Electronic Supplementary Material (ESM) for Publication:

A Highly Selective OFF-ON Fluorescent Sensor for Zinc in Aqueous Solution and Living Cell

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S1. Materials and general methods

S2. Supplementary results

Fig. S1. The fluorescence responses (I_{500nm}) of ***C1*** (20 μM) with various anions(800 μM) in Tris-HCl buffer(pH=5.0) (λex = 295 nm).

Fig. S2. The fluorescence responses (I_{500nm}) of ***C1*** (20 μM) with various amino acids (800 μM) in Tris-HCl buffer(pH=5.0) (λex = 295 nm).

Fig. S3. The fluorescence responses (I_{500nm}) of ***C1*** (20 μM) with various metal ions (800 μM) in Tris-HCl buffer(pH=5.0) (λex = 295 nm).

Fig. S4. The fluorescence responses (I_{500nm}) of ***C1*** (20 μM) with various metal ions and Zn^{2+} in Tris-HCl buffer (pH=5.0). (λex = 295 nm). (80μM for Zn^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, 800 μM for all other cations).

Fig. S5. The fluorescence responses (I_{500nm}) of ***C1*** (20 μM) with 20 amino acids and Zn^{2+} in Tris-HCl buffer (pH=5.0). (λex = 295 nm). (80μM for Zn^{2+} and amino acids).

Fig.S6. Excitation spectrum of ***C1***-Zn^{2+}

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Fig. S10. Luminescence of the acidificated MPIP with 1M HCl

Fig. S11. The fluorescence intensity of ***C1***-Zn^{2+} (λ_{ex} = 295 nm, λ_{em} = 500 nm) as a function of time (λ_{ex}=295 nm).

Fig. S12. Bright filed of confocal fluorescence images of C6 cells in the absence (a) and prensence of 50 μM ***C1***(b-e) . Cells treated with ***C1*** in the absence (b) and presence of 8 μM TPEN(c); after treatment with TPEN, 10 μM $ZnCl_2$ (d) and 100 μM $ZnCl_2$ (e), subsequent treatment of the cells with 50 μM ***C1***

Fig. S13. Confocal fluorescence images of C6 cells in the prensence of 50 μM ***C1*** and 50 μM $ZnCl_2$.

Table S1 Hydrogen binds in *C1*·2H_2O

Deleted: confocal fluorescence images of C6 cells in the prensence of 50 μM ***C1*** treatment with 10 μM $ZnCl_2$ and 100 μM $ZnCl_2$.¶
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S1. Materials and general methods

All the solvents were of analytic grade. The salts solutions of metal ions were NaCl, KCl, $MgCl_2·6H_2O$, $CaCl_2$, $CrCl_3·6H_2O$, $MnCl_2·4H_2O$, $FeCl_3·6H_2O$, $Co(NO_3)_2·6H_2O$, $NiCl_2·6H_2O$, $CuCl_2·2H_2O$, $LiCl·H_2O$, $ZnCl_2$, $Cd(NO_3)_2·4H_2O$, $PbCl_2$, $SnCl_4·5H_2O$, $Al(NO_3)_3·9H_2O$, $BaCl_2$. The salts solutions of anions were Na_3PO_4, Na_2CO_3, NaOAC, KBr, $K_2C_2O_4·H_2O$, Na_2S, NaCl, NaF, KNO_3, KSCN. The other reagents were purchased from Shanghai Reagents Ltd. MPIP was synthesized according to the reference.[R1]

S1.1 General instruments

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX300 spectrometer with DMSO as the internal standard. Electrospray ionization mass spectra (ESI-MS) were measured on a Finnigan LCQ system. UV-Vis spectra were recorded on a Hewlett Packard HP-8453 spectrohotometer. Fluorescence spectra were recorded on Varian Cary Eclipse spectrohotometer. The X-ray data of crystal ***C1*** were collected by ω-scan method on a SMART 1K CCD area detector single-crystal diffractometer that uses graphite monochromated Mo Kα radiation (λ=0.71073 Å). The structure was solved by direct methods and refined by least squares procedures on F^2 using SHELX-97 package.

S1.2 Procedures of metal ion sensing

Stock solutions of the metal ions (40 mM) and ***C1*** (2 mM) were prepared in deionized water, respectively. In titration experiments, a 20 μL of ***C1*** was added into 2 mL of Tris-HCl buffer (0.02 M, pH = 5) filled in a quartz optical cell of 1 cm optical path length, and the Zn^{2+} stock solution was added into the quartz optical cell gradually by using a micro-pipettes. The dilution of metal complex concentrations at the end of the titrations was negligible. In selectivity experiments, the test samples were prepared by placing appropriate amounts of metal ion stock into 2.0 mL of Tris-HCl buffer solution of ***C1*** (20 μM). For fluorescence measurements, excitation was provided at 295 nm, and emission was collected from 400 to 570 nm. Luminescence quantum yields in air-equilibrated solution were measured with reference to quinine sulfate (0.55 in 0.05 M H_2SO_4).

The binding constant was calculated from the emission intensity - titration curve according to the equations (S1a) and (S1b).[R2]

$$\frac{\varepsilon_a-\varepsilon_f}{\varepsilon_b-\varepsilon_f} = \frac{(b-(b^2-2K^2C_t[DNA]/s)^{1/2})}{2KC_t} \quad (S1a)$$

$$b=1+KC_t+K[DNA]/2s \quad (S1b)$$

where I is the emission intensity observed at a given Zn^{2+} concentration, I_f is the emission intensity of free ***C1*** in solution, I_b is the emission intensity of ***C1*** when fully bound to Zn^{2+} (it is assumed that when further addition of Zn^{2+} does not change the emission intensity, *K* is the equilibrium binding constant, Ct is the total ***C1*** concentration, $[Zn^{2+}]$ is the Zn^{2+} concentration, and *s* is the binding site size. Both *K* and *s* are obtained from the best fit line.

S1.3 Cell Culture

The C6 glioma cells were grown in DMEM (High Glucose) supplemented with 10 % FBS (Fetal Bovine Serum) at 37 ℃ and 5 % CO_2. Cells were plated on 0.17 mm glass coverslips and allowed to adhere for 24 hours. Cells were incubated for 20 min with TPEN, Zn^{2+} and ***C1***, respectively. Every time before the addition of species such as TPEN, Zn^{2+} and ***C1***, Cells were washed twice by DMEM solution.

S1.4 Fluorescence Imaging

Confocal fluorescence imaging was performed with Olympus FV1000 laser scanning microscopy and a 60x oil-immersion objective lens. Excitation of ***C1***-loaded cells at 405 nm was carried out with a semiconductor laser. Emission was collected at 490 ~550 nm for green channel.

S2. Supplementary results

S2.1 Selectivity experiment

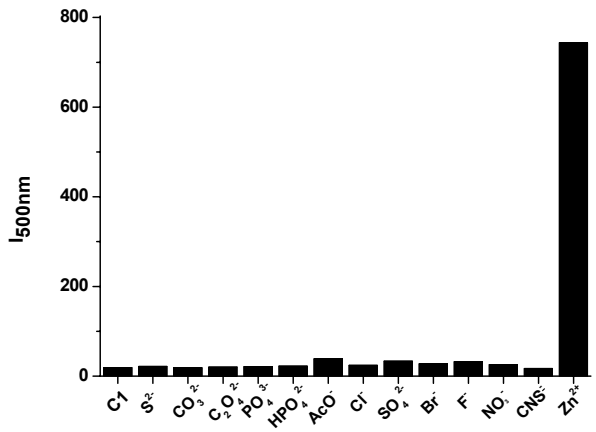

Fig. S1. The fluorescence responses (I_{500nm}) of ***C1*** (20 μM) with various anions(800 μM) in Tris-HCl buffer(pH=5.0) (λex = 295 nm).

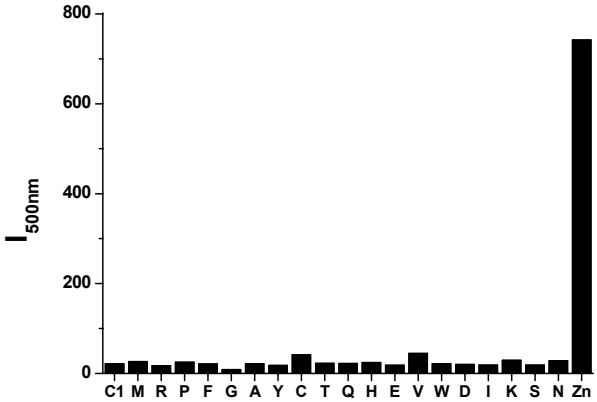

Fig. S2. The fluorescence responses (I_{500nm}) of ***C1*** (20 μM) with various amino acids (800 μM) in Tris-HCl buffer(pH=5.0) (λex = 295 nm).

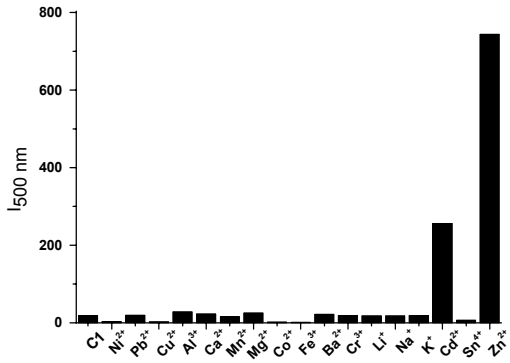

Fig. S3. The fluorescence responses (I_{500nm}) of ***C1*** (20 μM) with various metal ions (800 μM) in Tris-HCl buffer(pH=5.0) (λex = 295 nm).

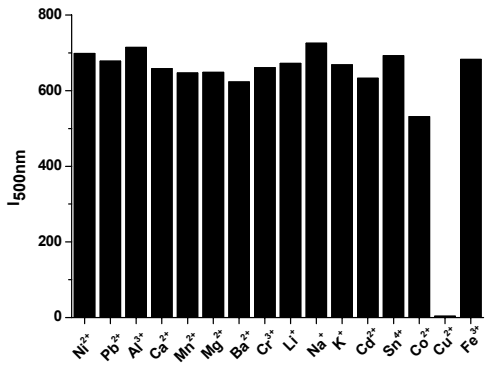

Fig. S4. The fluorescence responses (I_{500nm}) of ***C1*** (20 μM) with various metal ions and Zn^{2+} in Tris-HCl buffer (pH=5.0). (λex = 295 nm). (80μM for Zn^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, 800 μM for all other cations).

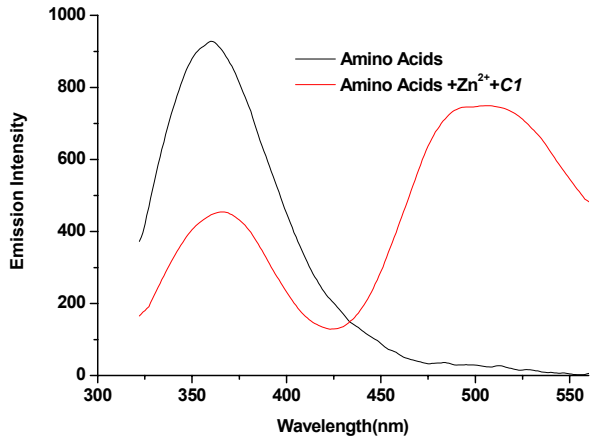

Fig. S5. The fluorescence responses (I_{500nm}) of ***C1*** (20 μM) with 20 amino acids and Zn^{2+} in Tris-HCl buffer (pH=5.0). (λex = 295 nm). (80μM for Zn^{2+} and amino acids).

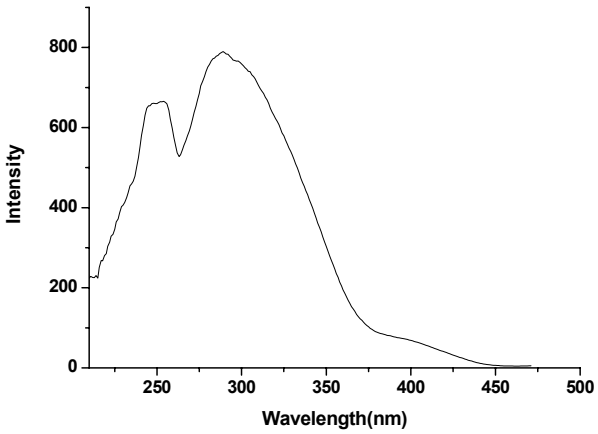

Fig.S6. Excitation spectrum of ***C1***-Zn^{2+}

S2.2 UV-Vis spectra:

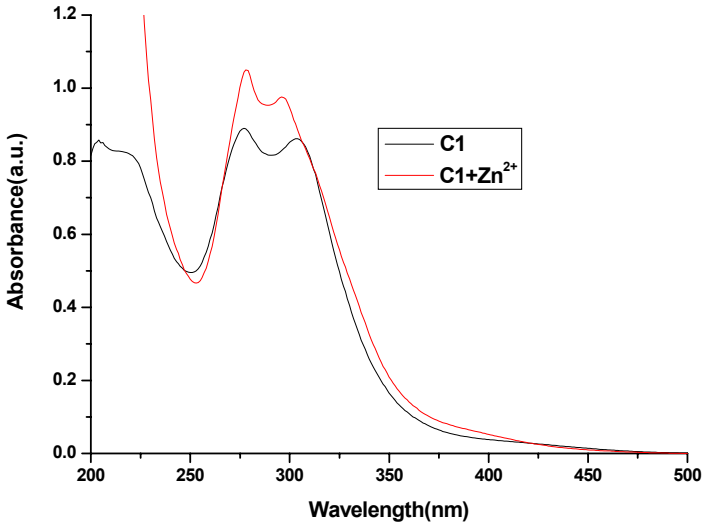

Fig.S7. The UV-Vis absorption spectra of ***C1*** (100 μM) and ***C1*** with excess Zn^{2+}.

S2.3 ESI-MS

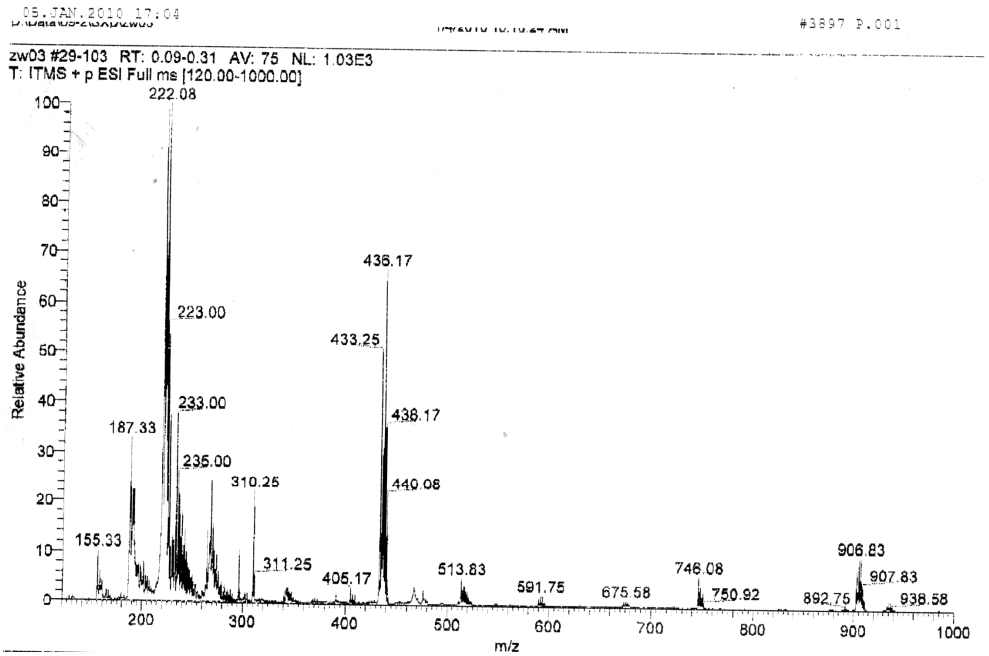

Fig. S8a ESI mass spectra of ***C1***,peaks 433.25 to 440.06 for [HMPIP·$HSeO_3H^+$],calc. 440.04

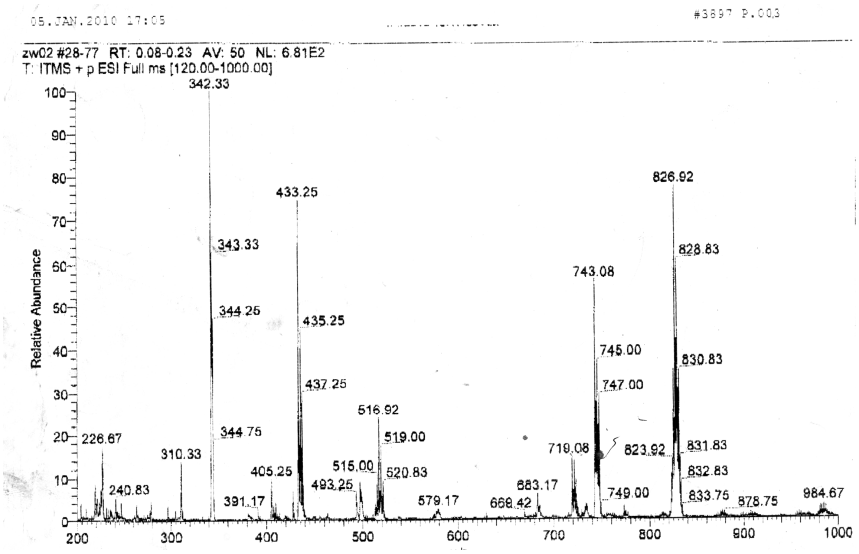

Fig. S8b. ESI mass spectra of ***C1*** upon addition of excess Zinc (III) chloride. The species with m/z 828.83 observed may be assigned to $\{[Zn(\boldsymbol{C1}\text{-H})]^{+}_{3}+3CH_3OH+CH_3O^{-}+H_2O\}^{2+}$ (m/z = 1653.99/2= 828.15); the species with m/z 743.08 may be assigned to $\{[Zn(\boldsymbol{C1}\text{-H})]^{+}_{4}+5CH_3OH+3H_2O\}^{4+}$ (m/z = 2229.09/4= 743.03); the species with m/z 433.25 may be assigned to $\{[Zn(\boldsymbol{C1}\text{-H})]^{+}_{4}+4CH_3OH+Na^{+}\}^{5+}$ (m/z = 2165.99/5= 433.20); the species with m/z 343.33 may be assigned to $\{[Zn(\boldsymbol{C1}\text{-H})]^{+}_{3}+5CH_3OH+2Na^{+}\}^{5+}$ (m/z = 1717.31/5= 343.46).

S2.4 Luminescence as functions of pH and time

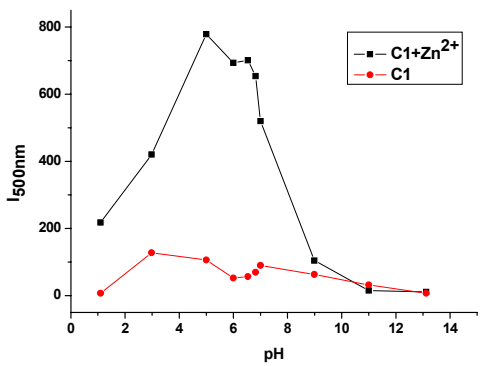

Fig. S9 luminescence of ***C1*** (20 μM) at 500 nm in various pH in the absence and presence of 80 μM Zn^{2+}. The pH of the solutions was adjusted by addition of 0.1 mol L^{-1} HCl (or 0.2 mol L^{-1} NaOH).

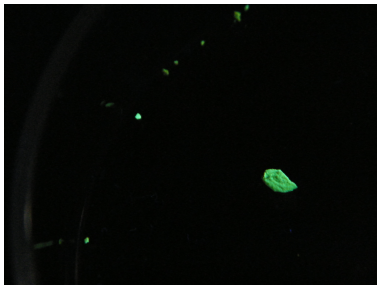

Fig. S10. Luminescence of the acidificated MPIP with 1M HCl

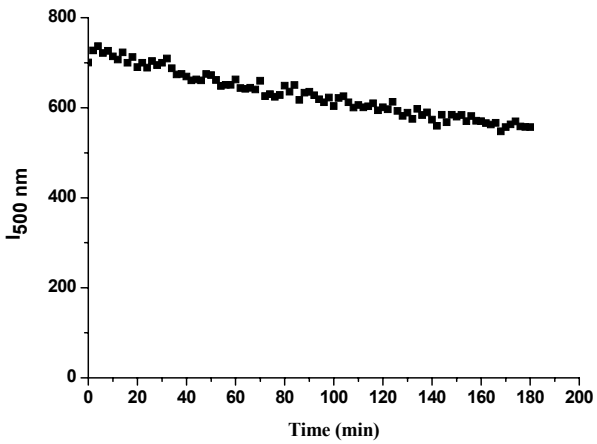

Fig. S11. The fluorescence intensity of ***C1***-Zn^{2+} (λ_{ex} = 295 nm, λ_{em} = 500 nm) as a function of time (λ_{ex}=295 nm).

S2. 6 Confocal fluorescence images of C6 cells

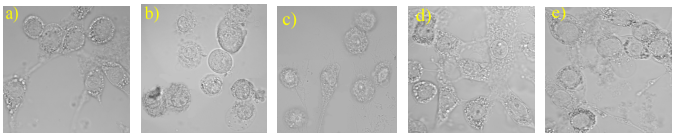

Fig. S12. Bright field of confocal fluorescence images of C6 cells in the absence (a) and prensence of 50 μM ***C1***(b-e) . Cells treated with ***C1*** in the absence (b) and presence of 8 μM TPEN(c), and after treatment with TPEN and 10 μM $ZnCl_2$ (d) and 100 μM $ZnCl_2$ (e) subsequent treatment of the cells with 50 μM ***C1***.

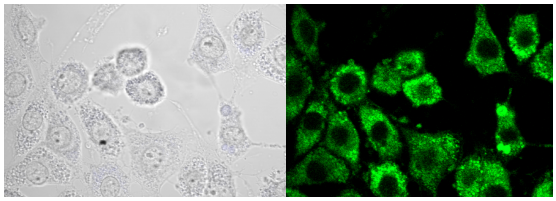

Fig. S13. Confocal fluorescence images of C6 cells in the prensence of 50 μM ***C1*** and 50 μM $ZnCl_2$.

Table S1 Hydrogen binds in ***C1·2H$_2$O***

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O5—H5B···O1	0.84	2.33	3.139(6)	161
O5—H5A···O1^{i}	0.84	2.27	2.866(5)	128
N3—H3B···O4^{ii}	0.86	1.93	2.787(5)	172
O4—H4B···O5^{iii}	0.85	2.30	2.936(6)	131
O4—H4A···O2^{iv}	0.85	2.00	2.794(7)	154
N1—H1A···N2	0.86	2.41	2.758(5)	105
N1—H1A···O1	0.86	1.85	2.657(5)	157
O3—H3···O2^{iv}	0.82	2.10	2.633(6)	122
C1—H1···O5	0.93	2.92	3.643(6)	135
C3—H3A···O4^{ii}	0.93	2.57	3.442(6)	156
C19—H19···O4^{ii}	0.93	2.58	3.484(6)	164
C15—H15···N4	0.93	2.62	2.927 (6)	100
C19—H19···O4^{ii}	0.93	2.58	3.484 (6)	164

Symmetry codes: (i) −*x*+2, −*y*+1, −*z*+1; (ii) *x*, *y*+1, *z*; (iii) *x*, −*y*+1/2, *z*−1/2; (iv) *x*, −*y*+1/2, *z*+1/2.

Reference:

R1:W.J. Mei, J. Liu, K.C. Zheng, L.J. Lin, H. Chao, A.X. Li, F.C. Yun and L.N. Ji, *Dalton Trans.*, 2003,1352.

R2: M.T. Carter, M. Rodriguez, and A.J. Bard, *J. Am. Chem. SOC.*, 1989, 111,8901