Supporting Information

A fast-responsive fluorescent turn-on probe for nitroreductase imaging in living cells

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1. Reported fluorescent probes

Table S1. Comparison of fluorescent probes for palladium detection

| Probe | $\lambda_{ex}/\lambda_{em}$ (nm) | Stokes shift (nm) | Response time (min) | Limit of detection (ng/mL) | Reference |
|-------|---------------------------------|------------------|---------------------|---------------------------|-----------|
| ![Probe 1](image1) | ![Probe 2](image2) | ![Probe 3](image3) | ![Probe 4](image4) | ![Probe 5](image5) | ![Probe 6](image6) |
2. The characterization of NTR-NO$_2$

Fig. S1: $^1$H NMR spectrum of NTR-NO$_2$
3. The measurement of fluorescence quantum yields

The quantum yield values were calculated by using coumarin-153 in ethanol ($\Phi = 0.38$) as a standard according to the following formula$^{1-3}$:

$$Y_u = Y_s \cdot \frac{F_u}{F_s} \cdot \frac{A}{A_s} \cdot \left[ \frac{G_u}{G_s} \right]^2$$
Where, $Y_u$ is the quantum yield of NTR-NH$_2$; $Y_s$ is the quantum yield of coumarin-153 ($\Phi = 0.38$) in ethanol; $F$ is the integrated emission intensity (peak area); $A$ is the absorbance at $\lambda_{ex}$.

**Table S2. Photophysical properties of NTR-NH$_2$**

(DMSO:PBS=1:5, pH = 7.4)

| Compound | $\lambda_{abs}$ (nm) | $\lambda_{em}$ (nm) | Stokes shift (nm) | $Y_u$ |
|----------|----------------------|---------------------|-------------------|-------|
| NTR-NH$_2$ | 430 | 541 | 111 | 0.43 |

4. The HRMS analysis of the products

![HRMS spectrum of NTR-NO$_2$](image)

Fig. S4: HRMS spectrum of NTR-NO$_2$
5. The fluorescent spectra of NTR-NO₂ responding with NaBH₄

![Fluorescent spectra graph]

**Fig. S5:** The fluorescence spectra of probe NTR-NO₂ (10μM) incubated with NTR (red) and NaBH₄ (black) in the presence of NADH (500μM)

6. Cytotoxicity assays of probe NTR-NO₂ at different concentrations

![Cytotoxicity assay graph]

**Fig. S6:** MTT assay for the viability of HeLa cells treated with various concentrations of probe NTR-NO₂ for 24h
7. Reference

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3. D. R. Haynes, A. Tokmakoff, S. M. George, Chemical Physics Letters, 1993, 214, 50-56.