

Supporting Information for

A fast-responsive two-photon fluorescent probe for in vivo imaging superoxide radical anion with a large stokes shift

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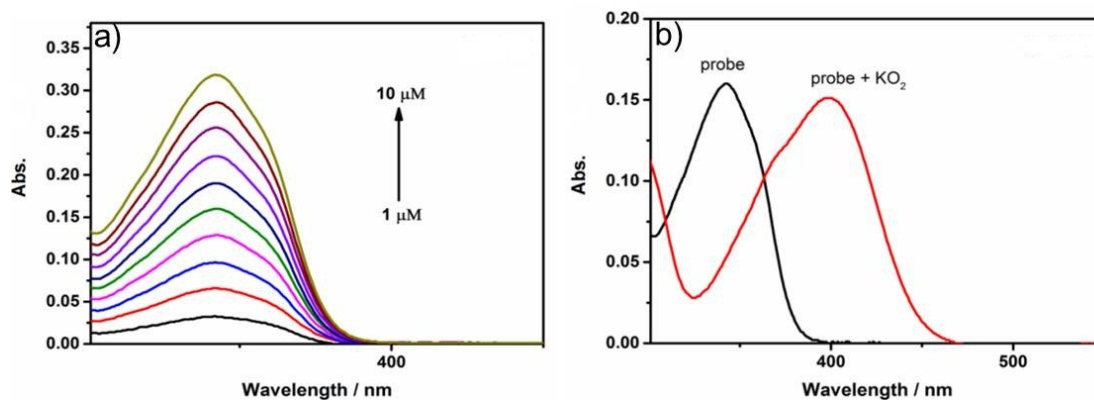


Fig. S1. The absorption spectra of **NS-O**: a) The absorption spectra of **NS-O** ($5 \mu\text{M}$) in pH 7.4 PBS/DMSO ($v/v = 1/1$) in the absence O_2^- . b) The absorption spectra of **NS-O** ($5 \mu\text{M}$) in pH 7.4 PBS/DMSO ($v/v = 1/1$) in the absence or presence of O_2^- (5 equiv)

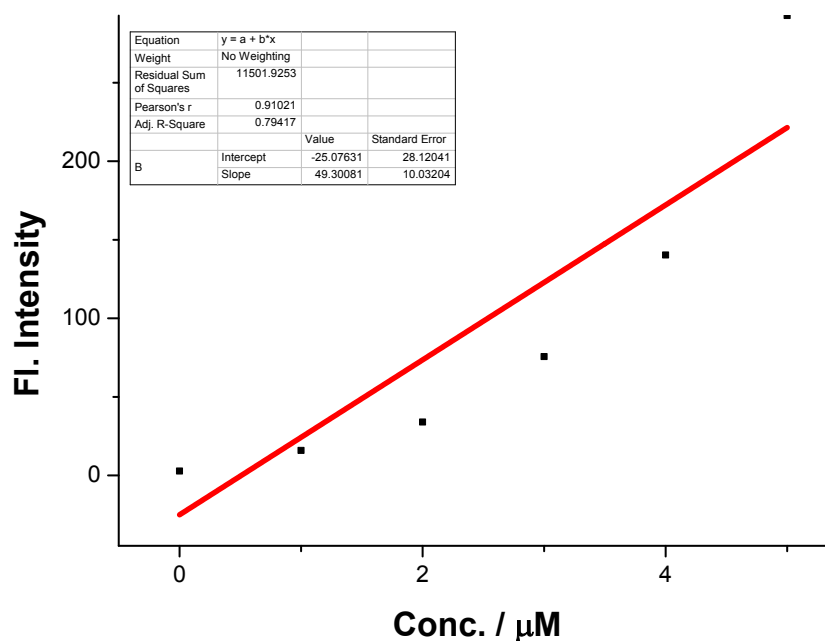


Fig. S2. The linear fit of **NS-O** ($5 \mu\text{M}$) in pH 7.4 PBS buffer (50% DMSO) in the absence or presence of KO_2 (0-1.0 equiv).

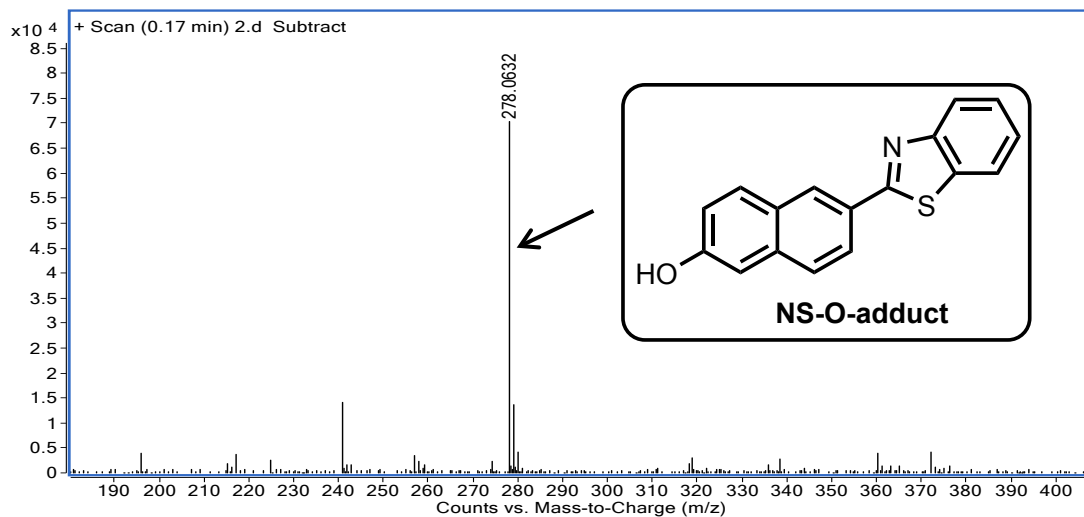


Fig. S3. HRMS (positive ion mode) spectrum of NS-O (20 μM) after treatment with KO_2 (200 μM) in pH 7.4 PBS/DMSO (1: 1) for 60 min. The peak at m/z 278.0632 corresponds to NS-O-adduct.

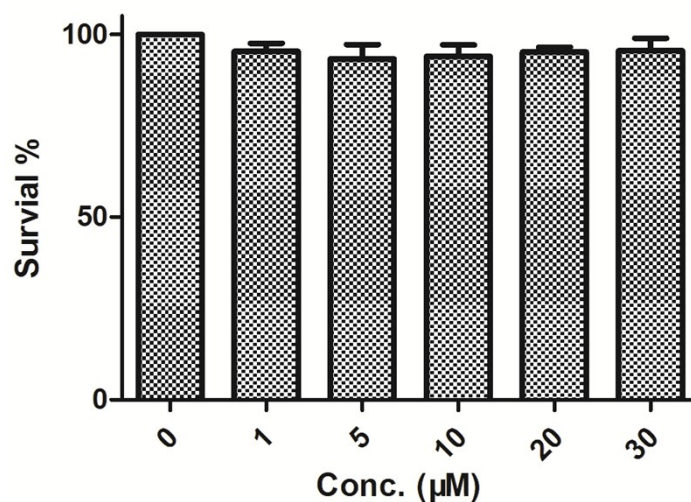


Fig. S4. Cytotoxicity assays of NS-O at different concentrations (0 μM ; 1 μM ; 5 μM ; 10 μM ; 20 μM ; 30 μM) for HeLa cells

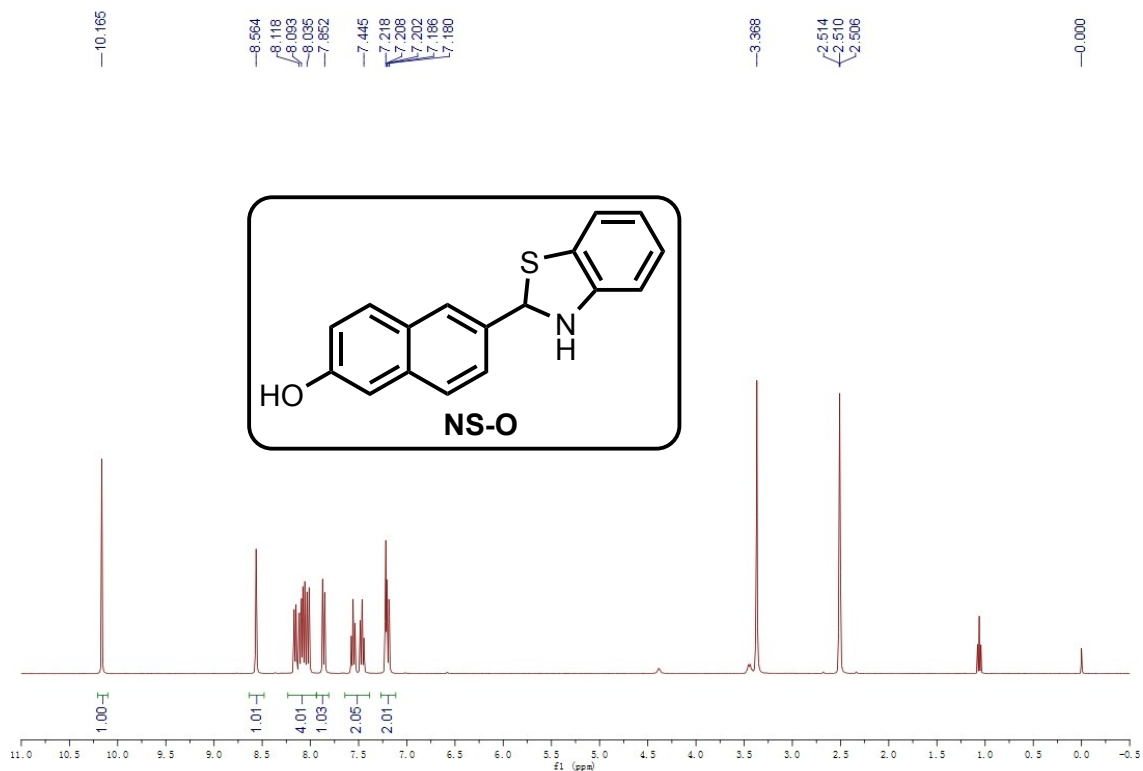


Fig. S5. $^1\text{H NMR}$ (DMSO- d_6) spectrum of NS-O.

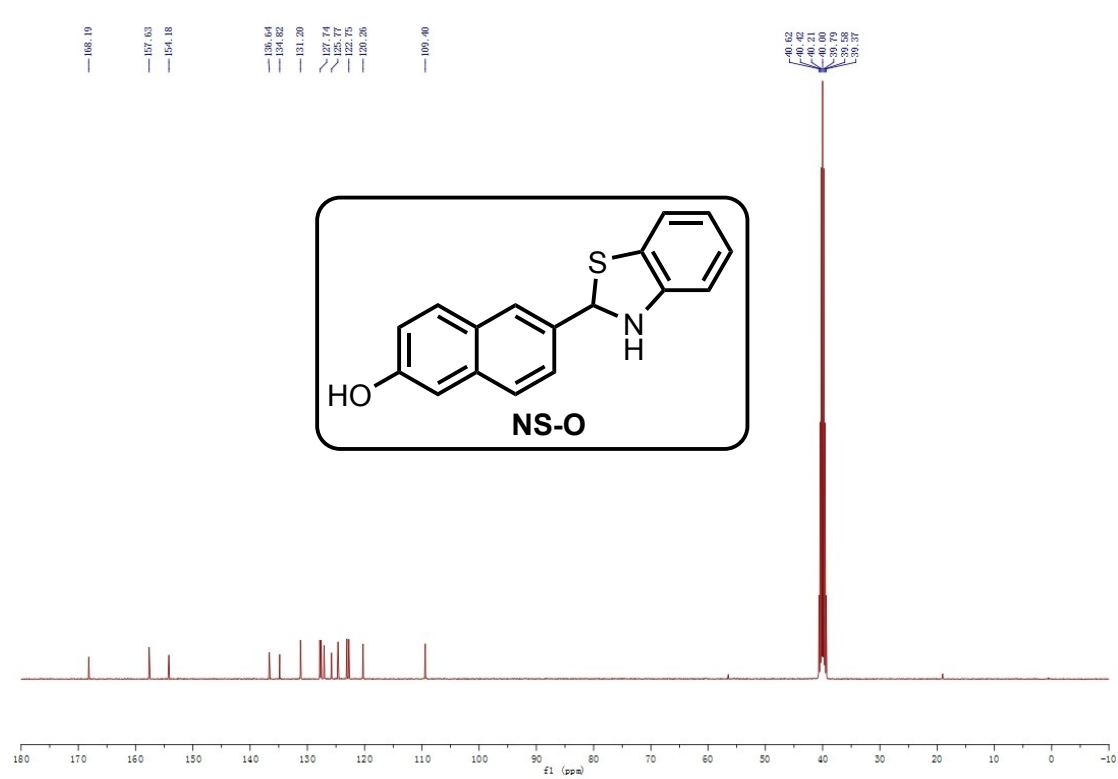


Fig. S6. $^{13}\text{C-NMR}$ (DMSO- d_6) spectrum of NS-O.

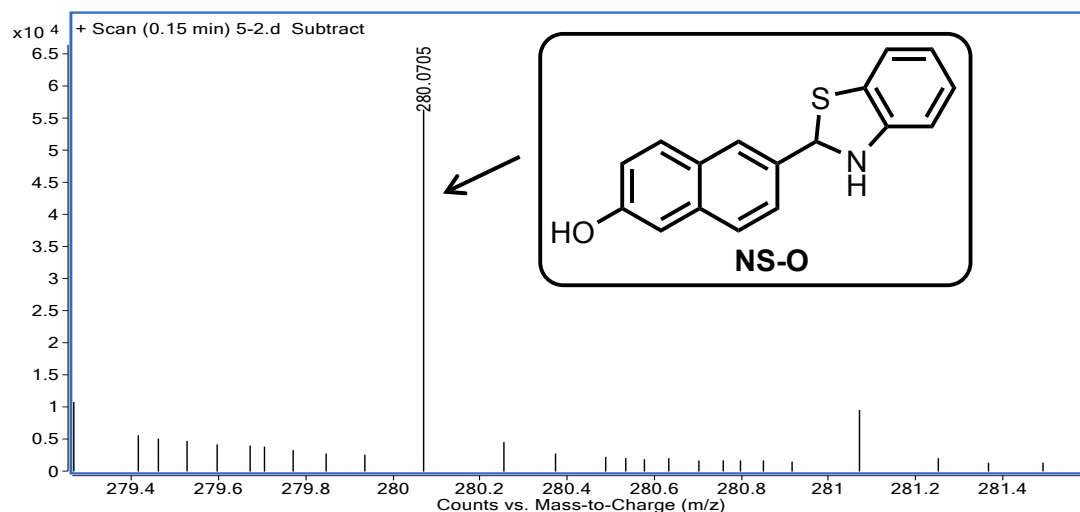


Fig. S7. HRMS spectrum of the probe NS-O.

Probes	Rf.	Response time	Selectivity and application	Detection limit
<p>TPE-CLA</p>	8a	-	Determination of O ₂ ⁻ in PBS Buffer, Living Cells and mice.	0.21nM for FL and 0.38 nM for CL
<p>HQ</p>	8b	About 3 min	Determination of O ₂ ⁻ in PBS Buffer, Living Cells, tissues and Zebrafish.	-
<p>CyR</p>	8c	10 min	Determination of O ₂ ⁻ in HEPES Buffer, Living Cells, Mouse, and Zebrafish.	9.9 nM

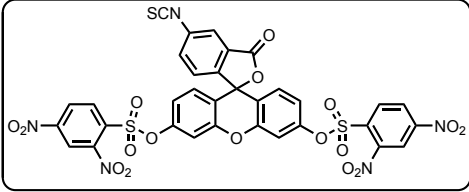
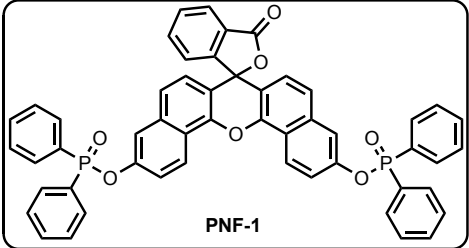
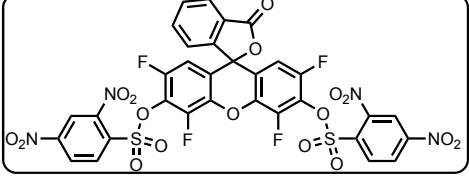
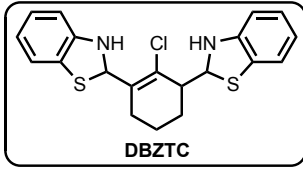
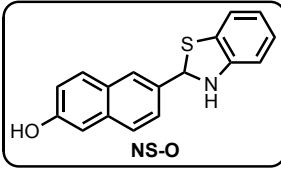
	8d	10 min	Determination of O ₂ ⁻ in PBS Buffer and Living Cells	-
 <p style="text-align: center;">PNF-1</p>	8e	10 min	Determination of O ₂ ⁻ in HEPS Buffer and Living Cells	9.9 nM
	8f	10 min	Not mentioned	1.0 pM
 <p style="text-align: center;">DBZTC</p>	8g	10 min	Determination of O ₂ ⁻ in HEPS Buffer and Living Cells	1.68 nM
 <p style="text-align: center;">NS-O</p>	this work	2 min	Determination of O ₂ ⁻ in PBS Buffer, Living Cells, tissues and Zebrafish.	1.71 μM

Table S1. Properties of the probe **NS-O** and the reported fluorescent probes.