Supporting Information

Ratiometric fluorescent imaging for endogenous selenocysteine in cancer cell matrix

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## 1. Comparison of previous probes for selenocysteine detection

### Table S1. Comparison of previous probes for selenocysteine detection

<table>
<thead>
<tr>
<th>Probe</th>
<th>Detection condition</th>
<th>Detection limit</th>
<th>Response time</th>
<th>$\lambda_{ex}/\lambda_{em}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Probe 1" /></td>
<td>pH 5.8 PBS/0.1% EtOH</td>
<td>4 nM</td>
<td>10 min</td>
<td>450/580 nm</td>
<td>Angew. Chem. Int. Ed., 2006, 45, 1810–1813</td>
</tr>
<tr>
<td><img src="image2.png" alt="Probe 2" /></td>
<td>pH 7.4 PBS/1% DMSO</td>
<td>62 nM</td>
<td>5 min</td>
<td>370/502 nm</td>
<td>J. Am. Chem. Soc., 2015, 137, 757–769</td>
</tr>
<tr>
<td><img src="image3.png" alt="Probe 3" /></td>
<td>pH 7.4 PBS/1% DMSO</td>
<td>7 nM</td>
<td>30 min</td>
<td>460/580 nm</td>
<td>Chem. Commun., 2015, 51, 3102–3105</td>
</tr>
<tr>
<td><img src="image4.png" alt="Probe 4" /></td>
<td>pH 7.4 PBS</td>
<td>1.5×10^{-7} M</td>
<td>5 min</td>
<td>360/550 nm</td>
<td>Anal. Chem., 2016, 88, 7259–7267</td>
</tr>
<tr>
<td><strong>This work</strong></td>
<td>pH 7.4 PBS/5% DMSO</td>
<td>12 nM</td>
<td>1 min</td>
<td>380/420, 546 nm</td>
<td></td>
</tr>
</tbody>
</table>
2. Synthesis of Rat-Sec

Nap-OH (269.3 mg, 1 mmol) and triethylamine (101.2 mg, 0.3 mmol) were dissolved in 10 mL anhydrous dichloromethane, and then acryloyl chloride (135.8 mg, 1.5 mmol) was added into the solution, the system was stirred at room temperature for 6h under argon protection. After reaction was over, the solvent was removed under reduced pressure, and the residues were purified by silica gel chromatography with dichloromethane to obtain the desired product Rat-Sec (171.3 mg, 0.53 mmol), a white solid. Yield was 53%. ¹H NMR (400 MHz, CDCl₃, ppm) δ: 0.98 (t, 3H), 1.41-1.50 (m, 2H), 1.65-1.78 (m, 2H), 4.18 (t, 2H), 6.20 (d, 1H), 6.50 (dd, 1H), 6.79 (d, 1H), 7.59 (d, 1H), 7.76 (t, 1H), 8.24 (d, 1H), 8.57-8.667 (m, 2H). ESI-MS calcd. for C₁₉H₁₈NO₄ [M+H⁺]: 324.1158; found: 324.1283.

Scheme S1 Synthetic route of Rat-Sec
3. Characterization of Rat-Sec

Fig. S1 $^1$H NMR spectrum of Rat-Sec

Fig. S2 HRMS spectrum of Rat-Sec
4. Measurement of quantum yield for Rat-Sec and Nap-OH

The quantum yield of Rat-Sec and Nap-OH were detected in PBS/5% DMSO solution. The standard samples of quinine sulfate ($\Phi=0.54$, in $0.5 \text{ M H}_2\text{SO}_4$) and rhodamine 6G ($\Phi=0.94$, in ethanol) were used as reference for Rat-Sec and Nap-OH respectively. The quantum yield can be calculated via the following equation:

$$\Phi_x = \Phi_s \times \left( \frac{G_x}{G_s} \right) \times \left( \frac{\eta_s^2}{\eta_x^2} \right)$$

Where the subscripts x and s denote test and standard sample respectively, $\Phi$ is the fluorescence quantum yield, $G$ the gradient from the plot of integrated fluorescence intensity vs absorbance, and $\eta$ the refractive index of the solvent.

The plot of integrated fluorescence intensity vs absorbance is given below, then the values of quantum yield are 0.077 (Rat-Sec) and 0.32 (Nap-OH).

Fig. S3 The linear plots of integrated fluorescence intensity vs absorbance for quinine sulfate and Rat-Sec (a); Rhodamine 6G and Nap-OH (b).

5. UV-vis responses of Rat-Sec toward various concentration of Sec

Fig. S4 Absorption spectra of Rat-Sec (5 µM) upon addition of Sec (0–50 µM).
6. HPLC chromatogram of Rat-Sec and Nap-OH

![HPLC chromatogram](image)

**Fig. S5** HPLC chromatogram of Rat-Sec before and after 5 min reaction with Sec, and Nap-OH. Eluent solvent: methanol/H$_2$O (v/v, 8/2), flow rate = 1 mL min$^{-1}$, detection wavelength at 380 nm.

7. ESI-MS spectra characterization of Rat-Sec reaction with Sec

![ESI-MS spectra](image)

**Fig. S6** ESI-MS spectra characterization of Rat-Sec reaction with Sec.
8. Photos of Rat-Sec toward various analytes

Fig. S7 Photos of Rat-Sec toward various analytes under daylight.

9. Semiquantitative determination of exogenous and endogenous Sec in living cells

Fig. S8 Semiquantitative determination of endogenous Sec in Hela cells according to the ratio of averaged fluorescence intensity of green-yellow channel (500–600 nm) to blue channel (410–460 nm).
10. Calibration curve of Sec and quantification of Sec in cells

Fig. S9 Calibration curve for quantification of Sec concentration from the ratio value. Ratio value is from signals between green-yellow channel (500–600 nm) and blue channel (410–460 nm).