Electronic Supplementary Information

Dual Functional Luminescent Nanoprobe for Monitoring Oxygen and Chloride Concentration Changes in Cells

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Reagents

Pluronic F-127 (F127), disodium hydrogen phosphate, sodium dihydrogen phosphate monohydrate, sodium chloride, potassium chloride, sodium nitrate, sodium fluoride, sodium pyrophosphate, adensine 5'-triphosphate disodium (ATP), adensine 5'-diphosphate disodium (ADP), adensine 5'-monophosphate disodium (AMP), potassium nitrate, magnesium nitrate hexahydrate, calcium nitrate tetrahydrate, calcium chloride, magnesium chloride hexahydrate, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), poly(dimethysiloxane-co-methyhydrosiloxane), trimethylsilyl terminated (PMHS, average Mn≈13000, methylhydrosiloxane 3-4 mol %), trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) and valinomycin were purchased from Sigma-Aldrich. 6-methoxy-quinoline and 11-bromo-1-undecene were purchased from Bide Pharmatech. Ltd., Shanghai, China. Pd(II) Meso-tetra(pentafluorophenyl)porphine (Pd) was purchased from Frontier Scientific incorporation. platinum(0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane complex solution was purchased from 3A Chemicals. Sylgard 184 base and the curing agents were purchased from Dow Corning. Poly(styrene)-graft-poly (ethylene oxide) (PS-PEO, product ID P15020A-SEOcomb, Mw/Mn = 1.5) was purchased from Polymer Source, Ltd., and used as received. LysoTracker DeepRed, MitoTracker DeepRed, ERTracker Blue-White, monensin, and nigericin were obtained from Thermal Fisher Scientific. Tetrahydrofuran (THF), dichloromethane (DCM), methanol (HPLC grade) toluene, dimethyl sulfoxide-d6 containing 99.9 atom % D, 1% (v/v) TMS (DMSO-d_6) were purchased from J&K Scientific Ltd. in China. Deionized water was used to prepare aqueous solutions after purification by Milli-Q Integral 5. Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin solution (100×), sterile phosphate buffered saline (PBS) were purchased from Corning. Cell Counting Kit-8 (CCK-8) was obtained from MedChem Express in China.

Synthesis of 6-methoxy-1-(undec-10-en-1-yl) quinolinium bromide (Q^+Br^-)

25 mL of toluene was added in a mixture of 300 mg of 6-methoxy-quinoline and 400 mg of 11-bromo-1-undecene and the reaction under N_2 atmosphere was conducted at 120 ℃ for 16 hours in the dark. After cooling down, toluene was evaporated and the crude product was purified by silica gel column with DCM as eluent to obtain 6-methoxy-1-(undec-10-en-1-yl) quinolinium bromide (Q^+Br^-). 4 mg of Q^+Br^- was dissolved in 600 μL of DMSO-d_6 for the ^{1}H-NMR characterization and 10 mg of Q^+Br^- in 600 μL of DMSO-d_6 for the ^{13}C-NMR characterization on a 400 MHz NMR spectrometer (AVANCE NEO, Bruker). 0.1 mg/mL Q^+Br^- in methanol was determined by Q-Exactive electrospray ionization (ESI) mass spectrometer in the positive ion mode from Thermo Fisher Scientific, USA.

Preparation of the Pd-Q^+@PDMS luminescent probes

6.4 mg of Sylgard 184 base, 0.64 mg of the curing agent, 1.6 mg of F127, 17 mg of PS-PEO, 2 mg of Q^+Br^- and 0.6 mg of Pd were dissolved in 5 mL of THF. 0.5 mL of the mixture was pipetted and injected into 5 mL of deionized water with vortexing at 1000 rpm. After compressed air was used to remove THF, and the mixture was quickly heated to 80 °C and kept for 3.5 h in an oil bath for the crosslinking. Then, the sample was dialyzed with a dialysis bag of molecular weight cut-off 5000 against deionized water for 3 days to obtain the Pd-Q^+@PDMS probes. The probes were diluted to 5 mL and denoted as the stock.

Morphologic Characterization of the Pd-Q^+@PDMS luminescent probes

The Pd-Q^+@PDMS probes were characterized with transmission electron microscopy (TEM, HT-

7700, Hitachi) at 100 kV acceleration voltage. The sample was prepared by dropping 5 μL of Pd-Q^+@PDMS suspension on a copper grid and drying in the air. 5X Hydrodynamic size distribution and zeta-potential were measured on the analyzer Zetasizer Nano ZS (Malvern Inc.).

Characterization of the Q^+@PMHS complex through Gel permeation chromatography and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

60 mg PMHS, 2 mg Q^+Br^- and 0.5 mol % platinum (0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane were mixed and added in 15 mL THF, and the reaction under N_2 atmosphere was conducted at 75 ℃ for 6 hours in the dark. After cooling down, the product (Q^+@PMHS) solution was collected for following usage.

The products Q^+@PMHS in THF were determined by GPC (Waters ACQUITY APC equipped with a Waters ACQUITY TUV detector and 3 series connected chromatographic columns (ACQUITY APC XT 450, 200, 45, 4.6×150 mm)) with THF as the eluent at a flow rate of 0.5 ml min^{-1}.2 μL, 1.0 mg/L Q^+@PMHS or PMHS THF solution was mixed with 2 μL, 5.0 mg/mL DCTB in methanol. Next, 2 μL mixture was added to the sample plate for MALDI-TOF. After drying in air at room temperature, the determination was conducted by Bruker Autoflex Speed.

Detection of Cl^- and O_2 with the Pd-Q^+@PDMS luminescent probes

For O_2 detection, a 50 mL three-necked round-bottomed flask was used to hold 35 mL of Pd-Q^+@PDMS suspension. A pre-calibrated dissolved-oxygen electrode (JPBJ-608, INESA Scientific Instrument CO. Ltd., Shanghai, China.) was immersed in the solution to monitor [O_2] separately. O_2 partial pressure was adjusted with a commercial O_2&N_2 mixer (MF-2600, ZCBG Technology CO. Ltd., Sichuan, China.). Luminescence was collected with a Y-shaped optical fiber linked to a fluorescence spectrometer (Fluorolog-3, Horiba Jobin Yvon). Fluorescence spectra of Pd-Q^+@PDMS suspension containing 0.0, 0.2, 0.6, 1.2, 1.8, 2.4, 3.0, 3.6, 4.2, 4.9, 5.6, 6.4 mg/L dissolved oxygen were respectively measured. In addition, the fluorescence spectra of Pd-Q^+@PDMS suspension containing 10 and 100 mM of NaCl at same dissolved oxygen concentration were recorded, respectively. For Cl^- detection, the fluorescence spectra of the Pd-Q^+@PDMS suspensions were recorded with added NaCl at 0, 1, 3, 5, 10, 30, 50, 100, 130, 150, and 200 mM, respectively.

Test of cell viability

Hela cells were seeded in a 96 well plate (5000 cells/per well) and incubated in DMEM full culturing medium at 37 ℃ and with 5% CO_2 for 24 h. Then, after rinsing with PBS, Pd-Q^+@PDMS suspensions of different concentration were added to incubate cells at three repeats. 8 hours later, the CCK-8 reagents were added for another incubation of 3 h. Finally, the optical density at 450 nm of each well was read by a microplate reader (Cytation5, BioTek).

Cell imaging

The chloride-free calibration buffer at pH 7.4 was prepared with 150 mM of KNO_3, 10 mM of $NaNO_3$, 1 mM of $Ca(NO_3)_2$, 1 mM of $Mg(NO_3)_2$, 20 mM of HEPES, 20 μM of nigericin, 20 μM of valinomycin, 20 μM of monensin. To include Cl^-, the chloride-free calibration buffer was adjusted by replacing the nitrate salt with chloride salts.

Hela cells were seeded in a glass-bottomed 96 well plate (5000 cells/per well) and grown overnight in DMEM full culturing medium at 37℃ and with 5% CO_2. After rinsing with PBS, Pd-Q^+@PDMS (Pd@PDMS followed by ERTracker Blue-White) probes (8 times diluted from the stock) was used to stain the cells for 20 min; then 100 nM LysoTracker DeepRed was used to

incubate cell for 1 min (Similarly, 100 nM MitoTracker DeepRed was used to incubate cells for 1 min; 500 nM ERTracker blue-white was used to incubate cells for 20 min at 37 ℃). After rinsing, the cells were imaged by a confocal laser scanning microscope (CLSM, SP8, Leica) under de-oxygenated and air-equilibrated condition, respectively.

For intracellular Cl^- calibration, HeLa cells were stained with the Pd-Q^+@PDMS probes and fixed with 4% paraformaldehyde for 10 min at room temperature. After rinsing, the sterile calibration buffer containing 0, 10, 40, 80, 120, 154 mM of Cl^- (respectively) were used to incubate the cells for 2 h to obtain equilibrated concentrations of Cl^-. Finally, the cells were imaged by CLSM with excitation length at 405 nm.

To simulate ischemia environment, Pd-Q^+@PDMS-stained HeLa cells incubated in DMEM minimal medium were first imaged, then the medium was replaced with PBS in which oxygen was removed with a wet bag of sodium sulfide and pictures were captured as soon as possible under CLSM. After another 15 and 20 min, pictures at the same position were taken again to trace the dynamic response of intracellular changes of O_2 and Cl^-.

The blue channel signal (for Cl^- detection) and red channel signal (for O_2 detection) were excited with a laser at 405 nm and collected in the range of 420-500 nm and 630-710 nm, respectively. The signal from LysoTracker DeepRed, MitoTracker DeepRed or ERTracker Blue-White was excited with laser at 638 nm and collected from 655 to 690 nm or 405 nm and collected from 435 to 480 nm.

Monitoring the fluctuation of Cl^- in hypoxic Ramos cell

Ramos cells (about 10^6 cells) were rinsed with PBS and centrifuged at 1000 RPM for 3 min. After resuspension in 1 mL PBS, 100 μL was pipetted and stained with Pd-Q^+@PDMS at 37℃ for 20 min. After rinsing with PBS, the emissions of deoxygenated Pd-Q^+@PDMS-stained Ramos cells at 462 nm, 556 nm and 670 nm were monitored for evaluating the fluctuation of Cl^- in hypoxic Ramos cell.

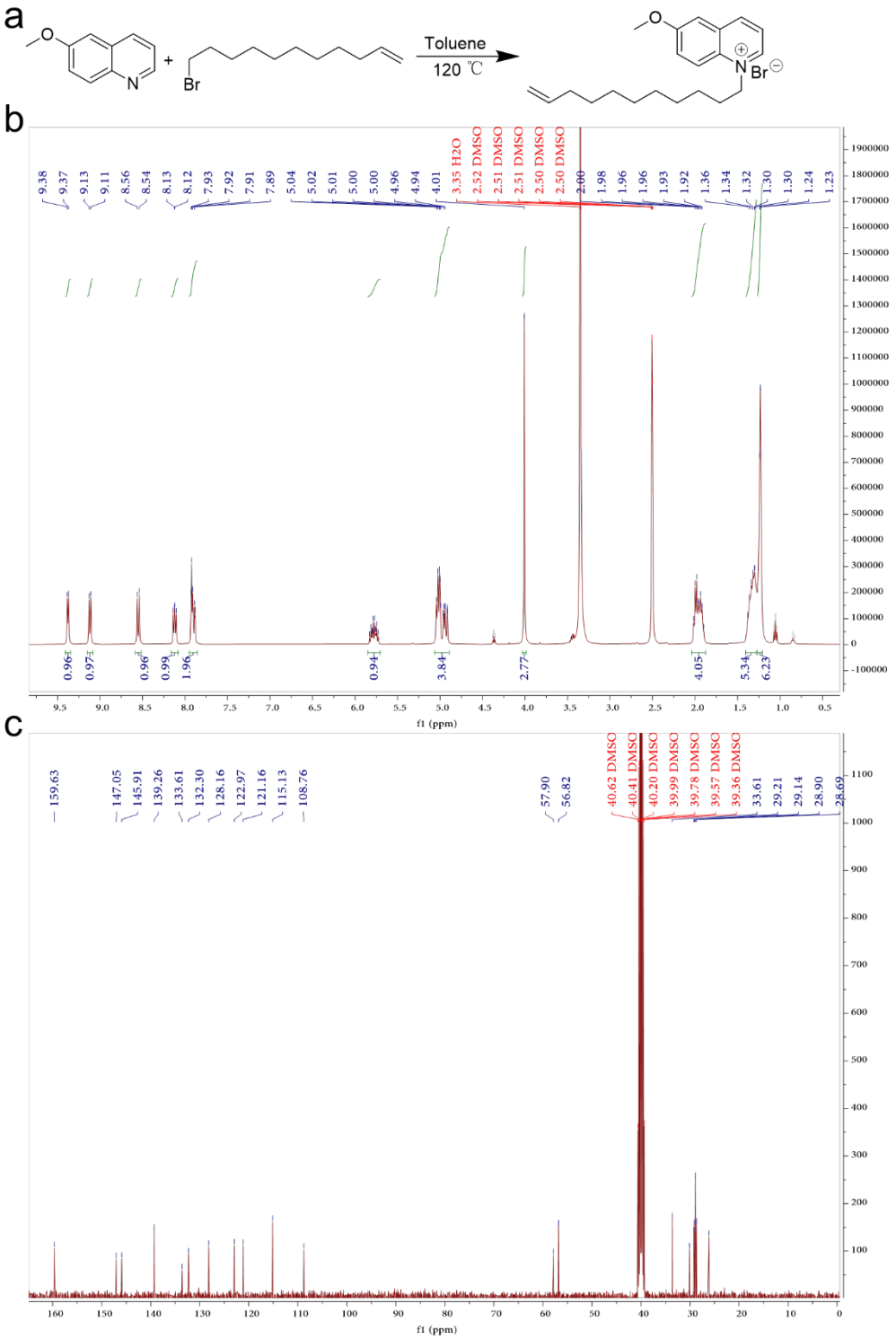

Figure S1. The synthetic route (a), ^{1}H-NMR spectrum (b), and ^{13}C-NMR spectrum (c) of $Q^{+}Br^{-}$.

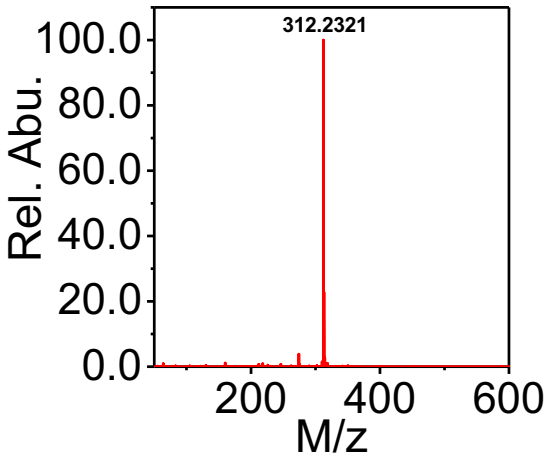

Figure S2. The ESI-Mass spectrometric characterization of Q^+Br^-. The exact mass of Q^+ equals to 312.2321, corresponding to highest relative abundance.

Table S1. A comparison of zeta-potential values between Pd@PDMS and Pd-Q^+@PDMS.

	Pd@PDMS	Pd-Q^+@PDMS
before dialysis	-19.7 ± 0.6 mV	+25.3 ± 1.5 mV
after dialysis	-19.7 ± 0.6 mV	+12.1 ± 0.4 mV

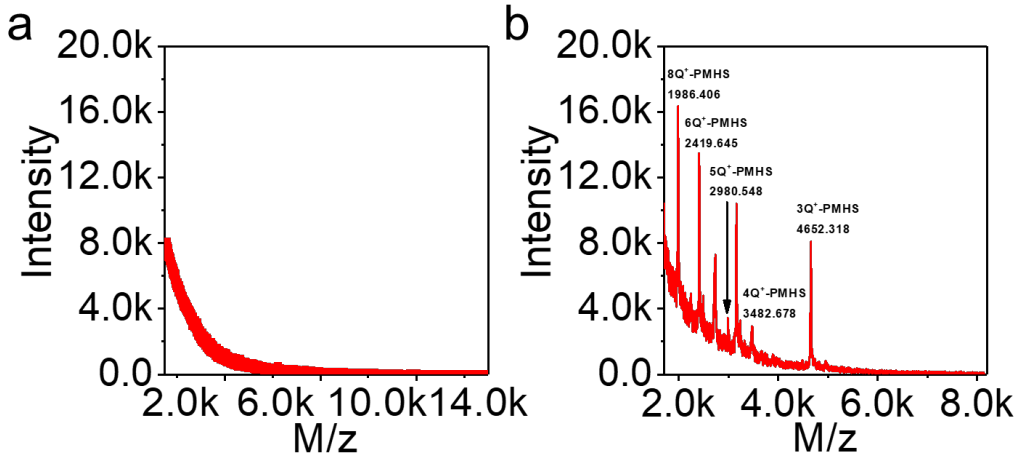

Figure S3. MALDI-TOF characterization of the commercial PMHS (a) and Q^+@PMHS (b). There are up to 8 reactive Si-H of per PMHS chain available for chemical graft of Q^+. Before modification there was no observation MS signals for PMHS, which is the main component of Sylgard 184. After attaching Q^+ through hydrosilylation, the MS was readily observed in the same matrix for MALDI.

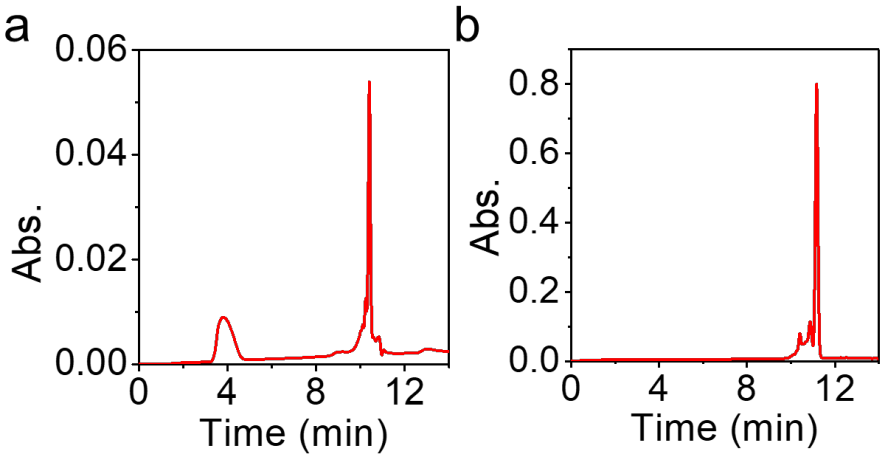

Figure S4. GPC characterization of the commercial PMHS reactant (a) and the product Q^+@PMHS (b). The product has higher molecular weight and more positive charges, explaining the longer retention time.

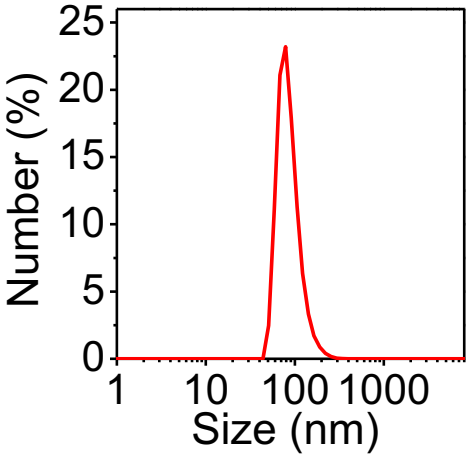

Figure S5. Hydrodynamic size distribution of Pd-Q^+@PDMS from DLS measurements.

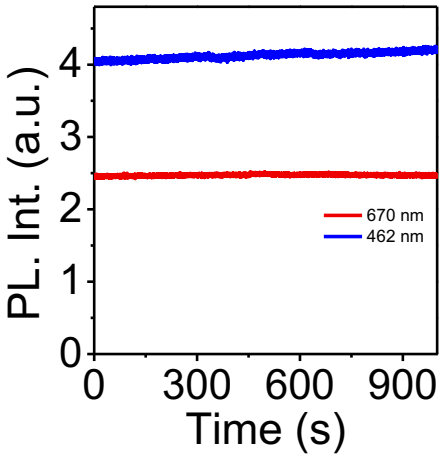

Figure S6. The photostability of Pd-Q^{+}@PDMS determined via monitoring emission at 462 nm and 670 nm during 1000s radiation (with 3/4 nm excitation/emission slit).

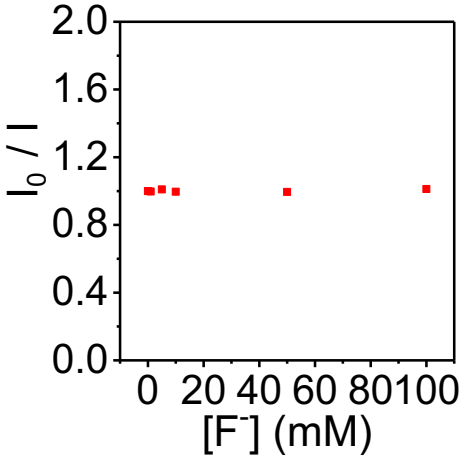

Figure S7. The response of Pd-Q^+@PDMS to F^- at 0, 1, 5, 10, 50, and 100 mM, respectively.

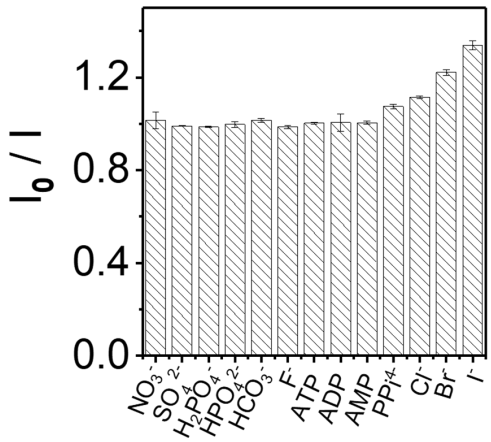

Figure S8. The selectivity of Pd-Q^+@PDMS to various anions at 1 mM.

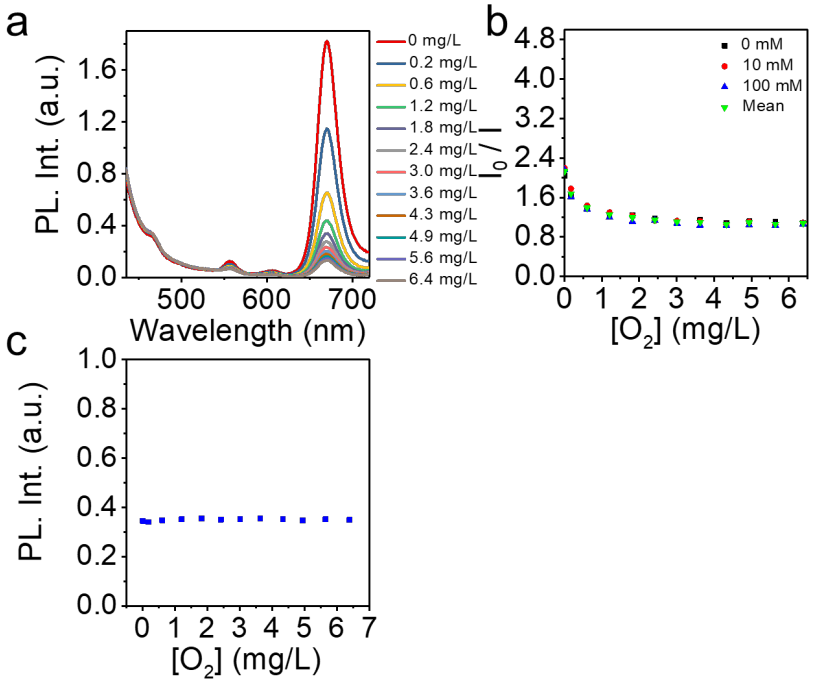

Figure S9. The fluorescence spectra (a) and emission at 462 nm (c) of Pd-Q^+@PDMS at different O_2 concentration. (b) The emission at 556 nm (b) of Pd-Q^+@PDMS at different Cl^-/O_2 concentration.

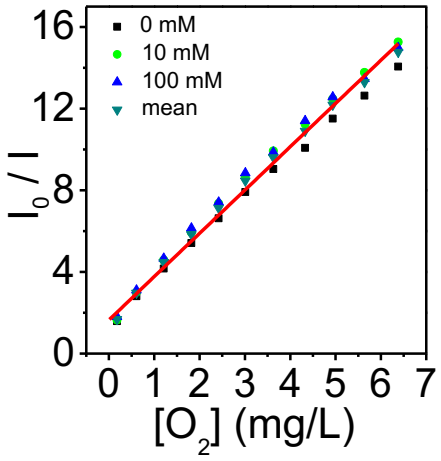

Figure S10. The Stern-Volmer plot of Pd-Q^+@PDMS at different concentrations of dissolved oxygen containing 0 mM, 10 mM, 100 mM NaCl as background, respectively. The mean values of I_0 / I were used for fitting (K_{SV} = 2.1 L/mg).

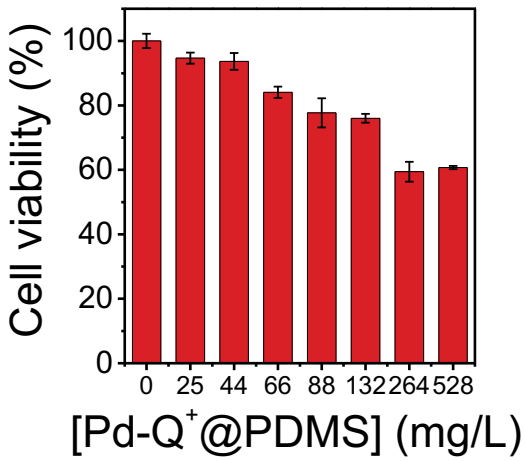

Figure S11. The viability of HeLa cells incubated with different mass concentrations of Pd-Q^+@PDMS.

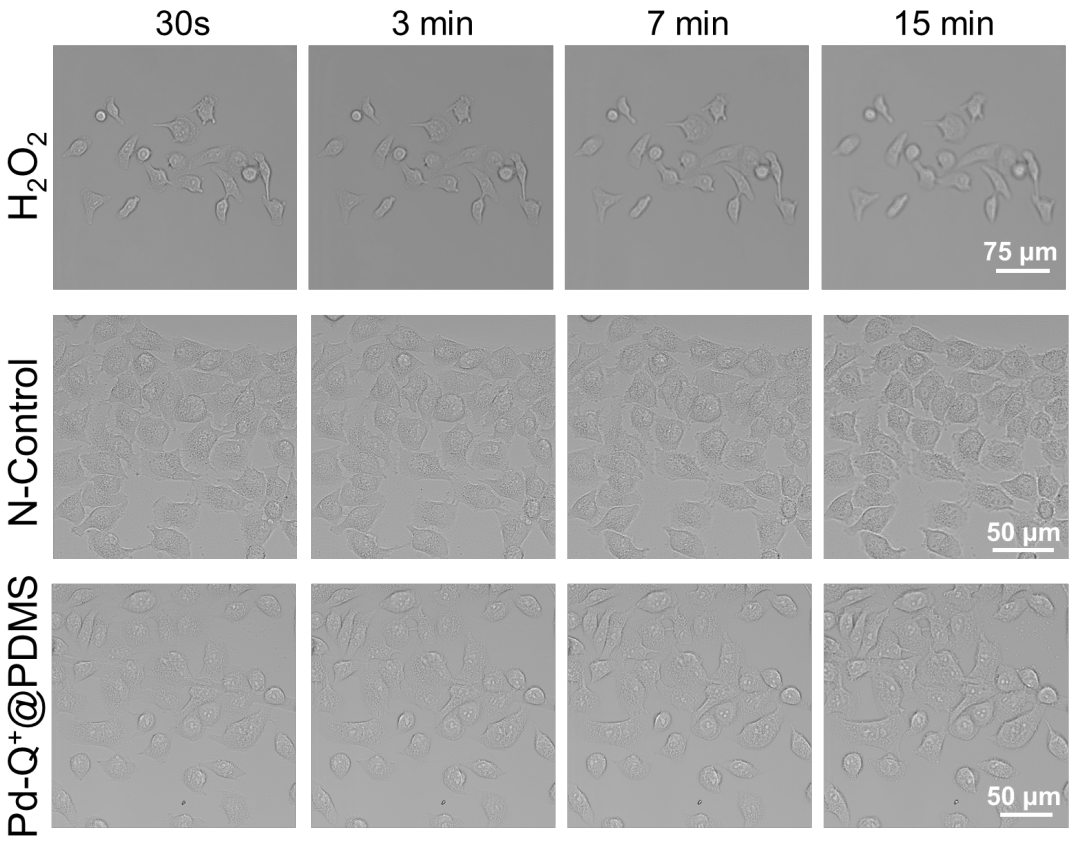

Figure S12. Images of Pd-Q^+@PDMS-stained, negative-control and 20 μM H_2O_2-incubated (without radiation) HeLa cells continuously radiated by 405 nm laser for 30 s, 3 min, 7 min, and 15 min.

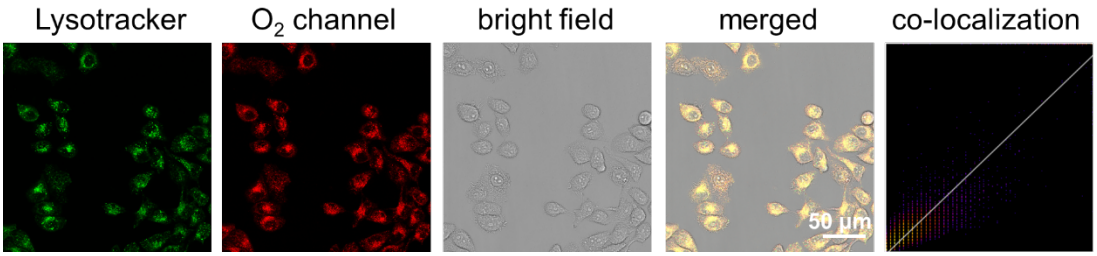

Figure S13. CLSM images of HeLa cells stained with Pd-Q^{+}@PDMS and LysoTracker DeepRed.

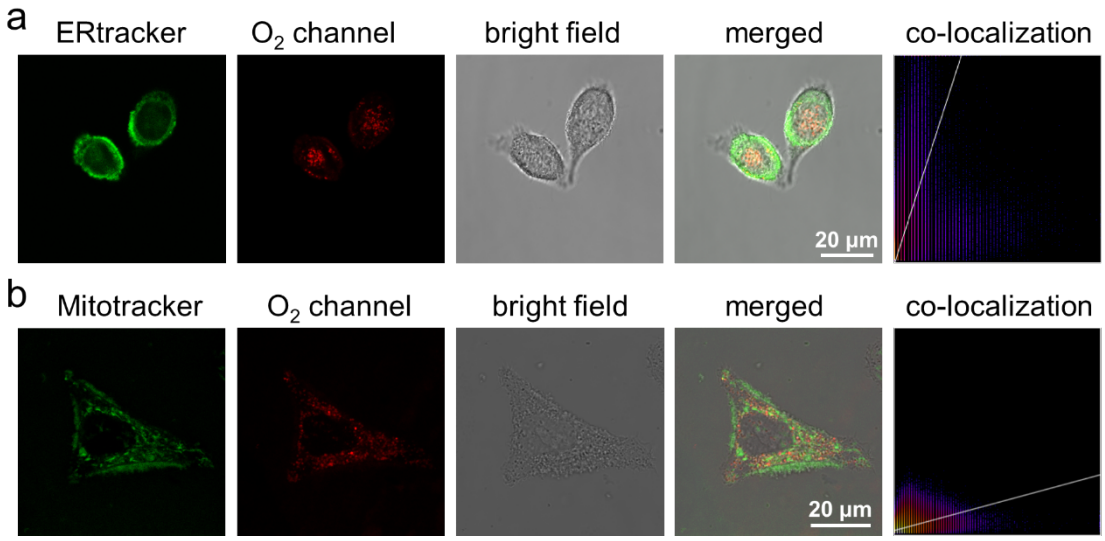

Figure S14. CLSM images of HeLa cells stained with Pd(-Q^+)@PDMS and ERTracker Blue-White (a) and MitoTracker DeepRed (b), respectively.

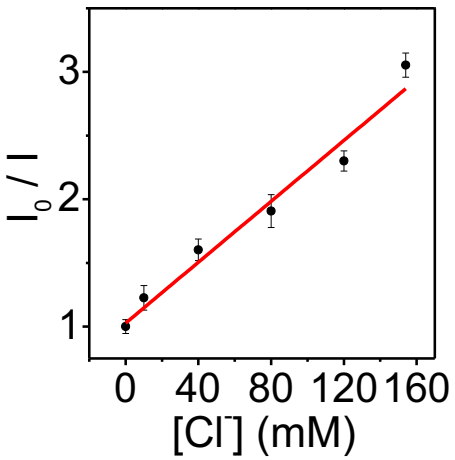

Figure S15. Cellular Cl^- calibration with Pd-Q^+@PDMS. Hela cells were equilibrated with different concentrations of Cl^-. K_{SV} equals to 12.0 M^{-1}.

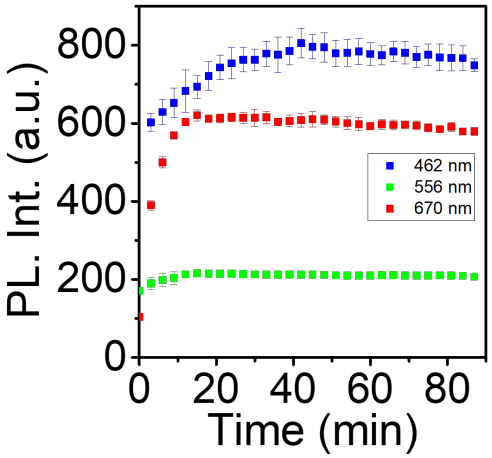

Figure S16. The fluctuation of Cl^- in hypoxic Ramos cell monitored via recording emission at 462 nm, 556 nm and 670 nm. The cells were exposed to a hypoxia shock with 6 mM Na_2SO_3, followed with consecutive recording the fluorescence intensity values.