Supporting Information for

Manganese displacement from Zinpyr-1 allows zinc detection by fluorescence microscopy and magnetic resonance imaging

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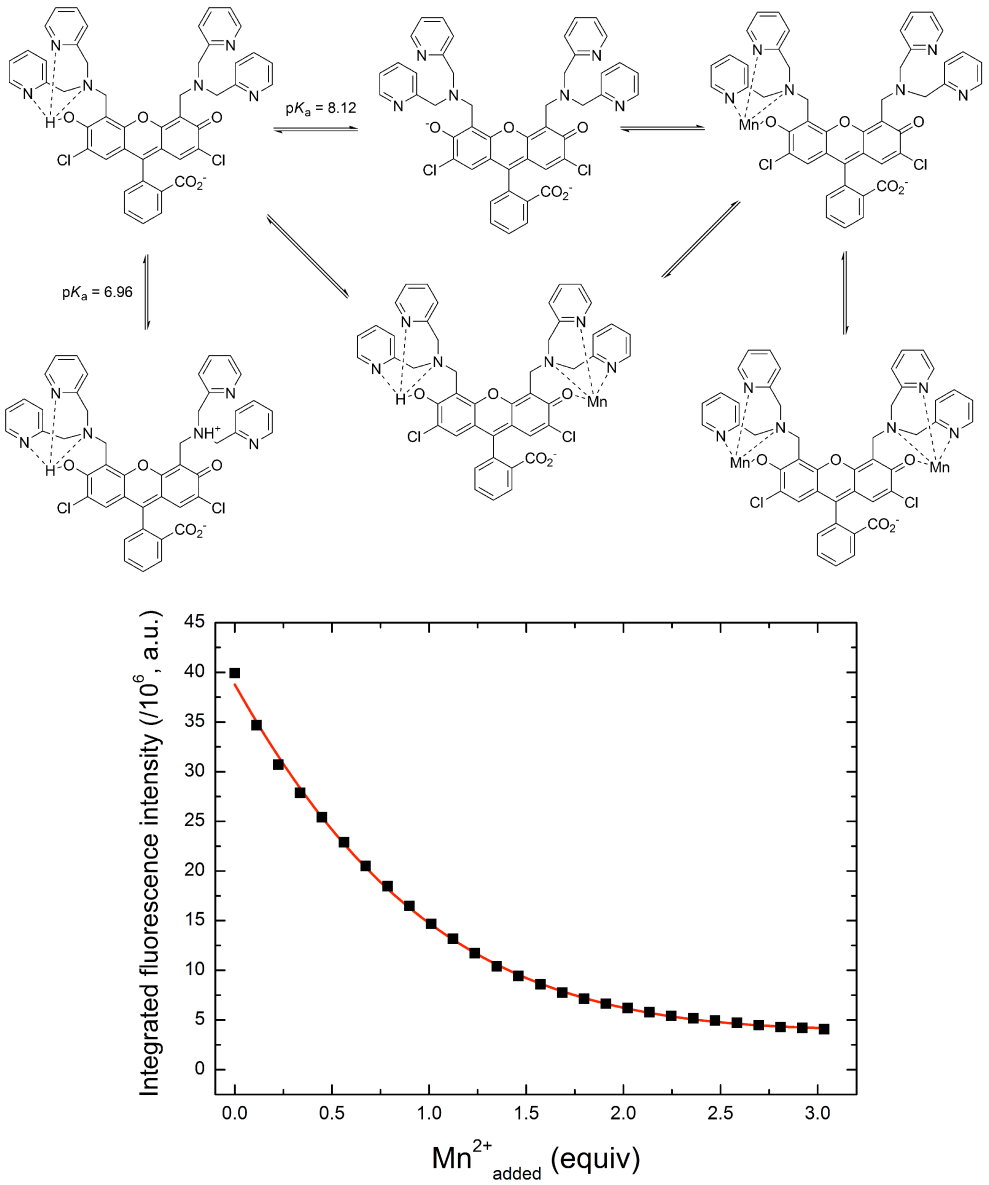
Materials. ZP1 was synthesized as described.[1,2] Samples for this study were obtained from at least two different batches. Buffer solutions, sensor stock solutions, and Zn^{2+} solutions were prepared as reported previously.[3] Buffer solutions were filtered through a 20 μm nylon membrane filter just before use. Mn^{2+} solutions were prepared by dissolving $MnCl_2 \cdot 4H_2O$ (Strem) in milli-Q water. Solutions for metal selectivity experiment were prepared similarly.

General Measurements. Absorption spectra were recorded with a Cary 1E UV-visible spectrophotometer from 230 to 600 nm. Fluorescence spectra were obtained with a QuantaMaster 30 spectrophotometer system (Photon Technology International) in the 450–700 nm range. A 10 μM solution was typically prepared for measuring absorption and fluorescence spectra. All photophysical measurements were performed in triplicate at 25 °C unless otherwise mentioned. K_d values for [ZP1Mn_2] complexation were determined from nonlinear least square fitting of the titration data. For this procedure, protonation equilibria were included in the model, which contained six (protonation + metal chelation) reactions altogether. The scheme for these equilibria was adopted from our previous report.[4] pK_a values of ZP1 were recently determined and used in this study.[4] The equation used for the fitting is shown in the caption to Fig. S2. Nonlinear least squares curve fitting was carried out by using a fitting module embedded in Microcal OriginPro software (OriginLab, Northampton, MA).

Cell Culture. HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Cellgro and Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), penicillin (100 units/mL), streptomycin (100 μg/mL), sodium pyruvate (1 mM, Cellgro), and pH 7.0 HEPES buffer (10 mM, Sigma-Aldrich). Two days before imaging, cells were passed and plated onto poly-D-lysine-coated glass-bottom culture dishes. Labeling was performed in the same growth media. [ZP1Mn_2] was prepared by combining a stock solution of $MnCl_2$ (1 mM) in milli-Q water and a stock solution of ZP1 sensor (1 mM) in DMSO. Cells were treated and incubated with either 10 μM ZP1 sensor or 10 μM of [ZP1Mn_2] at 37ºC under 5% CO_2 for 30 min. In the time-course experiment (Figure SI10), nuclear stain Hoechst 33258 (4.5 μM) was added to the growth media (data not shown). The cells were washed twice with DMEM containing no FBS prior to imaging and zinc addition. Zinc was introduced to the cultured cells as the pyrithione salt using a Zn(II)/pyrithione ratio of 1:1. Stock solutions of $ZnCl_2$ (10 mM) in water and sodium pyrithione (20 mM) in DMSO were combined and diluted to 1 mM with DMEM containing no FBS prior to addition. A stock solution of *N*,*N*,*N*',*N*'-tetrakis-(2-pyridylmethyl)-ethylenediamine (TPEN, 20 mM) was also diluted 20-fold with DMEM containing no FBS prior to cell treatment. In a typical experiment, a 100 μL aliquot of both the zinc-pyrithione and TPEN solutions was added to the cells in 2 mL of DMEM.

Fluorescence Microscopy and Data Processing. A Zeiss Axiovert 200M microscope equipped with a 63x oil-immersion objective was used to obtain fluorescence microscopy images. Samples were excited using an Exfo X-Cite 120 mercury halide lamp, and imaged using DIC and FITC settings. Addition of zinc pyrithione and TPEN was carried out directly to the plate media on the microscope stage. Fluorescence data were obtained using the Improvision Volocity software to select cellular regions and take measurements. Fluorescence of a single cell was measured as the maximum intensity within the cell region, and background fluorescence was calculated as the mean intensity of a region free of cells.

MRI Experiment. Sample solutions were prepared by mixing appropriate volumes of ZP1, Mn^{2+} and Zn^{2+} in 50 mM PIPES buffer (with either Cl^- or ClO_4^- as counter ion). They were pipetted into 384-well microtiter plates, which modified to fit into a 9 cm birdcage resonator probe (Bruker Instruments, Billerica, MA). The total volume of each well was 80 μL. Unused wells of the plates were filled with PIPES buffer to minimize susceptibility-related distortions in MRI. Imaging was performed on a 40 cm bore Bruker Avance 4.7 T scanner equipped with 26 G/cm triple-axis gradients. The samples were scanned at room temperature using a 200 MHz Avance console running Paravision 3.0 (Bruker Instruments) across a 2 mm horizontal slice selected at the middle of each microtiter plate well (parallel to the plate). Images were acquired using a multi-spin multi-echo pulse sequence. For T_2 determination repetition time was 2 s and echo times ranged from 20 ms to 320 ms, in 20 ms increments. In plane resolution was 0.467 mm. Data were acquired as matrices of 128 × 128 points and analyzed offline using custom routines implemented in Matlab (Mathworks, Natick, MA). Relaxation times were obtained by fitting monoexpoential decay functions to image intensities averaged over each microtiter well and relaxivities were calculated using the known sample concentrations.



S1. Fluorescence titration of ZP1 (10 μM) with $MnCl_2$ in pH 7.0 buffer (50 mM PIPES, 100 mM KCl). Black squares are experimental data points and red solid line is the theoretical fitting.

The equation used for this fitting can be derived in a manner similar to that reported previously,[2] but proton equilibria were considered together. Each equilibrium step shown in the upper scheme affords a simple relationship for K_a or K_d in terms of involved species. K_a values were potentiometrically determined earlier.[4] These relationships were used in the following mass balance equation for total concentration of sensor:

$$[ZP1]_{total} = [ZP1] + [ZP1 + H] + [ZP1 - H] + [ZP1 + Mn] + [ZP1 - H{+}Mn] + [ZP1 - H{+}2Mn]$$

.

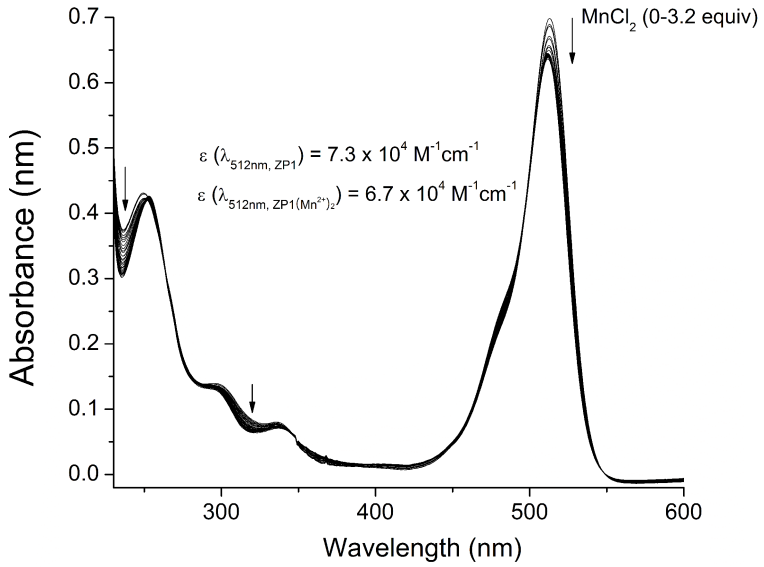
Then, rearrangement of this equation gave an expression of [ZP1] as a function of $[Mn^{2+}]$, which allows expression of the concentration of each species in terms of $[Mn^{2+}]$. The fluorescence intensity profile will be a linear combination of concentration of each species, if we ignore interchromophoric interactions among fluorescent species. The simplest expression is

$$\begin{aligned}\text{Total fluorescence intensity} = {} & a_{ZP1}\ [ZP1] + a_{ZP1+H}[ZP1 + H] + a_{ZP1\text{-}H}[ZP1 - H] \\ & + a_{ZP1+H+Mn}[ZP1 + Mn] + a_{ZP1\text{-}H+Mn}[ZP1 - H{+}Mn] + a_{ZP1\text{-}H+2Mn}[ZP1 - H{+}2Mn]\end{aligned}$$

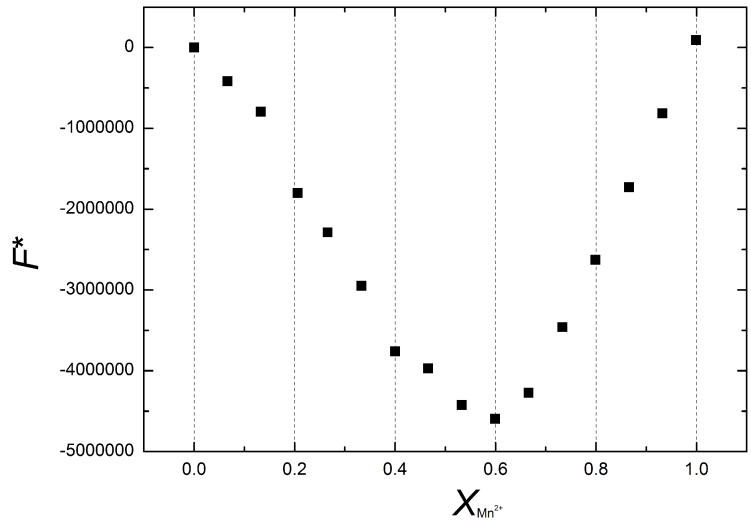
where a_X represents a proportionality parameter of species X which is again proportional to a product of fluorescence quantum yield and molar absorptivity. The proportionality parameter of [ZP1−H+2Mn] was calculated from fluorescence intensity at Mn^{2+}-saturated state, but initial guesses for others were put into the formula. Then, the equation was rearranged to a fractional equation that is quadratic in both denominator and numerator:

$$F_{total} = \frac{A+(B\cdot K_{d2}'+C\cdot K_{d1}')[Mn]+D\cdot K_{d2}'\cdot K_{d1}'[Mn]^2}{2.0865+(K_{d2}'+K_{d1}')[Mn]+K_{d1}'\cdot K_{d3}'[Mn]^2} \times [sensor]$$

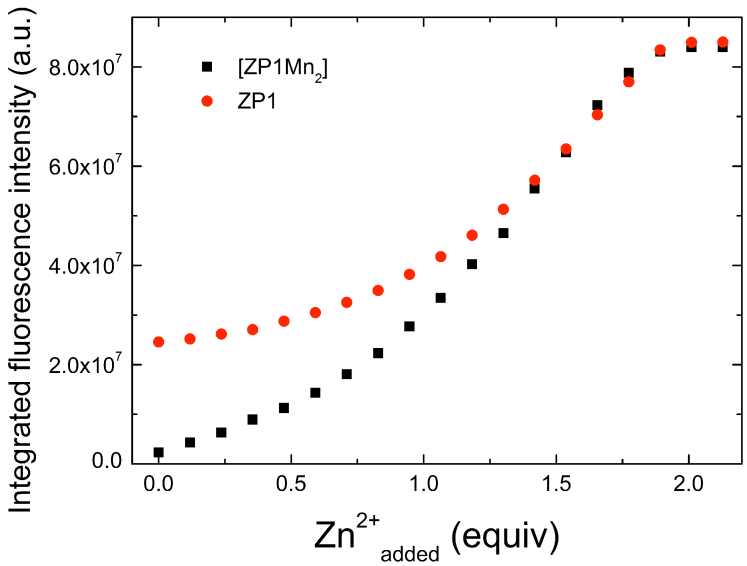
where K_{d1}', K_{d2}' and K_{d3}' are inverse of Mn^{2+} dissociation constant of ZP1−H+Mn, ZP1−H+2Mn and ZP1+Mn, respectively. Here, the first and second dissociation constants are considered. Finally, a nonlinear curve fitting module embedded in Microcal Origin 7.5 was used to get the fitted curve shown above.



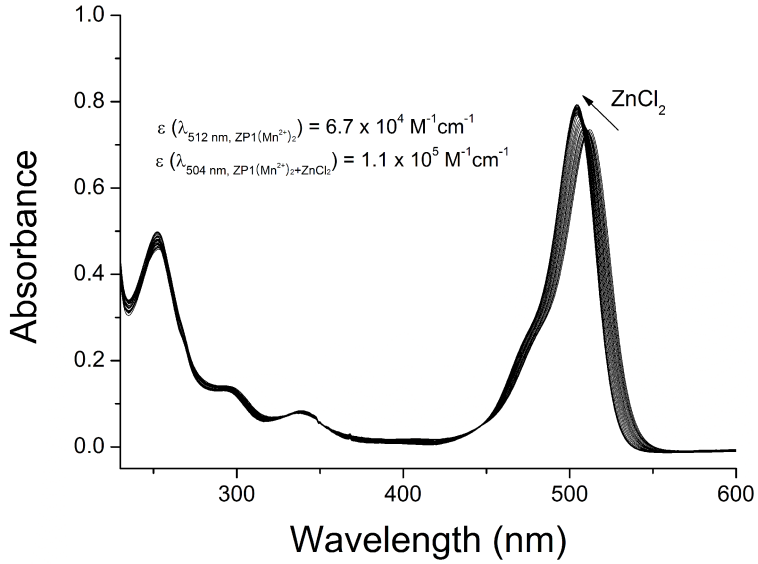
S2. Change in absorption spectra of ZP1 (10 μM) upon addition of Mn^{2+} ion at pH 7.0 (50 mM PIPES, 100 mM KCl).



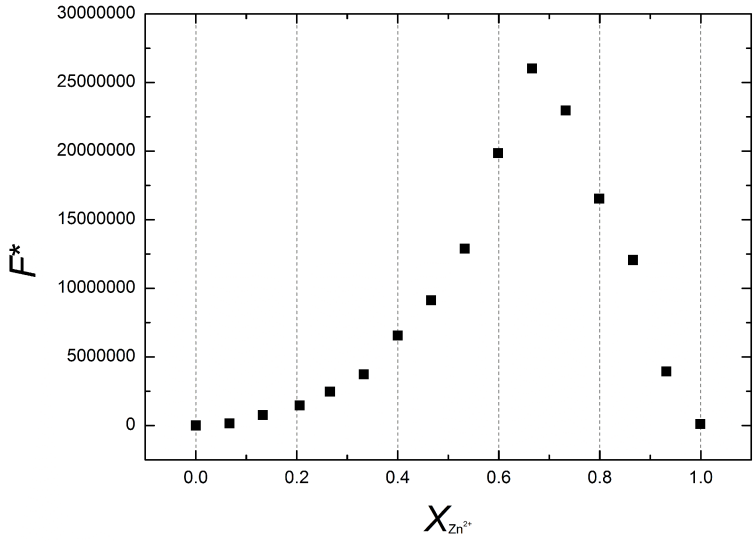
S3. Job's plot from fluorescence data of ZP1 complexation with Mn^{2+} (pH 7.0 buffer: 50 mM PIPES, 100 mM KCl). Total concentration was 10 μM.



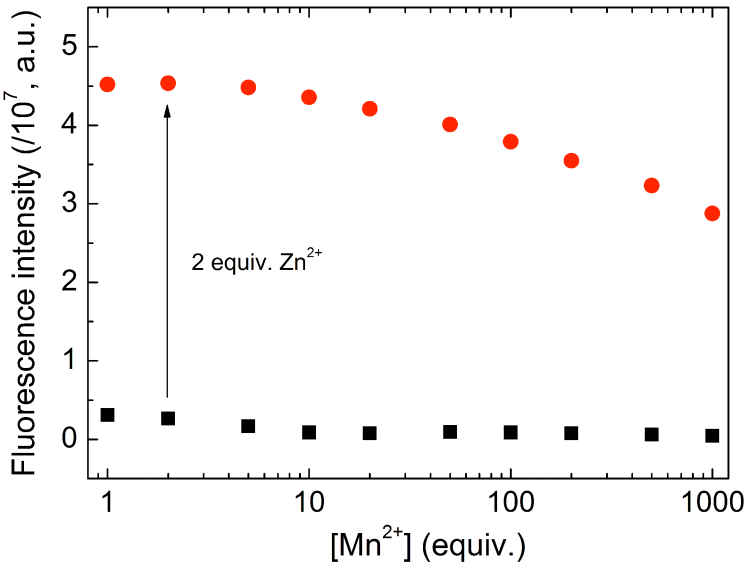
S4. Mole ratio plot for the fluorescence titrations of [ZP1Mn_2] (black squares, 10 μM) and ZP1 (red circles, 10 μM) with $ZnCl_2$ at pH 7.0 (50 mM PIPES, 100 mM KCl).



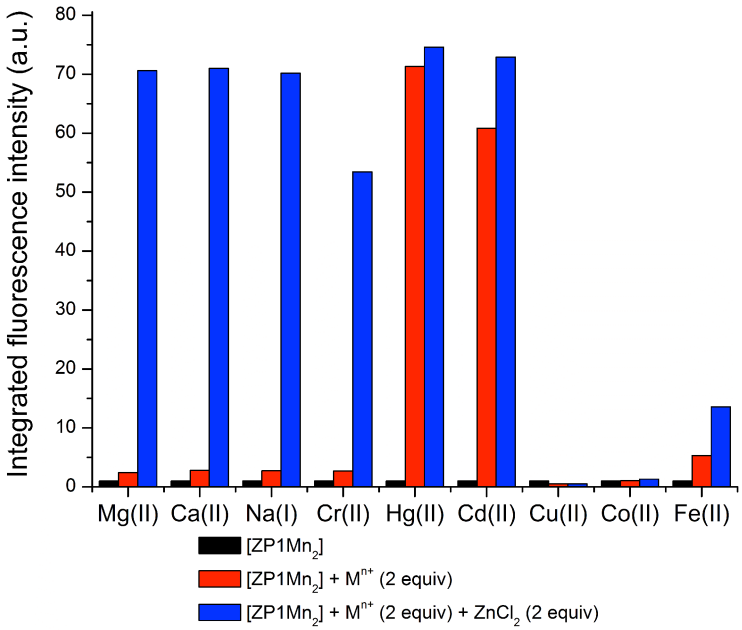
S5. Change in absorption spectra of [ZP1Mn_2] (10 μM) upon addition of Zn^{2+} ion at pH 7.0 (50 mM PIPES, 100 mM KCl).



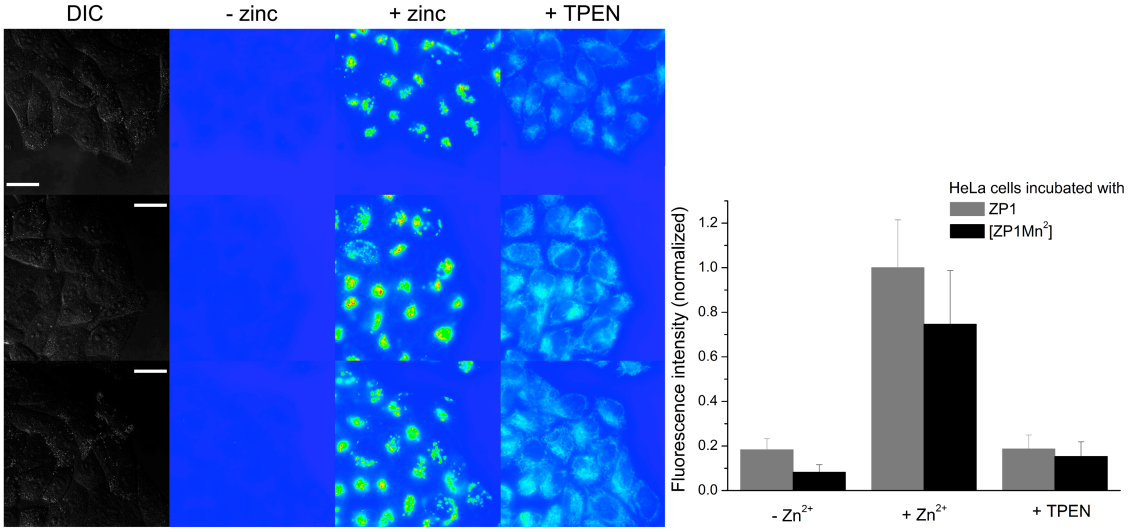
S6. Job's plot from fluorescence measurement results for Zn^{2+}-induced Mn^{2+} displacement of [ZP1Mn_2] (pH 7.0 buffer: 50 mM PIPES, 100 mM KCl). Total concentration was 10 μM.



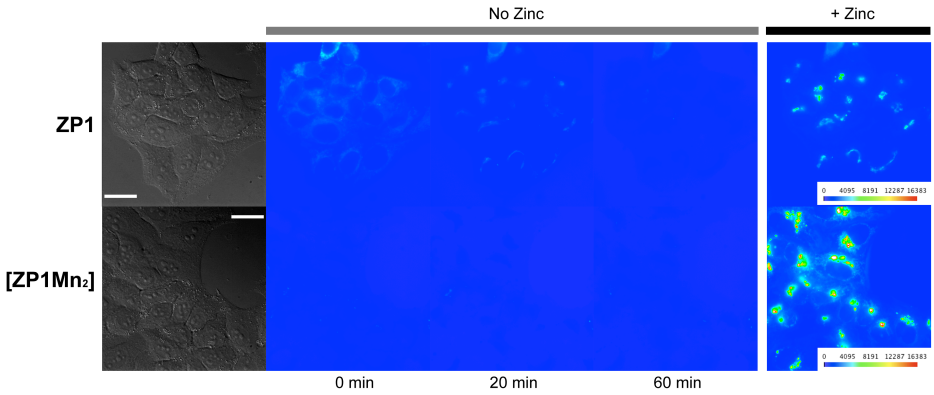
S7. Zn^{2+}-induced fluorescence turn-on of [ZP1Mn_2] (10μM) in the presence of various amounts of Mn^{2+} ion (1–1000 equiv) at pH 7.0 (50 mM PIPES, 100 mM KCl).



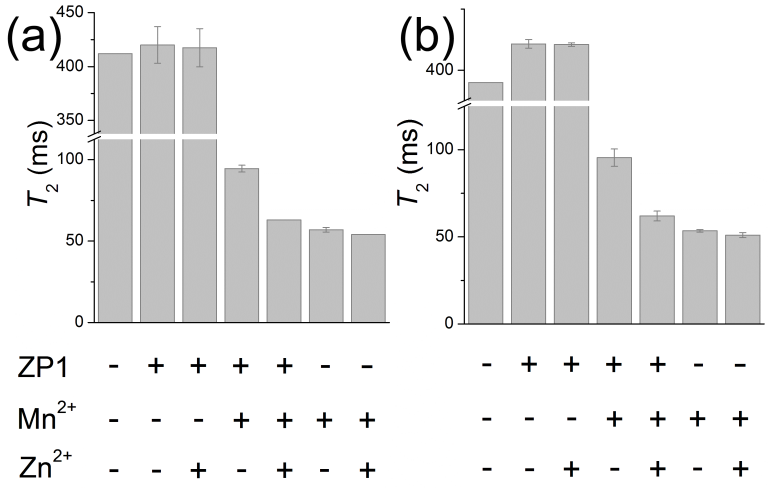
S8. Fluorescence metal selectivity of $[ZP1Mn_2]$ (pH 7.0 buffer: 50 mM PIPES, 100 mM KCl, 25 °C, 10 μM).



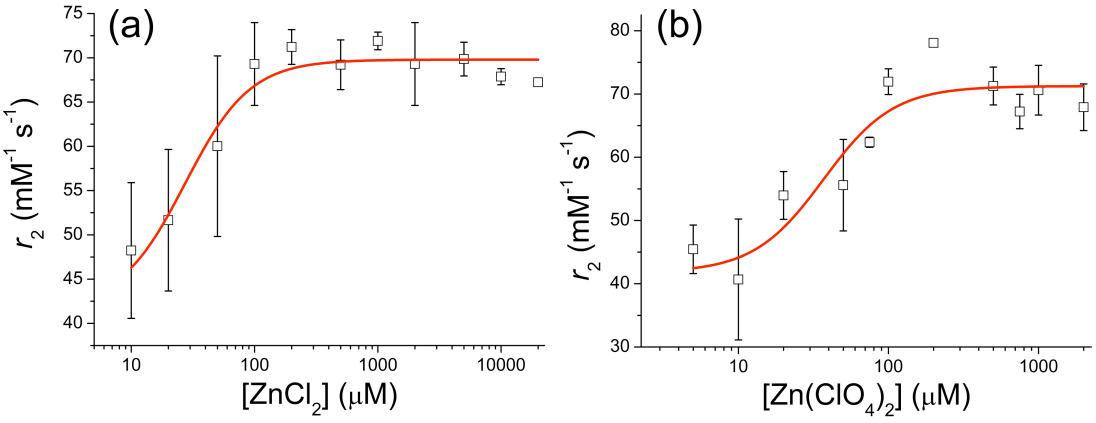
S9. Left: Zn^{2+}-responsive fluorescence micrographs of HeLa cells incubated with $[ZP1Mn_2]$. Images were acquired from three different cultures (Scale bar = 25 μm). Right: fluorescence intensity processed from the microscope images.



S10. Time course of fluorescence micrographs of HeLa cells incubated with ZP1 and [$ZP1Mn_2$] (0, 20, and 60 min) before the addition of zinc. We ascribe the progressive loss of signal over time to slow diffusion of the sensors out into the extracellular media. Manganese release from [$ZP1Mn_2$], which would cause a false positive turn-on, is not observed. Shown on the right are fluorescence micrographs after the addition of exogenous zinc/pyrithione.



S11. Acquired T_2 relaxation times of ZP1, Mn^{2+}, Zn^{2+} and their mixtures dissolved in pH 7.0 buffer (50 mM PIPES, 100 mM K^+ salt). Counter anions in solution are (a) Cl^- or (b) ClO_4^-. Concentrations: ZP1, 100 μM; Mn^{2+},200 μM; Zn^{2+}, 20 mM (a) and 2 mM (b).



S12. T_2 relaxivity changes as a function of Zn^{2+} ion concentration: (a) Cl^- and (b) ClO_4^- systems. [ZP1Mn$_2$] (100 μM) was dissolved in a buffer (50 mM PIPES containing 100 mM K^+ salt, pH 7.0). Data points at [Zn^{2+}] = 0 μM are not shown on the log scale representation. Refer to the main text for these values.

References

1. Walkup, G. K.; Burdette, S. C.; Lippard, S. J.; Tsien, R. J. *J. Am. Chem. Soc.* **2000**, *122*, 5644–5645.
2. Zhang, X.-a.; Hayes, D.; Smith, S. J.; Friedle, S.; Lippard, S. J. *J. Am. Chem. Soc.* **2008**, *130*, 15788–15789.
3. Burdette, S. C.; Walkup, G. K.; Spingler, B.; Tsien, R. Y.; Lippard, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 7831–7841.
4. Wong, B. A.; Friedle, S.; Lippard, S. J. *J. Am. Chem. Soc.* **2009**, *131*, 7142–7152.