Recent Endeavors on Molecular Imaging for Mapping Metals in Biology

Jing Gao¹, Yuncong Chen^{1,2}✉, Zijian Guo^{1,2}, Weijiang He¹✉

¹ State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

² Chemistry and Biomedicine Innovation Center, Nanjing University, Nanjing 210023, China

Received: 2 June 2020 / Accepted: 2 August 2020 / Published online: 4 October 2020

Abstract Transition metals such as zinc, copper and iron play vital roles in maintaining physiological functions and homeostasis of living systems. Molecular imaging, including two-photon imaging (TPI), bioluminescence imaging (BLI) and photoacoustic imaging (PAI), could act as non-invasive toolkits for capturing dynamic events in living cells, tissues and whole animals. Herein, we review the recent progress in the development of molecular probes for essential transition metals and their biological applications. We emphasize the contributions of metallostasis to health and disease, and discuss the future research directions about how to harness the great potential of metal sensors.

Graphic Abstract

Keywords Molecular imaging, Zinc, Copper, Iron

INTRODUCTION

Iron, zinc and copper, the top three abundant transition metals in the human body, were well recognized for their essential roles in maintaining homeostasis and sustaining life. There were numerous examples of clinical diseases that were closely associated with metal

misregulation, such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD) (Bleackley and MacGillivray 2011). In this regard, careful maintenance of transition metal homeostasis is required in most living systems.

Labile metal ions, part of the total metal pools, are weakly bound to ligands and can rapidly dissociate (Aron *et al.* 2015). Redox-active labile metals, such as copper and iron, can be harnessed to create new diagnostics and therapeutics (Chang 2015). Ferrous iron

✉ Correspondence: chenyc@nju.edu.cn (Y. Chen), hewei-j69@nju.edu.cn (W. He)

(Fe^{2+}) is oxidized into ferric iron (Fe^{3+}) through reaction with hydrogen peroxide (H_2O_2), leading to the formation of highly reactive hydroxyl radicals ($\text{OH}\cdot$), referred to as the Fenton reaction. Fe^{3+} is reduced back to Fe^{2+} via reaction with superoxide radicals ($\text{O}_2^{\cdot-}$). This redox cycle is called Harber–Weiss reaction (Hassannia *et al.* 2019). Like iron, copper (Cu^+ and Cu^{2+}) can transform between two oxidation states under biological conditions. These metals can also undergo kinetically appreciable ligand exchange with sensors that respond to metal binding or reaction with an alteration in signal (light or sound) output (Ackerman *et al.* 2017).

Fluorescence imaging (FLI) has been widely used in identifying diversified living processes. Providing a potentially non-invasive set of tools with spatial and temporal resolution, molecular imaging drives scientists to peer into biological environments, and unravels the mysteries of metal ions in a sophisticated milieu. Small-molecule indicators have been assorted into two basic categories for achieving specificity: (1) chelation-based and (2) reaction-based (Ackerman *et al.* 2017). Fundamental mechanisms such as fluorescence resonance energy transfer (FRET), photoinduced electron transfer (PET) and intramolecular charge transfer (ICT) integrate the foundation for most sensor designs (Chang *et al.* 2020). Traditional FLI normally utilizes excitation light < 600 nm, which can lead to autofluorescence as well as cell damage, and can be vigorously scattered in deep tissues. To circumvent the high background and low sensitivity, many efforts have been made to develop probes under near infra-red (NIR) light excitation and emission. Accordingly, other imaging modalities such as two-photon imaging (TPI), bioluminescence imaging (BLI) and photoacoustic imaging (PAI) have been

developed and attracted much attention. Table 1 points to a contrastive analysis of the three imaging modalities.

Compared with FLI, BLI does not need an excitation light source, resulting in minimized autofluorescence and much higher signal-to-noise ratio (Li *et al.* 2013). Beetle luciferases oxidize D-luciferin with ATP and O_2 , generating primarily yellow–green light, whereas marine luciferases release blue photons via the oxidation of imidazopyrazinone analogs (Yao *et al.* 2018). A growing field of “PAI”, which combines optical and ultrasound imaging, shows advantages of high resolution and high contrast at centimeter imaging depths. Theoretically, the design of molecular PAI sensors is primarily based on extending a large π -conjugated backbone with strong PA signal, for example, porphyrin, boron-dipyrromethene (BODIPY) and cyanine derivatives. Excellent reviews of Chan and coworkers discussing the development of PA agents can be found elsewhere (Knox and Chan 2018; Reinhardt and Chan 2018), and fluorescent sensors for measuring metal ions in living systems are also recommended (Carter *et al.* 2014; Liu *et al.* 2013; Park *et al.* 2020; Trusso Sfrazzetto *et al.* 2016).

METALLOSTASIS

Zinc

In the cellular environment, zinc is redox inert and is present in the + 2 oxidation state. As the second-most abundant metals in the human body following iron, Zn^{2+} is a major cofactor in DNA replication, protein synthesis and cell differentiation (Szewczyk 2013). Some evidence demonstrates that zinc homeostasis in mammals is controlled by the two major families

Table 1 Strengths and weaknesses of representative imaging modalities (Baker 2010)

Modality	Labels	Readout	Depth	Resolution	Pros	Cons
TPI	Fluorophores with two-photon absorption (700–1100 nm) cross section in GM units	Vis–NIR Light	~ 1 mm	1–2 μm	Less photodamage and photobleaching than the corresponding FLI	Relatively poor depth penetration
BLI	Bioluminescent enzymes (typically luciferases)	Light (500–630 nm emission)	~ 3 cm	3–5 μm	No external light, zero background emission	Inherently weak emission, expensive ($\sim 3 \times 10^5$ USD) charge-coupled device (CCD) camera
PAI	Probes ($\epsilon > 10^4/(\text{M} \times \text{cm})$) that absorb light (680–950 nm) and create sound signals	Sound	5–7 cm	~ 50 μm	Better depth than light	Information processing and machines still being optimized

denoted as ZIP (SLC39A) (Eide 2004) and CDF/ZnT (SLC30A) (Palmiter and Huang 2004).

ZIP transporters confer cytoplasmic Zn^{2+} uptake (Huang and Tepasamorndech 2013). Zinc is first absorbed from the diet by ZIP4 (Fig. 1), and is delivered to the basolateral membrane or bound to metallothioneins (MTs); the molecular mechanism behind this process has not yet been elucidated (Lazarczyk and Favre 2008; Nishito and Kambe 2018). An excess of unbound cytoplasmic Zn^{2+} is exported outside cells by ZnTs, predominantly ZnT1, and transported to peripheral tissues. Zn^{2+} possibly crosses the outer membrane of mitochondria through porin channels, and then bound to MTs in the intermembrane space or further shipped into the mitochondrial matrix via unknown proteins (Lazarczyk and Favre 2008; Nishito and Kambe 2018). Furthermore, a mutual exchange of Zn^{2+} between the ER and Golgi apparatus through antero- and retrograde vesicular transport has been postulated. Zn^{2+} is delivered in the opposite direction, from organelles to the cytosol by ZIPs; ZIP1 and ZIP7 (ER and Golgi apparatus) are closely related to this process. The ZIP and ZnT families are highly conserved, and their corresponding genes were found in zebrafish as well. The mRNA level

of zinc exporter ZnT1 was upregulated in fish subjected to zinc overload and downregulated through zinc deprivation (Zheng *et al.* 2008).

Copper

As the third-most abundant trace metal in the human body, copper not only plays a critical role in physiological processes (Valko *et al.* 2016), but also associates with cell proliferation and major diseases (Brady *et al.* 2014). It is revealed that biological functions of copper are essentially dependent on $\text{Cu}^{2+}/\text{Cu}^{+}$ transition, also associated with the production of reactive oxidative species (ROS). Abnormal $\text{Cu}^{+}/\text{Cu}^{2+}$ equilibrium can lead to dyshomeostasis, causing many genetic disorders such as Menkes (MNK) disease (Andersson *et al.* 2014), Wilson disease (WD) (Bandmann *et al.* 2015), neurodegenerative diseases (Müller *et al.* 2017) and cancers (Wang *et al.* 2015).

Cu^{2+} is reduced to Cu^{+} by an elusive mechanism and can enter cells through copper transporter 1 (CTR1), the principal high-affinity copper importer (Cotruvo *et al.* 2015). Upon entry, copper interacts with cellular ligands and chaperones that adjust its shipping to

Fig. 1 A simplified overview of zinc, copper and iron handling pathways in mammalian cells. ZIP Zrt/Irt-like protein; ZnT zinc transport protein; CTR1 copper transporter 1; ATOX1 anti-oxidant 1; ATP7 adenosine triphosphatase 7; CCS copper chaperones for superoxide dismutase; SOD1 superoxide dismutase 1; MTF1 metal regulatory transcription factor 1; COX cytochrome c oxidase; MT metallothionein; ISC iron-sulfur cluster; TF transferrin; TFR transferrin receptor; FPN ferroportin; FTN ferritin; NCOA4 nuclear receptor coactivator 4; STEAP3 six-transmembrane epithelial antigen of prostate 3; DMT1 divalent metal transporter 1; NRF2 nuclear factor erythroid 2-related factor 2; HMOX1 heme oxygenase 1

specific proteins. Glutathione (GSH) can buffer Cu^+ pools and mediate Cu^+ transfer between CTR1 and metallochaperones. Carriage of copper to cytosolic superoxide dismutase 1 (SOD1) proceeds through copper chaperones for superoxide dismutase (CCS). This metallochaperone for Cu/Zn SOD is a known marker for alterations in copper metabolism that inversely correlates with intracellular copper bioavailability (Bertinato *et al.* 2003; Prohaska *et al.* 2003). Metal regulatory transcription factor 1 (MTF1) transcriptionally activates MT genes and other targets. Cytosolic copper is delivered to mitochondria for loading onto cytochrome C oxidase (COX) (Festa and Thiele 2011). Atox1, homolog of Atx1, is responsible for copper delivery to two P-type ATPases, who also participate in copper efflux from cells. They are ATP7A and ATP7B in the trans-Golgi network, where most cuproproteins are metallated. MNK is an X-linked recessive disorder caused by mutations in genes coding for the copper-transport protein ATP7A, leading to copper deficiency. Conversely, WD is a rare genetic disorder characterized by excess copper stored in various body tissues (Bansagi *et al.* 2016).

Iron

As the most abundant transition metal in the human body, iron is essential and required for a variety of physiological and pathological processes (Theil and Goss 2009). Iron deficiency anemia (IDA) is imposed by a lack of iron, whereas hereditary hemochromatosis (HH) is an iron-overload disorder (Andrews 2000). Once iron is put into circulation, it is first bound to transferrin (TF) as Fe^{3+} and can subsequently enter cells, which maintain iron homeostasis through a balance of import proteins including transferrin receptor (TFR), divalent metal transporter 1 (DMT1), storage proteins like ferritin (FTN), and export proteins like ferroportin (FPN) (Aron *et al.* 2018). It is worth noting that metalloredoxase STEAP3 (six-transmembrane epithelial antigen of prostate 3) converts Fe^{3+} to Fe^{2+} . In mitochondria, iron-sulfur (Fe/S) cluster proteins (ISCs) are generated from iron and cysteine and are indispensable cofactors for proteins in mitochondrial respiration and other cellular activities (Braymer and Lill 2017).

Aberrant iron levels are implicated in ailments such as pyroptosis and ferroptosis (Zhou *et al.* 2018). Ferroptosis is a newly recognized cell death modality marked by the oxidative modification of phospholipid membranes via an iron-dependent mechanism and that has drawn constantly increasing attention in chemical biology (Conrad *et al.* 2016; Friedmann Angeli *et al.*

2019). Augmented levels of nuclear factor erythroid 2-related factor 2 (NRF2) protein after treatment with erastin and sorafenib (inducers of ferroptosis) render cells resistant to ferroptosis. This protection is traced to upregulation of NRF2 target genes like heme oxygenase 1 (HMOX1). Lysosomes can accumulate a large quantity of iron through degradation of FTN (coined ferritinophagy). Inhibition of lysosomal activity or silencing of nuclear receptor coactivator 4 (NCOA4), a cargo receptor recruiting FTN to autophagosomes for lysosomal degradation and iron release, suppresses ferroptosis (Hassannia *et al.* 2019). Remarkably, a novel quinolone-derived fluorescent turn-on probe IQ44 has been enabling the visualization of autophagosome-lysosome fusion in the process of autophagy (Lim *et al.* 2019).

MOLECULAR IMAGING

Zinc

Our laboratory reported a turn-on Zn^{2+} sensor NBD-TPEA, the first example for monitoring zinc ions in neuromasts of zebrafish via fluorescence (Qian *et al.* 2009). By replacing $-\text{NO}_2$ moiety with $-\text{SO}_2\text{NH}_2$, we devised an ICT ratiometric fluorescent probe, SBD-TPEA, for Zn^{2+} imaging in biological systems (Liu *et al.* 2014). In the presence of Zn^{2+} (50 mmol/L HEPES/DMSO = 99.85/0.15, pH 7.2), SBD-TPEA exhibits an emission band centered at 585 nm, with an excitation maximum at 466 nm. However, addition of Zn^{2+} to the probe leads to a distinct hypsochromic emission shift from 585 to 545 nm with a well-defined isoemission point at 585 nm. This change is attributed to ICT alteration when Zn^{2+} binds to the probe. The dissociation constant (K_d) of the SBD-TPEA/ Zn^{2+} complex was estimated to be 2.1 nmol/L and its limit of detection (LOD) for Zn^{2+} was 0.5 nmol/L. Because SBD-TPEA responded selectively to Zn^{2+} without interference from other metal ions, it was utilized to image Zn^{2+} in HepG2 cells and zebrafish larvae (Fig. 2).

Through integrating a Zn^{2+} ionophore N,N'-bis(pyridin-2-ylmethyl)ethane-1,2-diamine (BPEA) as the ICT donor of the ANaph fluorophore, we constructed Naph-BPEA with nuclear envelope penetrability (Zhang *et al.* 2013). Of note, this probe did not merely localize in the cell nucleus, it also fluoresced in the cytoplasm. More importantly, our group summarized photoluminescence Zn^{2+} imaging in living subjects before 2015 (Chen *et al.* 2015). Apart from biological functions and sensing mechanisms of labile Zn^{2+} , Zn^{2+} roles within an array of subcellular compartments, such as mitochondria,

Fig. 2 **A** Emission spectra of 3 mmol/L SBD-TPEA ($\lambda_{\text{ex}} = 460$ nm) in buffer (50 mmol/L HEPES/DMSO = 99.85/0.15, pH 7.2) obtained by adding aliquots of Zn^{2+} solution (1.2 mmol/L); Inset in **A**: chemical structure of SBD-TPEA. **B–E** Confocal fluorescence ratiometric Zn^{2+} imaging in the head of a 3-day-old zebrafish larva stained with SBD-TPEA. **B** Overlay of bright field and fluorescence image of the SBD-TPEA-stained zebrafish larva from the green channel. **C** Ratiometric image based on the fluorescence images of the zebrafish larva in **B**. **D** Overlay of bright field and fluorescence image of the SBD-TPEA-stained zebrafish larva from the green channel after feeding the larva with Zn^{2+} solution (100 mmol/L) for 1 h. **E** Ratiometric image of the larva in **D**. Green channel images obtained with a bandpass of 510–560 nm, and red channel images obtained with a bandpass of 580–630 nm upon excitation at 488 nm. Adapted from Liu *et al.* (2014)

lysosome, endoplasmic reticulum (ER), and Golgi apparatus were illuminated in detail, with corresponding Zn^{2+} sensors displayed. Localization is usually defined by comparing the colocalization of the sensor with a well-established organelle marker and quantifying the overlap using Pearson's correlation coefficient (PCC) given by a imaging software (Carter *et al.* 2014). Organelle-specific Zn^{2+} probes have been developed to study Zn^{2+} distribution in different subcellular compartments (Fig. 3); we will only introduce some representative works due to limited space of this review;

readers are encouraged to refer to another review for more detailed information (Zhu *et al.* 2016).

Golgi-Targeting

Golgi apparatus contain various metalloproteases and alkaline phosphatases, whose catalytic activities rely largely on the Zn^{2+} ions. In 2015, the Kim research group reported a Golgi-localized two-photon ratiometric probe for zinc ions (Singh *et al.* 2015). The dipicolylamine moiety within the compound SZnC selectively makes a complex with Zn^{2+} (30 mmol/L MOP/EtOH =

Fig. 3 Organelle-specific reversible Zn^{2+} probes. Golgi-targeting SZnC, ER-targeting ZR1, mitochondria-targeting DA-ZP1-TPP and lysosome-targeting LysoDPP-C4. Adapted from Zhu *et al.* (2016)

1:1, pH 7.2), with an emission enhancement peaked at 500 nm. The TP action cross-section values ($\Phi_{\delta\max}$) of SZnC in the absence and presence of excess Zn^{2+} were determined to be 16 and 92 GM, while the fluorescence quantum yield Φ were 0.12 and 0.93, respectively. Featuring low cytotoxicity and high photostability, this probe can be leveraged to monitor Zn^{2+} fluctuations in real time and getting a 3D distribution picture in the Golgi apparatus.

ER-Targeting

Metal ions, such as Cu^+ and Zn^{2+} , are required for ER functions. Recently, Lippard and coworkers synthesized a new red-emitting fluorescent probe ZR1 to monitor mobile Zn^{2+} in the ER, a benzoessorufin fluorophore functionalized with two 2,2'-dipicolylamine (DPA) arms (Loas *et al.* 2014). The free probe was non-fluorescent (50 mmol/L PIPES, pH 7.0) due to PET from the Zn^{2+} chelators, while Zn^{2+} -binding strongly elevated its emission maximum at 611 nm and an 18 nm Stokes shift. On average, the associated brightness values ($\epsilon\Phi$) enhanced 44-fold to 2.43×10^4 (L/(mol·cm)). In comparison with ZBR4, which anchors to the mitochondria, ZR1 spontaneously localizes to the endoplasmic reticulum of HeLa cells.

Mitochondria-Targeting

Using fluorescein fluorophore instead, Lippard and coworkers presented a reaction-based fluorescent sensor DA-ZP1-TPP, which selectively localized at the mitochondria (Chyan *et al.* 2014). Coordination of Zn^{2+} enhances fluorescence intensity by promoting ester hydrolysis and alleviating PET origination from the DPA motif. Adding nanomolar concentrations of free zinc ions resulted in large increases in both the absorption ($\lambda_{\text{abs}} = 510$ nm) and fluorescence ($\lambda_{\text{em}} = 529$ nm) spectral bands of DA-ZP1-TPP (50 mmol/L PIPES, pH 7.0). These optical changes combined to yield a > 140-fold increase in the fluorescence signal ($\Phi_{\text{Zn}} = 0.75$). With this probe, it is concluded that tumorigenic cancer cells lose the ability to accumulate Zn^{2+} within their mitochondria in contrast to healthy epithelial prostate cells.

Indeed, close correlations between prostatic Zn^{2+} levels and prostate cancer (PCa) have been reported (Costello *et al.* 2004). At least three ZIPs and six ZnTs are expressed in a lobe-dependent manner in the prostate (Kelleher *et al.* 2011). In malignant prostate tissues, there is a dramatic decline in Zn^{2+} concentration (500 nmol/g vs. 3000 nmol/g for cancerous and

healthy tissues, respectively), as well as a downregulation of ZIP1 (Kolenko *et al.* 2013).

Lysosome-Targeting

Very recently, LysoDPP-C4 has been developed for the evaluation of low pH and Zn^{2+} in an AND logic fashion to investigate lysosomal Zn^{2+} in prostate cancer cells (Du *et al.* 2019). A morpholine unit is appended for targeting lysosomes, as confirmed by HeLa cell fluorescence imaging studies. LysoDPP-C4 demonstrated an excellent selectivity toward Zn^{2+} and the resulting LysoDPP-C4/ Zn^{2+} complexes proved insensitive to pH 3.5–9. At low pH, Zn^{2+} chelation contributes to a large increase in the fluorescence intensity at 515 nm ($\lambda_{\text{ex}} = 430$ nm) ascribed to suppression of PET. The sensor formed a host-guest complex in 1:1 stoichiometry with $K_d = 1.91$ nmol/L. Besides, histological studies using a human sample revealed that LysoDPP-C4 could distinguish between cancerous prostate tissue and healthy one.

In view of the advances in Zn^{2+} -specific chelators (TPEA, BPEA, DPA, etc.) and Zn^{2+} -complexation-induced spirolactam ring-opening or hydrolysis (Jin *et al.* 2018), concurrent with phosphorescence lifetime imaging (PLIM) of labile Zn^{2+} (Zhang *et al.* 2018), it is not surprising that Zn^{2+} sensors constitute the largest family of fluorescent indicators for transition metals. Some of them are reversible and ratiometric, applied to appealing models. So far, the physiological participation of this ion is not completely clear in spite of the large arsenal of fluorescent Zn^{2+} sensors.

Copper

Molecular sensors are intriguing tools to visualize the distribution and speciation of labile copper. Nevertheless, there are added challenges posed by aiming at labile copper over Zn^{2+} due to the need for discrimination between different oxidation states, the quenching nature of Cu^{2+} , and the fact that probes must have enough affinities to compete for copper within its biological window ($10^{-21} - 10^{-17}$ M) (Wegner *et al.* 2011). In consequence, only a fraction of copper sensors has been utilized for biologically accessible copper. The Kim group shed light on the pathophysiological part of copper ions and sensors for the measurement thereof (Peter *et al.* 2015).

Cu^+

Although Cu^+ is prone to disproportionation and acquires stabilization by specific ligands in aqueous

media (Paredes and Das 2011), it is considered to be the dominant intracellular copper oxidation state of labile copper pools (Festa and Thiele 2011). Most of the probes designed for biological systems target Cu^+ engaged in two common aspects as follows (Fig. 4).

(A) NS_4 -based chelation.

Thioether (NS_4) receptors can be modified to develop fluorescent Cu^+ sensors. One notable example is CTAP-1, the first small-molecule sensor for mapping labile Cu^+ in cells (Yang *et al.* 2005). Soon after this study, the Chang group presented coppersensor 1 (CS1) with visible excitation and emission profiles (Zeng *et al.* 2006). Tuning the BODIPY fluorophore of CS1 to a rhodol scaffold produced a more hydrophilic Cu^+ sensor called copper Fluor-3 (CF3) (Dodani *et al.* 2014). As described earlier, fluorescent sensors that excited at short wavelengths succumb to limited penetration depths, high autofluorescence and inevitably induce photobleaching and photodamage. These flaws hinder their use for long-term imaging in tissues and organisms. One way to remove these limitations is by NIR single-photon excitation (Guo *et al.* 2014). Cao Cu-3 represents a novel NIR fluorescent turn-on probe suitable for imaging endogenous Cu^+ ions in living systems (Cao *et al.* 2012).

Another approach is to use two-photon microscopy (TPM), employing NIR photons of lower energy as the excitation source to possess the advantage of deepened penetration ($> 500 \mu\text{m}$) (Kim *et al.* 2014). Our laboratory presented the first-generation ER-localized TP Cu^+ sensor CNSB (Guo *et al.* 2019). There is a distinct overlap between coumarin-A (donor) emission and 4-amino-7-sulfamoyl benzoxadiazole (ASBD, acceptor) absorption, implying these two compounds are qualified candidates as a FRET pair to compose ratiometric sensors. CNSB exhibits $\sim 7.44 \times 10^{-11} \text{ M}$ K_d value (10 mmol/L Tris-HCl/DMF = 6:4, pH 7.2) and the Cu^+ -enhanced emission ratio F_{470}/F_{565} is stable from pH 4.0 to 8.0. The sensor was applied to assessing Cu^+ fluctuation in MCF-7 cells pre-incubated with tunicamycin,

proving the relationship between Cu^+ augmentation and ER stress. Meanwhile, the spatial distribution of Cu^+ in the heart slice of a 2-week rat was observed via TPI owing to the two-photon ability of coumarin fluorophore. Negligible enhancement of coumarin emission was observed during the fluorescent titration of CNSB.

(B) O_2 -dependent C–O bond cleavage.

Inspired by the O_2 activation for copper-dependent enzymes, the Chang group adapted the Cu^+ chelator tris(2-pyridylmethyl)amine (TPA) to develop copper-caged luciferin-1 (CCL-1) (Heffern *et al.* 2016), a bioluminescent reporter for monitoring labile Cu^+ levels in a diet-induced mouse model of non-alcoholic fatty liver disease (NAFLD) that manifests as a hepatic copper deficiency, revealing altered expression levels of central copper trafficking proteins that accompany symptoms of glucose intolerance and weight gain (Fig. 5). As supported by Western blot, CCS levels were elevated in the high-fat diet (HFD) mice over control diet mice, and the major copper exporter proteins ATP7A and ATP7B were also upregulated.

Not long ago, linking fluorescein donor and rhodamine acceptor through the TPA bridge, they reported reaction-based ratiometric FRET copper probe FCP-1. Together with its 2-color response (F_{526}/F_{576}), collective results indicate that oncogene-driven changes in the metabolism of GSH, a major cellular redox buffer, leads to a labile Cu^+ deficiency with differential expression of CTR1 (Chung *et al.* 2019). This work connects Cu^+ dysregulation and GSH stress in cancer, providing a roadmap for studying the crosstalk between metal and redox pathways in physiology and pathology with probes.

Cu^{2+}

As synthetic fluorescent probes for monovalent copper have been thoroughly reviewed by others (Cotruvo *et al.* 2015; Fahrni 2013), we focus our discussion on Cu^{2+}

Fig. 4 Design approaches and selected structures for Cu^+ detection. **A** NS_4 -based chelation: CNSB. O_2 -dependent C–O bond cleavage: CCL-1

Fig. 5 CCL-1 imaging reveals hepatic copper deficiency in a diet-induced murine model of NAFLD. **A** Representative images of mice from HFD and normal diet groups before the study (day 0) and at the end of the 8-week feeding period (day 56). **B** Western blot analysis of liver extracts of three mice fed normal diets and three HFD mice. Adapted from Heffern *et al.* (2016)

probes that compensate for these areas of copper sensing. A large library of luminescent chemodosimeters for Cu^{2+} bioimaging has been collected by Fuyou Li *et al.* (Yang *et al.* 2013). Most of such probes recognize Cu^{2+} by three principal categories: chelation, hydrolysis and oxidation (Fig. 6). Among them, the oxidation pattern draws in the fewest probes while the other two seem more attractive.

Chelation As mentioned previously, 2,2'-dipicolylamine (DPA) can be manipulated to design probes selective for Cu^{2+} over Zn^{2+} (Ballesteros *et al.* 2009). Modifying the FRET pair of CNSB with DPA unit forms a reversible ratiometric sensor CSBPA for intracellular Cu^{2+} imaging (Chen *et al.* 2013). Later we achieved *in vivo* fluorescence imaging for Cu^{2+} in live mice for the first time by a BODIPY-derived NIR fluorescent sensor

BDPA (Xue *et al.* 2016), profiting from its large Stokes shift (~ 100 nm), excellent photostability and high quantum yield ($\Phi = 0.85$).

Wang *et al.* developed a series of activatable PA probes with low molecular weights (< 438 Da), and could specifically chelate with Cu^{2+} to form radicals displaying turn-on PA signals in the NIR region (Wang *et al.* 2019a). Introducing the electron-donating group N,N-dimethylaniline into the probe was found to significantly enhance the radical stability and PA intensity. The best probe in the series, RPS1, produced a fast response (within seconds) to Cu^{2+} with a low LOD of 90.9 nmol/L (0.02 mol/L PBS, pH 7.4). Owing to the low molecular weight and amphiphilic structure, RPS1 could effectively cross the blood-brain barrier (BBB) and thus maps Cu^{2+} in the brains of AD mice via PAI for the first time (Fig. 7).

Fig. 6 Selected structures of Cu^{2+} -responsive probes based on chelation: CSBPA (A), hydrolysis: NRh (B) and oxidation: FluHMPP (C)

Hydrolysis The group headed by Chan reported the synthesis and photoacoustic properties of APC-1 and APC-2, two ratiometric probes for mobile Cu^{2+} (Li *et al.* 2015). Upon binding Cu^{2+} , the sensors display 89- and 101-fold enhancements of normalized ratiometric turn-on responses, respectively. Both APCs are equipped with a 2-picolinic ester sensing module that is readily hydrolyzed in the presence of Cu^{2+} but not by other divalent metal ions. Additionally, ratiometric PAI was realized by using an aza-BODIPY dye scaffold exhibiting two spectrally resolved NIR absorbance bands, which correspond to the 2-picolinic ester capped and uncapped phenoxide forms.

Combining Cu^{2+} -promoted hydrolysis with spirolactam ring-opening generates an effective strategy to construct fluorescent Cu^{2+} chemodosimeters. A ratiometric TP probe for Cu^{2+} trafficking based on through-

Fig. 7 A Experimental design of target PA probe RPS1 for Cu^{2+} analysis. B Calibration curve of the PA intensity at 710 nm for measuring Cu^{2+} in the range of 0–35 $\mu\text{mol/L}$. C Schematic diagram of RPS1 crossing the BBB and detecting Cu^{2+} , as observed by PAI. Adapted from Wang *et al.* (2019a)

bond energy transfer (TBET) was designed by directly conjugating BODAN (donor, green emission) and rhodamine spirolactam (acceptor, red emission) (Fig. 8)

Fig. 8 Two-photon TBET probe Np-Rh. TPI of a rat liver frozen slice stained with 10 $\mu\text{mol/L}$ Np-Rh at $\sim 95 \mu\text{m}$ for 60 min (A), followed by treatment with 100 $\mu\text{mol/L}$ Cu^{2+} and incubated for another 60 min (B). Green channel images obtained with a bandpath of 450–530 nm (A), and red channel images obtained with a bandpath of 540–650 nm upon excitation at 780 nm with femtosecond pulses (B). Adapted from Zhou *et al.* (2014)

(Zhou *et al.* 2014). Np-Rh elicits a ratiometric response ($\text{EtOH}/\text{H}_2\text{O} = 1:9$) upon addition of Cu^{2+} , with highly efficient energy transfer (93.7%) and two well-resolved emission peaks separated by 100 nm. Moreover, the Cu^{2+} distribution in Np-Rh-labeled HeLa cells as well as rat liver frozen slices pretreated with Cu^{2+} was visualized using TPM in the 450–530 and 540–650 nm collection windows.

Similarly, Fuyou Li and coworkers introduced a frequency upconversion luminescence (UCL) Cu^{2+} chemodosimeter NRh (Liu *et al.* 2016), lighting up efficient single-photon upconversion emission at 730 nm under NIR excitation at 808 nm. This probe can serve as an ideal Cu^{2+} sensor for ex vivo and in vivo assay in a WD mouse model. Impressively, the exploitation of UCL small-molecule probes and their bioimaging applications have been attracting much attention (Dong *et al.* 2018; Yang *et al.* 2016; Zhang *et al.* 2017).

Oxidation Subsequently, the same group developed an easy-to-use probe CYDA based on UV-vis-NIR absorption changes with excellent sensitivity and selectivity for Cu^{2+} (Shi *et al.* 2018). The mechanism of CYDA oxidation by Cu^{2+} was first explored (Fig. 9). In aqueous solution ($\text{EtOH}/\text{H}_2\text{O} = 1:1$), addition of Cu^{2+} to CYDA

Fig. 9 **A** Oxidation of CYDA by Cu^{2+} in acidic solution (pH 6.8) and alkaline solution (pH 8.0), respectively. **B** UV–Vis–NIR absorption spectra (WD patients' urine/EtOH/HEPES = 10:10:1) of 10 $\mu\text{mol/L}$ CYDA when the pH value was 6.8 (left), 8.0 (right), from top to bottom: CYDA/Urine_{start}, CYDA/Urine_{end} and Urine. Adapted from Shi *et al.* (2018)

induced significant changes in the absorption spectra. When the solution was mildly acidic (pH 6.8), the absorbance at 670 nm went down, with the absorbance at 553 nm ($\epsilon = 1.09 \times 10^5 \text{ L}/(\text{mol}/\text{cm})$) up, accompanied by the solution color changes from blue to red. In alkaline solutions (pH 8.0), the absorbance at 670 nm ($\epsilon = 2.14 \times 10^5 \text{ L}/(\text{mol}/\text{cm})$) dropped gradually, whereas the absorbance at 823 nm ($\epsilon = 0.22 \times 10^5 \text{ L}/(\text{mol}/\text{cm})$) rose with an isosbestic point at $\lambda = 793 \text{ nm}$. In the meantime, the solution color tuned into grayish blue. These phenomena confirmed the formation of CY1 and CY2. They further demonstrated that the probe was able to quantify Cu^{2+} in urine from WD patients.

Even a bit earlier than the above study, oxidative C–O bond cleavage turn-on probe FluHMPP has been prepared (Shi *et al.* 2013). On addition of 20 equiv of $\text{Cu}(\text{NO}_3)_2$ (10 mmol/L Tris–HCl/ CH_3CN = 7:3, pH 7.2), there was a strong emission enhancement (30-fold) after 2 h. Confocal microscopy experiments have demonstrated that FluHMPP was membrane permeable and capable of tracing exogenous CuCl_2 in HeLa cells. Nevertheless, such C = N structures are unstable and sensitive to reactive oxygen species.

Iron

Despite great interest in labile iron pool (LIP), its biological function remains insufficiently understood, in part due to a relative lack of tools for directly assessing labile iron in living specimens (Dixon and Stockwell 2014). Molecular imaging using iron selective sensors opens up a broad avenue for better understanding the complex handling of iron in biology. Ideally, probes should be able to track biological iron status, selectively detecting either Fe^{2+} or Fe^{3+} or responding to iron species such as heme–iron or ISCs. Because Fe^{2+} and Fe^{3+} are potent emission quenchers, iron imaging within living cells encountered many challenges. Two early sensors, Calcein and Phen Green SK, exhibit turn-off responses to iron binding (Carter *et al.* 2014). However, both probes cannot adequately distinguish the two oxidation states of iron but can interact with other metal ions, leading to an interfering signal. In the past few years, a substantial body of efforts has been devoted toward construction of iron probes that may make a stunning breakthrough regarding cellular iron homeostasis.

Fe^{2+}

The growing palette of chemodosimeters for Fe^{2+} mapping exploits the potent redox activity of this metal ion, and the last several years have witnessed a tremendous number of novel Fe^{2+} chemoprobes suitable for live-cell imaging experiments. Recent reviews by Hirayama, Aron and coworkers exhaustively profiled the development of molecular probes for Fe^{2+} (Aron *et al.* 2018; Hirayama 2019). A palette of Fe^{2+} -mediated principles have been reported, including N-oxide or nitroxide reduction, cyclization, O_2 activation and endoperoxide cleavage (Fig. 10).

N-oxide reduction The deoxygenation of tertiary amine N-oxide is preferably mediated by Fe^{2+} as it has a lower redox state than Fe^{3+} . Utilizing the reaction, Hirayama and coworkers developed the first turn-on fluorescent Fe^{2+} probe RhoNox-1 (Hirayama *et al.* 2013), which has been commercially available and applied to detecting Fe^{2+} changes during ferroptosis (Wang *et al.* 2019b). To date, this Fe^{2+} sensing strategy has emerged as the most widely used one, establishing a series of sensors. For instance, HMRhoNox for visualization of intracellular Fe^{2+} delivered by TF (Niwa *et al.* 2014), CoNox-1 and FluNox-1 for scrutiny of intracellular redox equilibrium shift toward Fe^{2+} in hypoxic HepG2 cells (Hirayama *et al.* 2017), and a membrane-anchoring probe Mem-

Fig. 10 Reaction-based sensing probes for Fe^{2+} detection. **A** N-oxide: RhoNox-1. **B** Nitroxide reduction: Rh-T. **C** Cyclization: L1. **D** O_2 activation: LCy7. **E** Endoperoxide cleavage: Trx-puro

RhoNox to scrutinize Fe^{2+} release during endocytotic iron uptake (Niwa *et al.* 2018).

Recent research expanded the applicability to establish a series of organelle-specific fluorescent probes selective to Fe^{2+} , *i.e.*, MtFluNox, Lyso-RhoNox, ER-SiRhoNox and Gol-SiRhoNox. The former three chemosensors (Fig. 11) demonstrated similar off/on contrast and reaction rates ($2.1 \times 10^{-3} \text{ s}^{-1}$, $2.2 \times 10^{-3} \text{ s}^{-1}$, and $1.7 \times 10^{-3} \text{ s}^{-1}$), investigated Fe^{2+} specifically at the targeted organelles ($PCC = 0.81, 0.80, 0.80$) and depicted fluorescence enhancement at 535 nm (green, 100-fold), 575 nm (orange, 60-fold) and 660 nm (magenta, 60-fold), respectively. Furthermore, these probes indicated the aberrant elevation of labile Fe^{2+} levels in the lysosomes and ER but no Fe^{2+} fluctuation in the mitochondria prior to HT1080 cell death initiated by erastin (Hirayama *et al.* 2019b).

As depicts in Fig. 1, DMT1 serves as a primary transporter of Fe^{2+} . Over the course of the endocytotic process, directing DMT1 toward the cellular membrane via the trans-Golgi network is partially regulated by a retromer-mediated protein-sorting machinery comprising vacuolar protein-sorting proteins (VPSs) (Burd and

Cullen 2014). Therefore, the Golgi organelle functions as a node in this dynamic system for intracellular Fe^{2+} species along with DMT1. Dysfunction of VPSs, particularly the mutation of VPS35, can activate the aberrant delivery of DMT1 to lysosomes concomitantly with Fe^{2+} ions (Tabuchi *et al.* 2010). The last one Gol-SiRhoNox revealed an abnormal cellular Fe^{2+} distribution induced by dysfunction of VPS35 with a molecular chaperone R55 (Hirayama *et al.* 2019a).

Nitroxide reduction In parallel studies, a related Fe^{2+} -promoted nitroxide reduction reaction has been employed for reaction-based sensing of Fe^{2+} (Maiti *et al.* 2015). A rhodamine-linked nitroxide probe, Rh-T, bears a pendant paramagnetic 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) group that quenches fluorescence. Fe^{2+} reduces the receptor radical to a diamagnetic hydroxylamine, resulting in a significant fluorescence turn-on (~ 2.5 -fold) with a LOD $\sim 0.75 \mu\text{mol/L}$. Fe^{2+} -dependent reduction of the TEMPO radical of Rh-T triggers both a fluorescence turn-on and a change in electron paramagnetic resonance (EPR) signal. Following this example, Zhu group reported NT-Fe with the fastest

Fig. 11 Chemical structures of the fluorescent Fe^{2+} probes used MtFluNox, Lyso-RhoNox and ER-SiRhoNox. And fluorescence microscopic analysis for multi-color detection of Fe^{2+} at each targeted organelle. Adapted from Hirayama *et al.* (2019b)

response (< 50 s), as well as the sensitive LOD of 89 nmol/L (Zhang *et al.* 2020). In zebrafish, NT-Fe responds to the addition of exogenous Fe^{2+} , and its signal decreases when pretreated with Fe^{2+} scavenger 2,2'-bipyridine (Bpy).

Cyclization By exploiting the Lewis acidity of Fe^{2+} , a recent report highlighted the application of an Fe^{2+} -specific cyclization of a phenolic unit adjacent to a C = N bond appended to a coumarin derivative (Long *et al.* 2018). Upon cyclization, generation of a benzoxazole ring handicaps C = N bond isomerization and rotation, turning on fluorescence in aqueous solution and HepG2 cells, which has also been confirmed by TD-DFT calculation. Significantly, the sensing reaction completed in 2 min with high sensitivity (LOD = 45 nmol/L). However, more analogous probes should be rationally constructed to verify the validity of the proposed mechanism.

O_2 activation Prior work by Chang laboratory on creating Cu^+ indicators presaged the general utility of biomimetic oxygen activation for Fe^{2+} surveillance. Inspired by the O_2 chemistry of Fe^{2+} , and in particular, the 2-His-1-carboxylate triad motif found in mononuclear non-heme Fe^{2+} enzymes (cytochrome P450), they engineered iron probe 1 (IP1) (Au-Yeung *et al.* 2013). IP1 was capable of monitoring elevated intracellular

labile Fe^{2+} levels in HepG2/C3A cells caused by treatment with hepcidin or ascorbic acid.

Replacement of the fluorescein alcohol substituent with a cyanine dye (Cy7), the Tang group yielded another NIR fluorescent probe (LCy7) (Wu *et al.* 2020), comprising a tris(pyridine) carboxylate-type ligand cage as an iron recognition and activity site. Upon Fe^{2+} coordination, O_2 activation triggers intramolecular C–O bond cleavage and oxidation to release the fluorophore, resulting in an increase (~ 3.7 -fold) in the fluorescence emission peaked at 690 nm. Utilizing LCy7, dynamic Fe^{2+} enhancement was distinctly monitored in HL-7702 cells under ER stress by acetaminophen (APAP) stimulation. *in vivo* FLI disclosed the conspicuous Fe^{2+} ascent in the liver of mice during drug-induced liver injury (DILI).

Endoperoxide cleavage Motivated by Fe^{2+} -reactive endoperoxide activation observed in antimalarial drugs (e.g., Artemisinin), Renslo and coworkers designed Trx-puro, a histochemical stain for labile Fe^{2+} comprised of a trioxalane-caged puromycin (Spangler *et al.* 2016). Upon reaction of the probe with Fe^{2+} , free puromycin is released, which can be translated onto nascent peptides and quantified via a conventional immunostaining method by a puromycin antibody after fixation. Application of Trx-puro to a panel of cancer cells (PC-3, U-2 OS, MCF10A and RKO) reveals FTN/FPN overexpression and declined Fe^{2+} stores. Linking fluorescein and Cy3 through this bioinspired endoperoxide trigger, Chang *et al.* envisioned FRET iron probe 1 (FIP-1) for ratio-metric fluorescence Fe^{2+} imaging upon 35MEW28-induced ferroptosis in MDA-MB-231 cells (Aron *et al.* 2016).

Expanding the scope of oxidative cleavage to BLI, the same group then reported the first bioluminescent indicator for Fe^{2+} (Aron *et al.* 2017), iron-caged luciferin-1 (ICL-1), to surveil labile Fe^{2+} accumulation in a luciferase-expressing murine (FVB-Luc⁺) model of *Acinetobacter* (*A.*) *baumannii* infection (Fig. 12). The probe also retains high selectivity and sensitivity for Fe^{2+} over a variety of biologically relevant metal ions, oxidants and reductants and is feasible for detecting both Fe^{2+} rise and fall in PC3M-luc cells and other Fe^{2+} -supplemented/depleted models.

Despite the exquisite selectivity toward Fe^{2+} for bioimaging, these reaction-based probes were not shown to have a reversible monitoring mode, hampering the capability to probe Fe^{2+} fluxes. If a probe is able to synchronously measure Fe^{2+} and Fe^{3+} without interference from other metal ions, the privileged part of iron taking in ferroptosis will no longer remain a mystery.

Fig. 12 A Fe^{2+} -selective cleavage of ICL-1, an endoperoxide-luciferin conjugate and *in vivo* probe of Fe^{2+} . B Representative images of FVB-Luc⁺ mice mock-infected (PBS) or infected with *A. baumannii* through retroorbital injection (dorsal images at 30 min postinjection of ICL-1) and imaged with ICL-1 (25 nmol) at 24 h postinfection. Adapted from Aron *et al.* (2017)

Fe^{3+}

In contrast to the growing usage of chemical probes for bioimaging of Fe^{2+} , measurement of Fe^{3+} in biological environments is rarer. Sahoo *et al.* reviewed advances on Fe^{3+} -specific fluorescent probes (Sahoo and Crisponi 2019; Sahoo *et al.* 2012). Due to the paramagnetic nature of ferric iron, its previous recognition by fluorescent probes involves different quenching mechanisms, which is improper for bioimaging applications. A new class of turn-on Fe^{3+} sensors can be classified into two major categories, chelation and spirolactam ring-opening (Fig. 13). Furthermore, some ratiometric sensors recognizing the metal with an appropriate ligand group have been proposed to interrogate intracellular Fe^{3+} ions.

Turn-on Fe^{3+} probes *Chelation* The modularity of the PET platform has been applied to developing Fe^{3+} -triggered fluorescent turn-on probes with enhanced properties. A novel PET-based reversible fluorescence turn-on probe L2 for selective assay of Fe^{3+} was introduced (Sui *et al.* 2014). The probe was comprised of BODIPY fluorophore with a 1,10-diaza-18-crown-6-based cryptand that acted as the analyte binding unit.

Upon addition of Fe^{3+} in aqueous media ($\text{H}_2\text{O}/\text{CH}_3\text{CN} = 9:1$), the weakly fluorescent L2 (PET process is active) displayed 23-fold fluorescence enhancement at 512 nm ($\lambda_{\text{ex}} = 480$ nm). The significant fluorescence enhancement occurred due to inhibition of PET from cryptand to BODIPY fluorophore upon complexation with Fe^{3+} . The probe displayed a LOD of 0.13 $\mu\text{mol/L}$ and was applied to sensing intracellular Fe^{3+} ions in HCT-116 cells.

Our group introduced a simple anthracene-based fluorescent turn-on probe L3 containing N^2 -hydroxyethylthiendiethylenetriamine (HEDTA) for inferring Fe^{3+} concentrations (Qiu *et al.* 2014). When excited at 373 nm, the [2 + 2] macrocycle fluorescent sensor (20 mmol/L Tris-HCl/MeOH = 1:1, pH 7.2) exhibits weak emission at 398, 421 and 447 nm. In the presence of Fe^{3+} , the formation of a L3- Fe^{3+} complex in 1:2 ratio restricts the PET process, causing significant fluorescence enhancement. Probe L3 shows a linear response ranging from 1 to 10 $\mu\text{mol/L}$ with the LOD of 0.58 $\mu\text{mol/L}$ Fe^{3+} . Confocal imaging disclosed that the probe possesses the ability of tracing cytosolic Fe^{3+} in SKOV-3 cells.

Spirolactam ring-opening Subsequently, we took advantage of the HEDTA ligand to develop another turn-on Fe^{3+} probe Mito-RhFe by appending the HEDTA receptor onto rhodamine B as a cationic, lipophilic tag to localize the probe to mitochondria (Zhu *et al.* 2018). In UV-Vis Fe^{3+} titration of this probe (20 mmol/L Tris-HCl/MeOH = 1:1, pH 7.2), the solution color changes from colorless to magenta, thereby offering Mito-RhFe as an Fe^{3+} -induced 'naked-eye' chemosensor. With this probe, the mitochondrial labile Fe^{3+} fluctuation in adherent HeLa cells ($PCC = 0.90$) upon ferric ammonium citrate (FAC) incubation was visualized via confocal imaging, and the flow cytometric assay for mitochondrial Fe^{3+} in suspension MEL cells, which is difficult to be monitored in an imaging manner, was also developed. Erythroid differentiation is normally accompanied by hemoglobin biosynthesis and upregulation of the mitochondrial iron importing protein mitoferrin-1 (MFRN1) containing ISC (Shaw *et al.* 2006). Interestingly, the labile Fe^{3+} drop in mitochondria of human K562 erythroleukemia cells undergoing the DMSO-stimulated erythroid differentiation was observed for the first time (Fig. 14).

In addition to Zn^{2+} and Cu^{2+} , Fe^{3+} can trigger the hydrolysis and spirolactam ring-opening process of rhodamine derivatives, which has provided a valuable method to design Fe^{3+} -selective luminescent sensors with a turn-on response. Lee *et al.* have presented six rhodamine-derived Schiff base probes' selectivity of the Fe^{3+} ion in biological systems (Lee *et al.* 2016). After

Fig. 13 Selected structures of Fe^{3+} probes. Turn-on sensors. **A** Chelation: L2 and L3. **B** Spirolactam ring-opening: L4, and ratiometric probe TPE-o-Py

Fig. 14 **A** Chemical structure of probe Mito-RhFe. **B** Photograph of Mito-RhFe solution before (left) and after (right) Fe^{3+} addition (20 mmol/L Tris-HCl/MeOH = 1:1, pH 7.2). **C** Colocalization of Mito-RhFe with MitoTracker deep red 633 in MCF-7 cells. The cells were stained firstly by 10 $\mu\text{mol/L}$ probe in PBS for 30 min at 37 °C. **D** Labile Fe^{3+} flow cytometric assay of K562 cells incubated in RPMI1640 medium containing 2% DMSO. **E** Western blot analysis of the cells in **D**. The cells were cultured at 37 °C for 4 days, and stained with Mito-RhFe at 0 °C for 30 min before assay. Cells cultured without DMSO were adopted as the control. Adapted from Zhu *et al.* (2018)

exposure to Fe^{3+} , the probes appeared a new absorption peak at 526 nm, with a concomitant emission maximum at 551 nm. Confocal microscopic analysis showcased that these probes underwent turn-on changes caused by cellular Fe^{3+} ions, and demonstrated a largely enhanced fluorescence upon iron overloading of the HepG2 cell line. They also found that the Fe^{3+} -mediated fluorescence augmentation was mainly localized in the ER. Among them, probe L5 exhibited the highest selectivity for ER localization ($PCC = 0.7854$) over other organelles.

Ratiometric sensors for Fe^{3+} Ratiometric probes are highly valued because their intrinsic internal standard

can correct potential variations in light intensity, dye localization and other experimental imaging conditions (Ackerman *et al.* 2017). The aforementioned two turn-on platforms can be adapted to quantify labile Fe^{3+} concentrations in a ratiometric readout, such as a bithiazole derivative (Geng *et al.* 2016), and a spirolactam analog RQBTE (Das *et al.* 2016).

It has been reported that ftn-1 (encoding the iron sequestering protein FTN) is upregulated in the presence of exogenous FAC supplementation, and down-regulated upon treatment with an iron chelator deferoxamine (DFO) in the nematode *Caenorhabditis* (*C.*) *elegans* (Valentini *et al.* 2012). Goel *et al.* created the first dual colorimetric and ratiometric fluorescent Fe^{3+}

probe NAP-3 (Goel *et al.* 2015), consisting of a naphtho[2,1-*b*]-[1,10]-phenanthroline (NAP). After exposure to increasing concentrations of Fe^{3+} ions (DMSO/ H_2O = 1:9, pH 7.4), a new peak with maximum at 605 nm emerged and the peak at 544 nm decreased in the emission spectrum, with a final enhancement factor (I_{605}/I_{544}) over 8.5-fold. It can approximately measure as low as 9.1 nmol/L Fe^{3+} . Possibly due to steric hindrance, Fe^{3+} chelator DFO failed to remove Fe^{3+} from the complex (NAP-3/ Fe^{3+}). Common ions do not imply any significant interference in the determination of Fe^{3+} ion. The indicator enabled direct visualization of exogenous Fe^{3+} in HepG2 cells, and endogenous Fe^{3+} in a multicellular organism, *ftn-1* silenced *C. elegans* (Fig. 15).

In fact, most conventional organic dyes exhibit aggregation-caused quenching (ACQ) when aggregated. Aggregation-induced emission (AIE) phenomena, originating from the restriction of intramolecular motions (RIM), is thus a unique turn-on approach for addressing the ACQ issue (Chen *et al.* 2018). AIE luminogens (AIEgens) tend to behave in a non-emissive manner when well dissolved in some solvents, but emit intense fluorescence when poorly dissolved by means of forming aggregates (Mei *et al.* 2015). AIEgens have emerged as a powerful methodology for the assay of transition metal ions such as Zn^{2+} (Jung *et al.* 2015), Cu^{2+} (Feng *et al.* 2014) and Hg^{2+} (Chen *et al.* 2016).

By making use of the position-dependent substituent effects, an AIE featured Fe^{3+} probe (TPE-o-Py) from ortho-substituted pyridinyl-functionalized tetraphenylethylene is synthesized by Tang's research group (Feng *et al.* 2018). From weak blue to strong red

emission (peak 472 nm to 573 nm) under 365 nm UV irradiation, TPE-o-Py displays high metal selectivity and oxidation-state specificity ($\text{THF}/\text{H}_2\text{O}$ = 3:7) arising from the position isomer of ortho-substitution. And it has a low acid dissociation constant ($\text{pK}_a \sim 3.27$) that is close to that of hydrolyzed Fe^{3+} . More importantly, the light-up fluorescent probe TPE-o-Py can be applied to sensing Fe^{3+} in HeLa cells with a pronounced red shift in fluorescence emission.

It should be noted that the reduction potential in the cytosol favors Fe^{2+} over Fe^{3+} (Aron *et al.* 2018), and Fe^{3+} is poorly soluble at neutral pH in aqueous media. On the other hand, the vast majority of Fe^{3+} probes have not been well suited for endogenous investigations due to a lack of high sensitivity. In this context, exploring improved motifs to decode Fe^{3+} roles in biology is of tremendous significance.

SUMMARY AND PERSPECTIVES

In this review, we summarized endeavors made in molecular imaging for transition metal cations. Specifically, we highlighted synthetic sensors that are utilized to image zinc, copper and iron in biological systems (Table 2). Great challenges still remain in the field to be confronted with:

- (A) Expanding metal probes bioavailable. It is the quality of probes, including solubility, sensitivity and selectivity (3S), not the quantity that matters. Deliberate design of metal sensors with improved bioavailability is of paramount importance. In addition, other transition metals like manganese

Fig. 15 **A** Ratiometric fluorescence spectra (DMSO/ H_2O = 1:9, pH 7.4) of 25 $\mu\text{mol/L}$ NAP-3 (λ_{ex} = 365 nm) after 1 h upon addition of increasing concentrations of Fe^{3+} (0–0.68 mmol/L). Inset in **A**: chemical structure of NAP-3. **B** Wild-type and *ftn-1* silenced *C. elegans* treated with NAP-3 (3 $\mu\text{mol/L}$, 24 h). Green channel images obtained with a bandpass of 505–550 nm upon excitation at 405 nm, red channel images obtained with 575 nm long pass upon excitation at 561 nm, and the corresponding ratiometric images ($F_{\text{red}}/F_{\text{green}}$). Adapted from Goel *et al.* (2015)

Table 2 Summary of selected probes for metal detection

Probe	Modality	Readout	Cells	Biological highlights
DA-ZP1-TPP	FLI	F ₅₂₉	HeLa, WPE-1, RWPE-2, PC-3	Mobile Zn ²⁺ in mitochondria of healthy vs cancerous prostate cells
LysoDPP-C4	FLI	F ₅₁₅	HeLa, PC-3, DU145	Cancerous prostate nodules via Zn ²⁺ -induced fluorescence signaling
CNSB	TPI	F ₄₇₀ / F ₅₆₅	MCF-7	Fluctuation and distribution of Cu ⁺ under ER stress in MCF-7 cells
CCL-1	BLI	n.d	PC3M-luc	Cu ⁺ deficiency in a murine model of non-alcoholic fatty liver disease
RPS1	PAI	PA ₇₁₀	bEnd.3	Cross the blood-brain barrier for Cu ²⁺ visualization in mice with Alzheimer's disease
NRh	UCL	UCL ₇₃₀	HeLa	<i>In vivo</i> detection of Cu ²⁺ in Wilson disease
LCy7	FLI	F ₆₉₀	HL-7702	Labile Fe ²⁺ in ER stress-mediated drug-induced liver injury
ICL-1	BLI	n.d	PC3M-luc	Labile Fe ²⁺ accumulation in a murine model of <i>A. baumannii</i> infection
Mito-RhFe	FLI	F ₅₈₀	MCF-7	Labile Fe ³⁺ drop in mitochondria of K562 cells undergoing the DMSO-stimulated erythroid differentiation
NAP-3	FLI	F ₆₀₅ / F ₅₄₄	HepG2	Endogenous labile Fe ³⁺ pools in ftn-1 silenced <i>C. elegans</i>

*n.d.: not determined. TPI and UCL belong to FLI

(Mn) also take active part in health and disease (Avila *et al.* 2013). Yet Mn sensors are still on an early stage, and there have been far fewer small-molecule probes prepared for Mn²⁺ detection (Bakthavatsalam *et al.* 2015; Liang and Canary 2010).

- (B) Combining super-resolution techniques. Super-resolution techniques, such as stimulated emission depletion (STED), structured illumination microscopy (SIM) and stochastic optical reconstruction microscopy (STORM), provide a valuable starting point to study analytes in nano-dimension (Kozma and Kele 2018). Application of this technique to molecular probes for mapping metals is an important and arduous task.
- (C) Designing dual-responsive probes. Using two selective probes simultaneously to monitor multiple species is troublesome, and it is therefore preferable to pursue dual-responsive single probes, operating as a logic gate in response to two elements (Kolanowski *et al.* 2018). In this case, interrelations between multiple elements in biology can be deciphered. For instance, if a probe is able to synchronously measure Cu⁺ and GSH without any interference from other analytes, more straightforward data would be offered with less work in NAFLD mentioned above.
- (D) Mining multi-mode imaging. Given that diverse imaging modes can be complementary, our laboratory recently reported a FLI/PAI dual-modality probe HS-CyBz (Chen *et al.* 2019a, b), realizing ratiometric in/ex vivo tracking for stimulated H₂S

fluctuations in mice. Integrating several imaging modes into a single metal probe should be appropriately achieved in the longer term.

To sum up, roles for labile metals in cells and tissues have been elucidated to a certain extent using these molecular probes. Continued innovations in exploiting chemical reagents, in cooperation with physicists and biologists, presage further opportunities for uncovering how metal cations work in biology.

Acknowledgements The work was under financial supports from the National Natural Science Foundation of China (21977044, 21907050, 21731004, 91953201), the Natural Science Foundation of Jiangsu Province (BK20190282) and the Excellent Research Program of Nanjing University (ZYJH004). We thank Dr. Chengcheng Zhu, Dr. Lin Qiu and Ms. Jungu Guo for offering the original images shown in abstract. We also thank sincere friends Miss Xiaotong Li (NJUST) and Mr. Dong Han (USTC) for polishing up the writing.

Compliance with Ethical Standards

Conflict of interest Jing Gao, Yuncong Chen, Zijian Guo and Weijiang He declare that they have no conflict of interest.

Human and animals rights and informed consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit

line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Ackerman CM, Lee S, Chang CJ (2017) Analytical methods for imaging metals in biology: from transition metal metabolism to transition metal signaling. *Anal Chem* 89(1):22–41
- Andersson M, Mattle D, Sitsel O, Klymchuk T, Nielsen AM, Møller LB, White SH, Nissen P, Gourdon P (2014) Copper-transporting P-type ATPases use a unique ion-release pathway. *Nat Struct Mol Biol* 21(1):43–48
- Andrews NC (2000) Iron metabolism: iron deficiency and iron overload. *Annu Rev Genomics Hum Genet* 1(1):75–98
- Aron A, Heffern M, Lonergan Z, Wal M, Blank B, Spangler B, Zhang Y, Park HM, Stahl A, Renslo A, Skaar E, Chang C (2017) *In vivo* bioluminescence imaging of labile iron accumulation in a murine model of *Acinetobacter baumannii* infection. *Proceedings of the National Academy of Sciences USA* 114:201708747
- Aron AT, Loefer MO, Bogena J, Chang CJ (2016) An endoperoxide reactivity-based FRET probe for ratiometric fluorescence imaging of labile iron pools in living cells. *J Am Chem Soc* 138(43):14338–14346
- Aron AT, Ramos-Torres KM, Cotruvo JA, Chang CJ (2015) Recognition- and reactivity-based fluorescent probes for studying transition metal signaling in living systems. *Acc Chem Res* 48(8):2434–2442
- Aron AT, Reeves AG, Chang CJ (2018) Activity-based sensing fluorescent probes for iron in biological systems. *Curr Opin Chem Biol* 43:113–118
- Au-Yeung HY, Chan J, Chantarojsiri T, Chang CJ (2013) Molecular imaging of labile iron(II) pools in living cells with a turn-on fluorescent probe. *J Am Chem Soc* 135(40):15165–15173
- Avila DS, Puntel RL, Aschner M (2013) Manganese in health and disease. In: Sigel H, Sigel RKO, et al. (eds) *Sigel A. Book. Manganese in health and disease*. Dordrecht, Springer, Netherlands, pp 199–227
- Baker M (2010) The whole picture. *Nature* 463(7283):977–979
- Bakthavatsalam S, Sarkar A, Rakshit A, Jain S, Kumar A, Datta A (2015) Tuning macrocycles to design 'turn-on' fluorescence probes for manganese(II) sensing in live cells. *Chem Commun* 51(13):2605–2608
- Ballesteros E, Moreno D, Gómez T, Rodríguez T, Rojo J, García-Valverde M, Torroba T (2009) A new selective chromogenic and turn-on fluorogenic probe for copper(II) in water–acetonitrile 1:1 solution. *Org Lett* 11(6):1269–1272
- Bandmann O, Weiss KH, Kaler SG (2015) Wilson's disease and other neurological copper disorders. *The Lancet Neurology* 14(1):103–113
- Bansagi B, Lewis-Smith D, Pal E, Duff J, Griffin H, Pyle A, Müller JS, Rudas G, Aranyi Z, Lochmüller H, Chinnery PF, Horvath R (2016) Phenotypic convergence of Menkes and Wilson disease. *Neurology Genetics* 2(6):e119
- Bertinato J, Iskandar M, Labbe MR (2003) Copper deficiency induces the upregulation of the copper chaperone for Cu/Zn superoxide dismutase in weanling male rats. *J Nutr* 133(1):28–31
- Bleackley MR, MacGillivray RTA (2011) Transition metal homeostasis: from yeast to human disease. *Biomaterials* 24(5):785–809
- Brady DC, Crowe MS, Turski ML, Hobbs GA, Yao X, Chaikuad A, Knapp S, Xiao K, Campbell SL, Thiele DJ, Counter CM (2014) Copper is required for oncogenic BRAF signalling and tumorigenesis. *Nature* 509(7501):492–496
- Braymer JJ, Lill R (2017) Iron-sulfur cluster biogenesis and trafficking in mitochondria. *The Journal of biological chemistry* 292(31):12754–12763
- Burd C, Cullen PJ (2014) Retromer: a master conductor of endosome sorting. *Cold Spring Harbor Perspectives in Biology* 6(2):a016774
- Cao X, Lin W, Wan W (2012) Development of a near-infrared fluorescent probe for imaging of endogenous Cu⁺ in live cells. *Chem Commun* 48(50):6247–6249
- Carter KP, Young AM, Palmer AE (2014) Fluorescent sensors for measuring metal ions in living systems. *Chem Rev* 114(8):4564–4601
- Chang CJ (2015) Searching for harmony in transition-metal signaling. *Nat Chem Biol* 11(10):744–747
- Chang CJ, James TD, New EJ, Tang BZ (2020) Activity-based sensing: achieving chemical selectivity through chemical reactivity. *Acc Chem Res* 53(1):1–1
- Chen Y, Bai Y, Han Z, He W, Guo Z (2015) Photoluminescence imaging of Zn²⁺ in living systems. *Chem Soc Rev* 44(14):4517–4546
- Chen Y, Lam J, Kwok R, Liu B, Tang B (2019a) Aggregation-induced emission: fundamental understanding and future developments. *Materials Horizons* 6:428–433
- Chen Y, Zhang W, Cai Y, Kwok RTK, Tang BZ (2016) AIEgens for dark through-bond energy transfer: design, synthesis, theoretical study and application in ratiometric Hg(2+) sensing. *Chemical science* 8(3):2047
- Chen Y, Zhu C, Cen J, Li J, He W, Jiao Y, Guo Z (2013) A reversible ratiometric sensor for intracellular Cu²⁺ imaging: metal coordination-altered FRET in a dual fluorophore hybrid. *Chem Commun* 49(69):7632–7634
- Chen Z, Mu X, Han Z, Yang S, Zhang C, Guo Z, Bai Y, He W (2019b) An optical/photoacoustic dual-modality probe: ratiometric *in/ex vivo* imaging for stimulated H2S upregulation in mice. *J Am Chem Soc* 141(45):17973–17977
- Chung CY-S, Posimo JM, Lee S, Tsang T, Davis JM, Brady DC, Chang CJ (2019) Activity-based ratiometric FRET probe reveals oncogene-driven changes in labile copper pools induced by altered glutathione metabolism. *Proceedings of the National Academy of Sciences USA* 116(37):18285–18294
- Chyan W, Zhang D, Lippard S, Radford R (2014) Reaction-based fluorescent sensor for investigating mobile Zn²⁺ in mitochondria of healthy versus cancerous prostate cells. *Proc Natl Acad Sci USA* 111(1):143–148
- Conrad M, Angeli JPF, Vandenabeele P, Stockwell BR (2016) Regulated necrosis: disease relevance and therapeutic opportunities. *Nat Rev Drug Discovery* 15(5):348–366
- Costello LC, Feng P, Milon B, Tan M, Franklin RB (2004) Role of zinc in the pathogenesis and treatment of prostate cancer: critical issues to resolve. *Prostate Cancer and Prostatic Diseases* 7(2):111–117
- Cotruvo JJA, Aron AT, Ramos-Torres KM, Chang CJ (2015) Synthetic fluorescent probes for studying copper in biological systems. *Chem Soc Rev* 44(13):4400–4414
- Das S, Aich K, Goswami S, Quah CK, Fun H-K (2016) FRET-based fluorescence ratiometric and colorimetric sensor to discriminate Fe³⁺ from Fe²⁺. *New J Chem* 40(7):6414–6420
- Dixon SJ, Stockwell BR (2014) The role of iron and reactive oxygen species in cell death. *Nat Chem Biol* 10(1):9–17

- Dodani SC, Firl A, Chan J, Nam CI, Aron AT, Onak CS, Ramos-Torres KM, Paek J, Webster CM, Feller MB, Chang CJ (2014) Copper is an endogenous modulator of neural circuit spontaneous activity. *Proceedings of the National Academy of Sciences USA* 111(46):16280–16285
- Dong Z, Han Q, Mou Z, Li G, Liu W (2018) A reversible frequency upconversion probe for real-time intracellular lysosome-pH detection and subcellular imaging. *Journal of Materials Chemistry B* 6(9):1322–1327
- Du C et al (2019) Diketopyrrolopyrrole-based fluorescence probes for the imaging of lysosomal Zn²⁺ and identification of prostate cancer in human tissue. *Chem Sci* 10:5699–5704
- Eide DJ (2004) The SLC39 family of metal ion transporters. *Pflügers Archiv* 447(5):796–800
- Fahrni CJ (2013) Synthetic fluorescent probes for monovalent copper. *Curr Opin Chem Biol* 17(4):656–662
- Feng H-T, Song S, Chen Y-C, Shen C-H, Zheng Y-S (2014) Self-assembled tetraphenylethylene macrocycle nanofibrous materials for the visual detection of copper(II) in water. *Journal of Materials Chemistry C* 2(13):2353–2359
- Feng X, Li Y, He X, Liu H, Zhao Z, Kwok RTK, Elsegood MRJ, Lam JYW, Tang BZ (2018) A substitution-dependent light-up fluorescence probe for selectively detecting Fe³⁺ ions and its cell imaging application. *Adv Func Mater* 28(35):1802833
- Festa RA, Thiele DJ (2011) Copper: an essential metal in biology. *Curr Biol* 21(21):R877–R883
- Friedmann Angeli JP, Krysko DV, Conrad M (2019) Ferroptosis at the crossroads of cancer-acquired drug resistance and immune evasion. *Nat Rev Cancer* 19(7):405–414
- Geng J, Liu Y, Li J, Yin G, Huang W, Wang R, Quan Y (2016) A ratiometric fluorescent probe for ferric ion based on a 2,2'-bithiazole derivative and its biological applications. *Sensors and Actuators B: Chemical* 222:612–617
- Goel A, Umar S, Nag P, Sharma A, Kumar L, Shamsuzzama, Hossain Z, Gayen JR, Nazir A (2015) A dual colorimetric-ratiometric fluorescent probe NAP-3 for selective detection and imaging of endogenous labile iron(III) pools in *C. elegans*. *Chemical Communications* 51(24):5001–5004
- Guo J, Yuan H, Chen Y, Chen Z, Zhao M, Zou L, Liu Y, Liu Z, Zhao Q, Guo Z, He W (2019) A ratiometric fluorescent sensor for tracking Cu(I) fluctuation in endoplasmic reticulum. *Science China Chemistry* 62:465–474
- Guo Z, Park S, Yoon J, Shin I (2014) Recent progress in the development of near-infrared fluorescent probes for bioimaging applications. *Chem Soc Rev* 43(1):16–29
- Hassannia B, Vandenabeele P, Vanden Berghe T (2019) Targeting ferroptosis to iron out cancer. *Cancer Cell* 35(6):830–849
- Heffern MC, Park HM, Au-Yeung HY, Van de Bittner GC, Ackerman CM, Stahl A, Chang CJ (2016) *In vivo* bioluminescence imaging reveals copper deficiency in a murine model of nonalcoholic fatty liver disease. *Proceedings of the National Academy of Sciences USA* 113(50):14219–14224
- Hirayama T (2019) Fluorescent probes for the detection of catalytic Fe(II) ion. *Free Radical Biol Med* 133:38–45
- Hirayama T, Inden M, Tsuboi H, Niwa M, Uchida Y, Naka Y, Hozumi I, Nagasawa H (2019a) A Golgi-targeting fluorescent probe for labile Fe(II) to reveal an abnormal cellular iron distribution induced by dysfunction of VPS35. *Chemical science* 10(5):1514–1521
- Hirayama T, Miki A, Nagasawa H (2019b) Organelle-specific analysis of labile Fe(II) during ferroptosis by using a cocktail of various colour organelle-targeted fluorescent probes. *Metallomics* 11(1):111–117
- Hirayama T, Okuda K, Nagasawa H (2013) A highly selective turn-on fluorescent probe for iron(II) to visualize labile iron in living cells. *Chemical science* 4(3):1250–1256
- Hirayama T, Tsuboi H, Niwa M, Miki A, Kadota S, Ikeshita Y, Okuda K, Nagasawa H (2017) 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. *Chemical science* 8(7):4858–4866
- Jin X, Wu X, Wang B, Xie P, He Y, Zhou H, Yan B, Yang J, Chen W, Zhang X (2018) A reversible fluorescent probe for Zn²⁺ and ATP in living cells and *in vivo*. *Sensors and Actuators B: Chemical* 261:127–134
- Jung SH, Kwon K-Y, Jung JH (2015) A turn-on fluorogenic Zn(II) chemoprobe based on a terpyridine derivative with aggregation-induced emission (AIE) effects through nanofiber aggregation into spherical aggregates. *Chem Commun* 51(5):952–955
- Kelleher SL, McCormick NH, Velasquez V, Lopez V (2011) Zinc in specialized secretory tissues: roles in the pancreas, prostate, and mammary gland. *Advances in Nutrition* 2(2):101–111
- Kim D, Ryu HG, Ahn KH (2014) Recent development of two-photon fluorescent probes for bioimaging. *Org Biomol Chem* 12(26):4550–4566
- Knox HJ, Chan J (2018) Acoustogenic probes: a new frontier in photoacoustic imaging. *Acc Chem Res* 51(11):2897–2905
- Kolanowski JL, Liu F, New EJ (2018) Fluorescent probes for the simultaneous detection of multiple analytes in biology. *Chem Soc Rev* 47(1):195–208
- Kolenko V, Teper E, Kutikov A, Uzzo R (2013) Zinc and zinc transporters in prostate carcinogenesis. *Nature Reviews Urology* 10(4):219–226
- Kozma E, Kele P (2018) Fluorogenic probes for super-resolution microscopy. *Org Biomol Chem* 17:215–233
- Lazarczyk M, Favre M (2008) Role of Zn²⁺ ions in host-virus interactions. *J Virol* 82:11486–11494
- Lee MH, Lee H, Chang MJ, Kim HS, Kang C, Kim JS (2016) A fluorescent probe for the Fe³⁺ ion pool in endoplasmic reticulum in liver cells. *Dyes Pigm* 130:245–250
- Li H, Zhang P, Smaga LP, Hoffman RA, Chan J (2015) Photoacoustic probes for ratiometric imaging of copper(II). *J Am Chem Soc* 137(50):15628–15631
- Li J, Chen L, Du L, Li M (2013) Cage the firefly luciferin! – a strategy for developing bioluminescent probes. *Chem Soc Rev* 42(2):662–676
- Liang J, Canary JW (2010) Discrimination between hard metals with soft ligand donor atoms: an on-fluorescence probe for manganese(II). *Angew Chem Int Ed* 49(42):7710–7713
- Lim B, Baek B, Jang K, Lee NK, Lee JH, Lee Y, Kim J, Kang SW, Park J, Kim S, Kang N-W, Hong S, Kim D-D, Kim I, Hwang H, Lee J (2019) Novel turn-on fluorescent biosensors for selective detection of cellular Fe³⁺ in lysosomes: thiophene as a selectivity-tuning handle for Fe³⁺ sensors. *Dyes Pigm* 169:51–59
- Liu Y, Su Q, Chen M, Dong Y, Shi Y, Feng W, Wu Z-Y, Li F (2016) Near-infrared upconversion chemodosimeter for *in vivo* detection of Cu(2+) in Wilson disease. *Advanced materials (Deerfield Beach, Fla.)* 28(31):6625–6630
- Liu Z, He W, Guo Z (2013) Metal coordination in photoluminescent sensing. *Chem Soc Rev* 42(4):1568–1600
- Liu Z, Zhang C, Chen Y, Qian F, Bai Y, He W, Guo Z (2014) *In vivo* ratiometric Zn²⁺ imaging in zebrafish larvae using a new visible light excitable fluorescent sensor. *Chem Commun* 50(10):1253–1255
- Loas A, Radford RJ, Lippard SJ (2014) Addition of a second binding site increases the dynamic range but alters the cellular localization of a red fluorescent probe for mobile zinc. *Inorg Chem* 53(13):6491–6493

- Long L, Wang N, Han Y, Huang M, Yuan X, Cao S, Gong A, Wang K (2018) A coumarin-based fluorescent probe for monitoring labile ferrous iron in living systems. *Analyst* 143(11):2555–2562
- Maiti S, Aydin Z, Zhang Y, Guo M (2015) Reaction-based turn-on fluorescent probes with magnetic responses for Fe²⁺ detection in live cells. *Dalton Trans* 44(19):8942–8949
- Mei J, Leung N, Kwok R, Jacky W, Tang B (2015) Aggregation-induced emission: together we shine, united we soar! *Chem Rev* 115(21):11718–11940
- Müller UC, Deller T, Korte M (2017) Not just amyloid: physiological functions of the amyloid precursor protein family. *Nat Rev Neurosci* 18(5):281–298
- Nishito Y, Kambe T (2018) Absorption mechanisms of iron, copper, and zinc: an overview. *J Nutr Sci Vitaminol* 64(1):1–7
- Niwa M, Hirayama T, Okuda K, Nagasawa H (2014) 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* 12(34):6590–6597
- Niwa M, Hirayama T, Oomoto I, Wang DO, Nagasawa H (2018) Fe(II) ion release during endocytotic uptake of iron visualized by a membrane-anchoring Fe(II) fluorescent probe. *ACS Chem Biol* 13(7):1853–1861
- Palmiter RD, Huang L (2004) Efflux and compartmentalization of zinc by members of the SLC30 family of solute carriers. *Pflügers Archiv* 447(5):744–751
- Paredes E, Das SR (2011) Click chemistry for rapid labeling and ligation of RNA. *ChemBioChem* 12(1):125–131
- Park S, Kwon N, Lee J, Yoon J, Shin I (2020) Synthetic ratiometric fluorescent probes for detection of ions. *Chem Soc Rev* 49(1):143–179
- Peter V, Kyong S, Jong Seung K (2015) The role of copper ions in pathophysiology and fluorescent sensors for the detection thereof. *Chem Commun* 46(21):5556–5571
- Prohaska JR, Broderius M, Brokate B (2003) Metallochaperone for Cu, Zn-superoxide dismutase (CCS) protein but not mRNA is higher in organs from copper-deficient mice and rats. *Arch Biochem Biophys* 417(2):227–234
- Qian F, Zhang C, Zhang Y, He W, Gao X, Hu P, Guo Z (2009) Visible light excitable Zn²⁺ Fluorescent sensor derived from an intramolecular charge transfer fluorophore and its *in vitro* and *in vivo* application. *J Am Chem Soc* 131(4):1460–1468
- Qiu L, Zhu C, Chen H, Hu M, He W, Guo Z (2014) A turn-on fluorescent Fe³⁺ sensor derived from an anthracene-bearing bisdiene macrocycle and its intracellular imaging application. *Chem Commun* 50(35):4631–4634
- Reinhardt CJ, Chan J (2018) Development of photoacoustic probes for *in vivo* molecular imaging. *Biochemistry* 57(2):194–199
- Sahoo SK, Sharma D, Bera RK, Crisponi G, Callan JF (2012) Iron(III) selective molecular and supramolecular fluorescent probes. *Chem Soc Rev* 41(21):7195–7227
- Shaw GC, Cope JJ, Li L, Corson K, Hersey C, Ackermann GE, Gwynn B, Lambert AJ, Wingert RA, Traver D, Trede NS, Barut BA, Zhou Y, Minet E, Donovan A, Brownlie A, Balzan R, Weiss MJ, Peters LL, Kaplan J, Zon LI, Paw BH (2006) Mitoferrin is essential for erythroid iron assimilation. *Nature* 440(7080):96–100
- Shi Y, Wang R, Yuan W, Liu Q, Shi M, Feng W, Wu Z, Hu K, Li F (2018) Easy-to-use colorimetric cyanine probe for the detection of Cu²⁺ in Wilson's disease. *ACS Appl Mater Interfaces* 10(24):20377–20386
- Shi Z, Tang X, Zhou X, Cheng J, Han Q, Zhou J-A, Wang B, Yang Y, Liu W, Bai D (2013) A Highly selective fluorescence “turn-on” probe for Cu(II) based on reaction and its imaging in living cells. *Inorg Chem* 52(21):12668–12673
- Singh H, Lee HW, Heo CH, Byun JW, Sarkar AR, Kim HM (2015) A Golgi-localized two-photon probe for imaging zinc ions. *Chem Commun* 51(60):12099–12102
- Spangler B, Morgan CW, Fontaine SD, Vander Wal MN, Chang CJ, Wells JA, Renslo AR (2016) A reactivity-based probe of the intracellular labile ferrous iron pool. *Nat Chem Biol* 12(9):680–685
- Sui B, Tang S, Liu T, Kim B, Belfield KD (2014) Novel BODIPY-based fluorescence turn-on sensor for Fe³⁺ and its bioimaging application in living cells. *ACS Appl Mater Interfaces* 6(21):18408–18412
- Szewczyk B (2013) Zinc homeostasis and neurodegenerative disorders. *Frontiers in Aging Neuroscience* 5(33)
- Tabuchi M, Yanatori I, Kawai Y, Kishi F (2010) Retromer-mediated direct sorting is required for proper endosomal recycling of the mammalian iron transporter DMT1. *J Cell Sci* 123:756–766
- Theil EC, Goss DJ (2009) Living with iron (and oxygen): questions and answers about iron homeostasis. *Chem Rev* 109(10):4568–4579
- Trusso Sfrassetto G, Satriano C, Tomaselli GA, Rizzarelli E (2016) Synthetic fluorescent probes to map metallostasis and intracellular fate of zinc and copper. *Coord Chem Rev* 311:125–167
- Valentini S, Cabreiro F, Ackerman D, Alam M, Kunze M, Kay C, Gems D (2012) Manipulation of *in vivo* iron levels can alter resistance to oxidative stress without affecting ageing in the nematode *C. elegans*. *mechanisms of ageing and development* 133: 282–290
- Valko M, Jomova K, Rhodes CJ, Kuča K, Musílek K (2016) Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. *Arch Toxicol* 90(1):1–37
- Wang J, Luo C, Shan C, You Q, Lu J, Elf S, Zhou Y, Wen Y, Vinkenberg JL, Fan J, Kang H, Lin R, Han D, Xie Y, Karpus J, Chen S, Ouyang S, Luan C, Zhang N, Ding H, Merckx M, Liu H, Chen J, Jiang H, He C (2015) Inhibition of human copper trafficking by a small molecule significantly attenuates cancer cell proliferation. *Nature Chemistry* 7(12):968–979
- Wang S, Sheng Z, Yang Z, Hu D, Long X, Feng G, Liu Y, Yuan Z, Zhang J, Zheng H, Zhang X (2019a) Activatable small-molecule photoacoustic probes that cross the blood-brain barrier for visualization of copper(II) in mice with Alzheimer's disease. *Angewandte Chemie (International ed. in English)* 58(36):12415–12419
- Wang X, Chen F, Zhang J, Sun J, Zhao X, Zhu Y, Wei W, Zhao J, Guo Z (2019) A ferroptosis-inducing iridium(III) complex. *Science China Chemistry*. <https://doi.org/10.1007/s11426-019-9577-3>
- Wegner SV, Sun F, Hernandez N, He C (2011) The tightly regulated copper window in yeast. *Chem Commun* 47(9):2571–2573
- Wu L, Ding Q, Wang X, Li P, Fan N, Zhou Y, Tong L, Zhang W, Zhang W, Tang B (2020) Visualization of dynamic changes in labile iron(II) pools in endoplasmic reticulum stress-mediated drug-induced liver injury. *Anal Chem* 92(1):1245–1251
- Xue X, Fang H, Chen H, Zhang C, Zhu C, Bai Y, He W, Guo Z (2016) *In vivo* fluorescence imaging for Cu²⁺ in live mice by a new NIR fluorescent sensor. *Dyes and Pigments* 130(116–121)
- Yang H, Han C, Zhu X, Liu Y, Zhang KY, Liu S, Zhao Q, Li F, Huang W (2016) Upconversion luminescent chemodosimeter based on NIR organic dye for monitoring methylmercury *in vivo*. *Adv Func Mater* 26(12):1945–1953
- Yang L, McRae R, Henary MM, Patel R, Lai B, Vogt S, Fahrni CJ (2005) Imaging of the intracellular topography of copper with a fluorescent sensor and by synchrotron X-ray fluorescence microscopy. *Proc Natl Acad Sci USA* 102(32):11179–11184

- Yang Y, Zhao Q, Feng W, Li F (2013) Luminescent chemodosimeters for bioimaging. *Chem Rev* 113(1):192–270
- Yao Z, Zhang BS, Prescher JA (2018) Advances in bioluminescence imaging: new probes from old recipes. *Curr Opin Chem Biol* 45:148–156
- Zeng L, Miller EW, Pralle A, Isacoff EY, Chang CJ (2006) A selective turn-on fluorescent sensor for imaging copper in living cells. *J Am Chem Soc* 128(1):10–11
- Zhang C, Liu M, Liu S, Yang H, Zhao Q, Liu Z, He W (2018) Phosphorescence lifetime imaging of labile Zn^{2+} in mitochondria via a phosphorescent iridium(III) complex. *Inorg Chem* 57(17):10625–10632
- Zhang C, Liu Z, Li Y, He W, Gao X, Guo Z (2013) In vitro and *in vivo* imaging application of a 1,8-naphthalimide-derived Zn^{2+} fluorescent sensor with nuclear envelope penetrability. *Chem Commun* 49(97):11430–11432
- Zhang S, Chen T-H, Lee H-M, Bi J, Ghosh A, Fang M, Qian Z, Xie F, Ainsley J, Christov C, Luo F-T, Zhao F, Liu H (2017) Luminescent probes for sensitive detection of pH changes in live cells through two near-infrared luminescence channels. *ACS Sensors* 2(7):924–931
- Zhang X, Chen Y, Cai X, Liu C, Jia P, Li Z, Zhu H, Yu Y, Wang K, Li X, Sheng W, Zhu B (2020) A highly sensitive rapid-response fluorescent probe for specifically tracking endogenous labile Fe^{2+} in living cells and zebrafish. *Dyes Pigm* 174:108065
- Zheng D, Feeney G, Kille P, Hogstrand C (2008) Regulation of ZIP and ZnT zinc transporters in zebrafish gill: zinc repression of ZIP10 transcription by an intronic MRE cluster. *Physiol Genomics* 34:205–214
- Zhou B, Zhang J-Y, Liu X-S, Chen H-Z, Ai Y-L, Cheng K, Sun R-Y, Zhou D, Han J, Wu Q (2018) Tom20 senses iron-activated ROS signaling to promote melanoma cell pyroptosis. *Cell Res* 28(12):1171–1185
- Zhou L, Zhang X, Wang Q, Lv Y, Mao G, Luo A, Wu Y, Wu Y, Zhang J, Tan W (2014) Molecular engineering of a tbt-based two-photon fluorescent probe for ratiometric imaging of living cells and tissues. *J Am Chem Soc* 136(28):9838–9841
- Zhu C, Wang M, Qiu L, Hao S, Li K, Guo Z, He W (2018) A mitochondria-targeting fluorescent Fe^{3+} probe and its application in labile Fe^{3+} monitoring via imaging and flow cytometry. *Dyes Pigm* 157:328–333
- Zhu H, Fan J, Du J, Peng X (2016) Fluorescent probes for sensing and imaging within specific cellular organelles. *Acc Chem Res* 49(10):2115–2126