Electronic Supplementary Information

Phosphorescent soft salt for ratiometric and lifetime imaging of intracellular pH variations

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Fig. S1 Normalized absorption spectrum of S1 in acetonitrile solution.
Fig. S2 Plot of $I_{625\text{nm}}$ versus pH value.
Fig. S3 pH reversibility study of S1 between pH 2 and 8.
**Fig. S4** Phosphorescence spectra of S1 (2.0 × 10^{-5} M) in the presence of 1.0 × 10^{-4} M of oxidative-stress-associated redox chemicals and metal ions (Cys, Hcy, GSH, H_2O_2, K^+, Na^+, Zn^{2+}, Cu^{2+}, Ca^{2+}, Mn^{2+}, Mg^{2+}, Fe^{2+}, and Fe^{3+}) in CH_3CN/buffer (1 : 9, v : v).
**Fig. S5** Relative ratio of phosphorescence intensity ($I_{625\ nm}/I_{451\ nm}$) changes of S1 in the pH range of 2–8 at 37 °C.
Fig. S6 Cell viability values (%) assessed using an MTT test versus incubation concentrations of S1. HepG-2 cells were cultured in the presence of 0–200 μM S1 at 37 °C for 24 h.
**Fig. S7** (a, b) Confocal luminescence, (c) overlay images and (d) bright-field of living HepG-2 cells. HepG-2 cells were incubated with 10 μM S1 for 1 h at 37 °C.
Fig. S8 Real-time monitoring of live cells stained with A1 and C1, respectively.
Fig. S9 Real-time monitoring of live cells stained with S1.
<table>
<thead>
<tr>
<th>pH</th>
<th>3.98</th>
<th>5.02</th>
<th>6.08</th>
<th>7.01</th>
<th>8.01</th>
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</thead>
</table>

**A1**

**C1**

**Fig. S10** Phosphorescence images of **A1** and **C1** in HepG-2 cells clamped at pH 3.98, 5.02, 6.08, 7.01 and 8.0, respectively.
Fig. S11 Average intracellular emission intensity of A1 and C1 at different pH values.
**Fig. S12** Phosphorescence images of S1 (1 μM) in living cells clamped at pH 4.02, 5.03, 5.99, 7.06 and 8.04, respectively. The excitation wavelength was 405 nm and the images of the first row (blue channel) and second row (red channel) were collected in the ranges of 430–480 nm and 600–700 nm, respectively. Overlay images (third row) and ratio images obtained from the red and blue channels (fourth row).
Fig. S13 Phosphorescence decay curves of S1 (10 μM) at 625 nm at different pH values.
**Fig. S14** Phosphorescence decay curves in cells stained with S1 (10 μM) at different pH values, obtained by confocal TCSPC-PLIM.
**Fig. S15** Phosphorescence decay curves of S1 (10 μM) in RPMI 1640 at different pH values, obtained by confocal TCSPC-PLIM.
**Fig. S16** Phosphorescence lifetime images of S1 (10 μM) in RPMI 1640 at different pH values.
**Fig. S17** Emission lifetime changes of S1 (10 μM) at different pH values in the cells and RPMI 1640, respectively, obtained by confocal TCSPC-PLIM.
**Fig. S18** Phosphorescence lifetime images of S1 (1 μM) in living cells at different pH values.
Fig. S19 Emission lifetime changes of S1 (1 μM) at different pH values in the cells, obtained by confocal TCSPC-PLIM.
**Fig. S20** Phosphorescence intensity images collected at different time ranges (0-700 ns).
Fig. S21 $^1$H NMR (400 MHz, DMSO-$d_6$) and mass spectra of A1.
Fig. S22 ¹H NMR (400 MHz, acetonitrile-$d_3$) and mass spectra of C1.
Fig. S23 $^1$H NMR (400 MHz, acetonitrile-$d_3$) spectrum of S1.
## Table S1 Photophysical properties of complexes A1, C1 and S1

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Solvent</th>
<th>λ&lt;sub&gt;abs&lt;/sub&gt; nm (logε)</th>
<th>λ&lt;sub&gt;PL&lt;/sub&gt; nm</th>
<th>τ&lt;sub&gt;PL&lt;/sub&gt;</th>
<th>Φ&lt;sub&gt;em&lt;/sub&gt;</th>
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<tr>
<td>A1</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;CN</td>
<td>251 (5.21) 293 (4.83) 364 (4.31)</td>
<td>451, 475</td>
<td>1329</td>
<td>0.62</td>
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<tr>
<td>C1</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;CN</td>
<td>249 (4.99) 291 (4.73) 386 (4.10)</td>
<td>633</td>
<td>413</td>
<td>0.18</td>
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<tr>
<td>S1</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;CN</td>
<td>249 (5.21) 289 (4.90) 364 (4.34)</td>
<td>453, 480, 633</td>
<td>819/398</td>
<td>0.22</td>
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</table>