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

<table>
<thead>
<tr>
<th>Probe</th>
<th>$\lambda_{ex}/\lambda_{em}$ (nm)</th>
<th>Stokes shift (nm)</th>
<th>Response time (min)</th>
<th>Limit of detection (ng/mL)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Probe 1" /></td>
<td>564/586</td>
<td>22</td>
<td>90</td>
<td>——</td>
<td>Dyes and Pigments 2019, 171.</td>
</tr>
<tr>
<td><img src="image2.png" alt="Probe 2" /></td>
<td>467/526</td>
<td>59</td>
<td>60</td>
<td>27</td>
<td>Sensors and Actuators B: Chemical 2018, 276, 397-403.</td>
</tr>
<tr>
<td><img src="image5.png" alt="Probe 5" /></td>
<td>470/520</td>
<td>50</td>
<td>5</td>
<td>9.6</td>
<td>Analyst, 2015, 140,</td>
</tr>
</tbody>
</table>
2. The characterization of NTR-NO$_2$

![NMR spectra](image)

**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\(^1\sim^3\):

\[
Y_u = Y_S \cdot \frac{F_u}{F_s} \cdot \frac{A}{A_u} \cdot \left[ \frac{G_u}{G_S} \right]^2
\]
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)

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{abs}$ (nm)</th>
<th>$\lambda_{em}$ (nm)</th>
<th>Stokes shift (nm)</th>
<th>$Y_u$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTR-NH$_2$</td>
<td>430</td>
<td>541</td>
<td>111</td>
<td>0.43</td>
</tr>
</tbody>
</table>

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](image)

**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](image)

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