Electronic Supplementary Information for

Hypoxia imaging in living cells, tissues and zebrafish with a

nitroreductase-specific fluorescent probe

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Contents:

- Fig. S1 ¹H NMR and ¹³C NMR spectra of compound 1 and NTNO.
- Fig. S2 HR-MS analysis of NTNO.
- Fig. S3 The pH effect on the fluorescence emission changes of NTNO (5 μ M) in the absence and presence of NTR containing NADH in different pH PBS buffer, respectively
- Fig. S4 Cytotoxic effect of NTNO on HeLa cells





Fig. S1 ¹H NMR, ¹³C NMR spectra of compound **1** and **NTNO**.





Fig. S2 HR-MS analysis of $[NTNO+H]^+$ and the reaction product $([NTNH+H]^+)$ of 5 μ M NTNO with 10 μ g/mL NTR in the presence of 500 μ M NADH.



Fig. S3 The pH effect on the fluorescence emission changes of NTNO (5 μ M) in the absence and presence of NTR (5.0 μ g/mL) containing NADH (500 μ M) in different pH PBS buffer, respectively.



Fig. S4 Cell viability of HeLa cells treated with different concentration of NTNO (0, 1, 2, 5, 10 and 15 μ M) for 24 h in fresh medium.