Supporting Information

Subtle Modification of 2,2-Dipicolylamine Lowers the Affinity and Improves the Turn-On of Zn(II)-Selective Fluorescent Sensors

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Synthesis. The syntheses of ZP1, ZP1B, 2',7'-difluorofluorescein (DFF), ZP3, and (2-picolyl)(4-picolyl)amine (2,4-DPA) were previously reported.[1-3] Use of pure rather than aqueous MeCN in the Mannich reaction yields high-purity sensors. Slow evaporation of the reaction solvent affords diffraction-quality crystals of each of these sensors. Solvents were supplied by Mallinckrodt and used as received. All other reagents were purchased from Aldrich and used as received. The X-ray crystal structure of ZP1B was reported previously and the structure of ZP3B is described below.

General Methods. NMR spectra were obtained on a Bruker 400 MHz spectrometer at ambient temperature and referenced to the residual proton resonance of the deuterated solvent. High-resolution mass spectra were provided by staff at the MIT Department of Chemistry Instrumentation Facility using a Bruker Daltonics APEXIV 4.7 Tesla FT-ICR mass spectrometer.

ZP3B. A suspension of 2,4-DPA (238 mg, 1.20 mmol) and paraformaldehyde (31.0 mg, 1.03 mmol) in 10 mL of MeCN was heated at 70 °C for 30 min before adding a suspension of DFF (138 mg, 0.376 mmol) in 12 mL of MeCN. After heating overnight, the solution was allowed to cool to room temperature and diffraction-quality crystals formed on standing. Filtration and washes with MeCN, Et_2O, and pentane resulted in 237 mg (80 %) of pale ochre crystals. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.76 (4H, s), 3.92 (4H, s), 4.08 (4H, s), 6.32 (2H, d, J = 11.2 Hz), 7.21-7.27 (5H, m), 7.29-7.36 (2H, m), 7.38 (2H, d, J = 8.0 Hz), 7.68 (1H, app t, J = 7.2 Hz), 7.72-7.83 (3H, m), 7.93 (1H, d, J = 8.0 Hz), 8.37 (4H, d, J = 6.0 Hz), 8.57 (2H, d, J = 4.8 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 49.11, 57.15, 58.59, 83.42, 108.36, 112.90, 113.11, 114.06, 123.41, 123.97, 124.03, 124.66, 125.63, 126.73, 131.02, 136.27, 138.20, 146.36, 147.06, 148.09, 148.29, 148.43, 148.93, 149.44, 150.13, 151.97, 157.80, 168.87. ^{19}F NMR (DMSO-d_6, 376.5 MHz) δ -139.7 (2F, d, J = 10.9 Hz). HRMS (ESI) calcd for $[M-H]^-$, 789.2642; found, 789.2616.

X-ray Crystallographic Studies. A single crystal of ZP3B was coated with paratone-N oil, mounted at room temperature on the tip of a glass fiber (Oxford magnetic mounting system), and cooled under a stream of cold N_2 maintained by a KRYO-FLEX low-temperature apparatus. Intensity data were collected on a Bruker APEX CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) controlled by a Pentium-based PC running the SMART software package.[4] A total of

2800 frames were acquired. The structure was solved by direct methods and refined on F^2 by using the SHELXTL software.[5,6] Empirical absorption correction was applied with SADABS[7] and the structure was checked for higher symmetry with PLATON.[8] All non-hydrogen atoms were refined anisotropically. In general, hydrogen atoms were assigned idealized positions and given thermal parameters equivalent to either 1.5 (methyl hydrogen atoms) or 1.2 (all other hydrogen atoms) times the thermal parameter of the atom to which they were attached. The two hydrogen atoms (H1 and H3) of O1 and O3 of the hydroxyl groups were located from the electron density map. Compound ZP3B crystallizes as pale-yellow blocks with 1.5 molecules of MeCN.

Table S1. Crystallographic parameters for **ZP3B**

	ZP3B•1.5MeCN
Empirical formula	$C_{49}H_{40.5}N_{7.5}O_5F_2$
Formula weight	852.39
Crystal System	Triclinic
Space group	$P\bar{1}$
a (Å)	12.4844(15)
b (Å)	13.9419(17)
c (Å)	14.7210(18)
α (deg)	114.010(2)
β (deg)	103.594(2)
γ (deg)	103.639(2)
V (Å^3)	2110.6(4)
Z	2
$\rho_{calc,}$ g/cm^3	1.341
Temperature (K)	110
μ (Mo Kα), mm^{-1}	0.095
θ range (deg)	2.46 to 26.37
Crystal size (mm)	0.15 x 0.15 x 0.10
Total no. of data	32603
No. of unique data	8583
Completeness to θ	99.5 %
max, min peaks, e/Å^3	0.747 and -0.504
Goodness-of-fit on F^2	1.042
R_1 (%)[a]	6.53
wR_2 (%)[b]	16.14

[a] $R_1 = \sum||F_o|-|F_c||/\Sigma|F_o|$, [b] $wR_2 = \{\Sigma[w(F_o^2-F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$

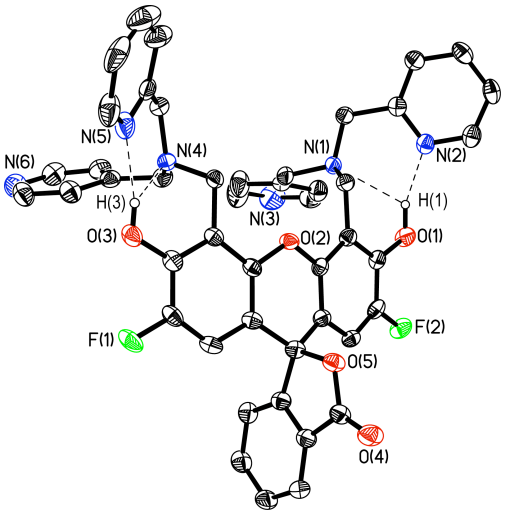

Figure S1. ORTEP diagram of ZP3B showing 50% probability thermal ellipsoids on all non-hydrogen atoms. Dashed lines designate hydrogen bonding contacts.

Table S2. Summary of Distances (Å) between Hydrogen Bond Donor and Acceptor Atoms in ZP3B.

Bond Lengths	Å
O1–H1...N1	2.940(3)
O1–H1...N2	2.709(3)
O3–H3...N4	2.932(3)
O3–H3...N5	2.761(3)

Atoms are labeled in Figure S1.

Spectroscopic Measurements. PIPES, piperazine-*N,N'*-bis(2-ethanesulfonic acid), and 99.999% KCl were purchased from Calbiochem. Optical absorption spectra were collected with a Cary 1E spectrophotometer and fluorescence emission spectra were recorded with a Photon Technology International QM-4/2003 fluorimeter. Measurements at pH 7.0 were performed in aqueous buffer containing 50 mM PIPES and 100 mM KCl. Extinction coefficients were obtained at pH 7.0 using dye solutions in the 0.1-1.0 μM range. All quantum yield measurements were performed with a dye concentration of 0.3 μM, exciting each fluorophore at its wavelength of maximal absorbance. Emission spectra were integrated from 490-700 nm after subtracting the signal caused by scattered excitation light. Dissociation constant (K_d) values for Zn(II) were determined at a sensor concentration of 1 μM in the above mentioned pH 7.0 buffer and titrating with aliquots of 1-10 mM $ZnCl_2$. The integrated emission data were treated using a binding equation previously described.[1] Fluorescence pH titration curves were acquired by using a solution containing 10 mM KOH, 100 mM KCl, and 1.0 μM dye, to which was added aliquots of dilute aqueous HCl. Readings at several points between pH 2-10 were recorded with an Orion 720A pH meter. Samples were maintained at 25 ± 1 °C by a circulating water bath for all spectroscopic measurements.

Potentiometric Titrations. A Mettler Toledo T70 Automated Titrator equipped with a DG-111-SG glass electrode, calibrated against standard buffers, was used for all potentiometric titrations. Solutions were prepared from Millipore water that had been degassed by boiling under low pressure for at least three hours.[9] Titration solutions contained 100 mM KCl as the electrolyte to maintain constant ionic strength and log(K_w) was defined as 13.78, which is appropriate for these conditions.[10] Samples were maintained at 25 ± 1 °C by circulating temperature-regulated water through the jacketed titration vessel during all experiments. ZP3 (0.76 mM) and ZP3B (1 mM) were pre-dissolved in 1 mL of 1.0 M HCl and diluted with water. Samples were titrated with ca. 0.1 M NaOH, standardized against potassium hydrogen phthalate before each series of titrations. Analysis of the data was performed by using the HYPERQUAD2006 v3.1.48 computer program.[11] For each compound, the proton dissociation constants (pK_a values) were determined from three separate titrations. The number of protonation equilibria was allowed to vary during the construction of computer models. In all cases, the triplicate data sets for each compound were combined for determination of the dissociation constants, which in turn were used to compute theoretical titration curves for comparison with the experimental ones.

As described previously, the titration data for ZP1 and ZP1B were best fit to a six proton model,[2] whereas the data for ZP3 and ZP3B were best fit to a five proton model. This difference has little effect on the evaluation of the higher pK_a values, as seen from a comparison between, e.g., pK_{a2} through pK_{a4} of ZP1B and ZP3B. In order to simplify comparison between the various compounds, we assign the lowest dissociation constant for ZP3B and ZP3 as pK_{a2} so that pK_{a6} represents the equilibrium between the monoprotonated (LH^-) and fully deprotonated (L^{2-}) forms in all cases.

Table S3. Comparison of pK_a values as determined by potentiometric titration

	ZP1B[a]	ZP3B	ZP1[a]	ZP3
pK_{a6}	7.473(5)	7.378(3)	8.12(2)	7.96(2)
pK_{a5}	6.35(1)	6.22(1)	6.96(1)	6.81(2)
pK_{a4}	5.205(8)	5.22(1)	4.59(3)	4.62(4)
pK_{a3}	4.51(2)	4.45(5)	3.810(9)	3.78(1)
pK_{a2}	3.10(3)	3.15(7)	2.8(3)	2.9(2)
pK_{a1}	2.67(5)	--	2.3(2)	--

[a]Values from ref. 2. Numbers in parentheses represent standard deviations in the last significant digit.

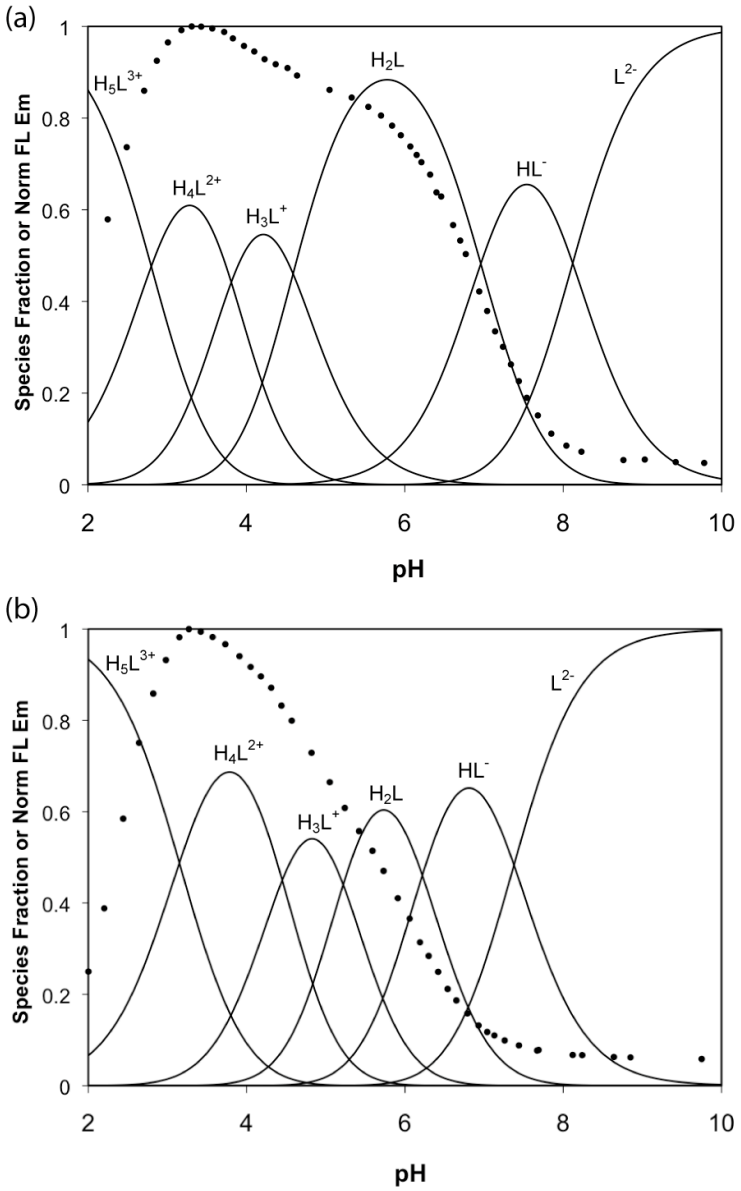

Figure S2. Fluorescence pH titration data (points) for (a) ZP3 and (b) ZP3B overlaid on speciation plots created from calculated acid dissociation constants.

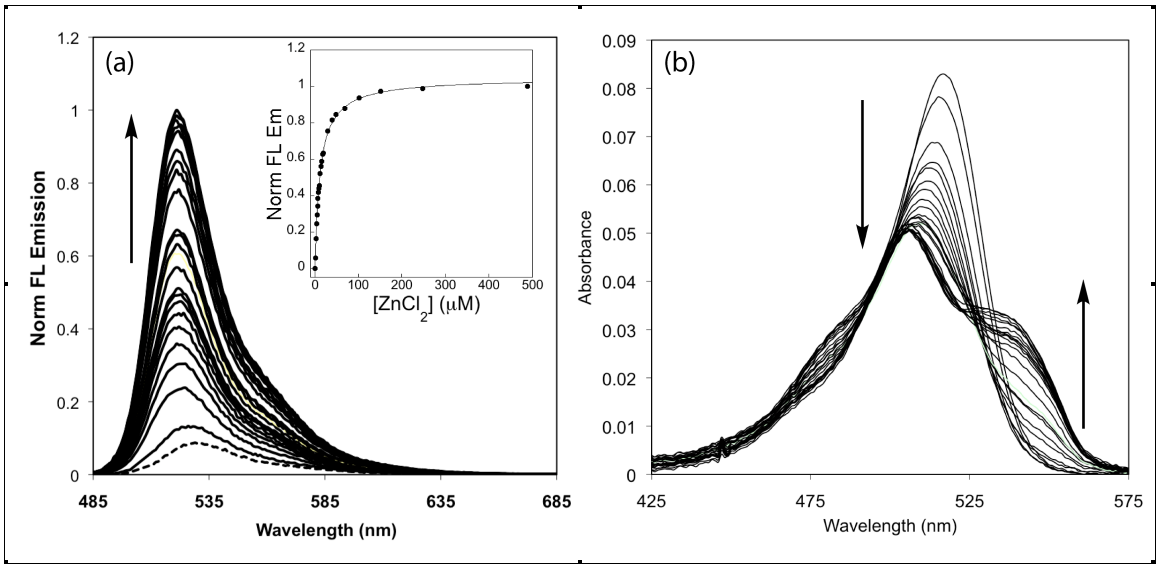

Figure S3. Fluorescence (a) and absorbance (b) titration of 1 μM ZP1B with increasing amounts of $ZnCl_2$ at pH 7 (50 mM PIPES, 100 mM KCl). Emission increases from basal fluorescence (dashed line) in the presence of 1, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 29, 39, 49, 68, 97, 102, 151, 248, and 489 μM total $ZnCl_2$. The absorption band at 529 nm begins to emerge at ~7 μM $ZnCl_2$.

Cell culture and Microscopy. Min6 cells were cultured at 37 °C in Dulbecco's Modification of Eagle's Medium (DMEM, Mediatech), containing 4.5 g/L of glucose and L-glutamine and supplemented with 10 % fetal bovine serum (HyClone), penicillin (1 unit/mL), and streptomycin (1 μg/mL). For live cell imaging, cells were plated onto poly-D-lysine coated glass-bottom culture dishes (MatTek) and grown for 2-3 days. Cells were incubated with 40 μM of either ZP1B or ZP3B and 5 μM Hoechst 33258 (Aldrich) for 1-4 hours at 37 °C, washed with DMEM twice, and imaged in PBS. After collecting images in both the green and blue channels, *N,N,N',N'*-tetrakis(2-pyridylmethyl)-ethylenediamine (TPEN) was added to the dish to a final concentration of 40 μM and cells were imaged again after 5 min.

Microscopy was performed using a Zeiss Axiovert 200M inverted epifluorescence microscope and images were collected with an EM-CCD digital camera (Hamamatsu). Differential Interference Contrast (DIC) and fluorescence images were acquired with an oil-immersion 63x objective lens. An EXFO metal-halide lamp was used for fluorescence excitation, with a GFP filter (450-490 nm excitation, 500-550 nm emission) for the green channel and a DAPI filter (320-380 nm excitation, 420-470 nm emission) used for the blue channel. Microscope operation and image processing were performed using Volocity software (Improvision, Lexington, MA).

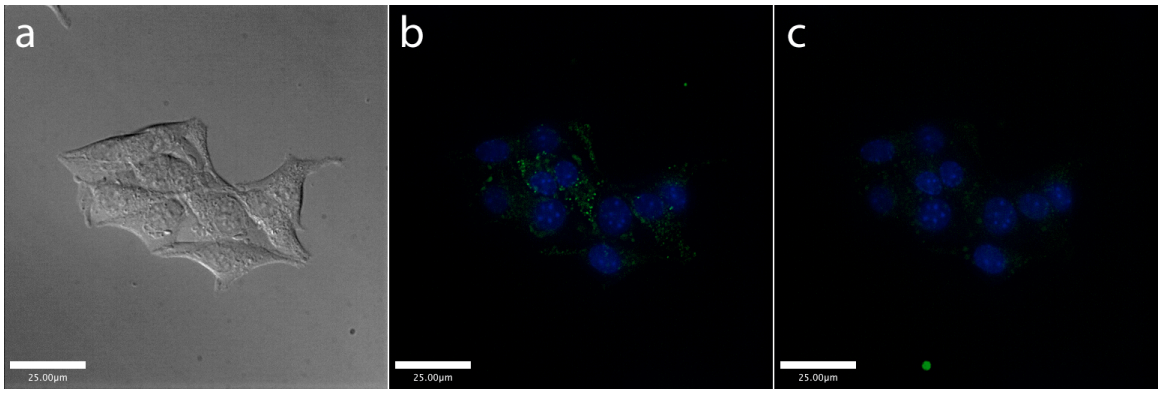

Figure S4. Live cell images of Min6 cells after incubating with ZP1B (green) and Hoechst 33258 nuclear stain (blue) for 1 h. DIC image (a) and merged green and blue channels before (b) and after (c) the addition of TPEN.

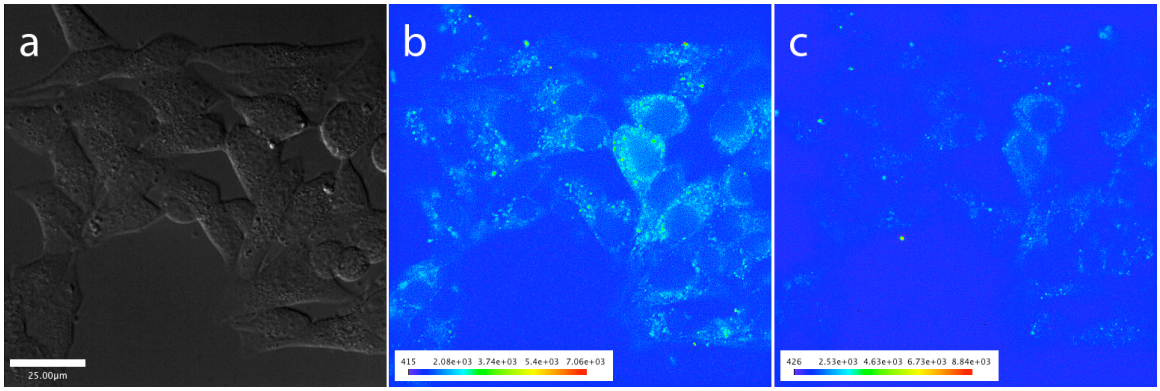

Figure S5. Live cell images of Min6 cells after incubating with ZP3B for 3 h. DIC image (a) and green fluorescence channel, depicted on a pseudocolor scale for clarity, before (b) and after (c) the addition of TPEN.

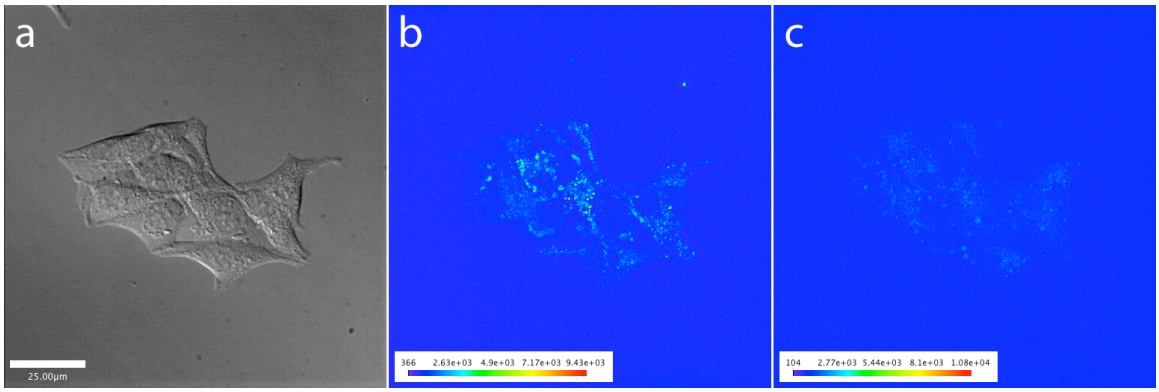

Figure S6. Live cell images of Min6 cells after incubating with ZP1B for 1 h. DIC image (a) and green fluorescence channel, depicted on a pseudocolor scale for clarity, before (b) and after (c) the addition of TPEN.

References.

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