

*Electronic Supporting Information*

**FRET-based sensor for visualizing pH variation with colorimetric/ratiometric and application for bioimaging in living cells, bacteria and zebrafish**

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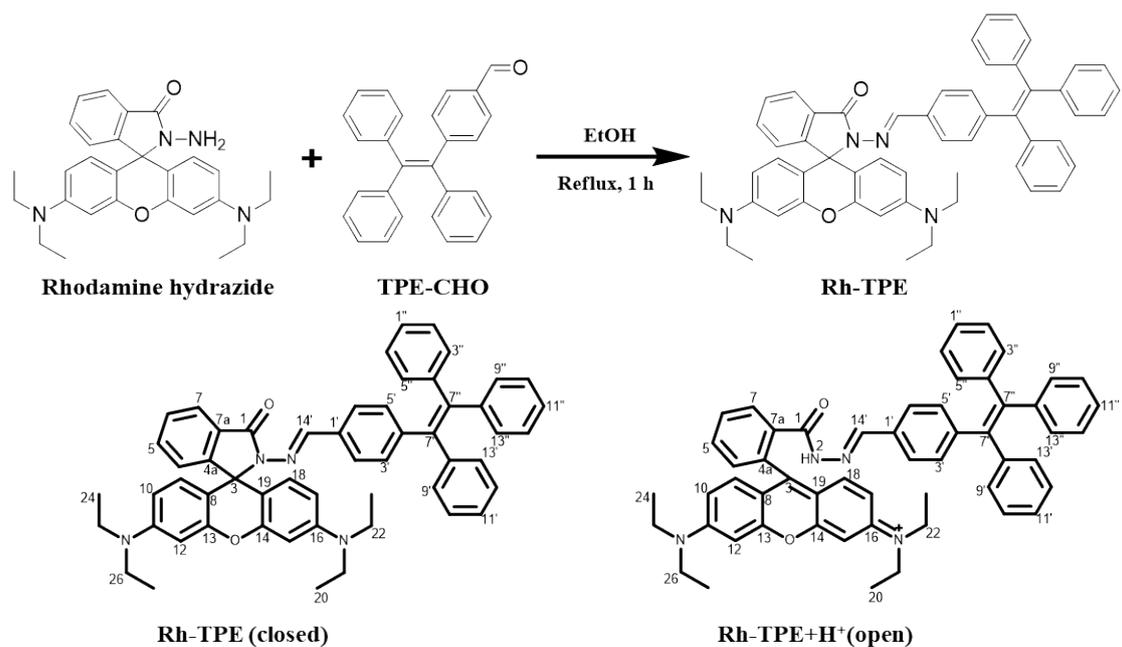
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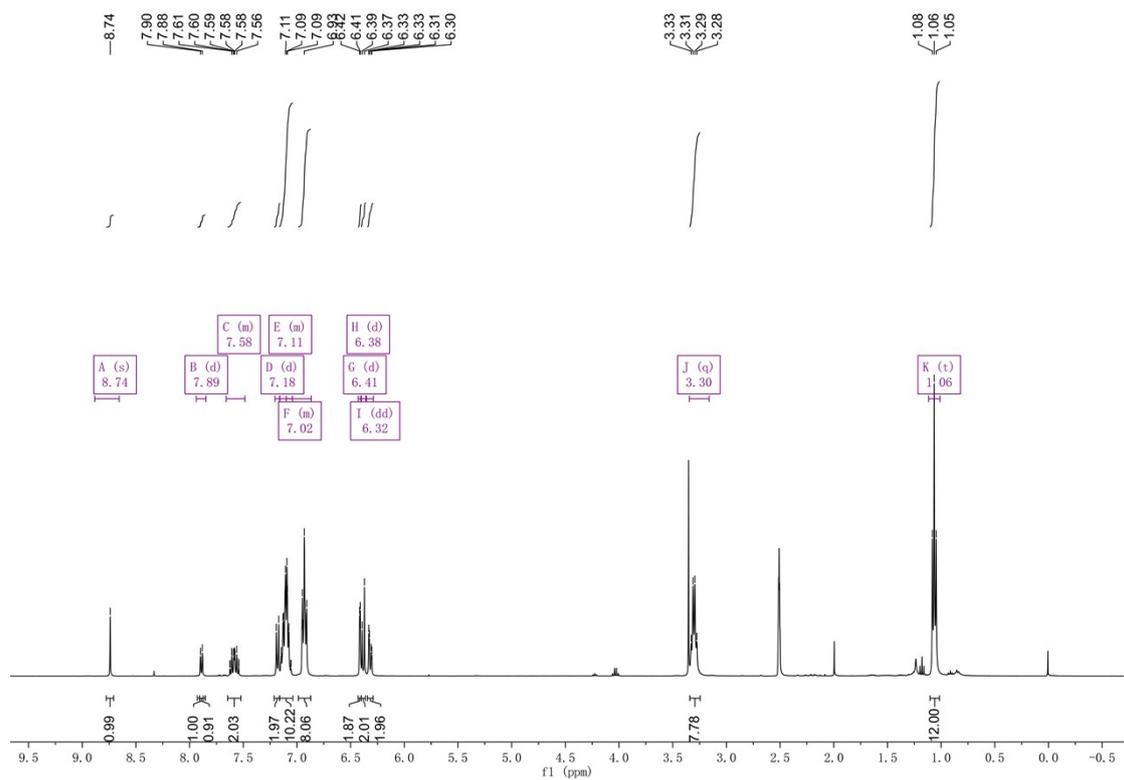
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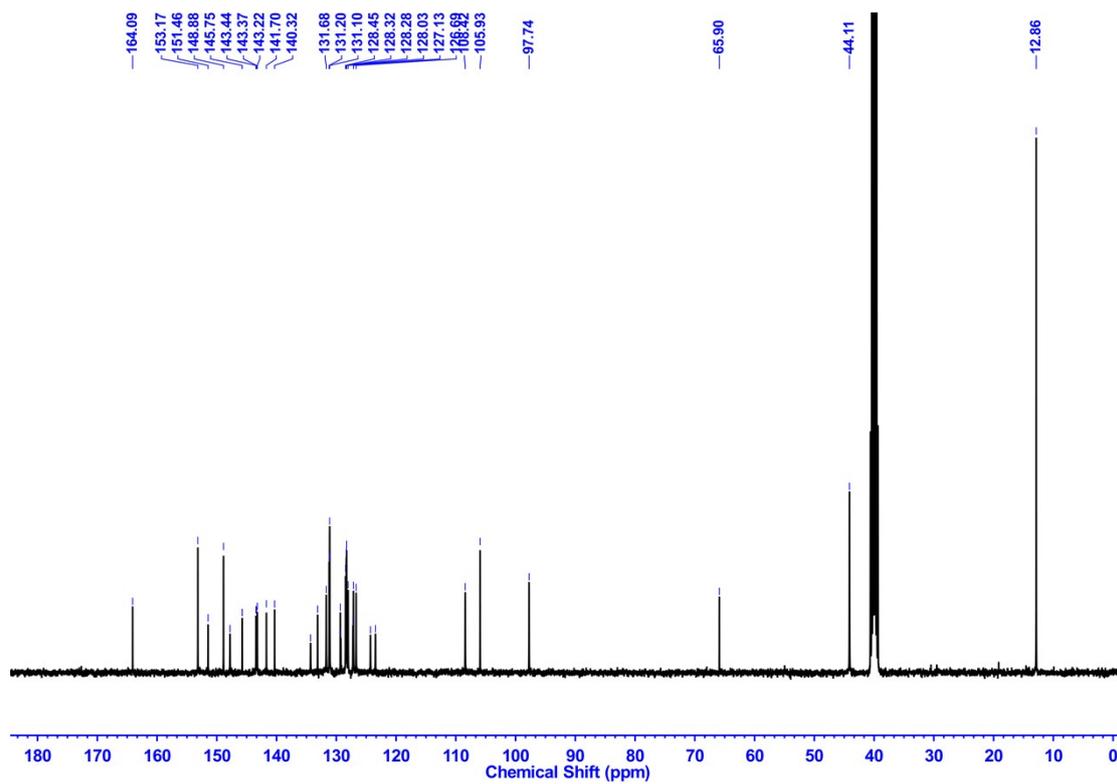
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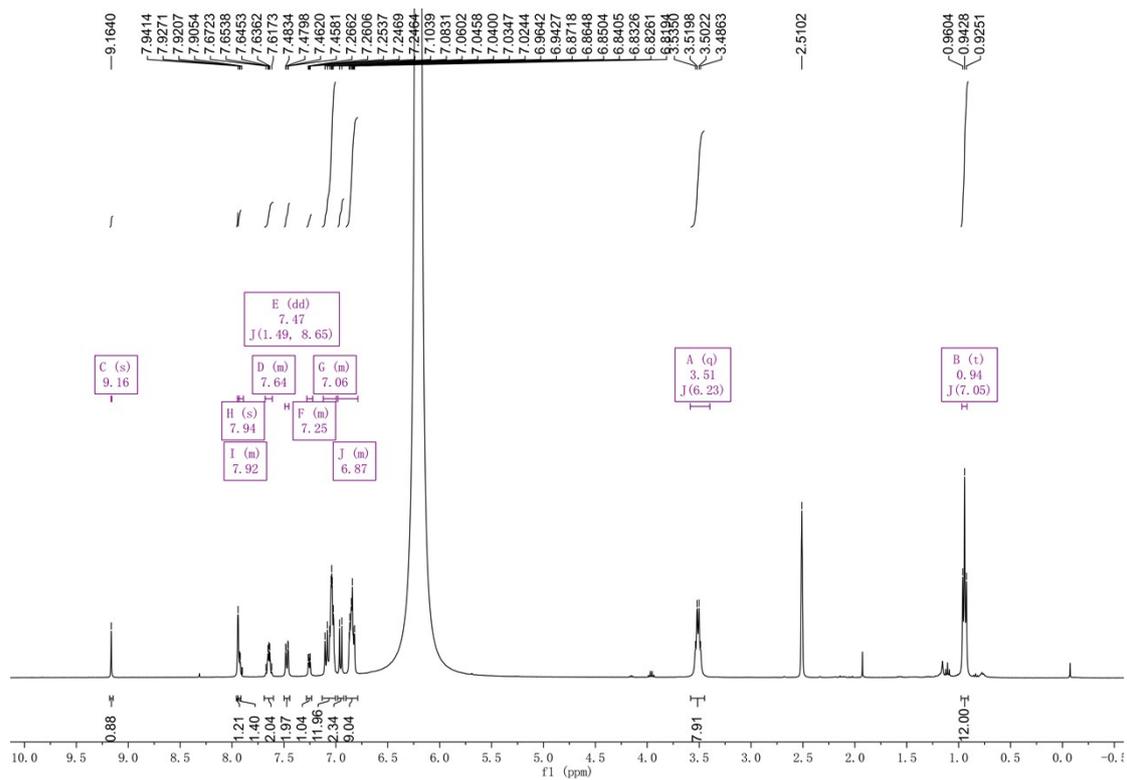
**Scheme S1** Synthetic routes of **Rh-TPE** sensor, and the structure of **Rh-TPE** in neutral and acidic condition.



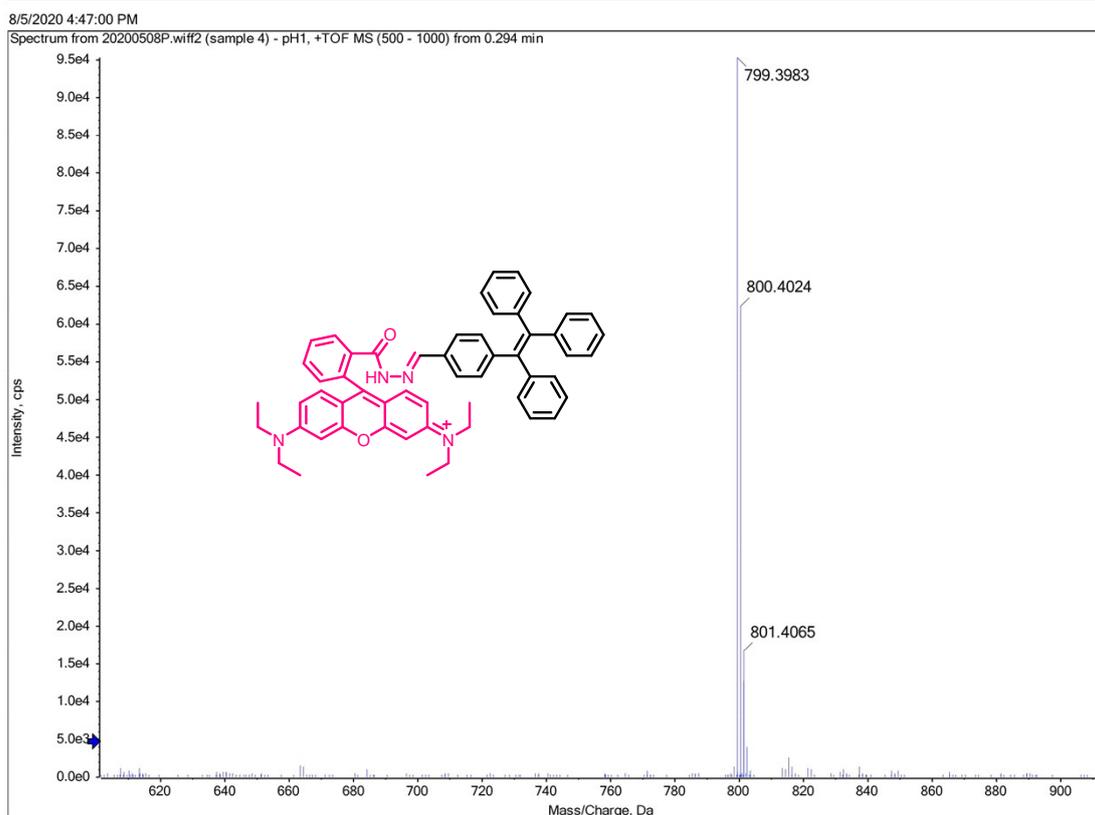
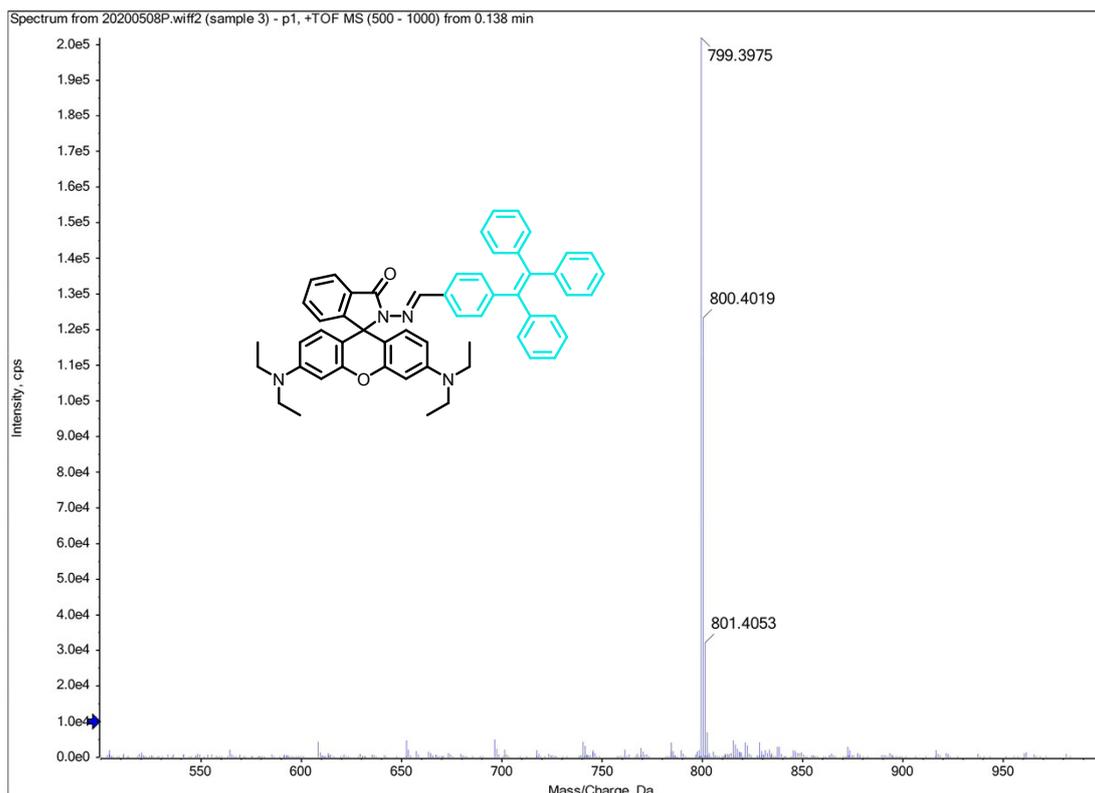
**Figure S1**  $^1\text{H}$  NMR spectra of Rh-TPE sensor in  $\text{DMSO-}d_6$ .



**Figure S2**  $^{13}\text{C}$  NMR spectra of **Rh-TPE** sensor in  $\text{DMSO-}d_6$ .

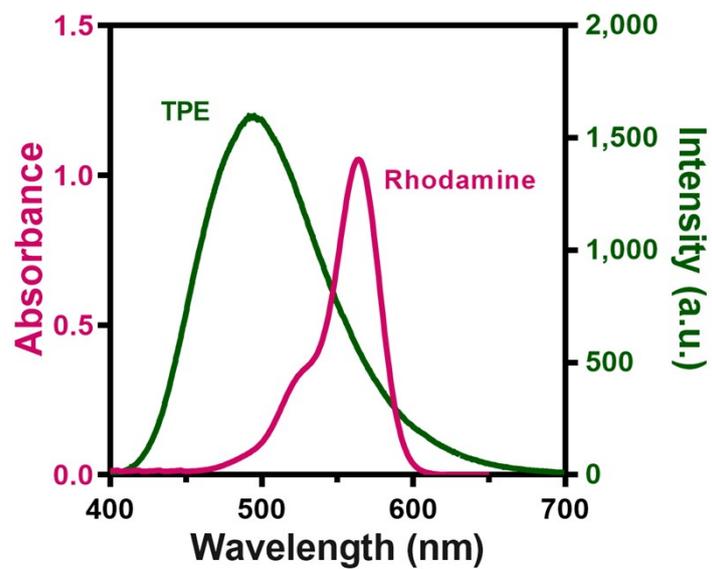


**Figure S3**  $^1\text{H}$  NMR spectra of Rh-TPE sensor after addition of  $\text{H}^+$  in  $\text{DMSO-}d_6$ .

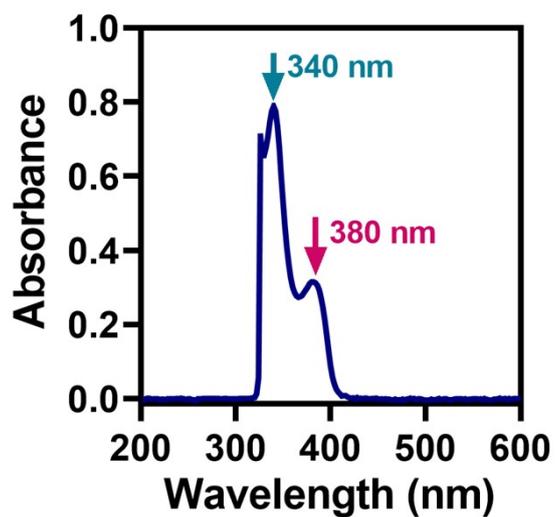


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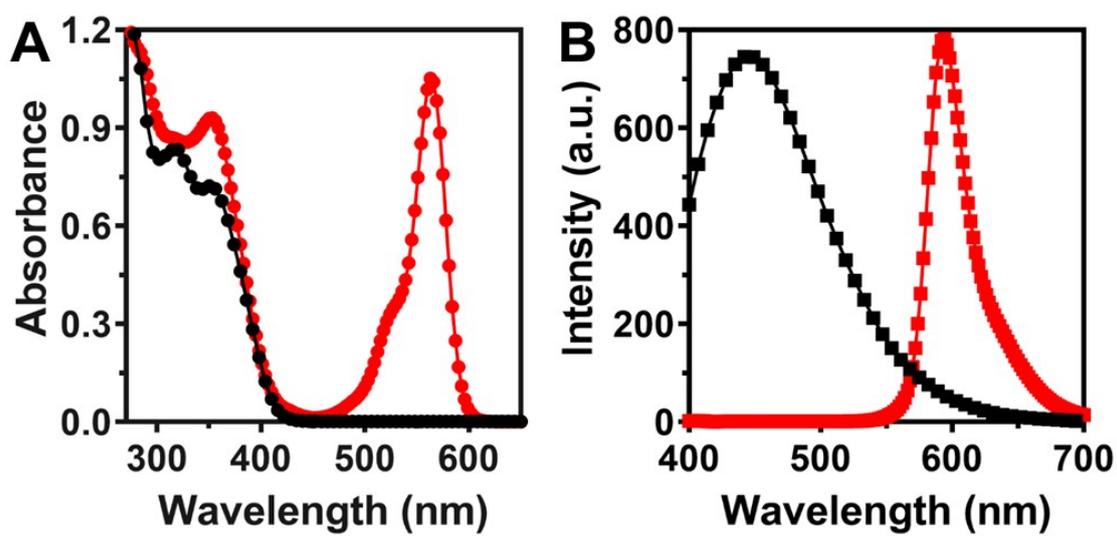
**Figure S4** Mass spectrum of Rh-TPE with and without addition of H<sup>+</sup> in EtOH.



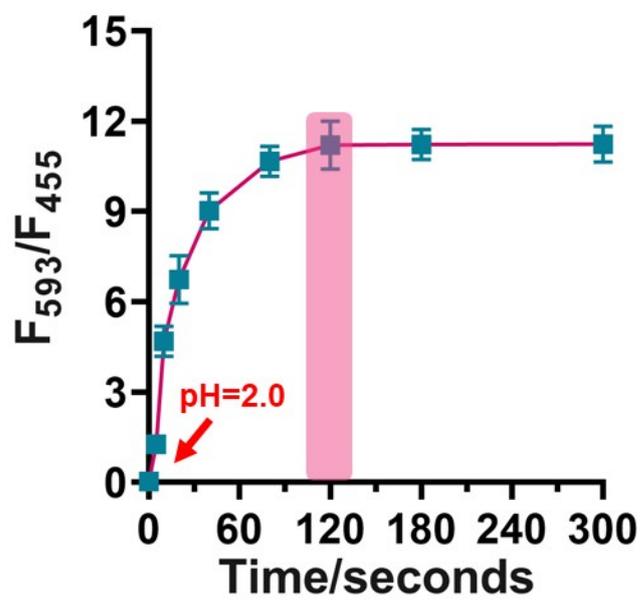
**Figure S5** The overlap spectra of the absorption of Rhodamine and emission of TPE.



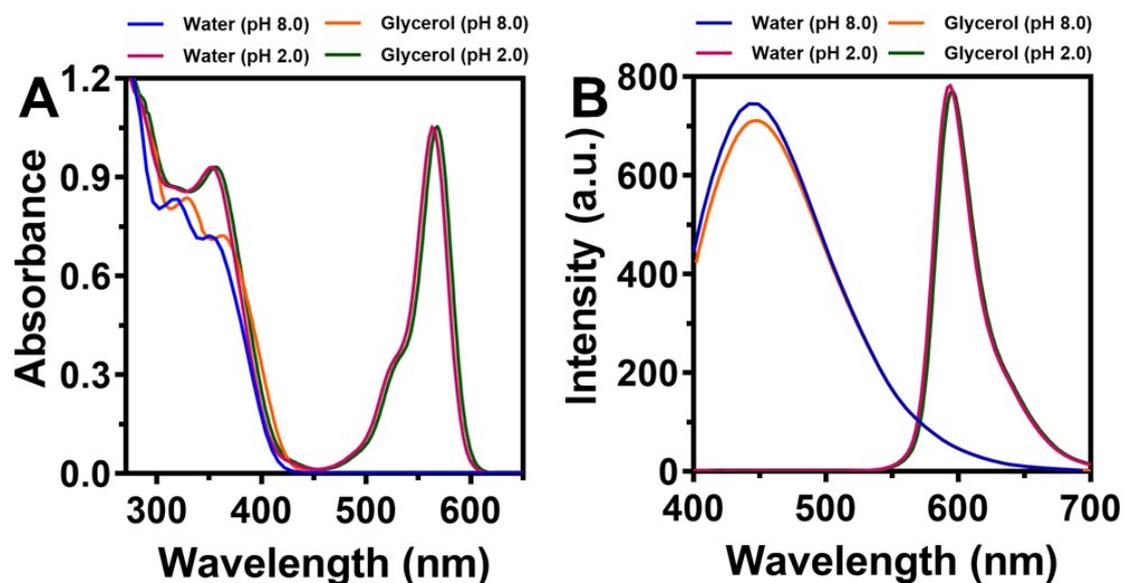
**Figure S6** The excitation spectrum of Rh-TPE in buffer (1% DMSO) at pH 8.4.



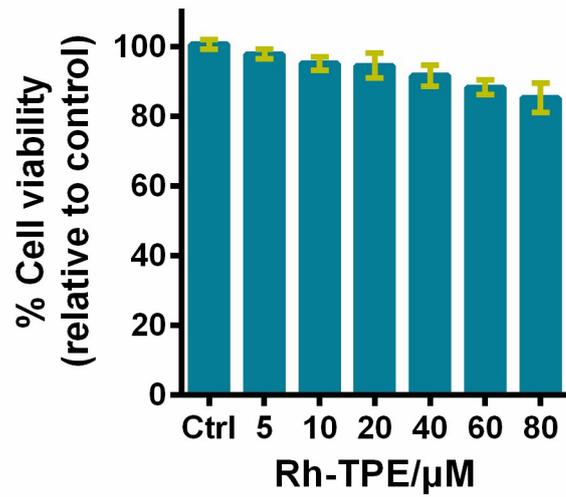
**Figure S7** (A) Absorption spectra (10  $\mu\text{M}$ ) and (B) fluorescence spectra (5  $\mu\text{M}$ ) of **Rh-TPE** in BR buffer (include 1% DMSO) at pH 8.4 (black line) and pH 2.0 (red line), respectively.



**Figure S8** Time-depend fluorescence responses ( $F_{593}/F_{455}$ ) of 5  $\mu\text{M}$  **Rh-TPE** at pH 2.0 buffer solution.



**Figure S9** Detection the effect of viscosity on the sensor in water-glycerol system. (A) Absorption spectra of **Rh-TPE** (10  $\mu\text{M}$ ) in the water (containing 1% DMSO) and glycerol (containing 1% DMSO) at pH 8.0 and pH 2.0. (B) Fluorescence spectra of **Rh-TPE** (5  $\mu\text{M}$ ) in the water (containing 1% DMSO) and glycerol (containing 1% DMSO) at pH 8.0 and pH 2.0.



**Figure S10** Cell viability test using the MTT assay in hPDLCs cells at 24 h of culture. PBS treatment as a control group. The concentration of **Rh-TPE** sensor varied from 5 to 80  $\mu\text{M}$ . Results are presented as the mean of the three measurements  $\pm$  standard deviation. (n = 3)