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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.

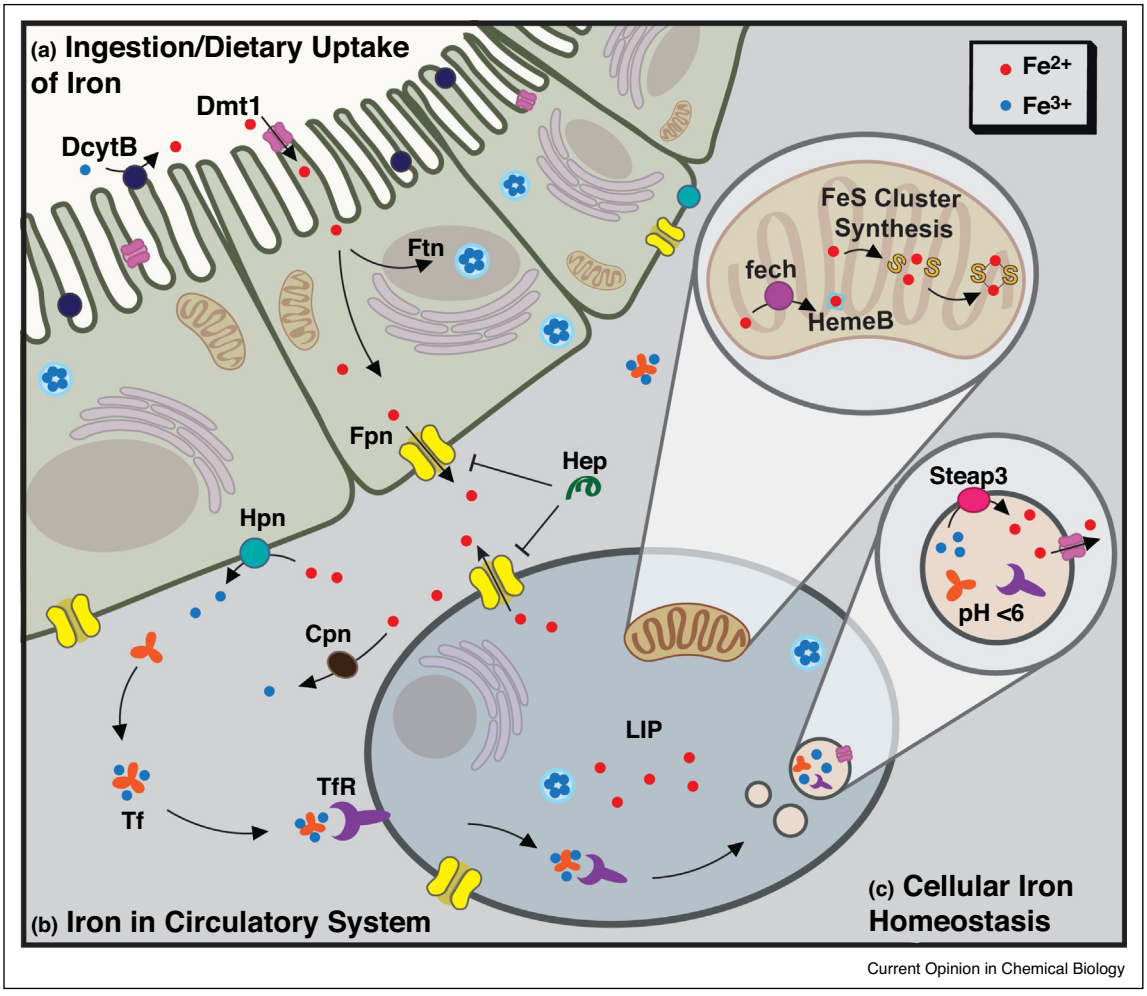
At the systemic whole body level, the hormone hepcidin is a main driver of iron homeostasis in mammals, regulating absorption of dietary iron, release of hepatic iron stores, and recycling of iron by macrophages [6,7]. At the cellular level, the transferrin receptor and divalent metal transporter-1 import 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+}