SUPPORTING INFORMATION FOR

QZ1 and QZ2: Rapid, Reversible Quinoline-Derivatized Fluoresceins for Sensing Biological Zn(II)

Elizabeth M. Nolan,[1] Jacek Jaworski,[2,4] Ken-Ichi Okamoto,[2,3] Yasunori Hayashi,[2,3] Morgan Sheng,[2,3,4] and Stephen J. Lippard*[,1]

Contribution from the [1]*Department of Chemistry,* [2]*Picower Center for Learning and Memory,* [3]*RIKEN-MIT Neuroscience Research Center, and* [4]*Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

Received April 00, 2005

*To whom the correspondence should be addressed: lippard@mit.edu

INCLUDED ARE:

Full listing, showing all authors, for reference 14 cited in the main text:

Henshall, S. M.; Afar, D. E. H.; Rasiah, K. K.; Horvath, L. G.; Gish, K.; Caras, I.; Ramakrishnan, V.; Wong, M.; Jeffry, U.; Kench, J. G.; Quinn, D. I.; Turner, J. J.; Delprado, W.; Lee, C.-S.; Golovsky, D.; Brenner, P. C.; O'Neil, G. F.; Kooner, R.; Stricker, P. D.; Grygiel, J. J.; Mack, D. H.; Sutherland, R. L. *Oncogene*, **2003**, *22*, 6005-6012.

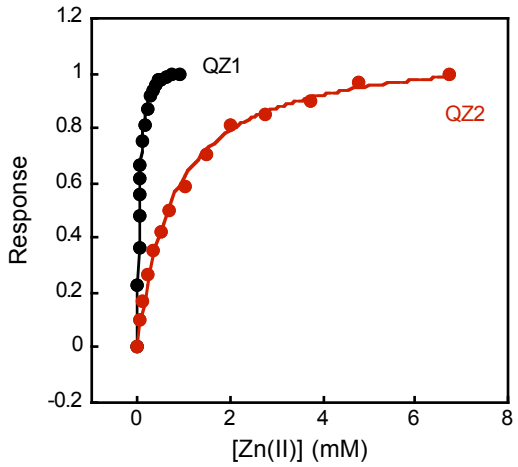

Figure S1. Direct comparison of the Zn(II) response of QZ1 and QZ2 as a function of Zn(II) concentration. Normalized integrated emission versus [Zn(II)] for QZ1 and QZ2 at pH 7 (50 mM PIPES, 100 mM KCl). These data correspond to the titrations presented in Figure 2. A dissociation constant of 33 ± 2 μM was obtained for QZ1 by fluorimetric titration (1:1 binding model) at 25 ºC., A K_d v value of 48 ± 3 μM was obtained from stopped-flow kinetic experiments conducted at 4.3 ºC. A K_{d1} of 41 ± 3 μM was obtained for QZ2 by stopped-flow kinetic studies at 4.3 ºC. The QZ2 fit above is for a 1:1 binding model, which was used only to determine the concentration of Zn(II) required for ~50% of the total fluorescence change (~770 μM zinc) to compare this value to the K_d of QZ1. The K_{d2} value for QZ2, of significantly lower affinity judging by data included in the panel above, was not determined.

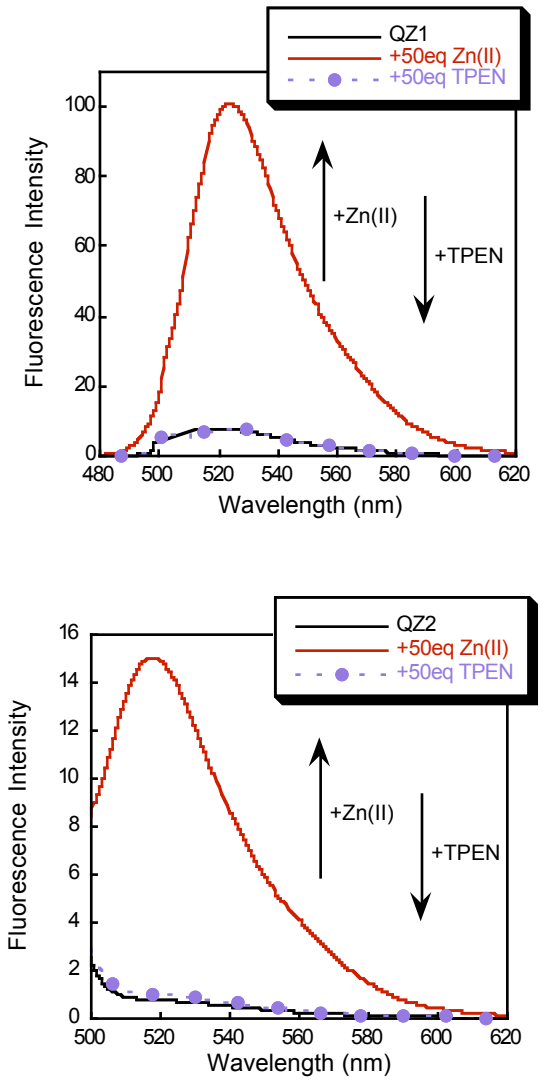

Figure S2. Reversibility of QZ1 (top) and QZ2 (bottom) binding to Zn(II) by addition of TPEN. A solution of 1 μM QZ was prepared in 50 mM PIPES, 100 mM KCl, pH 7 and the emission spectrum collected. Subsequently, 50 equiv of $ZnCl_2$ were added and the emission change was recorded. Addition of 50 equiv of TPEN to the solution containing QZ and Zn(II) resulted in fluorescence decrease to the background value immediately upon mixing. Samples were excited at 495 nm (QZ1) and at 490 nm (QZ2). T = 25 ± 1 °C.

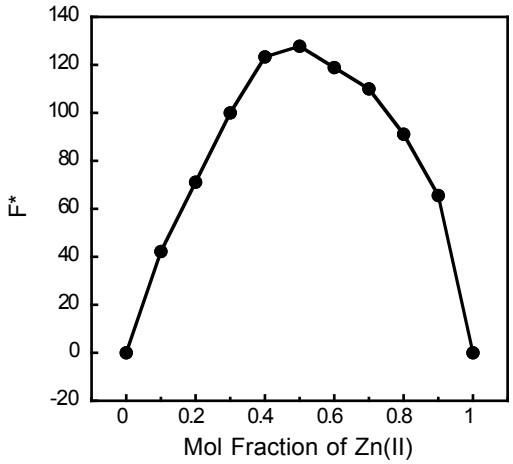

Figure S3. Job plot for the formation of the QZ1:Zn(II) complex determined by using fluorescence spectroscopy (50 mM PIPES, 100 mM KCl, pH 7). F* is the fluorescence change associated with Zn(II) binding. The inflection point at 0.5 equivalents of Zn(II) indicates formation of a 1:1 complex. The concentration of the initial QZ1 and Zn(II) solutions were 2 μM and samples were excited at 500 nm.

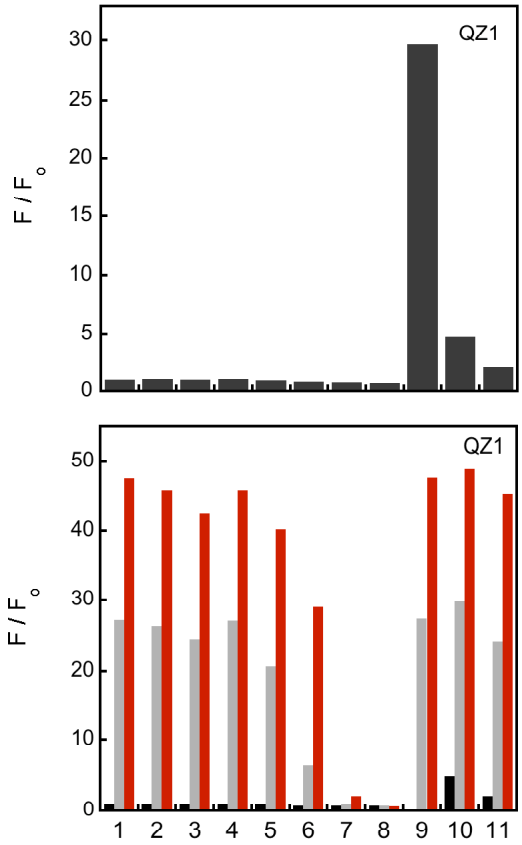

Figure S4. Selectivity of QZ1 for Zn(II) at pH 7 (50 mM PIPES, 100 mM KCl). Top: Fluorescence response of QZ1 following addition of 50 equiv of the metal ion of interest: **1**, Na(I); **2**, Ca(II); **3**, Mg(II); **4**, Mn(II); **5**, Fe(II); **6**, Co(II); **7**, Ni(II); **8**, Cu(II); **9**, Zn(II); **10**, Cd(II); **11**, Hg(II). Bottom: Selectivity of QZ1 for Zn(II) over the metal ions of interest. Black bars: QZ1 + 50 equiv cation; light grey bars: addition of 50 equiv Zn(II) to the solution containing QZ1 and the cation of interest; red bars: introduction of an additional 500 equiv Zn(II) to the solution containing QZ1 and the cation of interest. The emission spectra were integrated from 510-650 nm and samples were excited at 495 nm. All data (F) are normalized with respect to the emission of the free dye (F_o). [QZ1] = 1 μM.

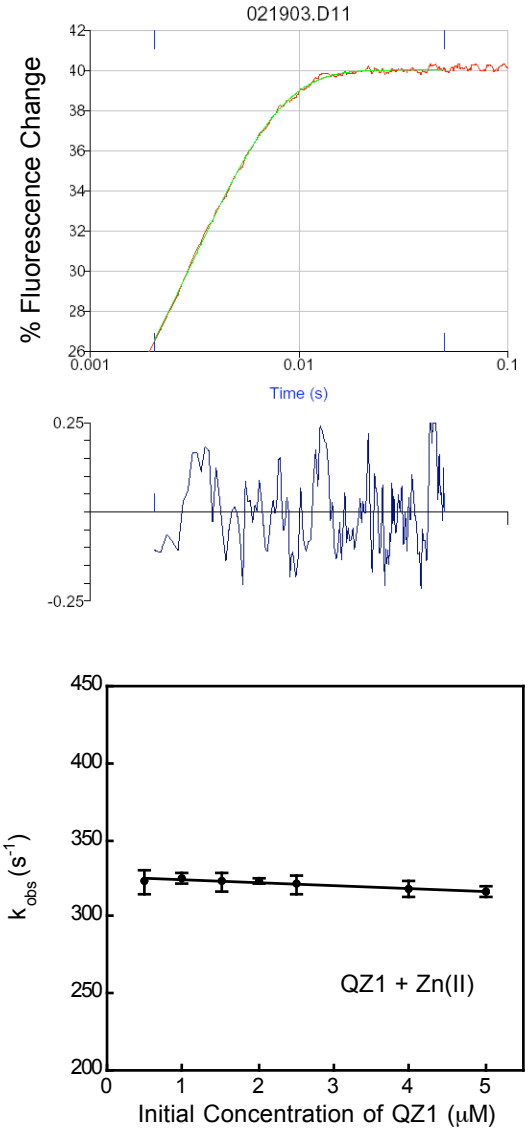

Figure S5. Example of stopped-flow kinetics data for Zn(II) binding to QZ1 with the concentration of QZ1 varied at pH 7 (50 mM PIPES, 100 mM KCl). [Zn(II)] = 75 μM after mixing. [QZ1] = 0.5, 1, 1.5, 2.5, 4 and 5 μM after mixing. Top: Example kinetic trace of fluorescence change vs. time for [QZ1] = 1.5 μM after mixing, monoexponential fit and residuals from the fit. Bottom: Plot of k_{obs} versus concentration of QZ1. Samples were excited at 495 nm and the emission was monitored from 455 to 700 nm. Data were collected at 4.3 ± 0.1 °C.

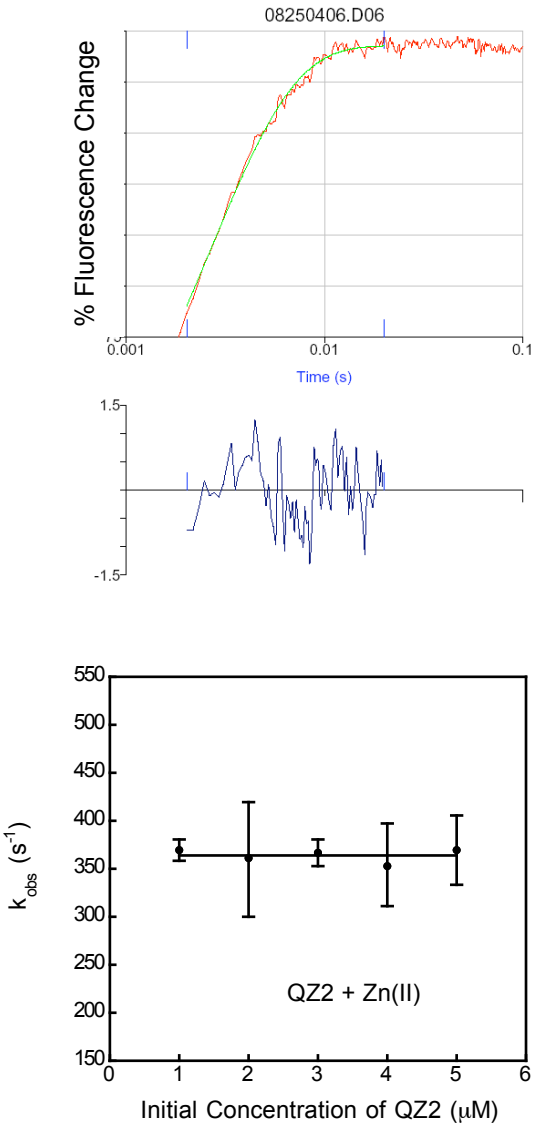

Figure S6. Example of stopped-flow kinetics data for Zn(II) binding to QZ2 with the concentration of QZ2 varied at pH 7 (50 mM PIPES, 100 mM KCl). [Zn(II)] = 50 μM after mixing. [QZ2] = 1, 2, 3, 4 and 5 μM after mixing. Top: Example kinetic trace of fluorescence change vs. time for [QZ2] = 3 μM after mixing, monoexponential fit and residuals from the fit. Bottom: Plot of k_{obs} versus concentration of QZ2. Samples were excited at 490 nm and the emission was monitored from 455 to 700 nm. Data were collected at 4.3 ± 0.1 °C.

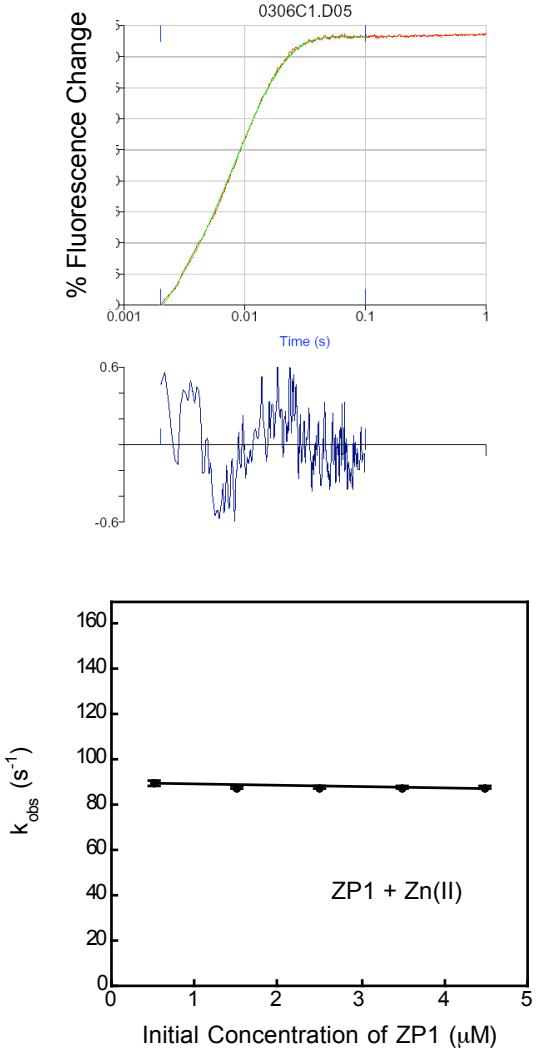

Figure S7. Example of stopped-flow kinetics data for Zn(II) binding to ZP1 with the concentration of ZP1 varied (50 mM PIPES, 100 mM KCl). [Zn(II)] = 150 μM after mixing. [ZP1] = 0.5, 1.5, 2.5, 3.5, and 4.5 μM after mixing. Top: Example kinetic trace of fluorescence change vs. time for [ZP1] = 0.5 μM after mixing, monoexponential fit and residuals from the fit. Bottom: Plot of k_{obs} versus concentration of ZP1. Samples were excited at 505 nm and the emission was monitored from 455 to 700 nm. Data were collected at 4.3 ± 0.1 °C

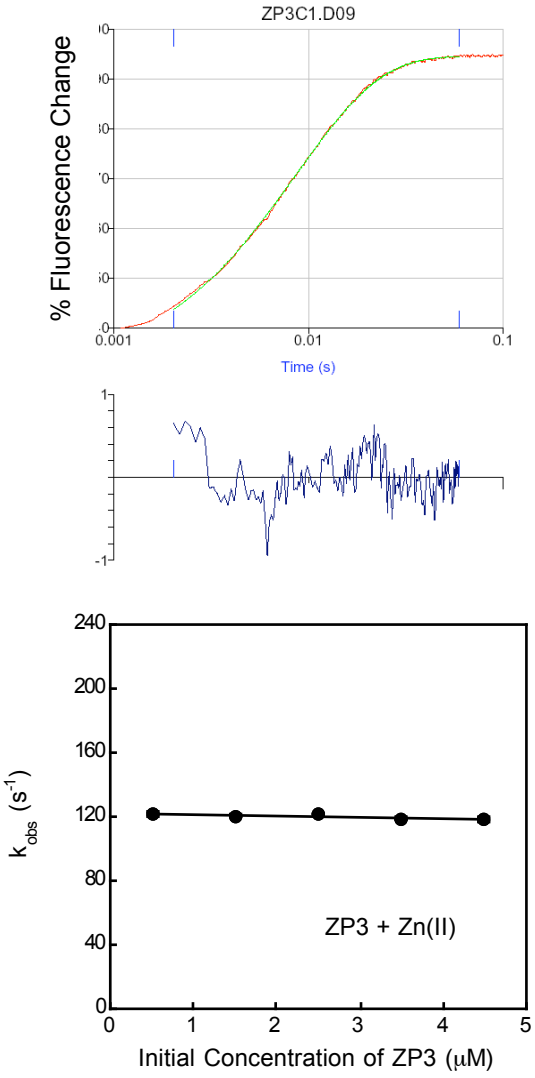

Figure S8. Example of stopped-flow kinetics data for Zn(II) binding to ZP3 with the concentration of ZP3 varied at pH 7 (50 mM PIPES, 100 mM KCl). [Zn(II)] = 150 μM after mixing. [ZP3] = 0.5, 1.5, 2.5, 3.5, and 4.5 μM after mixing. Top: Example kinetic trace of fluorescence change vs. time for [ZP1] = 0.5 μM after mixing, monoexponential fit and residuals from the fit. Bottom: Plot of k_{obs} versus concentration of ZP3. Samples were excited at 492 nm and the emission was monitored from 455 to 700 nm. Data were collected at 4.3 ± 0.1 ºC

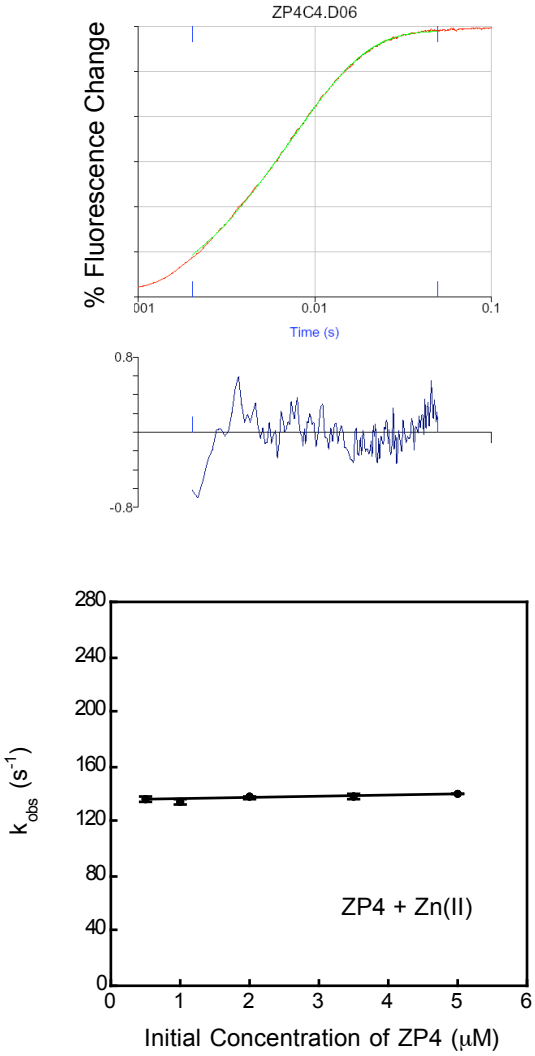

Figure S9. Example of stopped-flow kinetics data for Zn(II) binding to ZP4 with the concentration of ZP4 varied at pH 7 (50 mM PIPES, 100 mM KCl). [Zn(II)] = 150 μM after mixing. [ZP4] = 0.5, 1.5, 2.5, 3.5, and 4.5 μM after mixing. Top: Example kinetic trace of fluorescence change vs. time for [ZP4] = 4.5 μM after mixing, monoexponential fit and residuals from the fit. Bottom: Plot of k_{obs} versus concentration of ZP4. Samples were excited at 495 nm and the emission was monitored from 455 to 700 nm. Data were collected at 4.3 ± 0.1 ºC

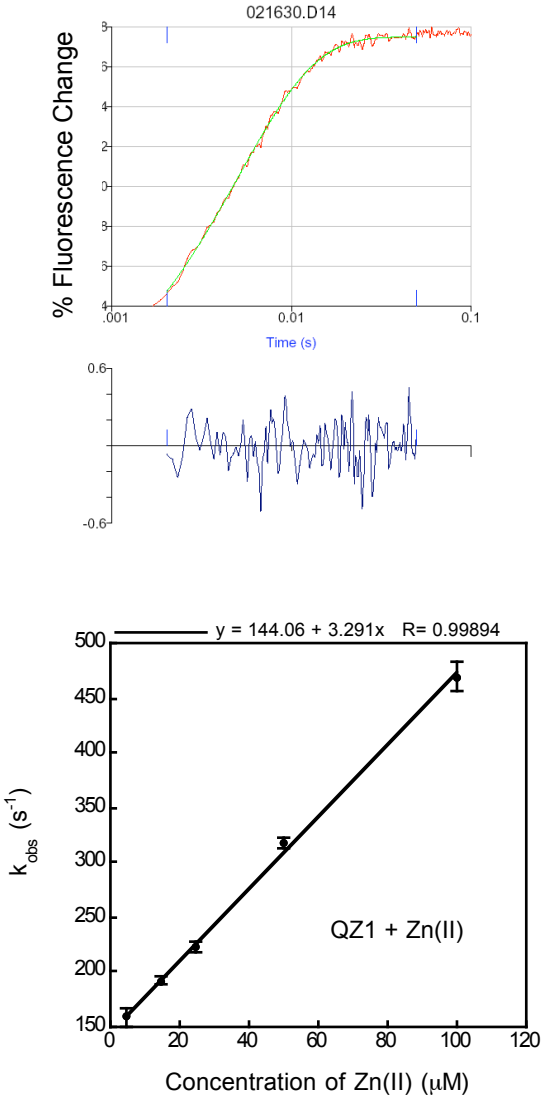

Figure S10. Example of stopped-flow kinetics data for Zn(II) binding to QZ1 at pH 7 (50 mM PIPES, 100 mM KCl). [QZ1] = 0.5 μM after mixing. [Zn(II)] = 5, 15, 25, 50 and 100 μM after mixing. Top: Example kinetic trace of fluorescence change vs. time for [Zn(II))] = 15 μM, monoexponential fit, and residuals from the fit. Bottom: Plot of k_{obs} versus concentration of Zn(II). Samples were excited at 495 nm and the emission was monitored from 455 to 700 nm. Data were collected at 4.3 ± 0.1 ºC

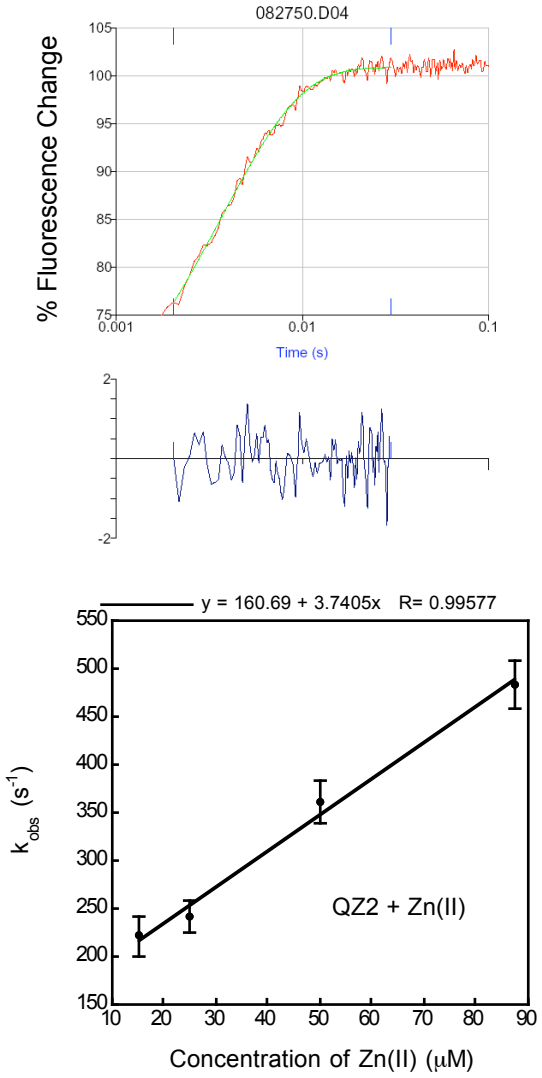

Figure S11. Example of stopped-flow kinetics data for Zn(II) binding to QZ2 at pH 7 (50 mM PIPES, 100 m M KCl). [QZ2] = 0.5 μM after mixing. [Zn(II)] = 15, 25, 50 and 87 μM after mixing. Top: Example kinetic trace of fluorescence change vs. time for [Zn(II)] = 25 μM after mixing, monoexponential fit and residuals from the fit. Bottom: Plot of k_{obs} versus concentration of Zn(II). Samples were excited at 490 nm and the emission was monitored from 455 to 700 nm. Data were collected at 4.3 ± 0.1 °C

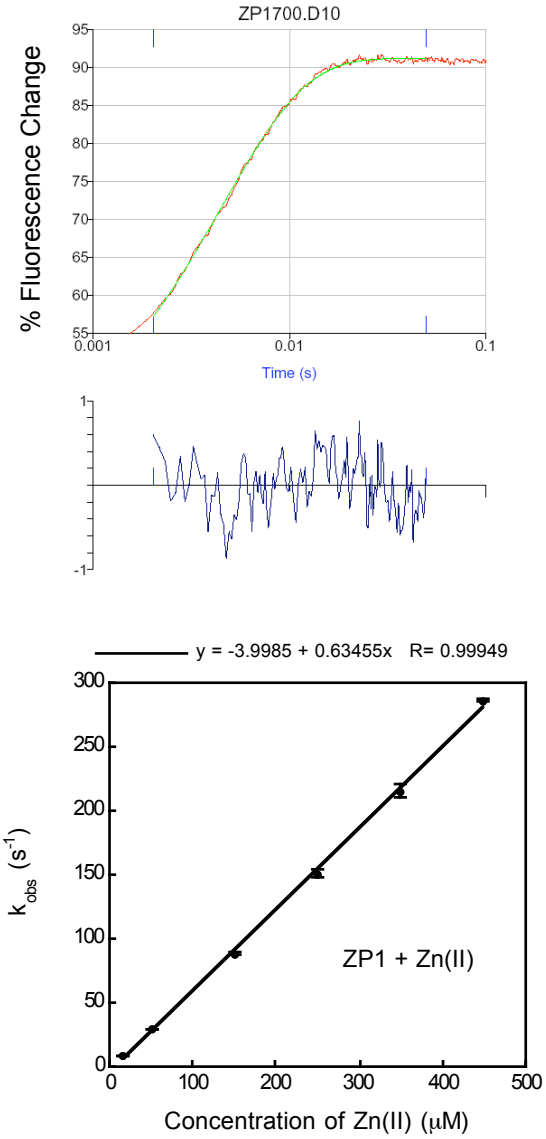

Figure S12. Example of stopped-flow kinetics data for Zn(II) binding to ZP1 at pH 7 (50 mM PIPES, 100 mM KCl). [ZP1] = 0.5 μM after mixing. [Zn(II)] = 15, 50, 150, 250, 350 and 450 μM after mixing. Top: Example kinetic trace of fluorescence change vs. time for [Zn(II)] = 350 μM, monoexponential fit and residuals from the fit. Bottom: Plot of k_{obs} versus concentration of Zn(II). [ZP1] = 0.5 μM after mixing. Samples were excited at 505 nm and the emission was monitored from 455 to 700 nm. Data were collected at 4.3 ± 0.1 °C

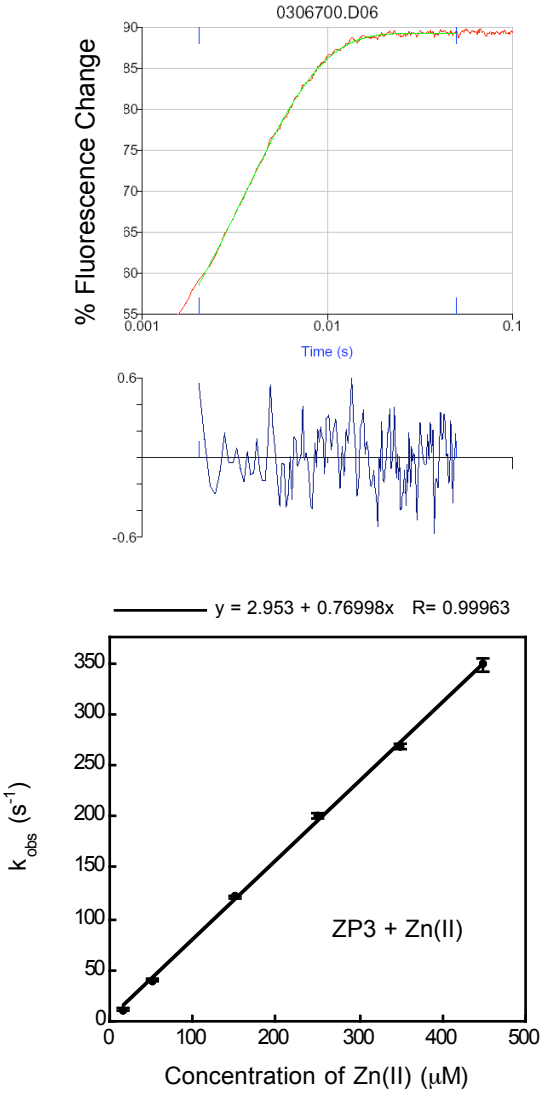

Figure S13. Example of stopped-flow kinetics data for Zn(II) binding to ZP3 at pH 7 (50 mM PIPES, 100 mM KCl). [ZP3] = 0.5 μM after mixing. [Zn(II)] = 15, 50, 150, 250, 350 and 450 μM after mixing. Top: Example kinetic trace of fluorescence change vs. time for [Zn(II)] = 350 μM after mixing, monoexponential fit and residuals from the fit. Bottom: Plot of k_{obs} versus concentration of Zn(II). Samples were excited at 492 nm and the emission was monitored from 455 to 700 nm. Data were collected at 4.3 ± 0.1 °C

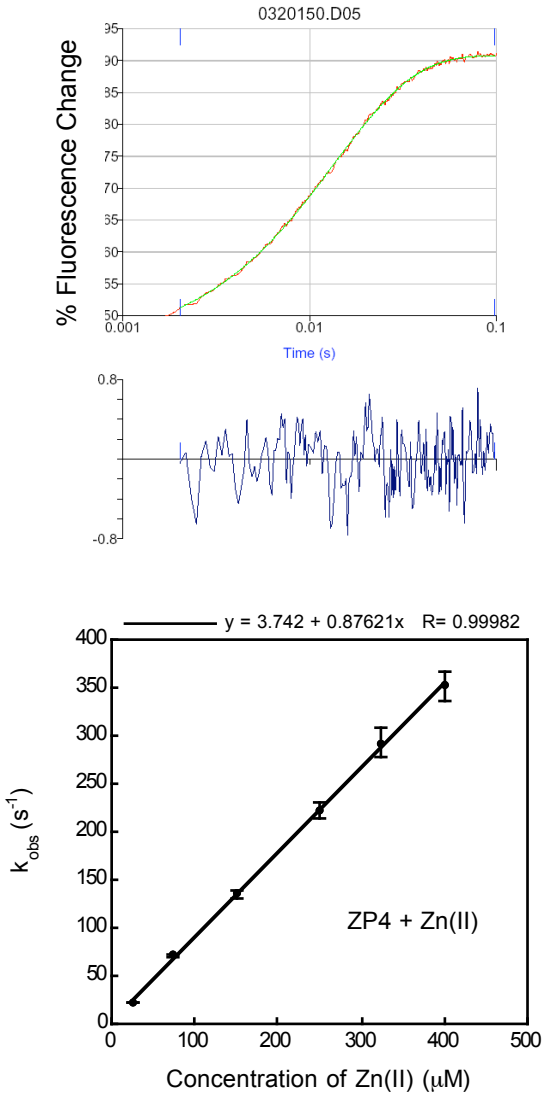

Figure S14. Example of stopped-flow kinetics data for Zn(II) binding to ZP4 at pH 7 (50 mM PIPES, 100 mM KCl). [ZP4] = 0.5 μM after mixing. [Zn(II)] = 25, 75, 150, 250, 350 and 450 μM after mixing. Top: Example kinetic trace of fluorescence change vs. time [Zn(II)] = 75 μM after mixing, monoexponential fit and residuals from the fit. Bottom: Plot of k_{obs} versus concentration of Zn(II). Samples were excited at 495 nm and the emission was monitored from 455 to 700 nm. Data were collected at 4.3 ± 0.1 °C

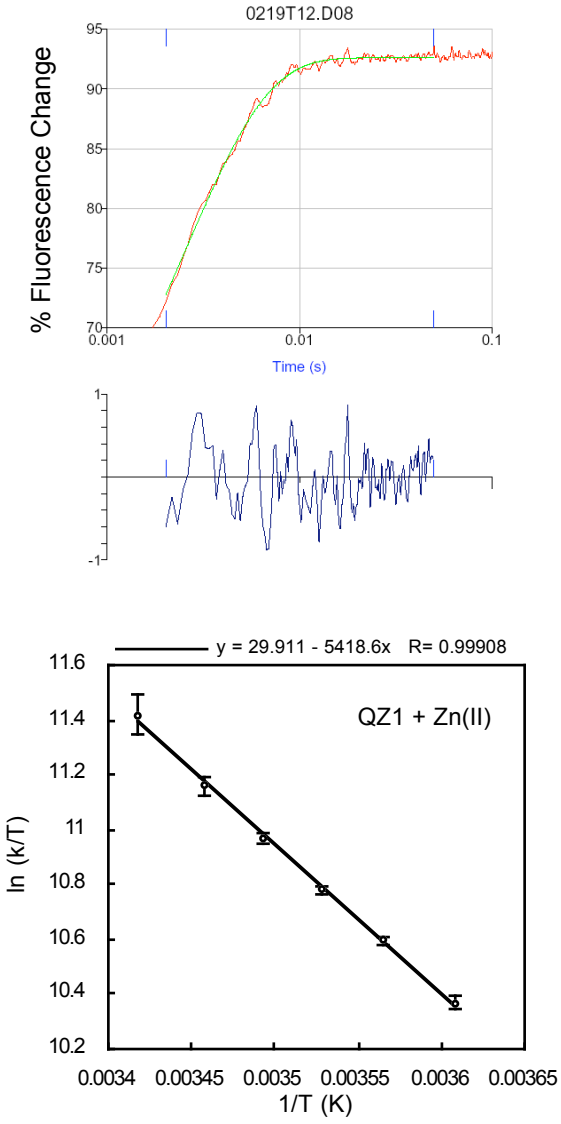

Figure S15. Example kinetic trace and Eyring plot from temperature-dependent stopped-flow fluorescence studies of QZ1 binding to Zn(II) at pH 7 (50 mM PIPES, 100 mM KCl). [QZ1] = 1 μM and [Zn(II)] = 25 μM after mixing. The temperature was varied from ~4 to ~16 °C and the samples were excited at 495 nm. Emission was monitored from 455 to 700 nm. Top: Example kinetic trace of fluorescence change vs. time for T = 11.9 °C, monoexponential fit and residuals from the fit. Bottom: Eyring plot.

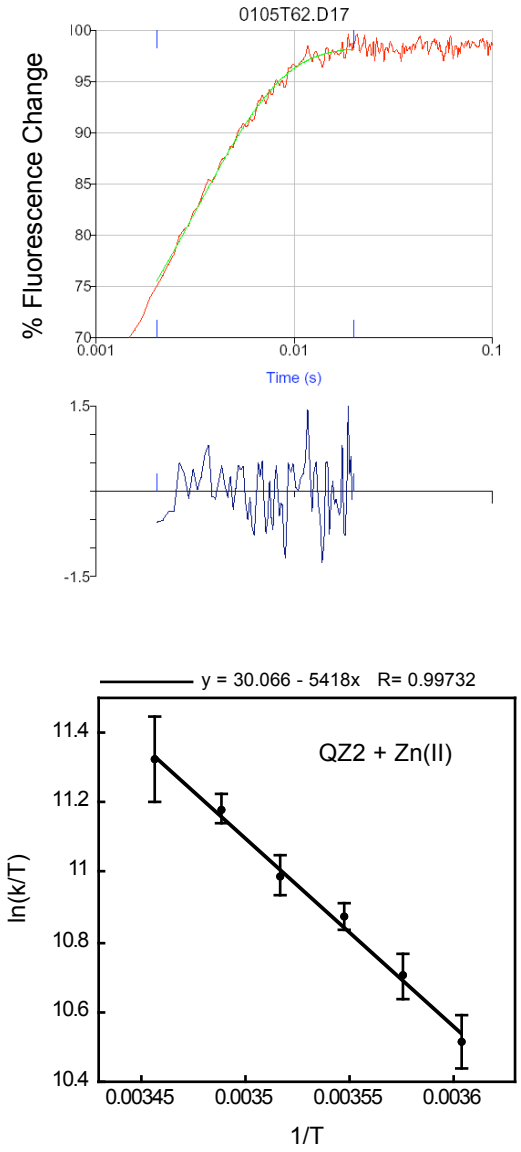

Figure S16. Example kinetic trace and Eyring plot from temperature-dependent stopped-flow fluorescence studies of QZ2 binding to Zn(II) at pH 7 (50 mM PIPES, 100 mM KCl). [QZ2] = 1.5 μM and [Zn(II)] = 25 μM after mixing. The temperature was varied from ~4 to ~16 °C and the samples were excited at 490 nm. Emission was monitored from 455 to 700 nm. Top: Example kinetic trace of fluorescence change vs. time for T = 25 °C, monoexponential fit and residuals from the fit. Bottom: Eyring plot.

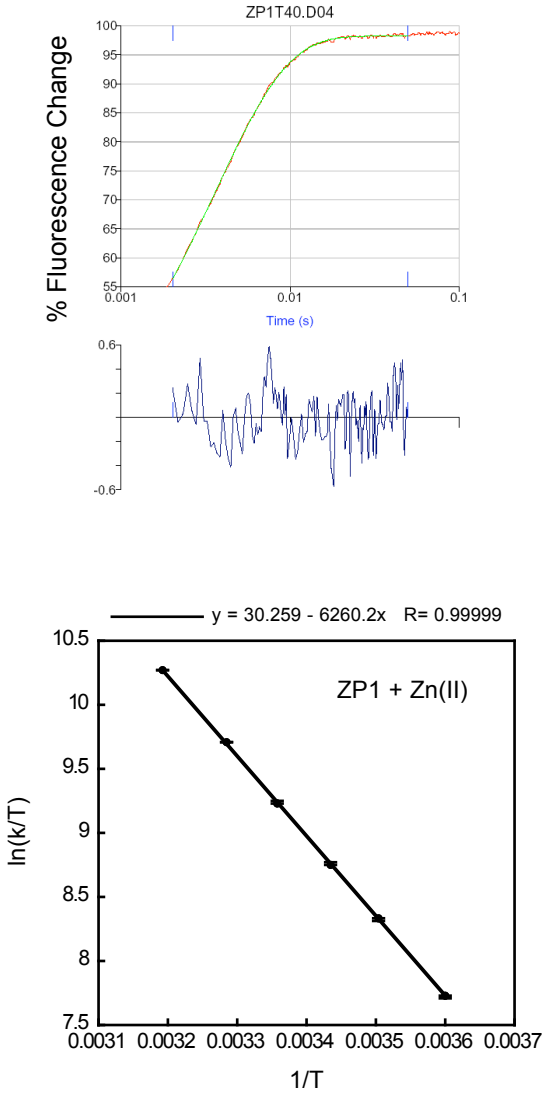

Figure S17. Example kinetic trace and Eyring plot from temperature-dependent stopped-flow fluorescence studies of ZP1 binding to Zn(II) at pH 7 (50 mM PIPES, 100 mM KCl). [ZP1] = 1 μM and [Zn(II)] = 25 μM after mixing. The temperature was varied from ~4 to ~40 °C and the samples were excited at 490 nm. Emission was monitored from 455 to 700 nm. Top: Example kinetic trace of fluorescence change vs. time for T = 40.2 °C, monoexponential fit and residuals from the fit. Bottom: Eyring plot.

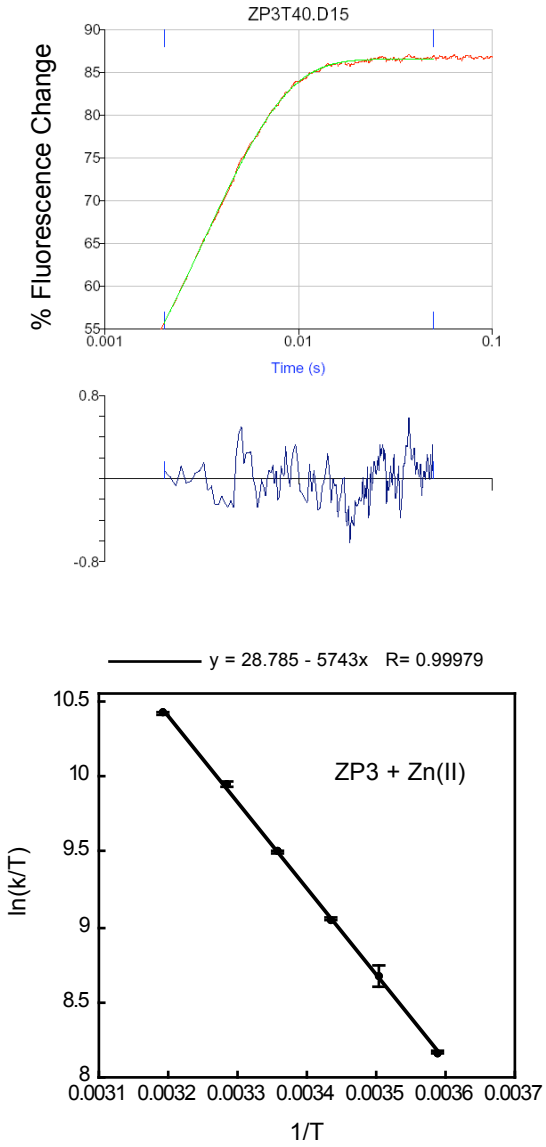

Figure S18. Example kinetic trace and Eyring plot from temperature-dependent stopped-flow fluorescence studies of ZP3 binding to Zn(II) at pH 7 (50 mM PIPES, 100 mM KCl). [ZP3] = 1 μM and [Zn(II)] = 25 μM after mixing. The temperature was varied from ~4 to ~40 °C and the samples were excited at 492 nm. Emission was monitored from 455 to 700 nm. Top: Example kinetic trace of fluorescence change vs. time for T=40.2 °C, monoexponential fit and residuals from the fit. Bottom: Eyring plot.

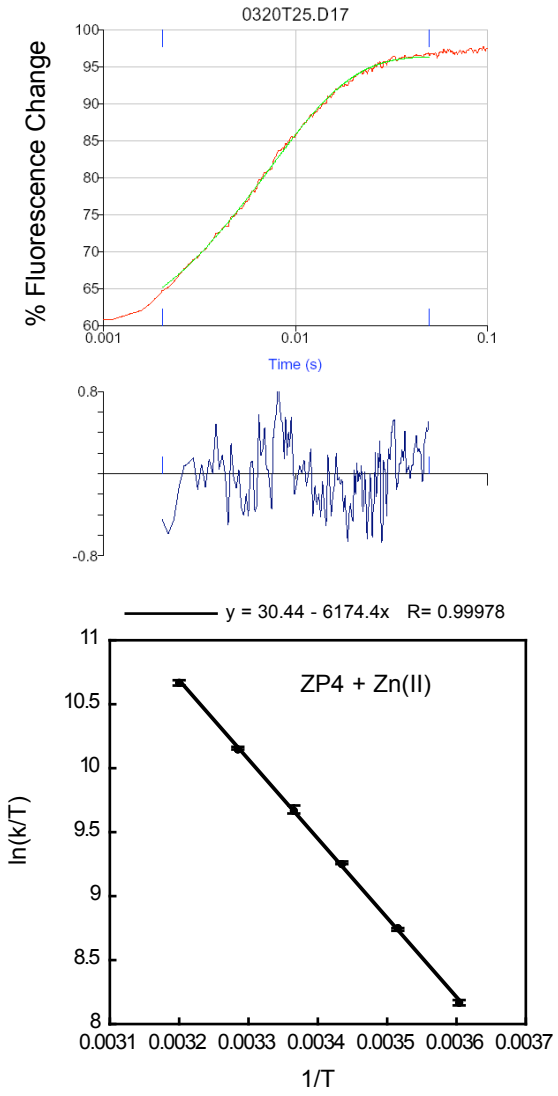

Figure S19. Example kinetic trace and Eyring plot from temperature-dependent stopped-flow fluorescence studies of ZP4 binding to Zn(II) at pH 7 (50 mM PIPES, 100 mM KCl). [ZP4] = 1 μM and [Zn(II)] = 25 μM after mixing. The temperature was varied from ~4 to ~40 °C and the samples were excited at 495 nm. Emission was monitored from 455 to 700 nm. Top: Example kinetic trace of fluorescence change vs. time for T = 25 °C, monoexponential fit and residuals from the fit. Bottom: Eyring plot.

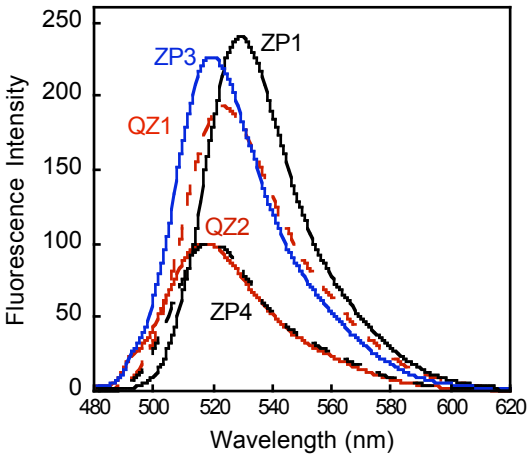

Figure S20. Relative fluorescence emission of Zn(II) bound sensors ZP1, ZP3, ZP4, QZ1 and QZ2 (50 mM PIPES, 100 mM KCl, pH 7). Solutions of 1 μM free sensor were prepared and excess Zn(II) was added to saturate the signal. Samples were excited at 490 (QZ2, ZP3), 495 (ZP1, QZ1), or 505 (ZP3) nm. See Fig. 9 for a comparison of the fluorescence emission of the unbound sensors.

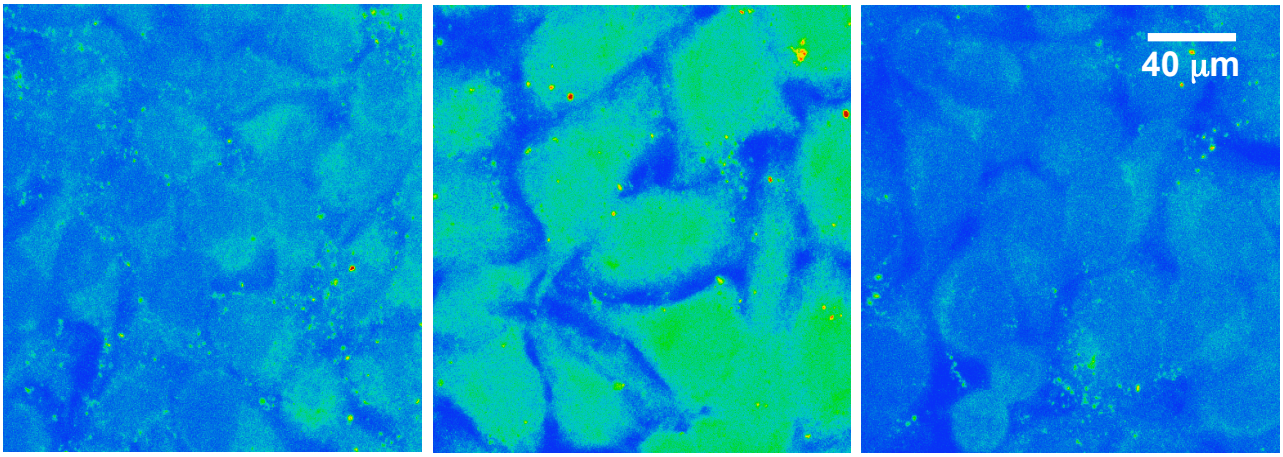

Figure S21. Confocal imaging of HeLa cells treated with QZ2. Left panel: HeLa cells incubated with 10 μM QZ2 for 4 h. Middle panel: Fluorescence change upon introduction of 100 μM Zn(II) to the QZ2-treated HeLa cells. A 10:2 Zn(II)/pyrithione ratio was employed and the cells were incubated with Zn(II) for 10 min at 37 °C. Right panel: HeLa cells treated as described for the middle panel with subsequent addition of 100 μM TPEN (10 min, 37 °C). All cells were fixed (PBS with 4% PFA, 4% sucrose) prior to imaging.

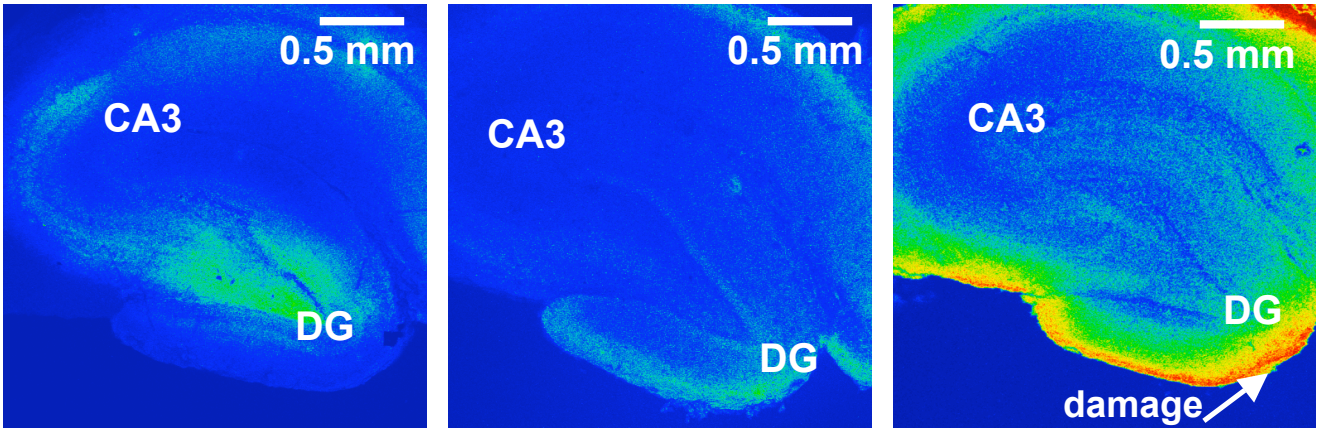

Figure S22. Confocal fluorescence microscopic images of hippocampal slices from adult rat stained with ZP3 (left), QZ1 (middle) or QZ2 (right). The slices were bathed in Zn(II)-free Krebs ringer buffer and incubated with 10 μM ZP3 or QZ prior to imaging. The slice treated with ZP3 shows intense fluorescence in the DG region and staining of the CA3 region. The slices treated with QZ show no differential staining in these substructures. The brighter ring around the QZ2 image is an artifact of the slice preparation and occurs because of the higher gain used to assure that any Zn(II) staining was not overlooked in the DG regions.

Experimental details:

Hippocampal Slice Preparation. The whole brains of 90-day old adult Sprague-Dawley rates were removed. The hippocampi were dissected, cut into 0.5-1.0 mm-thick slices, and washed twice with Zn(II)-free Krebs ringer buffer. Slices were incubated with 10 μM dye at 37 °C under 5% CO_2 in Zn(II)-free Krebs ringer buffer. The incubation time was varied from 10 min to 3 h depending on the experiment. Prolonged incubation did not result in increased QZ fluorescence in the DG and CA3 regions.

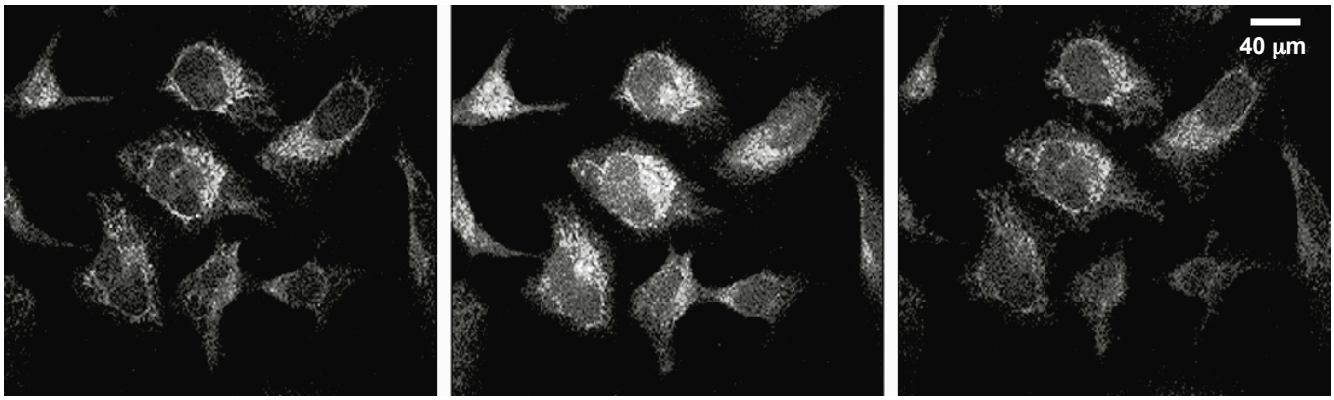

Figure S23. Images of live HeLa cells treated with 10 μM QZ2 and Zn(II) obtained by two-photon microscopy. Left panel: Hela cells treated with 10 μM QZ2 (4 h, 37 ºC). Middle panel: After addition of 100 μM Zn(II) with 10:2 Zn(II)/pyrithione for 5 min at 37 ºC. A fluorescence increase is observed. Right panel: After treatment with 100 μM TPEN for 5 min at 37 ºC the fluorescence decreases to baseline. Samples were excited at 780 nm. Emission was monitored from 0 to 570 nm.