Supporting Information for

A Targetable Fluorescent Probe for Imaging Exogenous and Intracellular Formed Nitroxy1 at Mitochondria in Living Cells

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**Figure S1** The absorption spectral changes of *Mito-HNO* (10 μM) upon addition of increasing concentrations of AS (0-10 equiv) in PBS buffer, pH 7.4, containing 5 % DMF as a cosolvent. Inset: Photographs showing the color changes of the probe *Mito-HNO* (1 mM) before and after addition of 10 equiv. AS to the solution.

**Figure S2** The emission intensity changes (at 545 nm) of compound 3 at different pH PBS buffer, containing 5 % DMF as a cosolvent (λ<sub>ex</sub> = 488 nm).
Figure S3  (A) The fluorescence intensities at 545 nm of Mito-HNO (5 μM) in the presence of AS (30 μM) at room temperature (25 °C) for continuously monitored at time intervals periods (0-60 min) in PBS buffer (pH 7.4, containing 5 % DMF as a cosolvent). (B) *Pseudo*-first-order kinetic plot of the reaction of Mito-HNO in the presence of AS at 25 °C.

Figure S4  (A) The fluorescence intensities at 545 nm of Mito-HNO (5 μM) in the presence of AS (30 μM) at 37 °C for continuously monitored at time intervals periods (0-7 min) in PBS buffer (pH 7.4, containing 5 % DMF as a cosolvent). (B) *Pseudo*-first-order kinetic plot of the reaction of Mito-HNO in the presence of AS at 37 °C.
**Figure S5** The fluorescence responses of the probe Mito-HNO (10.0 µM) to various relevant species (100 µM) in pH 7.4, PBS buffer (5 % DMF) at 37 °C for 20 min (\(\lambda_{ex} = 488\) nm).

**Figure S6** Cytotoxicity assays of Mito-HNO at different concentrations (0 µM; 5 µM; 10 µM; 20 µM; 30 µM) for HeLa cells in different time periods (A) 4h and (B) 24h.

**Figure S7** Brightfield and fluorescence images of HeLa cells stained with compound 4 and MitoTracker Red a) brightfield image; b) from green channel; c) from the red channel (mitochondria staining); d) overlay of brightfield, green and red channels; e) overlay of green and red channels; f) Intensity profile of linear region of interest across the HeLa cell costained with green channel of compound 4 imaging of HNO and red
channel of Mito Tracker Red; g) Intensity scatter plot of green and red channels.

**Figure S8** $^1$H-NMR (CDCl$_3$) spectrum of compound 3

**Figure S9** $^{13}$C-NMR (DMSO-$d_6$) spectrum of compound 3
Figure S10 $^1$H-NMR (DMSO-$d_6$) spectrum of compound 4.

Figure S11 $^{13}$C-NMR (DMSO-$d_6$) spectrum of compound 4.
**Figure S12** $^1$H-NMR (DMSO-$d_6$) spectrum of compound Mito-HNO.

**Figure S13** $^{13}$C-NMR (DMSO-$d_6$) spectrum of compound Mito-HNO.
**Figure S14** $^{31}$P-NMR (DMSO-$d_6$) spectrum of compound Mito-HNO without reference.

**Figure S15** The purity of the probe Mito-HNO was analyzed by HPLC. (A) Typical HPLC chromatogram with UV/vis detection (254 nm). The retention times of Mito-HNO is 14.5 min. (B) Integration peak list of the results of HPLC chromatogram.