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Activity-based sensing fluorescent probes for iron in biological systems

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Iron is an essential nutrient for life, and its capacity to cycle between different oxidation states is required for processes spanning oxygen transport and respiration to nucleotide synthesis and epigenetic regulation. However, this same redox ability also makes iron, if not regulated properly, a potentially dangerous toxin that can trigger oxidative stress and damage. New methods that enable monitoring of iron in living biological systems, particularly in labile Fe^{2+} forms, can help identify its contributions to physiology, aging, and disease. In this review, we summarize recent developments in activity-based sensing (ABS) probes for fluorescence Fe^{2+} detection.

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Introduction

Iron is the most abundant transition metal in the human body and its capacity to cycle between various oxidation states is required for oxygen transport in globins, electron transfer in iron-sulfur (FeS) clusters and cytochromes, C–H functionalization by P450 oxygenases and non-heme congeners, and nucleotide synthesis by ribonucleotide reductase [1–5]. However, this same potent redox capability also makes iron a potential danger through aberrant generation of reactive oxygen species (ROS) through Fenton chemistry [5]. As such, iron homeostasis is precisely controlled from the cellular to the whole body level (Figure 1), with local and global overload and/or deficiency both being detrimental.

At the systemic whole body level, the hormone hepcidin is a main driver of iron homeostasis in mammals, regulating absorption of dietary iron, release of hepatic iron stores, and recycling of iron by macrophages [6,7]. At the cellular level, the transferrin receptor and divalent metal transporter-1 import proteins, iron storage protein ferritin, and ferroportin-1 export protein are in dynamic equilibrium with a ‘labile iron pool’ (LIP) that exists in the center of this network and refers to a predominantly cytosolic pool of Fe^{2+} that is weakly bound to cellular ligands. Indeed, imbalances in LIPs are implicated in diseases ranging from cancer [8*,9,10,11] to cardiovascular [12] and prion/neurodegenerative disorders [11,12] to aging [13] and inflammation [14]. For example, proliferating cancer cells accumulate elevated concentrations of labile iron compared to normal cells owing to their increased metabolic activity, yet this expanded iron pool may also sensitize cancer cells to death by ferroptosis, a newly recognized iron-dependent cell-death pathway [15,16].

The broad contributions of iron status in health, aging, and disease provide motivation to develop new methods for biological iron detection [17*,18,19*], particularly in its labile Fe^{2+} forms. Fluorescence detection in particular offers the convenience of a real-time optical readout that can enable biological study across a variety of length scales. Because Fe^{2+} is a potent fluorophore quencher by energy and electron transfer [20,21] and a weak binder on the Irving-Williams series [22], conventional chelation-based probes for iron detection (Figure 2) suffer largely from a turn-off response and/or limitations in metal ion selectivity [23–28]. To address these issues, we [29**,30*] and others [31**,32,33,34**] have recently developed reagents for ‘turn-on’ detection of labile Fe^{2+} . These probes share a general design approach that we term ‘activity-based sensing’ (ABS), which relies on molecular reactivity, rather than molecular recognition, to achieve high chemical selectivity in complex biological systems. As such, ‘activity’ refers to the ability of the moiety present in a probe to confer reactivity with Fe^{2+} ; the probes in this review in particular exploit redox reactivity with Fe^{2+} to selectively detect this analyte. This review summarizes recent progress in the use of ABS for biological iron detection.

Design considerations for fluorescent Fe^{2+} probes

Detection of biological Fe^{2+} presents a number of challenges, including sensitivity to labile iron pools that span a

Figure 1

Iron homeostasis is highly regulated at both systemic whole body and cellular levels and proceeds in the following general sequence of pathways (a)–(c). Iron is first absorbed from the diet by the intestine (a), where it is then put into the circulatory system (b). Once in circulation, iron is bound to transferrin (Tf) as Fe^{3+} and can subsequently enter cells, which maintain iron homeostasis through a complex network of proteins (c). While Hepcidin (Hep) is a hormone that controls systemic homeostasis, cellular homeostasis is maintained through a dynamic network of import proteins including transferrin (Tf)/transferrin receptor (TfR) and divalent metal transporter-1 (Dmt1), storage proteins like ferritin (Ftn), and export proteins like ferroportin-1 (Fpn), in dynamic exchange with a central labile iron pool (LIP) that is comprised predominantly of Fe^{2+} coordinated to weakly-bound ligands.

wide range of potential concentrations (high nM to low μM) [24,35], the need for metal and oxidation state specificity, particularly over Fe^{3+} and divalent metals that are more abundant in biological systems or stronger in the Irving-Williams series, and a turn-on or ratiometric response to avoid non-specific quenching by electron and/or energy transfer. ABS is well-suited to meet these challenges, and recent advances have largely exploited the intrinsic redox activity of Fe^{2+} for its selective detection (Figure 2 and Table 1).

Iron probes via *N*-oxide reduction

Hirayama and Nagasawa introduced RhoNox-1, the first member in a series of reagents that harness Fe^{2+} -mediated *N*-oxide reduction for iron detection [31^{••}].

The *N*-oxide moiety quenches RhoNox-1 through both twisted internal charge transfer (TICT) and photo-induced electron transfer (PET) processes, which are relieved after reaction with Fe^{2+} to produce the parent rhodamine dye. RhoNox-1 is capable of detecting exogenous addition of Fe^{2+} to HepG2 liver cells in addition to visualizing decreases in basal Fe^{2+} upon 2,2'-bipyridine treatment. More recently, RhoNox-1 has been successfully applied to detect elevations in Fe^{2+} in A549 lung carcinoma cells treated with plasma-activated medium [36], in addition to a number of other biological models [37–40].

The *N*-oxide reduction strategy has proved broadly useful for developing an expanded toolbox of fluorescent Fe^{2+}

Figure 2

Chemical probes for fluorescent Fe^{2+} detection, divided into chelation-based probes (left) that undergo fluorescence turn-off quenching upon iron binding, and activity-based sensing (ABS) probes (right) that produce a turn-on increase or ratiometric shift upon reaction with iron, with iron-reactive triggers highlighted in red.

Table 1

Summary of activity-based sensing fluorescent Fe probes

Probe	λ_{ex} (nm)	λ_{em} (nm)	Response to Fe <i>in vitro</i>	Biological system examined
N-oxide reduction probes				
RhoNox-1 [31**]	540	575	30-fold turn-on to 20 μM Fe (2 μM probe, 1 h, 50 mM HEPES)	Cells (HepG2, A549)
HMRhoNox-M [32]	550	575	60-fold turn-on to 20 μM Fe (2 μM probe, 1 h, 50 mM HEPES)	Cells (HEPG2, endometrial stromal, epithelial, mesothelioma, or fibroblast)
CoNox-1 [33]	295/405	495	10-fold turn-on to 20 μM Fe (2 μM probe, 1 h, 50 mM HEPES)	Cells (HEPG2)
FluNox-1 [33]	450/488	530	30-fold turn-on to 20 μM Fe (2 μM probe, 1 h, 50 mM HEPES)	Cells (HEPG2)
SiRhoNox-1 [33]	575/630	660	60-fold turn-on to 20 μM Fe (2 μM probe, 1 h, 50 mM HEPES)	Cells (HEPG2)
Nitroxide reduction probes				
RhT [43]	557	580	2.5-fold turn-on to 24 μM FA (10 mM MOPS)	Cells (Ws1 fibroblasts)
Biomimetic oxygen activation probes				
IP1 [29**]	470	508	6-fold turn-on to 20 μM Fe (1 μM probe, 1 h, 50 mM Tris)	Cells (HepG2/C3A)
Peroxide activation probes				
Trx-puro [34**]	N/A	N/A	N/A – immunofluorescent readout <i>in cellulo</i>	Cells (U2OS, PC3)
FIP-1 [30*]	495 and 545	515 and 556	1.7-fold ratio-change to 10 μM Fe (1 μM probe, 1 h, 50 mM HEPES)	Cells (HEK 293, MDA-MB 231, U2OS, MCF10A)

probes through ABS. For example, HMRhoNox-M and related hydroxymethylrhodamine and hydroxymethylrhodol derivatives exhibit improved turn-on responses with reduced pH sensitivity, enabling visualization of

iron uptake *via* transferrin endocytosis [32] and iron accumulation in ovarian endometriosis [41], along with other applications [42]. In addition, SiRhoNox-1 is the most red-shifted and sensitive probe of an expanded color

palette and is capable of detecting endogenous iron pools by both microscopy and flow cytometry [33]. This reagent also reveals a shift in intracellular redox equilibrium towards labile iron in response to hypoxia in both living cells and in 3D tumor spheroids, an increase that appears to be independent of iron uptake pathways. Finally, a related Fe^{2+} -dependent nitroxide reduction reaction has been employed for ABS of Fe^{2+} [43]. Fe^{2+} -mediated reduction of the 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) radicals of CouT and RhT triggers both a fluorescence turn-on and a change in electron paramagnetic resonance (EPR) signal.

A fluorescent iron probe utilizing biomimetic oxygen activation

In parallel, our laboratory developed an alternative ABS strategy for Fe^{2+} detection by exploiting a biomimetic iron-binding motif to activate oxygen for oxidative C–O cleavage and release of a fluorescent product. Indeed, prior work by Taki [44] and our laboratory [45] on creation of fluorescent indicators for copper and cobalt, respectively, presaged the general utility of this strategy for redox-active metal detection [46[•]]. Inspired by heme P450, mononuclear non-heme enzymes containing a 2-His-1-carboxylate motif, and their corresponding model complexes, the first-generation reagent Iron Probe 1 (IP1) employs a receptor with a mixed nitrogen/oxygen donor set for Fe^{2+} coordination with an open site for O_2 binding and activation [29^{••}]. Binding of Fe^{2+} and O_2 at this recognition motif results in an oxidative cleavage of the C–O bond and release of a fluorescent fluorescein alcohol [45]. The Fe^{2+} -triggered turn-on response for IP1 is highly selective over other biologically relevant metals, noting that mammalian Co^{2+} exists primarily in tightly-bound vitamin B12/cobalamin forms [47].

IP1 is capable of detecting changes in iron status with iron supplementation and chelation in HepG2/C3A liver cells, as well as endogenous changes in labile Fe^{2+} upon treatment of these cells with ascorbic acid, a vitamin known to promote redox cycling and mobilization of iron. Interestingly, an increase in IP1 fluorescence was also observed upon treatment with hepcidin, the key iron regulatory hormone responsible for degrading iron export protein (ferroportin). The largely lysosomal localization of this probe in HepG2 models can potentially limit its signal-to-noise responses but also makes it attractive for reporting on LIPs in these cellular storage compartments.

ABS iron probes based on peroxide activation

IP-1 relies on an oxygen-dependent, three-component sensing mechanism (probe, Fe^{2+} , and O_2) for iron detection. As such, our laboratory has sought to explore alternative ABS strategies that would utilize a direct reaction between a probe and Fe^{2+} for sensing purposes. Inspired by endoperoxide motifs that are a common feature of natural product anticancer and antimalarial drugs such as

arteminisin [48–52], as well as synthetic compounds that enable specific targeting of tumors or parasites *via* local iron-dependent drug release [53–56], we turned our attention to developing ABS probes that exploit peroxide activation by Fe^{2+} . Indeed, recent work by our laboratory and Renslo has established the efficacy of this approach for Fe^{2+} detection.

Trx-puro is a puromycin-based probe that utilizes an endoperoxide-caged puromycin to report on intracellular iron levels in fixed samples through an immunofluorescence readout [34^{••}]. Specifically, Fe^{2+} -dependent cleavage of Trx-puro yields puromycin, which is incorporated into growing polypeptide chains to yield a covalent tag that can be detected with puromycin-specific antibodies upon cellular fixation. Trx-puro exhibits high selectivity for Fe^{2+} over a variety of biologically-relevant metals, reductants, and oxidants and can be used to compare intracellular Fe^{2+} levels across a variety of cell types, including identification of expanded labile iron pools in cancer cell lines as compared to their non-cancerous counterparts. The high sensitivity of this probe also enabled detection of changes in intracellular iron status when expression of the major iron storage protein (ferritin heavy chain, FHC) or export protein (ferroportin, FPN) were altered.

In parallel, our laboratory reported FRET Iron Probe 1 (FIP-1), a first-generation endoperoxide probe for use in live-cell settings [30[•]]. FIP-1 also represents a unique ratiometric indicator for Fe^{2+} . This dye makes use of an Fe^{2+} -cleavable endoperoxide linker to regulate FRET between donor and acceptor dyes. In the absence of Fe^{2+} , efficient FRET occurs between the covalently-linked 5-aminomethyl fluorescein (5-AMF) donor dye and the Cy3 acceptor dye; in the presence of Fe^{2+} , the endoperoxide linker is cleaved to separate the dyes into two fragments, reducing FRET efficiency. Owing to its endoperoxide ABS trigger, FIP-1 retains high selectivity and sensitivity for Fe^{2+} over a variety of biologically-relevant metal ions, oxidants, and reductants and is capable of detecting both increases and decreases in LIPs in HEK 293T cells and other models upon iron supplementation and/or depletion. Moreover, application of FIP-1 provides, to the best of our knowledge, the first direct imaging evidence for changes in labile iron levels during ferroptosis, opening the door to identifying and studying iron fluxes involved in other cell signaling pathways.

Conclusions and outlook

Iron is central to health, aging, and disease, and the development of chemical probes that enable monitoring of dynamic changes in iron pools across a variety of biological length and time scales can help contribute to a better understanding of iron physiology and pathology. In this context, activity-based sensing (ABS) offers an effective and general strategy for Fe^{2+} detection, and the

chemical triggers surveyed in this review rely largely on exploiting the inherent redox capacity of Fe^{2+} for its detection. Indeed, the early stages of turn-on and ratio-metric probe development for Fe^{2+} using ABS have already identified labile iron fluxes in ferroptosis and related cell death signaling pathways, alterations in labile iron status in cancer models, as well as hypoxia-dependent changes in iron redox balance. These findings motivate further efforts to increase sensitivity, add to the color palette, target reagents to specific biological locales, and expand to tissue and *in vivo* models, which will surely enable new studies of iron biology, particularly as a transition metal signal. Towards this end, our lab has recently reported the development of a bioluminescent probe for imaging iron *in vivo* [57]. Finally, in a larger context, the development of fluorescent iron probes offers another important example of the broad and emerging impact of activity-based sensing as a field at the interface between chemistry and biology [46,58–61].

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Cammack R, Wrigglesworth JM, Baum H: *Transport and Storage*. CRC Press; 1989.
2. Johnson DC, Dean DR, Smith AD, Johnson MK: **Structure, function, and formation of biological iron-sulfur clusters**. *Annu Rev Biochem* 2005, **74**:247-281.
3. Andrews NC: **Iron homeostasis: insights from genetics and animal models**. *Nat Rev Genet* 2000, **1**:208-217.
4. Xu W, Barrientos T, Andrews NC: **Iron and copper in mitochondrial diseases**. *Cell Metab* 2013, **17**:319-328.
5. Lippard SJ, Berg JM: *Principles of Bioinorganic Chemistry*. Mill Valley: University Science Book; 1994.
6. Ganz T: **Systemic iron homeostasis**. *Phys Rev* 2013, **93**:1721-1741.
7. Ganz T, Nemeth E: **Hepcidin and iron homeostasis**. *Biochim Biophys Acta* 2012, **1823**:1434-1443.
8. TS V, TF M: **Iron and cancer: more ore to be mined**. *Nat Rev Cancer* 2013, **13**:342-355.
This review provides a detailed discussion about various roles of iron in cancer.
9. Wu KJ, Polack A, Dala-Favera R: **Coordinated regulation of iron-controlling genes, H-ferritin and IRP2, by c-MYC**. *Science* 1999, **283**:676-679.
10. Pinnix ZK, Miller LD, Wang W, D'Agostino RJ, Kute T, Willingham MC, Hatcher H, Tesfay L, Sui G, Di X *et al.*: **Ferroportin and iron regulation in breast cancer progression and prognosis**. *Sci Transl Med* 2010, **2**:43ra56.
11. Toyokuni S: **Role of iron in carcinogenesis: cancer as a ferrotoxic disease**. *Cancer Sci* 2009, **100**:9-16.
12. von Haehling S, Jankowska EA, van Veldhuisen DJ, Ponikowski P, Anker SD: **Iron deficiency and cardiovascular disease**. *Nat Rev Cardiol* 2015, **12**:659-669.
13. James SA, Robert BR, Hare DJ, de Jonge MD, Birchall IE, Jenkin NL, Cherny RA, Bush AI, McColl G: **Direct in vivo imaging of ferrous iron dyshomeostasis in ageing *Caenorhabditis elegans***. *Chem Sci* 2015, **6**:2952-2962.
14. Wessling-Resnick M: **Iron homeostasis and the inflammatory response**. *Ann Rev Nutr* 2010, **30**:105-122.
15. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS *et al.*: **Ferroptosis: an iron-dependent form of nonapoptotic cell death**. *Cell* 2012, **149**:1060-1072.
16. Stockwell BR, Angeli JPF, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Hatzios SK, Kagan VE *et al.*: **Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease**. *Cell* 2017, **171**:273-285.
17. Carter KP, Young AM, Palmer AE: **Fluorescent sensors for measuring metal ions in living systems**. *Chem Rev* 2014, **114**:4564-4601.
This review of fluorescent sensors provides an extremely detailed survey of fluorescent sensors for visualizing metal ions in living cells.
18. Domaille DW, Que EL, Chang CJ: **Synthetic fluorescent sensors for studying to cell biology of metals**. *Nat Chem Biol* 2008, **4**:168-175.
19. Ackerman CM, Lee S, Chang CJ: **Analytical methods for imaging metals in biology: from transition metal metabolism to transition metal signaling**. *Anal Chem* 2017, **89**:22-41.
This review covers a number of analytical methods for visualizing metals in biological systems, including both mass spectrometry-based imaging and fluorescence imaging methods.
20. Kemlo JA, Shepherd TM: **Quenching of excited singlet states by metal ions**. *Chem Phys Lett* 1977, **47**:158-162.
21. Varnes AW, Dodson RB, Wehry EL: **Interactions of transition-metal ions with photoexcited states of flavines. Fluorescence quenching studies**. *J Am Chem Soc* 1972, **94**:946-950.
22. Irving H, Williams RJP: **The stability of transition-metal complexes**. *J Chem Soc* 1953:3192-3210.
23. Breuer W, Epsztejn S, Millgram P, Cabantchik IZ: **Transport of iron and other transition metals into cells as revealed by a fluorescent probe**. *J Am Phys Soc* 1995, **268**:C1354-C1361.
24. Petrat F, Rauen U, de Groot H: **Determination of the chelatable iron pool of isolated rat hepatocytes by digital fluorescence microscopy using the fluorescent probe, phen green SK**. *Hepatology* 1999, **29**:1171-1179.
25. Ma Y, de Groot H, Liu Z, Hider RC, Petrat F: **Chelation and determination of labile iron in primary hepatocytes by pyridinone fluorescent probes**. *Biochem J* 2006, **395**:49-55.
26. Ma Y, Liu Z, Hider RC, Petrat F: **Determination of the labile iron pool of human lymphocytes using the fluorescent probe, CP655**. *Anal Chem Insights* 2007, **2**:61-67.
27. Petrat F, Weisheit D, Lensen M, de Groot H, Sustmann R, Rauen U: **Selective determination of mitochondrial chelatable iron in viable cells with a new fluorescent sensor**. *Biochem J* 2002, **362**:137-147.
28. Rauen U, Springer A, Weisheit D, Petrat F, Korth H, de Groot H, Sustmann R: **Assessment of chelatable mitochondrial iron by using mitochondrion-selective fluorescent ironindicators with different iron-binding affinities**. *ChemBiochem* 2007, **8**:341-352.
29. Au-Yeung HY, Chan J, Chantarojsiri T, Chang CJ: **Molecular imaging of labile iron(II) pools in living cells with a turn-on fluorescent probe**. *J Am Chem Soc* 2013, **135**:15165-15173.
This paper presents a biomimetic activation of oxygen as a mode for iron detection.
30. Aron AT, Loehr MO, Bogena J, Chang CJ: **An endoperoxide reactivity-based FRET probe for ratiometric fluorescence imaging of labile iron pools in living cells**. *J Am Chem Soc* 2016, **138**:14338-14346.

This paper presents a strategy for ratiometric detection of ferrous iron in living cells using an endoperoxide moiety for detection.

31. Hirayama T, Okuda K, Nagasawa H: **A highly selective turn-on fluorescent probe for iron(II) to visualize labile iron in living cells.** *Chem Sci* 2013, **4**:1250-1256.

This article is the first example of iron detection using the *N*-oxide capping strategy.

32. Niwa M, Hirayama T, Okuda K, Nagasawa H: **A new class of high-contrast Fe(II) selective fluorescent probes based on spirocyclized scaffolds for visualization of intracellular labile iron delivered by transferrin.** *Org Biomol Chem* 2014, **12**:6590-6597.
33. Hirayama T, Tsuboi H, Niwa M, Miki A, Kadota S, Ikeshita Y, Okuda K, Nagasawa H: **A universal fluorogenic switch for Fe(II) ion based on N-oxide chemistry permits the visualization of intracellular redox equilibrium shift towards labile iron in hypoxic tumor cells.** *Chem Sci* 2017, **8**:4858-4866.
34. Spangler B, Morgan CW, Fontaine SD, Vander Wal MN, Chang CJ, Wells JA, Renslo AR: **A reactivity-based probe of the intracellular labile ferrous iron pool.** *Nat Chem Biol* 2016, **12**:680-685.

This paper utilizes immunofluorescence to present the first example of iron detection using an endoperoxide moiety.

35. Petrat F, de Groot H, Rauen U: **Subcellular distribution of chelatable iron: a laser scanning microscopic study in isolated rat hepatocytes and rat liver endothelial cells.** *Biochem J* 2001, **356**:61-69.
36. Adachi T, Nonomura S, Horiba M, Hirayama T, Kamiya T, Nagasawa H, Hara H: **Iron stimulates plasma-activated medium-induced A549 cell injury.** *Sci Rep* 2016, **6**:20928.
37. Ikeda Y, Horinouchi Y, Hamano H, Hirayama T, Kishi S, Izawa-Ishizawa Y, Imanishi M, Zamami Y, Takechi K, Miyamoto L *et al.*: **Dietary iron restriction alleviates renal tubulointerstitial injury induced by protein overload in mice.** *Sci Rep* 2017, **7**:10621.
38. Takenaka M, Suzuki N, Mori M, Hirayama T, Nagasawa H, Morishige KI: **Iron regulatory protein 2 in ovarian endometrial cysts.** *Biochem Biophys Res Commun* 2017, **487**:789-794.
39. Itoa F, Nishiyama T, Shia L, Moria M, Hirayama T, Nagasawa H, Yasuie H, Toyokuni S: **Contrasting intra- and extracellular distribution of catalytic ferrous iron in ovalbumin-induced peritonitis.** *Biochem Biophys Res Commun* 2016, **476**:600-606.
40. Wang Y, Okazaki Y, Shi L, Kohda H, Tanaka M, Taki K, Nishioka T, Hirayama T, Nagasawa H, Yamashita Y *et al.*: **Role of hemoglobin and transferrin in multi-wall carbon nanotube-induced mesothelial injury and carcinogenesis.** *Cancer Sci* 2016, **107**:250-257.
41. Mori M, Ito F, Shi L, Wang Y, Ishida C, Hattori Y, Niwa M, Hirayama T, Nagasawa H, Iwase A *et al.*: **Ovarian endometriosis-associated stromal cells reveal persistently high affinity for iron.** *Redox Biol* 2015, **6**:578-586.
42. Shi L, Ito F, Wang Y, Okazaki Y, Tanaka H, Mizuno M, Hori M, Hirayama T, Nagasawa H, Des R, Richardson DR *et al.*: **Non-thermal plasma induces a stress response in mesothelioma cells resulting in increased endocytosis, lysosome biogenesis and autophagy.** *Free Radic Biol Med* 2017, **108**:904-917.
43. Maiti S, Aydin Z, Zhang Y, Guo M: **Reaction-based turn-on fluorescent probes with magnetic responses for Fe²⁺ detection in live cells.** *Dalton Trans* 2015, **44**:8942-8949.
44. Taki M, Iyoshi S, Ojida A, Hamachi I, Yamamoto Y: **Development of highly sensitive fluorescent probes for detection of intracellular copper(I) in living systems.** *J Am Chem Soc* 2010, **132**:5938-5939.
45. Au-Yeung HY, New EJ, Chang CJ: **A selective reaction-based fluorescent probe for detecting cobalt in living cells.** *Chem Commun* 2012, **48**:5268-5270.

46. Aron AT, Ramos-Torres KM, Cotruvo JR Jr, Chang CJ: **Recognition- and reactivity-based fluorescent probes for studying transition metal signaling in living systems.** *Acc Chem Res* 2015, **48**:2434-2442.

This review presents an in-depth coverage of chelation- and activity-based sensing strategies, describing strengths, weaknesses, and examples associated with each strategy.

47. Stahlberg KG, Radner S, Norden A: **Liver B12 in subjects with and without vitamin B12 deficiency. A quantitative and qualitative study.** *Scand J Haematol* 1967, **4**:312-320.
48. Borstnik K, Paik IH, Shapiro TA, Posner GH: **Antimalarial chemotherapeutic peroxides: artemisinin, yingzhaosu A and related compounds.** *Int J Parasitol* 2002, **32**:1661-1667.
49. Wang X, Creek DJ, Schiaffo CE, Dong Y, Chollet J, Scheurer C, Wittlin S, Charman SA, Dussault PH, Wood JK *et al.*: **Spiroadamantyl 1,2,4-trioxolane, 1,2,4-trioxane, and 1,2,4-trioxepane pairs: relationship between peroxide bond iron(II) reactivity, heme alkylation efficiency, and antimalarial activity.** *Bioorg Med Chem Lett* 2009, **19**:4542-4545.
50. Creek DJ, Charman WN, Chiu FC, Prankerd RJ, Dong Y, Vennerstrom JL, Charman SA: **Relationship between antimalarial activity and heme alkylation for spiro- and dispiro-1,2,4-trioxolane antimalarials.** *Antimicrob Agents Chemother* 2008, **52**:1291-1296.
51. Creek DJ, Charman WN, Chiu FC, Prankerd RJ, McCullough KJ, Dong Y, Vennerstrom JL, Charman SA: **Iron-mediated degradation kinetics of substituted dispiro-1,2,4-trioxolane antimalarials.** *J Pharm Sci* 2007, **96**:2945-2956.
52. Tang Y, Dong Y, Wang X, Sriraghavan K, Wood JK, Vennerstrom JL: **Dispiro-1,2,4-trioxane analogues of a prototype dispiro-1,2,4-trioxolane: mechanistic comparators for artemisinin in the context of reaction pathways with iron(II).** *J Org Chem* 2005, **70**:5103-5110.
53. Deu E, Chen IT, Lauterwasser EM, Valderramos J, Li H, Edgington LE, Renslo AR, Bogoy M: **Ferrous iron-dependent drug delivery enables controlled and selective release of therapeutic agents in vivo.** *Proc Natl Acad Sci USA* 2013, **110**:18244-18249.
54. Fontaine SD, Spangler B, Gut J, Lauterwasser EMW, Rosenthal PJ, Renslo AR: **Drug delivery to the malaria parasite using an arterolane-like scaffold.** *ChemMedChem* 2015, **10**:47-51.
55. Fontaine SD, DiPasquale AG, Renslo AR: **Efficient and stereocontrolled synthesis of 1,2,4-trioxolanes useful for ferrous iron-dependent drug delivery.** *Org Lett* 2014, **16**:5776-5779.
56. Abrams RP, Carroll WL, Woerpel KA: **Five-membered ring peroxide selectively initiates ferroptosis in cancer cells.** *ACS Chem Biol* 2016, **11**:1305-1312.
57. Aron AT, Heffern MC, Lonergan ZR, Vander Wal MN, Blank BR, Spangler B, Zhang Y, Park HM, Stahl A, Renslo AR *et al.*: **In vivo bioluminescence imaging of labile iron accumulation in a murine model of *Acinetobacter baumannii* infection.** *Proc Natl Acad Sci USA* 2017, **114**:12669-12674.
58. Chan J, Dodani SC, Chang CJ: **Reaction-based small-molecule fluorescent probes for chemoselective bioimaging.** *Nat Chem* 2012, **4**:973-984.
59. Chen X, Tian X, Shin I, Yoon J: **Fluorescent and luminescent probes for detection of reactive oxygen and nitrogen species.** *Chem Soc Rev* 2011, **40**:4783-4804.
60. Yang Y, Zhao Q, Feng W, Li F: **Luminescent chemodosimeters for bioimaging.** *Chem Rev* 2013, **113**:192-270.
61. Cho DG, Sessler JL: **Modern reaction-based indicator systems.** *Chem Soc Rev* 2009, **38**:1647-1662.