Supporting Information for
Ratiometric Fluorescence Imaging of Lysosomal Zn$^{2+}$
Release under Oxidative Stress in Neural Stem Cells

Hao Zhu, a Jiangli Fan, *a Shiling Zhang, a Jianfang Cao, a Kedong Song, b Dan Ge, b Huijuan Dong, a Jingyun Wang, b and Xiaojun Peng *a

a State Key Laboratory of Fine Chemicals, Dalian University of Technology, No. 2 Linggong Road, High-tech District, Dalian 116024, China
b School of Life Science and Biotechnology, Dalian University of Technology, Dalian, China.

Email: fanjl@dlut.edu.cn; pengxj@dlut.edu.cn

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**Fig. S1** Fluorescence ratio ($F_{610\text{nm}}/F_{578\text{nm}}$) of **LysoZn-1** (1 µM) changes as a function of pH in ethanol/10 mM Tris-HCl = 4/6, v/v. Excitation wavelength was 545 nm.

**Fig. S2** Fluorescence spectra of 1 µM **LysoZn-1** in the absence (black line) and presence of 200 µM Zn$^{2+}$ (red line) and Cd$^{2+}$ (blue line) in ethanol/50 mM CH$_3$COOH-CH$_3$COONa = 9/1, v/v, pH 5.0.
**Fig. S3** The charge numbers of atoms on 1 (a) and LysoZn-1 (b), respectively, calculated by method of DFT (B3LYP/6-31g(d, p)) using Gaussian 09. The arrow pointed number indicate the charge numbers of the tertiary amine nitrogen atom of DPA in 1 and LysoZn-1.
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a) Ground State

LUMO -2.49664 ev

$\Delta E = 2.39161$ ev
$f = 1.2550$
$\lambda_{abs} = 558$ nm

HUMO -4.88825 ev

1+Cd$^{2+}$

b) Ground State

LUMO -3.13720 ev

$\Delta E = 2.58281$ ev
$f = 1.1673$
$\lambda_{abs} = 513$ nm

HUMO -5.72201 ev

1+Cd$^{2+}$

LUMO -3.21312 ev

$\Delta E = 2.35378$ ev
$f = 1.4223$
$\lambda_{em} = 610$ nm

HUMO -5.56690 ev
c) **Ground State**

![Ground State Diagram]

- LUMO: -3.04114 ev
- ΔE = 2.43297 ev
- f = 1.2912
- λ_{abs} = 554 nm
- HUMO: -5.47411 ev

**Excited State**

![Excited State Diagram]

- LUMO: -3.08305 ev
- ΔE = 2.22371 ev
- f = 1.5527
- λ_{em} = 655 nm
- HUMO: -5.30676 ev

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d) **Ground State**

![Ground State Diagram]

- LUMO: -2.57120 ev
- ΔE = 2.36875 ev
- f = 1.3033
- λ_{abs} = 562 nm
- HUMO: -4.93995 ev

**Excited State**

![Excited State Diagram]

- LUMO: -2.59651 ev
- ΔE = 2.11432 ev
- f = 1.5201
- λ_{em} = 684 nm
- HUMO: -4.71083 ev
Fig. S4 Frontier molecular orbital plots of 1 (a), 1+\text{Cd}^{2+} (b), 1+\text{Zn}^{2+} (c), LysoZn-1 (d), LysoZn-1+\text{Cd}^{2+} (e), LysoZn-1+\text{Zn}^{2+} (f); it is involved in the vertical excitation (UV/Vis
absorption, left column) and emission (right column). The vertical excitation related calculations are based on the optimised geometry of the ground state, and the emission related calculations were based on the optimised geometry of the excited state. B3LYP geometries and 6-31G (d, p) / LanL2DZ (for complex of LysoZn-1 with Cd$^{2+}$ or Zn$^{2+}$) basis set during the TD-DFT calculations.

**Fig. S5** a) Fluorescence spectra of 1 µM LysoZn-1 upon the titration of Zn$^{2+}$ (0-10 µM) in ethanol/10 mM Tris-HCl = 9/1, v/v, pH 7.2. b) Fluorescence ratio ($F_{578\text{nm}}/F_{647\text{nm}}$) changes as a function of Zn$^{2+}$ concentration (0-10 µM). Excitation wavelength was 545 nm.

**Fig. S6** Curve of fluorescence intensity at 578 nm ($F_{578\text{nm}}$) of LysoZn-1 versus increasing
concentration of Zn$^{2+}$. The concentration of LysoZn-1 was 1 µM. The dissociation constant $K_d$ is deduced to be $6.8 \pm 0.4 \times 10^{-5}$ M.

**Fig. S7** Curve of fluorescence ratio ($F_{578\text{nm}}/F_{680\text{nm}}$) of LysoZn-1 versus increasing concentration of Zn$^{2+}$. The concentration of LysoZn-1 was 1 µM. The dissociation constant $K_d$ is deduced to be $12.3 \pm 0.6 \times 10^{-5}$ M.

**Fig. S8** Fluorescence intensity at 578 nm ($F_{578\text{nm}}$) of LysoZn-1 versus increasing concentration of log[Zn$^{2+}$]. The concentration of LysoZn-1 was 1 µM. The fluorescence response fits to a Hill coefficient of 1(1.09046). It is consistent with the formation of a 1:1 stoichiometry for the LysoZn-1-Zn$^{2+}$ complex.

**Fig. S9** Fluorescence ratio ($F_{578\text{nm}}/F_{680\text{nm}}$) of LysoZn-1 versus increasing concentration of
log[Zn$^{2+}$]. The concentration of LysoZn-1 was 1 µM. The fluorescence response fits to a Hill coefficient of 1(1.08136). It is consistent with the formation of a 1:1 stoichiometry for the LysoZn-1-Zn$^{2+}$ complex.

**Fig. S10** Fluorescence spectra of 1 µM LysoZn-1 upon the titration of Zn$^{2+}$ (0, 5, 10, 20, 30, 50, 70, 90, 120, 150, 180, 200, 230, 300, 400, 500, 600 and 800 µM) in ethanol/50 mM CH$_3$COOH-CH$_3$COONa = 9/1, v/v, pH 5.0. Excitation wavelength was 545 nm.

**Fig. S11** Curve of fluorescence intensity at 578 nm ($F_{578nm}$) of LysoZn-1 versus increasing concentration of Zn$^{2+}$ at pH 5.0. The concentration of LysoZn-1 was 1 µM. The dissociation constant $K_d$ is deduced to be 8.1±0.9×10$^{-5}$ M.

**Fig. S12** Curve of fluorescence intensity ratio ($F_{578nm}/F_{680nm}$) of LysoZn-1 versus increasing concentration of Zn$^{2+}$ at pH 5.0. The concentration of LysoZn-1 was 1 µM. The dissociation
constant $K_d$ is deduced to be $16.0 \pm 0.7 \times 10^{-5}$ M.

**Fig. S13** The time courses of fluorescence intensity (578 nm) of LysoZn-1 (1 μM) in the presence of 200 μM Zn$^{2+}$ in ethanol/10 mM Tris-HCl = 9/1, v/v, pH 7.2. Excitation wavelength was 545 nm.

**Fig. S14** Fluorescence responses of LysoZn-1 (1 μM) toward 200 μM various cations in ethanol/10 mM Tris-HCl = 9/1, v/v, pH 7.2. From right to left: Ag$^+$, Ba$^{2+}$, Ca$^{2+}$, Cd$^{2+}$, Cu$^+$, Co$^{2+}$, Cr$^{3+}$, Fe$^{3+}$, Hg$^{2+}$, K$^+$, Mg$^{2+}$, Mn$^{2+}$, Na$^+$, Ni$^{2+}$, Pb$^{2+}$, Zn$^{2+}$. Excitation wavelength was 545 nm. Black bars represent the relative emission intensity ($F/F_0$, at 578 nm) of LysoZn-1+cations; light gray bars represent the fluorescence intensity of LysoZn-1+Zn$^{2+}$ in the presence of other cations.

**Fig. S15** Fluorescence responses of LysoZn-1 (1 μM) toward various anions (200 μM) in ethanol/50 mM CH$_3$COOH-CH$_3$COONa = 9/1, v/v, pH 5.0. From right to left: Br$^-$, CH$_3$COO$^-$, Cl$^-$,
ClO\textsuperscript{-}, ClO\textsubscript{4}\textsuperscript{-}, CO\textsubscript{3}\textsuperscript{2-}, HPO\textsubscript{4}\textsuperscript{2-}, H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-}, I\textsuperscript{-}, NO\textsubscript{3}\textsuperscript{-}, S\textsubscript{2}\textsuperscript{-}, Zn\textsuperscript{2+}. Excitation wavelength was 545 nm. Black bars represent the relative emission intensity ($F/F_0$, at 578 nm) of LysoZn-1+anions; gray bars represent the fluorescence intensity of LysoZn-1+Zn\textsuperscript{2+} in the presence of other anions.

**Fig. S16** Fluorescence responses of LysoZn-1 (1 µM) toward various anions (200 µM) in ethanol/10 mM Tris-HCl = 9/1, v/v, pH 7.2. From right to left: Br\textsuperscript{-}, CH\textsubscript{3}COO\textsuperscript{-}, Cl\textsuperscript{-}, ClO\textsubscript{4}\textsuperscript{-}, CO\textsubscript{3}\textsuperscript{2-}, HPO\textsubscript{4}\textsuperscript{2-}, H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-}, I\textsuperscript{-}, NO\textsubscript{3}\textsuperscript{-}, S\textsubscript{2}\textsuperscript{-}, Zn\textsuperscript{2+}. Excitation wavelength was 545 nm. Black bars represent the relative emission intensity ($F/F_0$, at 578 nm) of LysoZn-1+anions; gray bars represent the fluorescence intensity of LysoZn-1+Zn\textsuperscript{2+} in the presence of other anions.

**Fig. S17** Fluorescence ratio images ($F_{575-620 \text{ nm}}/F_{655-755 \text{ nm}}$) of LysoZn-1 (1 µM) labelled MCF-7 cells in the presence of 100 µM chloroquine at different time points: a) 0 min; b) 5 min; c) 10 min; d) 15 min; e) 20 min; f) 25 min; g) 30 min. Excitation wavelength is 559 nm. h) Bright field. i) Plot of the emission ratios as a function of time after chloroquine was added. The ratio values are extracted from 7 regions of each image, error bars are ±sem.
Fig. S18 Zn\textsuperscript{2+}-dependent ratio changes of LysoZn-1 in NSCs. Cells were treated with 1 μM LysoZn-1 for 30 min, then various concentrations of Zn\textsuperscript{2+} was added: a) 0 μM; b) 10 μM; c) 20 μM; d) 50 μM. After 10 min incubation, confocal fluorescence images were recorded; e) bright field; f) fluorescence ratio ($F_{575-620nm}/F_{655-755nm}$) changes as a function of Zn\textsuperscript{2+} concentration upon 559 nm excitation. The ratio values are extracted from 5 regions of each image, error bars are ±sem.

Fig. S19 Zn\textsuperscript{2+}-dependent ratio changes of LysoZn-1 in MCF-7 cells. Cells were treated with 1 μM LysoZn-1 for 30 min, then various concentrations of Zn\textsuperscript{2+} was added: a) 0 μM; b) 10 μM;
c) 20 μM; d) 50 μM. After 10 min incubation, confocal fluorescence images were recorded; e) bright field; f) fluorescence ratio \( (F_{575-620\ \text{nm}}/F_{655-755\ \text{nm}}) \) changes as a function of \( \text{Zn}^{2+} \) concentration upon 559 nm excitation. The ratio values are extracted from 10 regions of each image, error bars are ±sem.

Fig. S20 Ratiometric imaging \( (F_{575-620\ nm}/F_{655-755\ nm}) \) of \( \text{Zn}^{2+} \) in \textbf{LysoZn-1} labeled MCF-7 cells.

a) Cells were incubated with 1 μM \textbf{LysoZn-1} for 30 min; b) following a 10 min treatment of \( \text{Zn}^{2+} \) (20 μM); c) fluorescence ratio images of cells in b) treated further by TPEN solution (20 μM, 10 min); d) bright field; e) statistical analyses were performed with One-Way ANOVA (n =13 fields of cells). ***\( P < 0.001 \), and error bars are ±sem. Excitation wavelength is 559 nm.
**Fig. S21** Fluorescence spectra of LysoZn-1 (blue line) and LysoZn-1+H₂O₂ (red line) in ethanol/50 mM CH₃COOH-CH₃COONa = 9/1, v/v, pH 5.0. Excitation wavelength is 545 nm.

The concentrations of LysoZn-1 and H₂O₂ were 1 μM and 1 mM, respectively.

**Fig. S22** Cytotoxicity of LysoZn-1 on NSCs and MCF-7 cells. Cells were incubated with 1 μM LysoZn-1 in FBS buffer for 12 h or 24 h, cell viabilities were examined using Thermo Fisher Scientific.
Fig. S23 $^1$H-NMR of 2

Fig. S24 $^{13}$C-NMR of 2
Fig. S25 $^{1}$H-NMR of 3

Fig. S26 $^{13}$C-NMR of 3
Fig. S27 $^1$H-NMR of LysoZn-1

Fig. S28 $^{13}$C-NMR of LysoZn-1

Reference