

Imaging Mobile Zinc in Biology

Elisa Tomat and Stephen J. Lippard

Address

Department of Chemistry 18-498, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

Corresponding author: Lippard, Stephen J. (lippard@mit.edu)

Summary

Trafficking and regulation of mobile zinc pools influence cellular functions and pathological conditions in multiple organs, including brain, pancreas, and prostate. The quest for a dynamic description of zinc distribution and mobilization in live cells fuels the development of increasingly sophisticated probes. Detection systems that respond to zinc binding with changes of their fluorescence emission properties have provided sensitive tools for mobile zinc imaging, and fluorescence microscopy experiments have afforded depictions of zinc distribution within live cells and tissues. Both small-molecule and protein-based fluorescent probes can address complex imaging challenges, such as analyte quantification, site-specific sensor localization, and real-time detection.

Introduction

The divalent zinc cation, Zn^{2+} , plays structural and/or functional roles in a multitude of proteins and thereby influences a wide metabolic spectrum. Redox inert in biological media and adaptable to different coordination geometries, much of the zinc in eukaryotic cells resides in binding domains provided by metalloenzymes [1]. For proper functioning of the zinc proteome, specialized proteins efficiently regulate zinc uptake, storage, buffering, and efflux [2]. Metallothioneins and the ZIP and ZnT families of proteins redistribute zinc cations among different organelles and maintain tightly regulated zinc concentration gradients. In this context, the presence of a dynamic pool of *mobile* and chelatable zinc in cells is attracting increased attention. The zinc homeostatic machinery can produce transient zinc signals that stimulate and/or initiate cellular functions [3,4]. This mobilized zinc can play many physiological roles in zinc-enriched tissues, such as hippocampus [5], pancreas [6], and prostate [7]. Although insights about the biochemistry and physiology of mobile zinc in these and other biological settings accrue at a steady pace, a comprehensive map of mobile zinc in human biology is not yet available.

Efforts to describe intracellular zinc trafficking motivate the construction and improvement of tools for zinc visualization in live cells and animals. Synthetic and biochemical approaches aim to develop detection systems that provide: (i) selective binding of zinc over competing ions, such as Ca^{2+} , H^+ , or Fe^{2+} , (ii) a rapid and reversible response over a wide dynamic range of zinc concentrations, and (iii) biocompatibility in terms of toxicity, solubility, and stability. Mobile zinc bioimaging is currently dominated by fluorescent probes. In part because of technical advances in microscopy

instrumentation, fluorescent sensors have proved to be sensitive and versatile tools for zinc detection in live cells and tissues. Because ultraviolet (UV) irradiation causes biological tissue damage and elicits cellular autofluorescence, fluorescent indicators featuring visible absorption and emission profiles are strongly preferred. In order to minimize signal contamination and background contributions, current sensor design also aims to resist photobleaching and to minimize background fluorescence arising from pH and ionic strength effects. Control of probe distribution and zinc quantification in live cells are two additional objectives.

Because comprehensive surveys of zinc sensors have appeared in recent literature [8-11], the present review concentrates on detection systems reported since 2007. We focus on dynamic imaging of mobile zinc in living specimens, thereby excluding valuable imaging methods, such as autometallography and X-ray fluorescent microscopy, which do not employ zinc-responsive sensors and are generally not suited for live cell imaging [12].

Fluorescent small-molecule sensors

Fluorescent sensor molecules for zinc ions typically consist of a zinc-chelating unit, such as iminodiacetate (IDA) or di-2-picolylamine (DPA), and a fluorescent reporter that signals the occurrence of binding events through a change in emission properties, such as quantum yield and/or wavelength(s). Sensor systems typically implement a quenching mechanism, for example via photoinduced electron transfer or intramolecular charge transfer, in the metal-free state of the molecule. Fluorescent dyes of high quantum yield and molar absorptivity are exploited to engineer bright sensor responses

suited for imaging biological analytes in live cells with high sensitivity. Examples include the commercially available fluorescein-based probes ZP1 and Newport Green, which have imaged synaptic zinc translocation in neuronal cells [13,14].

Quinoline moieties were utilized in the very first fluorescent sensors for biological zinc and, despite their UV excitation energy, modest quantum yields, and generally poor water solubility, they continue to be employed in constructs having good selectivity for zinc. Two water-soluble systems, a carboxamidoquinoline derivative [15] and a β -cyclodextrine conjugate [16], were recently reported and tested as cell-permeable zinc-responsive sensors in yeast. A bisquinoline-based zinc sensor (**1**, Figure 1) displayed 40-fold fluorescence emission enhancement and negligible pH sensitivity [17]. Despite limited water solubility, this system could detect exogenous zinc in mammalian cells. Sensors based on other fluorophores requiring UV excitation were recently reported and tested in biological systems at a proof-of-concept level [18-20].

[Please insert Figure 1 here]

Following an initial report of a zinc sensor relying on visible fluorescence emission from 7-nitrobenz-2-oxa-1,3-diazole (NBD) [21], two groups [22,23] independently reported the NBD-based system **2**, NBD-TPEA (Figure 1). Despite low quantum yields in both the zinc-free ($\Phi = 0.003$) and zinc-bound ($\Phi = 0.046$) states, NBD-TPEA can detect exogenous zinc in several cell lines. In addition, this probe localizes to the Golgi apparatus and lysosomes of HeLa cells, and it can image intact zebrafish larvae with or without zinc supplementation [22].

Three new members of the Zinpyr family of sensors, ZP1B, ZP3B, and ZPP1 (Figure 1), were recently reported. In ZP1B and ZP3B, each zinc-binding unit contains coordinating and non-coordinating picolyl groups. The resulting sensors afford micromolar zinc affinity and an improved dynamic range over the parent sensors ZP1 and ZP3 [24]. These compounds were employed to stain zinc-rich granules in Min6 insulinoma cells. The related molecule ZPP1, which features two pyrazine-containing binding units, was used to image endogenous zinc in Min6 cells [25]. ZPP1 is remarkable in its ability to determine zinc concentrations via titration experiments in which increasing amounts of sensor are added to a zinc-containing solution. With increasing sensor, the fluorescence emission increases to a maximum when the molar ratio of sensor to zinc is 1:2, after which it diminishes as the 1:1, quenched complex forms. In this manner, ZPP1 was used to estimate mobile zinc released from Min6 cells following stimulation with high concentrations of glucose and KCl [25].

The quest for ratiometric detection systems has created a vibrant field of research in mobile zinc bioimaging [10]. Ratiometric fluorescent sensors respond to analyte binding by a shift of emission and/or absorption profiles. The goal is to correlate analyte concentration with the ratio of emission intensities at two different wavelengths in dual excitation or emission measurements. Unlike intensity-based sensors, ratiometric systems afford a signal that is virtually independent of probe concentration, illumination intensity, and photobleaching in the biological specimen.

An iminocoumarin scaffold has been adopted in the ratiometric probe ZnIC (**6**, Figure 1) [26], which exhibits spectral changes in the visible region upon zinc binding with picomolar affinity. In dual-emission fluorescence microscopy experiments, ZnIC

proved suitable for imaging exogenous zinc in cultured HEK293 cells and could stain zinc-enriched CA1 and CA3 regions in hippocampal tissue slices. Recently, a coumarin-based system **7** (Figure 1) [27] was reported that featured picomolar zinc affinity, moderate metal selectivity and spectral changes in the visible region, and good cell permeability in microscopy studies of RAW264 cells. In another study, a large zinc-induced emission spectral shift in the visible region was observed for the furoquinoline-based system FQ1 (**8**, Figure 1), although with limited water solubility and metal selectivity [28]. Finally, the red emitting indicator ICPBCZin (**9**, Figure 1) containing an iminodiacetate binding unit reported zinc concentration changes in the nanomolar range with with an ipsochromic shift and a decrease in emission intensity [29].

Small-molecule fluorescence sensors for zinc have also been conjugated to nano-sized materials such as carbon nanotubes [30] and mesoporous silica [31], and functionalized silica nanoparticles were used to detect exogenous zinc in live cells [31]. The applicability and utility of nano-sized systems for investigating mobile zinc biology await further evaluation.

Protein-based fluorescent sensors

Genetically encoded fluorescent biosensors composed entirely of amino acids can be generated directly in the living systems through protein expression following gene transfer procedures, such as liposome-mediated transfection or electroporation. An efficient design for such protein-based fluorescent sensors for Zn(II) ions features a cation-binding domain and a pair of autofluorescent proteins capable of undergoing fluorescence resonance energy transfer (FRET). Conformational changes induced by

zinc binding alter the orientation of the fluorescent protein domains with a concomitant change in FRET efficiency. Because the ratio of emission intensity for the two fluorophores is correlated to the zinc concentration levels, this sensing strategy allows for ratiometric quantification of zinc in live cells. In addition, by fusing the sensing sequence to proteins that localize to specific subcellular compartments, genetically encoded biosensors can be targeted to predefined locations in the cell. This feature facilitates the accurate depiction of the origin and fate of mobile zinc ions in live cells.

Following initial reports that established the potential of FRET-based biosensors for mobile Zn(II) ion detection [32,33], recent strategies have exploited protein-based systems to investigate the distribution of the ion. On such FRET-based biosensor relies on a zinc finger domain to bind zinc with micromolar affinity [34]. Targeted to the mitochondria of hippocampal neurons, this sensor revealed the accumulation and release of mobile zinc in response to different stimuli. Ratiometric biosensors spanning affinities from the pico- to nanomolar range revealed Zn(II) concentration levels in both the cytosol and the insulin-containing granules of pancreatic β -cells [35].

In a recent proof-of-concept study [36], we employed the SNAP-tagTM methodology, which exploits the reactivity of DNA repair enzyme O⁶-alkylguanine transferase (AGT), to target a sensor of the Zinpyr family to the mitochondria and the Golgi apparatus of HeLa cells (Figure 2). Such localization can overcome the limitation of optical microscopy to resolve subcellular compartments — only when mobile zinc arrives at a pre-programmed locale will bright fluorescence emission be observed. The combination of protein labeling techniques with intensity-based and ratiometric small-

molecule sensors to illuminate cellular compartments with genetically targeted synthetic fluorophores affords a powerful approach for tracking mobile zinc.

[Please insert Figure 2 here]

Emerging detection avenues

Non-linear optical microscopy [37] is a powerful technique in biomedical research. It allows deep tissue (> 100 μm) imaging with three-dimensional resolution, reduced phototoxicity and minimal cellular autofluorescence and light scattering. Multi-photon excitation techniques are particularly well suited to provide information about the behavior of cells in their natural environment in living tissue [38]. Zinc-responsive fluorescent probes suitable for two-photon excitation fluorescence emission (TPEM) studies have been recently described [39-41]. The sensor AZn2 (**10**, Figure 3) features relatively high two-photon cross-section and has proved suitable for TPEM imaging studies of acute rat hippocampal slices [41].

[Please insert Figure 3 here]

Further opportunities for zinc bioimaging are offered by fluorescence lifetime imaging microscopy (FLIM) [42]. Because image contrast is provided by the excited-state lifetime of a fluorescent probe, this technique generates a signal that is generally independent of probe concentration and provides high spatial resolution and low

background. One example of a lifetime-responsive probe for zinc is a cryptate-based system [43].

Finally, magnetic resonance imaging (MRI), a non-invasive technique important in medical diagnosis, could provide three-dimensional maps of biological zinc in opaque objects such as living animals. Although the sensitivity and spatial resolution for MRI detection are lower than those of fluorescence imaging techniques, the development of zinc-activated MRI contrast agents will extend mobile zinc imaging to whole animal studies [44]. In two recent examples of zinc-responsive contrast agents (Figure 3), zinc coordination increases the relaxivity of gadolinium(III) (**11**) [45] or manganese(II) ions (**12**) [46] by facilitating access of water molecules to the paramagnetic center. An alternative approach was employed in a Gd(III)-based system (**13**), in which zinc coordination increases the affinity of the small-molecule sensor for human serum albumin [47]. The resulting increased relaxivity of the contrast agent was attributed to a slower rotational correlation time for the protein-bound complex. To date, Zn(II)-activated MRI contrast agents have not been employed for *in vivo* imaging of endogenous zinc.

Conclusions

The study of mobile zinc functions in human health and disease requires effective tools for zinc bioimaging. In spite of rapid advances in zinc sensing, however, the current detection systems will need additional modification and improvement to overcome several remaining challenges. Poor brightness and water solubility, as well as high-energy excitation, are still areas where fluorescent sensor design could use

improvement. Of interest are systems operating in the near-infrared region and very bright probes that can be observed at concentrations well below their zinc binding affinity (K_d) values. At the forefront of fluorescence-based zinc imaging needs are the challenges of ratiometric detection and probe localization. These goals are the focus of active investigation, although considerable progress has been made in both small-molecule and protein-based sensor development. In the near future, zinc bioimaging will most likely feature sensors of varying affinities, dual-modality probes with different readouts, and an extended choice of probes for FLIM and MRI. Investigations of the mechanisms of zinc-induced response for currently available sensors [48-50], as well as a systematic evaluation of interfering species [51,52] and of kinetic parameters, will guide the rational design of more effective systems.

Studies interrogating the same biological specimen with different probes and/or detection techniques are likely to reveal unanticipated challenges in mobile zinc imaging. The potential of zinc-responsive probes to perturb zinc homeostasis needs further evaluation, and disparate estimates of transient and resting concentrations of mobile zinc need to be addressed [53]. In order to correlate zinc mobilization to cellular function, physiology, and pathology, progress in biological zinc detection must include real-time observation and quantification of transient zinc signals in the synaptic cleft, in intracellular compartments, and in specialized organs.

Acknowledgements

We acknowledge support from the National Institute of General Medical Sciences in the form of grant GM065519. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or the National Institutes of Health.

Figure captions

Figure 1. Selected small-molecule fluorescent sensors for imaging zinc in fluorescence microscopy experiments.

Figure 2. Labeling of a genetically encoded tag (AGT or SNAP-tagTM) with a small-molecule sensor of the Zinpyr family.

Figure 3. Sensors for mobile zinc detection based on a fluorescent probe for two-photon emission microscopy (**10**) and zinc-responsive contrast agents for magnetic resonance imaging (**11–13**).

References and recommended reading

1. Maret W, Li Y: **Coordination dynamics of zinc in proteins.** *Chem Rev* 2009, **109**:4682-4707.
2. Eide DJ: **Zinc transporters and the cellular trafficking of zinc.** *Biochim Biophys Acta* 2006, **1763**:711-722.

3. Franklin RB, Costello LC: **The important role of the apoptotic effects of zinc in the development of cancers.** *J Cell Biochem* 2009, **106**:750-757.
- A contemporary perspective on the interplay between zinc homeostasis and cell proliferation, this review includes important considerations on the complexity of experimental design in zinc sensing studies.
4. Maret W: **Molecular aspects of human cellular zinc homeostasis: redox control of zinc potentials and zinc signals.** *Biometals* 2009, **22**:149-157.
5. Frederickson CJ, Koh JY, Bush AI: **The neurobiology of zinc in health and disease.** *Nat Rev Neurosci* 2005, **6**:449-462.
6. Taylor CG: **Zinc, the pancreas, and diabetes: Insights from rodent studies and future directions.** *BioMetals* 2005, **18**:305-312.
7. Costello LC, Franklin RB: **The clinical relevance of the metabolism of prostate cancer; zinc and tumor suppression: connecting the dots.** *Mol Cancer* 2006, **5**.
8. Domaille DW, Que EL, Chang CJ: **Synthetic fluorescent sensors for studying the cell biology of metals.** *Nat Chem Biol* 2008, **4**:168-175.
9. Que EL, Domaille DW, Chang CJ: **Metals in neurobiology: Probing their chemistry and biology with molecular imaging.** *Chem Rev* 2008, **108**:1517-1549.
10. Carol P, Sreejith S, Ajayaghosh A: **Ratiometric and near-infrared molecular probes for the detection and imaging of zinc ions.** *Chem Asian J* 2007, **2**:338-348.

11. Nolan EM, Lippard SJ: **Small-molecule fluorescent sensors for investigating zinc metalloneurochemistry**. *Acc Chem Res* 2009, **42**:193-203.
12. McRae R, Bagchi P, Sumalekshmy S, Fahrni CJ: **In situ imaging of metals in cells and tissues**. *Chem Rev* 2009, **109**:4780-4827.
13. Ketterman JK, Li YV: **Presynaptic evidence for zinc release at the mossy fiber synapse of rat hippocampus**. *J Neurosci Res* 2008, **86**:422-434.
- Small-molecule fluorescent probe ZP1 was used to stain presynaptic vesicular zinc in the mossy fiber region of the hippocampus and to study zinc translocation into the cytosol of postsynaptic neurons.
14. Suh SW: **Detection of zinc translocation into apical dendrite of CA1 pyramidal neuron after electrical stimulation**. *J Neurosci Methods* 2009, **177**:1-13.
15. Zhang Y, Guo XF, Si WX, Jia LH, Qian XH: **Ratiometric and water-soluble fluorescent zinc sensor of carboxamidoquinoline with an alkoxyethylamino chain as receptor**. *Org Lett* 2008, **10**:473-476.
16. Liu Y, Zhang N, Chen Y, Wang LH: **Fluorescence sensing and binding behavior of aminobenzenesulfonamidoquinolino-beta-cyclodextrin to Zn(II)**. *Org Lett* 2007, **9**:315-318.
17. Mikata Y, Yamashita A, Kawamura A, Konno H, Miyamoto Y, Tamotsu S: **Bisquinoline-based fluorescent zinc sensors**. *Dalton Trans* 2009:3800-3806.
18. Dhara K, Karan S, Ratha J, Roy P, Chandra G, Manassero M, Mallik B, Banerjee P: **A two-dimensional coordination compound as a zinc ion selective**

- luminescent probe for biological applications. *Chem Asian J* 2007, **2**:1091-1100.
19. Liu Z, Zhang C, Li Y, Wu Z, Qian F, Yang X, He W, Gao X, Guo Z: **A Zn(II) fluorescent sensor derived from 2-(pyridin-2-yl)benzoimidazole with ratiometric sensing potential.** *Org Lett* 2009, **11**:795-798.
 20. Tamanini E, Katewa A, Sedger LM, Todd MH, Watkinson M: **A synthetically simple, click-generated cyclam-based zinc(II) sensor.** *Inorg Chem* 2009, **48**:319-324.
 21. Jiang W, Fu Q, Fan H, Wang W: **An NBD fluorophore-based sensitive and selective fluorescent probe for zinc ion.** *Chem Commun* 2008:259-261.
 22. Qian F, Zhang C, Zhang Y, He W, Gao X, Hu P, Guo Z: **Visible light excitable Zn(II) fluorescent sensor derived from an intramolecular charge transfer fluorophore and its in vitro and in vivo application.** *J Am Chem Soc* 2009, **131**:1460-1468.
 23. Xu Z, Kim G-H, Han SJ, Jou MJ, Lee C, Shin I, Yoon J: **An NBD-based colorimetric and fluorescent chemosensor for Zn(II) and its use for detection of intracellular zinc ions.** *Tetrahedron* 2009, **65**:2307-2312.
 24. Wong BA, Friedle S, Lippard SJ: **Subtle modification of 2,2-picolylamine lowers the affinity and improves the turn-on of Zn(II)-selective fluorescent sensors.** *Inorg Chem* 2009, **48**:7009-7011.
 25. Zhang X-a, Hayes D, Smith SJ, Friedle S, Lippard SJ: **New strategy for quantifying biological zinc by a modified Zinpyr fluorescence sensor.** *J Am Chem Soc* 2008, **130**:15788-15789.

- ● A fluorescein-based reagent is employed to quantitate mobile zinc using a fluorescence titration protocol.
26. Komatsu K, Urano Y, Kojima H, Nagano T: **Development of an iminocoumarin-based zinc sensor suitable for ratiometric fluorescence imaging of neuronal zinc.** *J Am Chem Soc* 2007, **129**:13447-13454.
 - A simple small-molecule probe demonstrates some of the advantages of ratiometric detection, such as low sensitivity to pH changes and improved staining of tissue slices.
 27. Mizukami S, Okada S, Kimura S, Kikuchi K: **Design and synthesis of coumarin-based Zn(II) probes for ratiometric fluorescence imaging.** *Inorg Chem* 2009, **48**:7630-7638.
 28. Xue L, Liu C, Jiang H: **A ratiometric fluorescent sensor with a large Stokes shift for imaging zinc ions in living cells.** *Chem Commun* 2009:1061-1063.
 29. Roussakis E, Voutsadaki S, Pinakoulaki E, Sideris DP, Tokatlidis K, Katerinopoulos HE: **ICPBCZin: A red emitting ratiometric fluorescent indicator with nanomolar affinity for Zn(II) ions.** *Cell Calcium* 2008, **44**:270-275.
 30. Dong Z, Yang B, Jin J, Li J, Kang H, Zhong X, Li R, Ma J: **Quinoline Group Modified Carbon Nanotubes for the Detection of Zinc Ions.** *Nanoscale Res Lett* 2009, **4**:335-340.
 31. Sarkar K, Dhara K, Nandi M, Roy P, Bhaumik A, Banerjee P: **Selective zinc(II)-ion fluorescence sensing by a functionalized mesoporous material**

- covalently grafted with a fluorescent chromophore and consequent biological applications.** *Adv Funct Mater* 2009, **19**:223-234.
32. Qiao W, Mooney M, Bird AJ, Winge DR, Eide DJ: **Zinc binding to a regulatory zinc-sensing domain monitored in vivo by using FRET.** *Proc Natl Acad Sci U S A* 2006, **103**:8674-8679.
33. van Dongen EMWM, Dekkers LM, Spijker K, Meijer EW, Klomp LWJ, Merkx M: **Ratiometric fluorescent sensor proteins with subnanomolar affinity for Zn(II) based on copper chaperone domains.** *J Am Chem Soc* 2006, **128**:10754-10762.
34. Dittmer PJ, Miranda JG, Gorski JA, Palmer AE: **Genetically encoded sensors to elucidate spatial distribution of cellular zinc.** *J Biol Chem* 2009, **284**:16289-16297.
35. Vinkenborg JL, Nicolson TJ, Bellomo EA, Koay MS, Rutter GA, Merkx M: **Genetically encoded FRET sensors to monitor intracellular Zn(II) homeostasis.** *Nat Meth* 2009, **6**:737-740.
- ● In a study of zinc-enriched pancreatic β cells, newly engineered FRET biosensors for zinc allow site-specific localization to genetically targeted cellular compartments and ratiometric detection of endogenous zinc.
36. Tomat E, Nolan EM, Jaworski J, Lippard SJ: **Organelle-specific zinc detection using Zinpyr-labeled fusion proteins in live cells.** *J Am Chem Soc* 2008, **130**:15776-15777.
- The SNAP-tagTM labeling methodology is employed to anchor a zinc-responsive fluorescent sensor to the mitochondria and Golgi apparatus of HeLa cells.

37. Helmchen F, Denk W: **Deep tissue two-photon microscopy**. *Nat Meth* 2005, **2**:932-940.
38. Kim HM, Cho BR: **Two-photon probes for intracellular free metal ions, acidic vesicles, and lipid rafts in live tissues**. *Acc Chem Res* 2009, **42**:863-872.
39. Sumalekshmy S, Henary MM, Siegel N, Lawson PV, Wu Y, Schmidt K, Bredas JL, Perry JW, Fahrni CJ: **Design of emission ratiometric metal-ion sensors with enhanced two-photon cross section and brightness**. *J Am Chem Soc* 2007, **129**:11888-11889.
40. Chen X-Y, Shi J, Li Y-M, Wang F-L, Wu X, Guo Q-X, Liu L: **Two-photon fluorescent probes of biological Zn(II) derived from 7-hydroxyquinoline**. *Org Lett* 2009, **11**:4426-4429.
41. Kim HM, Seo MS, An MJ, Hong JH, Tian YS, Choi JH, Kwon O, Lee KJ, Cho BR: **Two-photon fluorescent probes for intracellular free zinc ions in living tissue**. *Angew Chem, Int Ed* 2008, **47**:5167-5170.
- A small-molecule probe showcases the value of two-photon microscopy to image endogenous zinc at a depth of 80–150 μm in hippocampal tissue.
42. Levitt JA, Matthews DR, Ameer-Beg SM, Suhling K: **Fluorescence lifetime and polarization-resolved imaging in cell biology**. *Curr Opin Biotechnol* 2009, **20**:28-36.
43. Felton CE, Harding LP, Jones JE, Kariuki BM, Pope SJA, Rice CR: **A wavelength and lifetime responsive cryptate-containing fluorescent probe for zinc ions in water**. *Chem Commun* 2008:6185-6187.

44. Que EL, Chang CJ: **Responsive magnetic resonance imaging contrast agents as chemical sensors for metals in biology and medicine.** *Chem Soc Rev* 2010:DOI: 10.1039/b914348n.
45. Major JL, Parigi G, Luchinat C, Meade TJ: **The synthesis and in vitro testing of a zinc-activated MRI contrast agent.** *Proc Natl Acad Sci U S A* 2007, **104**:13881-13886.
46. Zhang XA, Lovejoy KS, Jasanoff A, Lippard SJ: **Water-soluble porphyrins as a dual-function molecular imaging platform for MRI and fluorescence zinc sensing.** *Proc Natl Acad Sci U S A* 2007, **104**:10780-10785.
47. Esqueda AC, Lopez JA, Andreu-de-Riquer G, Alvarado-Monzon JC, Ratnakar J, Lubag AJM, Sherry AD, De Leon-Rodriguez LM: **A new gadolinium-based MRI zinc sensor.** *J Am Chem Soc* 2009, **131**:11387-11391.
48. Wong BA, Friedle S, Lippard SJ: **Solution and fluorescence properties of symmetric dipicolylamine-containing dichlorofluorescein-based Zn(II) sensors.** *J Am Chem Soc* 2009, **131**:7142-7152.
49. Major JL, Boiteau RM, Meade TJ: **Mechanisms of Zn(II)-activated magnetic resonance imaging agents.** *Inorg Chem* 2008, **47**:10788-10795.
50. Cody J, Mandal S, Yang L, Fahrni CJ: **Differential tuning of the electron transfer parameters in 1,3,5-triarylpyrazolines: A rational design approach for optimizing the contrast ratio of fluorescent probes.** *J Am Chem Soc* 2008, **130**:13023-13032.

- Synthetic, photophysical, electrochemical and computational studies converge in a rational design approach that could be employed for the development of a variety of cation-responsive probes.
51. Zhao JF, Bertoglio BA, Gee KR, Kay AR: **The zinc indicator FluoZin-3 is not perturbed significantly by physiological levels of calcium or magnesium.** *Cell Calcium* 2008, **44**:422-426.
 52. Zhao JF, Bertoglio BA, Devinney MJ, Dineley KE, Kay AR: **The interaction of biological and noxious transition metals with the zinc probes FluoZin-3 and Newport Green.** *Anal Biochem* 2009, **384**:34-41.
 53. Frederickson CJ, Giblin LJ, Rengarajan B, Masalha R, Zeng YP, Lopez EV, Koh JY, Chorin U, Besser L, Hershinkel M, et al.: **Synaptic release of zinc from brain slices: Factors governing release, imaging, and accurate calculation of concentration.** *J Neurosci Methods* 2006, **154**:19-29.