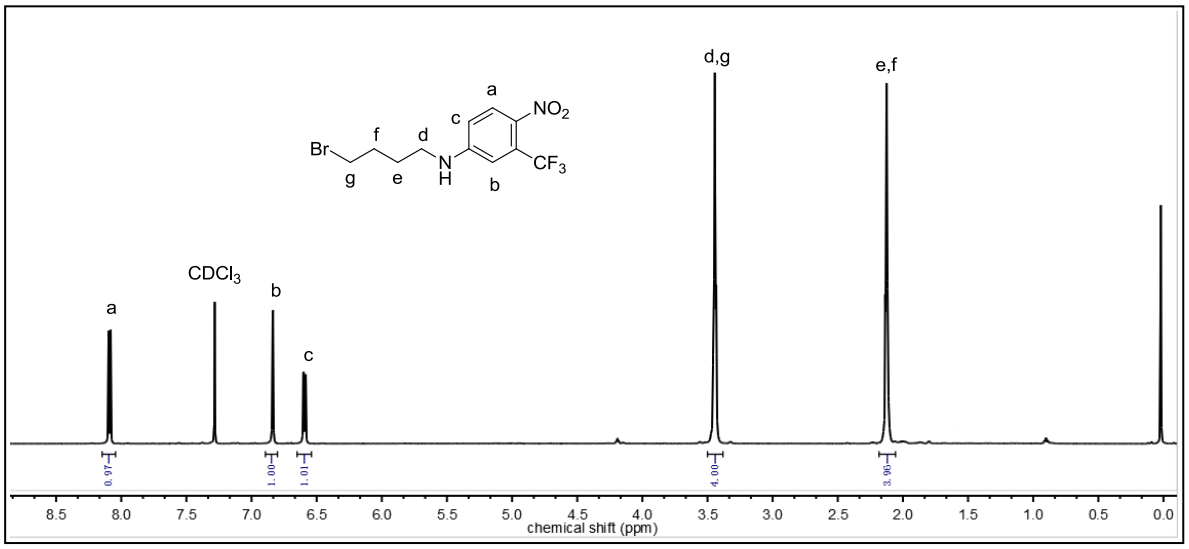
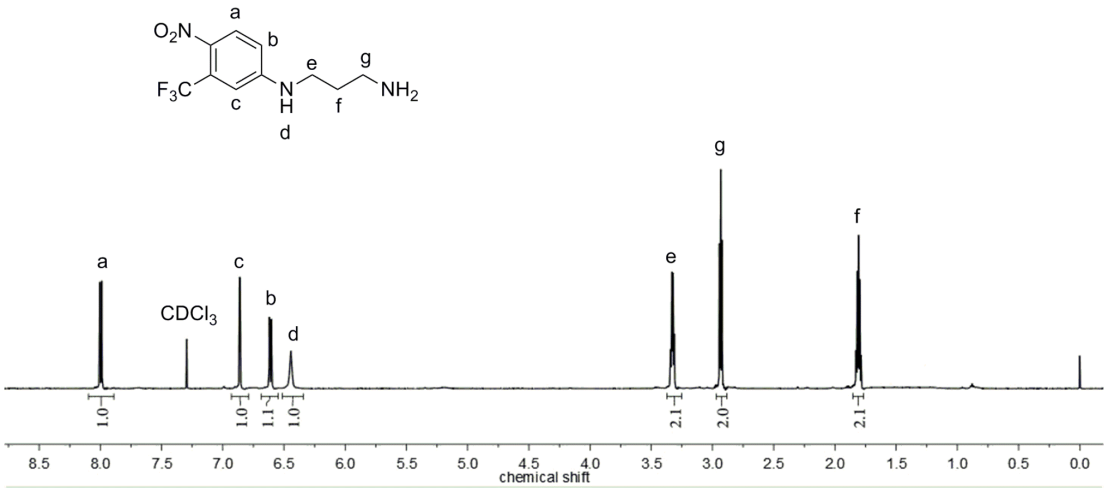
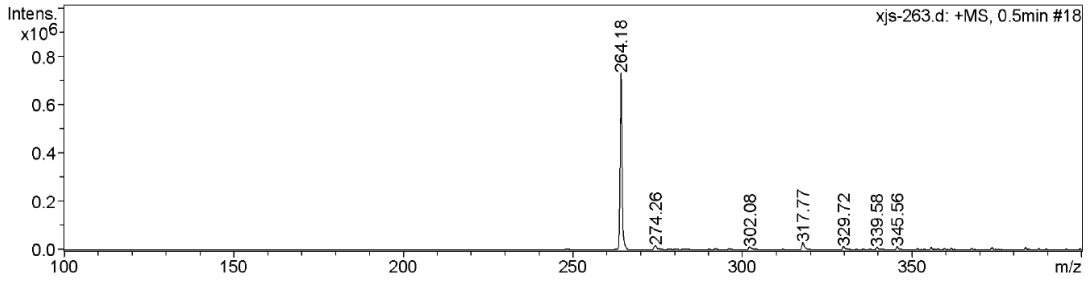


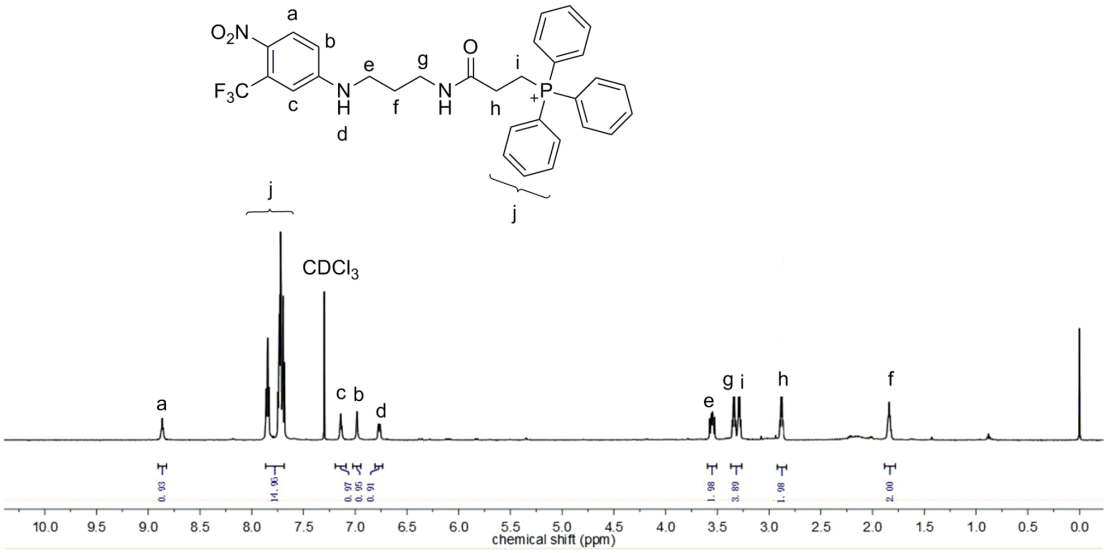
Figure S3. ^{1}H NMR spectrum for the Br-NF.

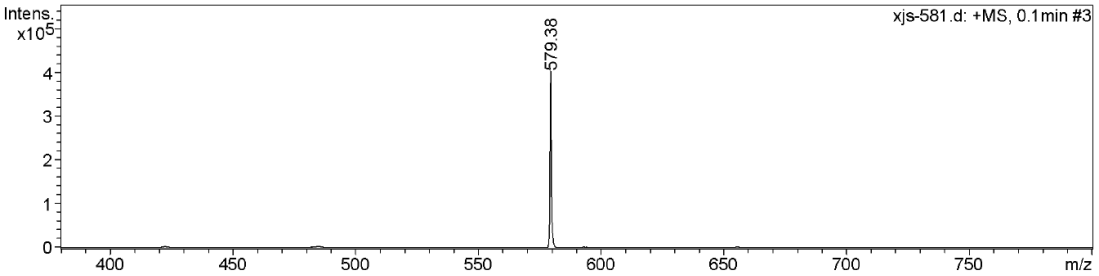




#	m/z	I	I %
1	264.18	729982	100.0
2	274.26	17473	2.4
3	302.08	11542	1.6
4	317.77	29906	4.1
5	329.72	12456	1.7
6	339.58	11130	1.5
7	345.56	12890	1.8
8	355.54	9414	1.3
9	373.49	7528	1.0
10	383.37	7982	1.1

Figure S4. ^{1}H NMR and mass spectra for the NH_2-NF.





#	m/z	I	I %
1	422.39	2006	0.5
2	448.23	329	0.1
3	481.20	338	0.1
4	481.97	464	0.1
5	484.54	1933	0.5
6	485.19	2060	0.5
7	579.38	402902	100.0
8	592.98	822	0.2
9	593.93	473	0.1
10	655.30	668	0.2

Figure S5. ^{1}H NMR and mass spectra for the Mito-NF.

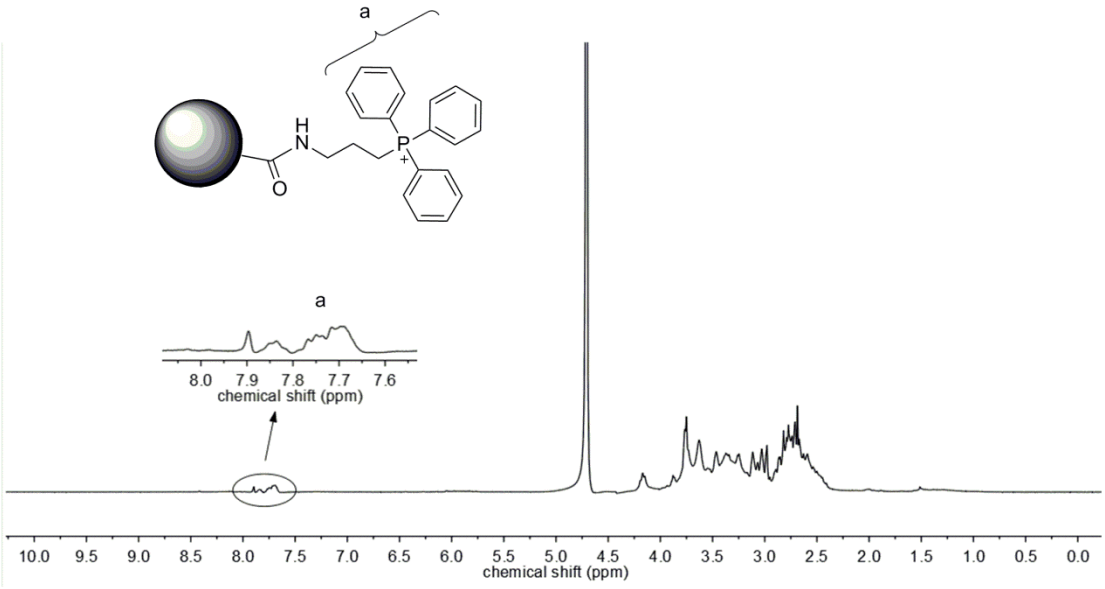


Figure S6. ^{1}H NMR spectrum (in D2O) for mitochondria-targeted Carbon dots (MitoCdot).

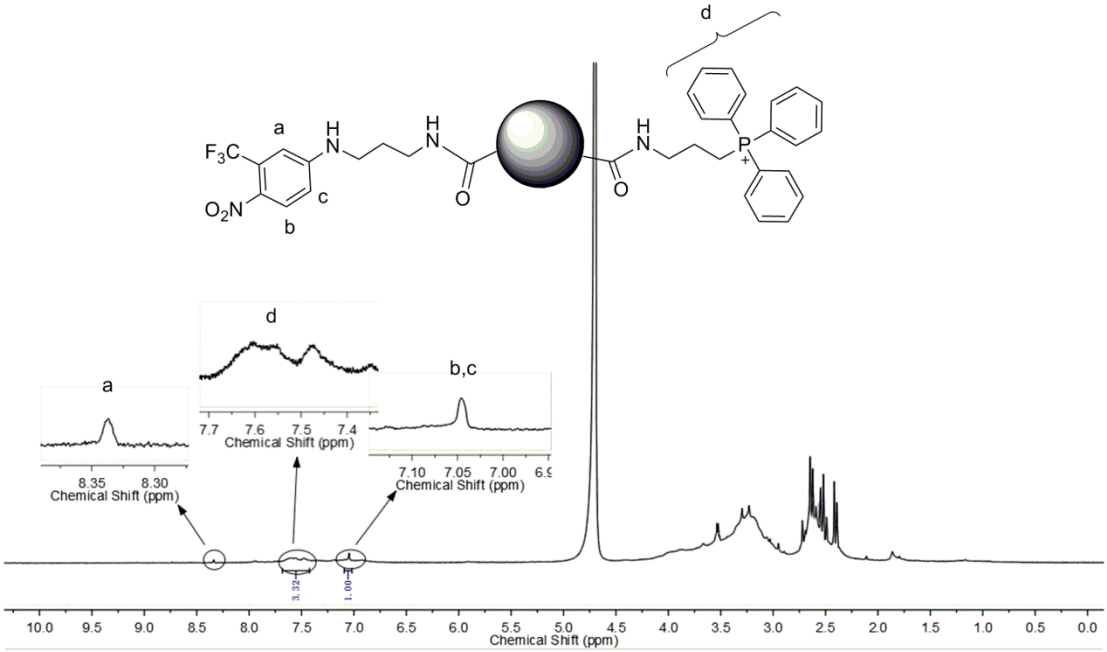


Figure S7. ^{1}H NMR spectrum for the MitoCdot-NF.

Determination of carboxyl group content on carbon dot

Determination of carboxyl group on carbon dot was performed according to the literature report based on the reaction of carboxyl groups with 4-bromomethyl-6,7-dimethoxycoumarin (BMMC). [1]

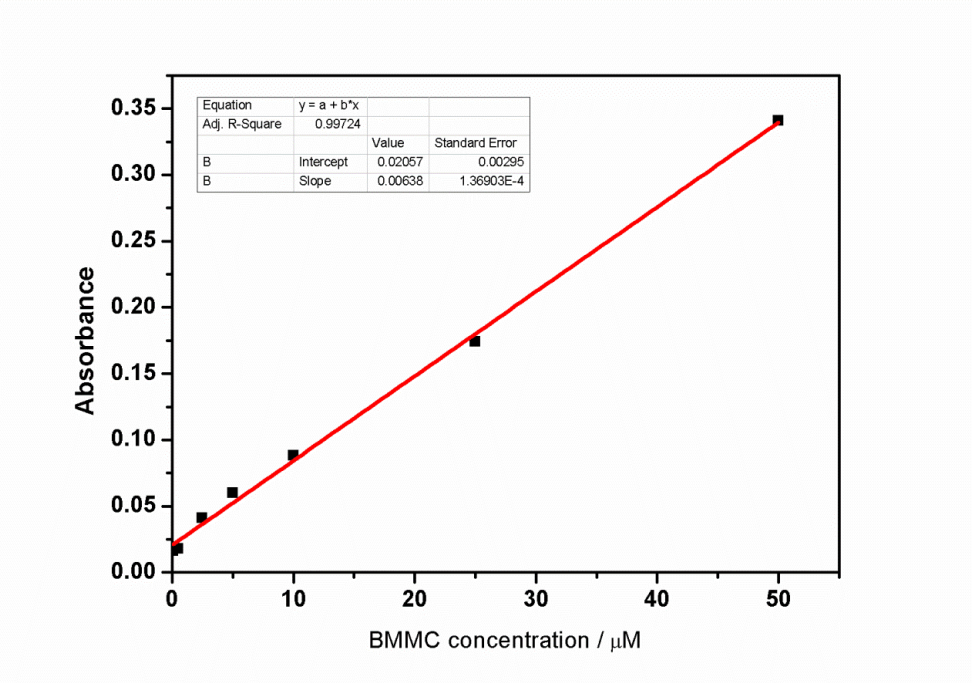


Figure S8. A standard plot of concentration of BMMC vs. its absorbance value at 350 nm.

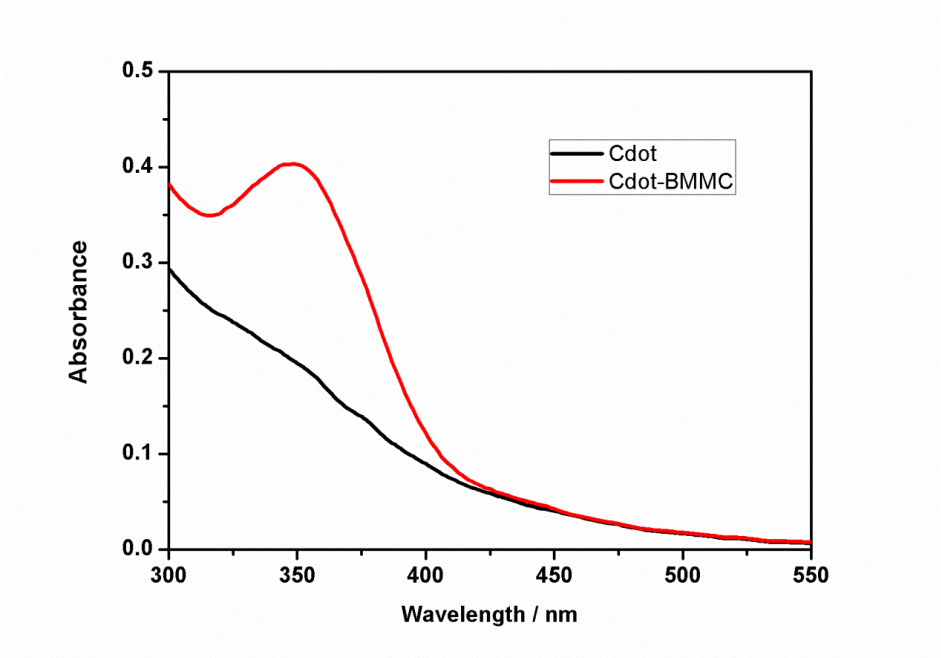


Figure S9. Absorption spectra for aqueous dispersion of Cdot and Cdot treated with BMMC.

To determine the concentration of carboxyl group on Cdot in aqueous solution, first a standard plot (Figure S8) was created by measuring the absorbance (at 350 nm) of BMMC solutions of known concentrations. This plot was subsequently used to calculate the concentration of BMMC moiety on Cdot. The determination was performed as follows:

(1) To the as-prepared Cdot solution, excessive amount of BMMC was added and allowed to react with carboxyl groups on Cdot surface. The reaction was allowed to proceed at 70 °C for two hours. The resultant solution was then dialyzed against double-distilled water for 48 hours to remove the residual BMMC.

(2) 1.0 mg dried Cdot-BMMC was dissolved in 2 mL of double distilled water (concentration: 0.5 g/L), and 0.4 mL Cdot-BMMC was added into quartz cell with 1.6 mL H_2O (The final concentration of Cdot-BMMC is 0.1 g/L) and the absorbance at 350 nm was measured as 0.2064.

(3) The relationship of C_{BMMC} and A_{350} according to the standard plot is Y = 0.02067 + 0.00638X (Y represents the absorbance at 350 nm, X the BMMC concentration), and in this experiment, Y = 0.2064, X = 29.11 μM.

(4) The concentration of BMMC moiety on Cdot was then calculated as $29.11/(0.1\text{-}299.1\times29.11\times10^{-6})$ = 318.87 μM/g carbon dots. (299.1 is the molecular weight of BMMC)

Reference

1. M. Markowitz, P. Schoen, P. Kust and B. Gaber, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 1999, **150**, 85.

Determination of 4-nitro-3-(trifluoromethyl)aniline derivative (-NF) moiety content in the nanosystem.

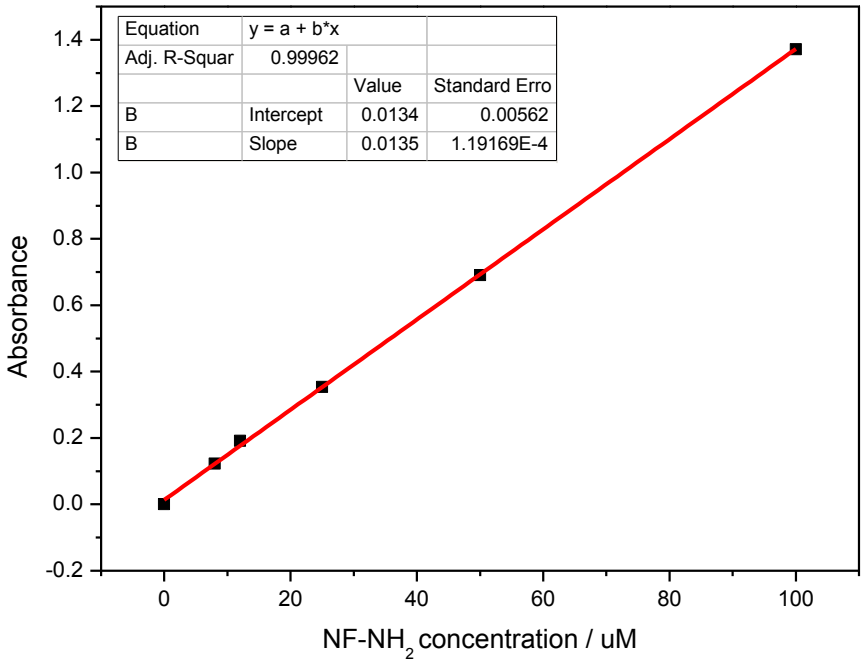


Figure S10. Relationship between concentration for NF-NH_2 and their absorbance.

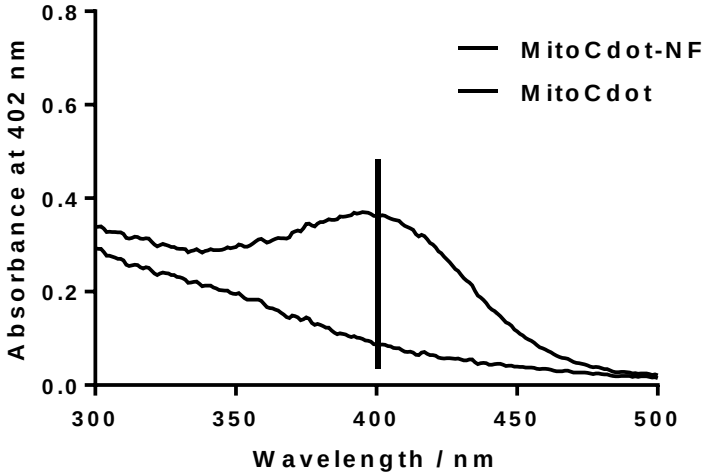


Figure S11. Absorption spectra for aqueous dispersion of MitoCdot and MitoCdot-NF.

The absorbance values at 402 nm were used to calculate the concentration of

4-nitro-3-(trifluoromethyl)aniline moiety in water. The calculation of the NO-precursor 4-nitro-3-(trifluoromethyl)aniline (-NF) concentration is as follows:

(1) 1.2 mg dried MitoCdot-NF was dissolved in 3 mL of H_2O (namely, the concentration 400 mg/L), 1 mL MitoCdot-NF was added into quartz cell with 2 mL H_2O and the absorbance at 402 nm was measured to be 0.2760.
(2) The calibration curve of NF-NH_2 is Y = 0.0134 + 0.0135X (Y represents the absorbance, X represents the -NF concentration), Y = 0.276, X = 19.5 μmol/L=5.148 mg/L.

-NF content was calculated as 5.148/(400/3) = 39.12 mg/g

The TPP content can be calculated based on the content of –NF and the ^{1}H NMR spectrum of the nanosystem (Figure S7)

$$\text{TPP (wt\%)} = \frac{\mathrm{Sd}/_{15}}{\mathrm{S(b+c)}/_{2}} \times (\text{-NF}) \times 319$$

$$= \frac{2/_{15}}{1/_{2}} \times \frac{39.12}{264} \times 252$$

$$= 9.96 \text{ mg/g}$$

Where $S_{(b+c)}$ is the integrated peak areas of the H(b+c) protons of –NF (as shown in Figure S7), S_d represents the integrated peak area of Hd protons of TPP, while –NF represents the calculated –NF content in the nanosystem as shown above. 264 is the molecular weight of –NF (N-(3-aminopropyl)-4-nitro-3-(trifluoromethyl) aniline (NF-NH_2)), and 252 is the molecular weight of TPP.

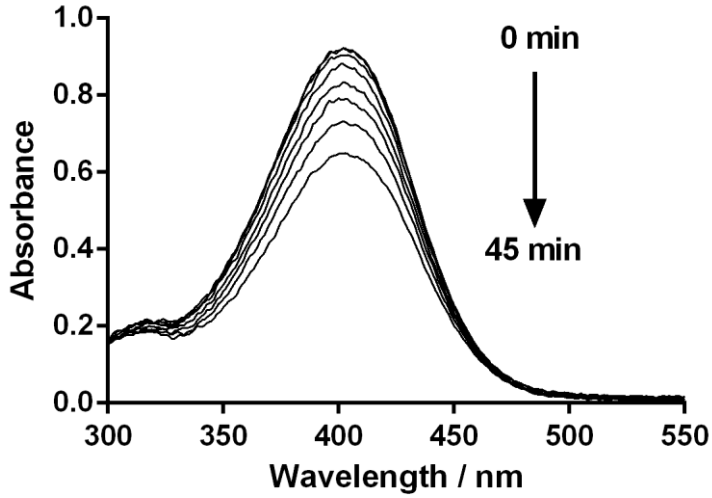


Figure S12. Absorption spectral changes of Mito-NF over different periods of time under irradiation, the arrows indicate the spectral evolution with irration time from 0 to 45 min.

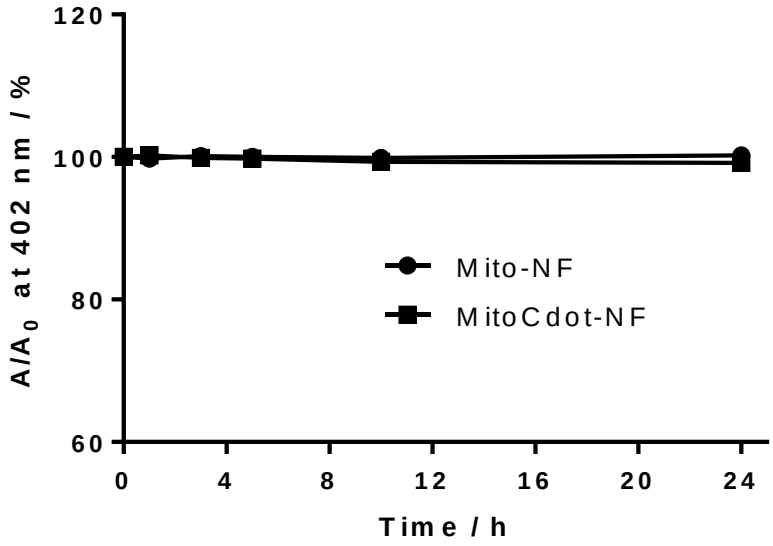


Figure S13. Absorbance values at 402 nm for Mito-NF and Mito-Cdot-NF plotted against time in pH = 7.4 buffers at 37 °C.

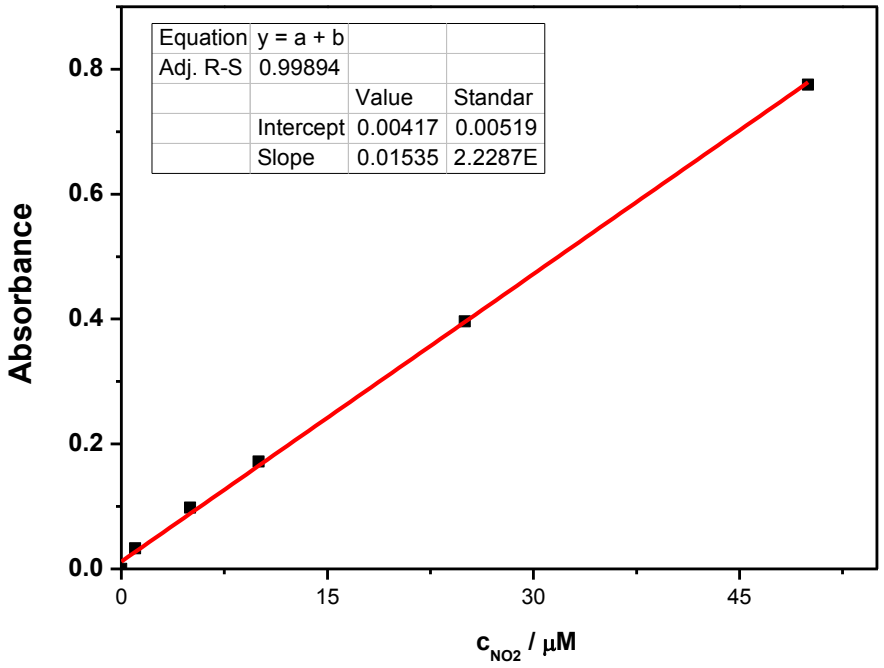


Figure S14. The standard curve demonstrating the relationship between concentration for NO_2^- and the absorbance of Griess agent.

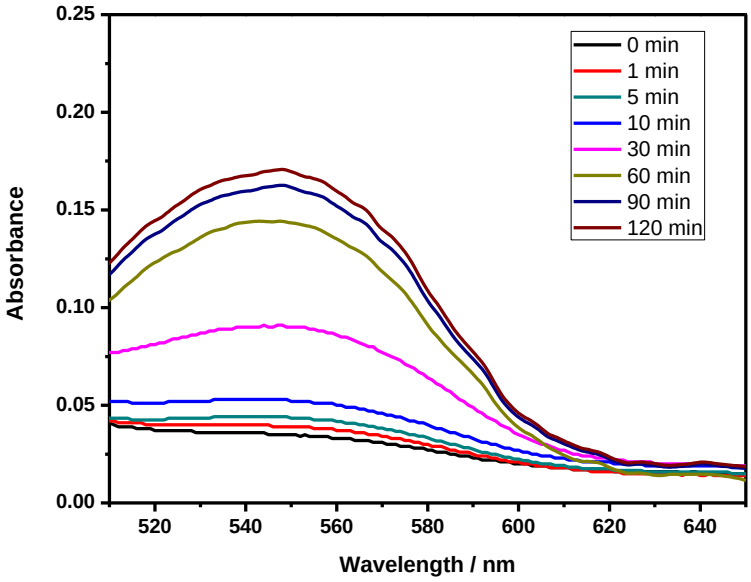


Figure S15. Absorption spectra for MitoCdot-NF treated by Griess agent over different periods of time under light irradiation in pH 7.4 PBS buffer at 37°C.
Note: No change in absorption spectrum was observed without light irradiation.

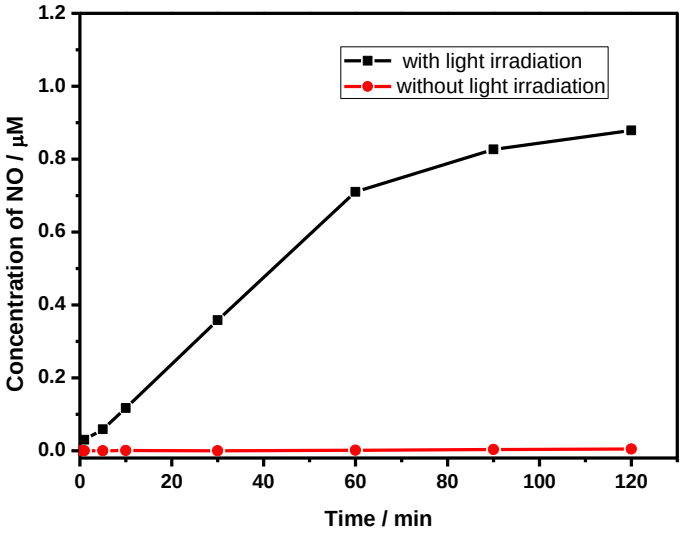


Figure S16. NO release profile for the nanosytem (MitoCdot-NF) incubated in PBS buffered water at 37 °C for varied time periods with or without light irradiation. The amount of released NO was determined using Griess assay.

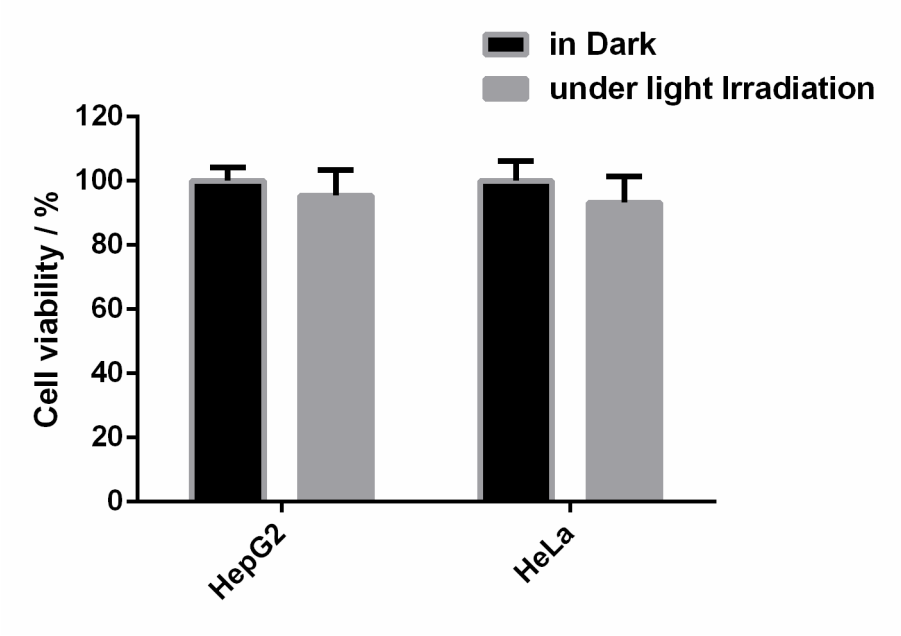


Figure S17. Viability assay for untreated (by nanosystem) cells incubated for 24 h in dark or under continuous light irradiation using a 12 W LED lamp with light intensity at 10 mW/cm^2 with the spectral peak position at 400 nm.

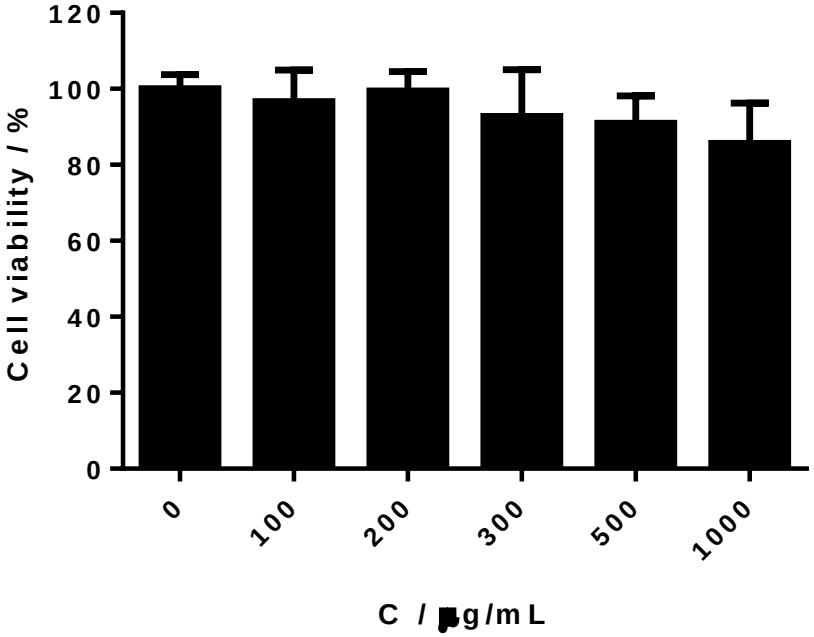


Figure S18. Viability assay for HeLa cells treated with MitoCdot (without NO-donor) under continuous light irradiation for 30 min using a 12 W LED lamp with light intensity at 10 mW/cm^2 with the spectral peak position at 400 nm.