## **Supporting Information**

## Real-time Imaging Viscosity in Mitochondrial Matrix by a Red-emissive Molecular Rotor

Lin Yuan,<sup>†</sup><sup>a</sup> Dong Wang,<sup>†</sup><sup>a</sup> Shan Shan,<sup>†</sup><sup>a</sup> Juan Chen,<sup>a</sup> Wei Huang,<sup>a</sup> Zhengjie Liu,<sup>\*</sup><sup>a</sup> Guangmei Han,<sup>a</sup> Xiaohe Tian,<sup>a</sup> Ruilong Zhang,<sup>a,b</sup> Zhongping Zhang<sup>a,b</sup>

<sup>a</sup>School of Chemistry and Chemical Engineering, and Institute of Physical Science and Information Technology, Anhui University, Hefei 230601, China. <sup>b</sup>Key Laboratory of Structure and Functional Regulation of Hybrid Materials (Anhui University), Ministry of Education, Hefei, Anhui 230601, China †These authors contributed equally to this work. \*E-mail address: zjliu@ahu.edu.cn



Fig. S1 <sup>1</sup>H-NMR spectrum of compound 1 in deuterated methanol.



Fig. S2 <sup>13</sup>C-NMR spectrum of compound 1 in deuterated methanol.



Fig. S3 HR-MS spectrum of BSP.



**Fig. S4** <sup>1</sup>H-NMR spectrum of BSP in d<sub>6</sub>-DMSO.



50 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 f1 (ppm) Fig. S5  $^{13}$ C-NMR spectrum of BSP in d<sub>6</sub>-DMSO.



Fig. S6 Absorption spectrum of BSP in PBS and glycerine.



Fig. S7 Fluorescence spectra of the BSP probe in PBS and glycerine.



Fig. S8 Temperature effects of probe BSP ( $10 \mu M$ ) in glycerine.



Fig. S9 pH effects of probe BSP ( $10 \mu M$ ) in PBS.



**Fig. S10** Hep G2 cell viability in the presence of BSP measured by the MTT assay. The error bars represent the mean errors from 5 measurements.



**Fig. S11** The fluorescence lifetime selectivity of BSP (10  $\mu$ M) to various analytes: 1: blank, 2: RNA, 3: DNA, 4: BSA, 5: Ca<sup>2+</sup>, 6: Mg<sup>2+</sup>, 7: H<sub>2</sub>O<sub>2</sub>, 8: HClO, 9: H<sub>2</sub>S, 10: Cys, 11: Lys, 12: Trp, 13: glycerine. The error bars represent the mean errors from 3 tests.



Fig. S12 Fluorescence lifetime decays spectra of BSP in PBS (red) and glycerine (green).