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Recognition- and Reactivity-Based Fluorescent Probes for Studying Transition Metal Signaling in Living Systems

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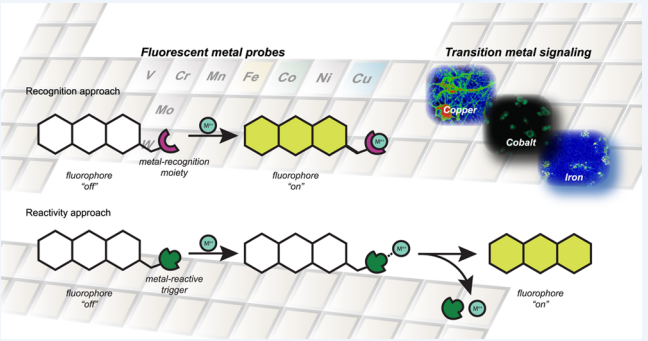
CONSPECTUS: Metals are essential for life, playing critical roles in all aspects of the central dogma of biology (e.g., the transcription and translation of nucleic acids and synthesis of proteins). Redox-inactive alkali, alkaline earth, and transition metals such as sodium, potassium, calcium, and zinc are widely recognized as dynamic signals, whereas redox-active transition metals such as copper and iron are traditionally thought of as sequestered by protein ligands, including as static enzyme cofactors, in part because of their potential to trigger oxidative stress and damage via Fenton chemistry. Metals in biology can be broadly categorized into two pools: static and labile. In the former, proteins and other macromolecules tightly bind metals; in the latter, metals are bound relatively weakly to cellular ligands, including proteins and low molecular weight ligands. Fluorescent probes can be useful tools for studying the roles of transition metals in their labile forms. Probes for imaging transition metal dynamics in living systems must meet several stringent criteria. In addition to exhibiting desirable photophysical properties and biocompatibility, they must be selective and show a fluorescence turn-on response to the metal of interest. To meet this challenge, we have pursued two general strategies for metal detection, termed "recognition" and "reactivity". Our design of transition metal probes makes use of a recognition-based approach for copper and nickel and a reactivity-based approach for cobalt and iron. This Account summarizes progress in our laboratory on both the development and application of fluorescent probes to identify and study the signaling roles of transition metals in biology. In conjunction with complementary methods for direct metal detection and genetic and/or pharmacological manipulations, fluorescent probes for transition metals have helped reveal a number of principles underlying transition metal dynamics. In this Account, we give three recent examples from our laboratory and collaborations in which applications of chemical probes reveal that labile copper contributes to various physiologies. The first example shows that copper is an endogenous regulator of neuronal activity, the second illustrates cellular prioritization of mitochondrial copper homeostasis, and the third identifies the "cuprosome" as a new copper storage compartment in *Chlamydomonas reinhardtii* green algae. Indeed, recognition- and reactivity-based fluorescent probes have helped to uncover new biological roles for labile transition metals, and the further development of fluorescent probes, including ones with varied K_d values and new reaction triggers and recognition receptors, will continue to reveal exciting and new biological roles for labile transition metals.



1. INTRODUCTION

Metals are necessary for sustaining all life, playing essential roles in all aspects of the central dogma of biology (e.g., DNA to RNA to proteins), as exemplified by the requirement of metal cofactors for function of all nucleic acids and an estimated one-third to one-half of all proteins, including DNA and RNA polymerases.[1] The biological chemistry of metals is remarkably versatile; metal ions provide permanent and transient structural reinforcement, mediate electron transfer, transport small-molecules, and act as Lewis-acid and redox catalysts.[1] In most cases, a specific metal is required for a particular role, so it is critical that the proper level of that metal be present in the right place at the right time. This process of metal homeostasis is inherently dynamic because metals, unlike organic products, can neither be created nor destroyed under ambient biological conditions (Figure 1).

Accordingly, rapid changes in concentration gradients of metal ions can be used to mediate signaling processes; redox-inactive alkali, alkaline earth, and transition metals—especially calcium, sodium, potassium, and zinc—have been extensively studied in this regard. In contrast, the ability of redox-active transition metals like copper and iron to act as signals has been relatively understudied. As these metals can aberrantly produce reactive oxygen species (ROS),[2] most research has considered redox-active transition metals as cofactors sequestered within enzyme active sites, despite the intricate cellular machinery regulating their concentration in labile forms. We have initiated a program to better understand the roles of transition metals in their labile

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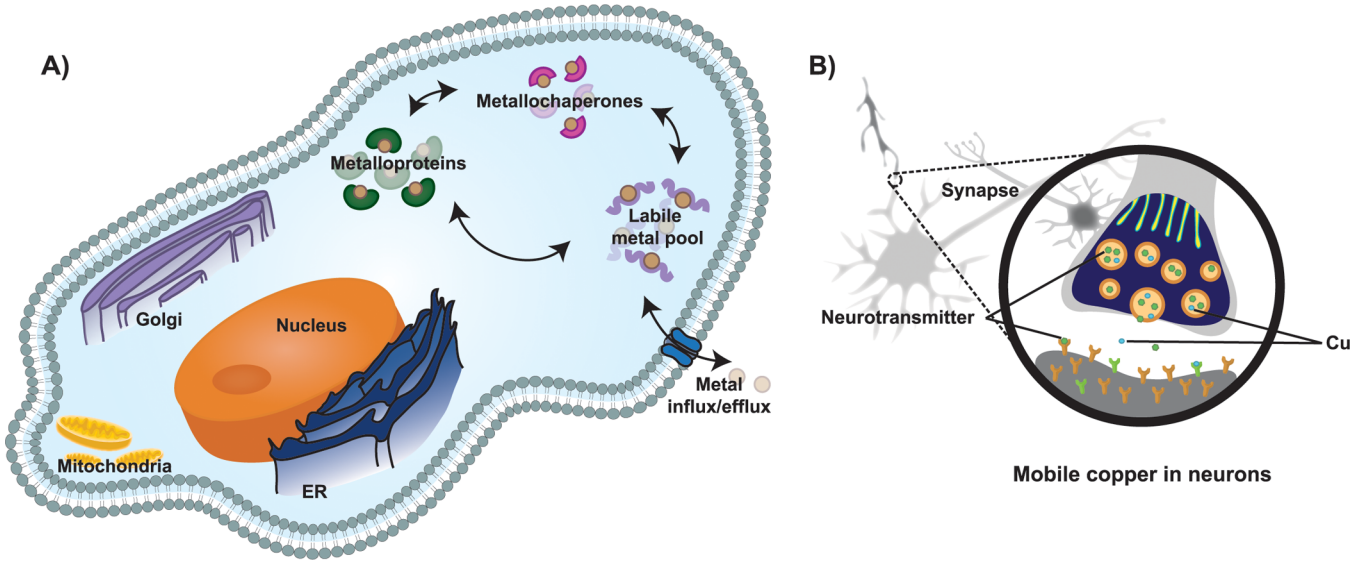

Figure 1. Metal homeostasis and transition metal signaling. (A) Simplified model of cellular metal homeostasis. (B) Mobile copper in neurons as an example of transition metal signaling.

forms and identify signaling roles for these elements. This Account summarizes progress in our laboratory on the development of fluorescent probes for transition metals using recognition and reactivity approaches and their application to identify principles of transition metal signaling.

2. RECOGNITION AND REACTIVITY APPROACHES TO FLUORESCENT METAL DETECTION

Metals in biology can be divided into two general pools: a static pool where metals are tightly bound by proteins and other macromolecules, and a labile pool where metals are bound relatively weakly to cellular ligands, including proteins and low molecular weight ligands (Figure 1A). These smaller ligands include glutathione for copper[3] and citrate, phosphate, and glutathione for iron.[4] Exchange can occur between static and labile metal pools, and tightly bound metal can exchange with variable kinetic parameters.[5] In some cases, this dynamic exchange can be transduced into cellular signaling events, as identified for copper in neuronal activity, an example of transition metal signaling (Figure 1B). Transition metal signaling can be defined as transition metal interactions with biomolecules that govern and coordinate biological activities in response to a stimulus. Fluorescent probes offer a potentially powerful set of tools for mapping labile metal pools with spatial and temporal resolution. However, as with any tool, there is no one-size-fits-all reagent; the diversity and chemical complexity of biological systems—including heterogeneities in pH, hydrophobicity, and local ligand concentrations—mean that it is important, but not sufficient, to characterize these probes in a controlled in vitro chemical setting. As no synthetic buffer or additives can faithfully mimic all aspects of a biological specimen, in vitro characterization should not be relied on solely for interpretation of probe efficacy. Application of a probe in a native biological context requires supporting experiments, including metal supplementation and chelation, genetic models of metal hyperaccumulation and depletion, bulk analysis of metal concentrations (e.g., XFM, Nano-SIMS, ICP), and comparative use of control dyes containing identical fluorophore scaffolds but minimal to no metal response.

Useful probes for imaging transition metal dynamics in living systems must meet several criteria. First and foremost is a selective response to the metal of interest. For transition metals, this criterion is especially challenging because these metals are far less abundant than their alkali/alkaline earth counterparts; in redox-active metal cases, specificity for a particular oxidation state is also required [e.g., Cu(I) vs Cu(II)]. Probes must also have a dissociation constant (K_d) appropriate to compete with the predominant ligand(s) in a given biological context, which in many cases is in flux or even uncertain. Suitable photophysical properties—a large turn-on response and high optical brightness, and excitation and emission in the visible region—minimize potential disruption of cellular homeostasis because lower probe concentrations are needed for imaging experiments. Transition metals pose an additional challenge for turn-on detection as they can act as potent quenchers of fluorescence through a variety of mechanisms, including quenching by an unfilled d shell via electron or energy transfer.[6,7] Finally, probes must be compatible with biological specimens (e.g., be nontoxic and water-compatible, and exhibit predictable biodistribution).

We have pursued two general strategies for metal detection, termed "recognition" and "reactivity" (Figure 2). Recognition-based sensing utilizes a fluorophore attached to a chelating group (receptor) specific to the metal of interest. Binding of analyte to the receptor results in a readily visualized optical change that is reversed upon analyte dissociation. A suitable receptor can often be designed based on coordination chemistry fundamentals, including hard-soft acid-base theory and preferred donor numbers and ligand field geometries. This strategy is best known for its successes in Ca(II)[8] and Zn(II) sensing,[9,10] and we have applied it most extensively to Cu(I) detection.[9,11,12] Alternatively, a reactivity approach can be taken when receptor selectivity is challenging and/or when binding of a metal of interest quenches fluorophore emission. In this approach, selectivity is derived from the ability of the analyte of interest to uniquely carry out chemistry that results in an optical change. Because this approach most commonly makes use of an irreversible reaction, fluorescent signal accumulates over time, and reversible sensing is not possible. We have employed this reactivity-based approach for the detection of transient biological analytes, such as hydrogen peroxide,[13] carbon monoxide,[14] and hydrogen sulfide,[15] as well as redox-active Co(II) and Fe(II).[16] In both recognition- and reactivity-based modes, the probe provides information based on a chemical interaction rather than on an intrinsic property of the metal.

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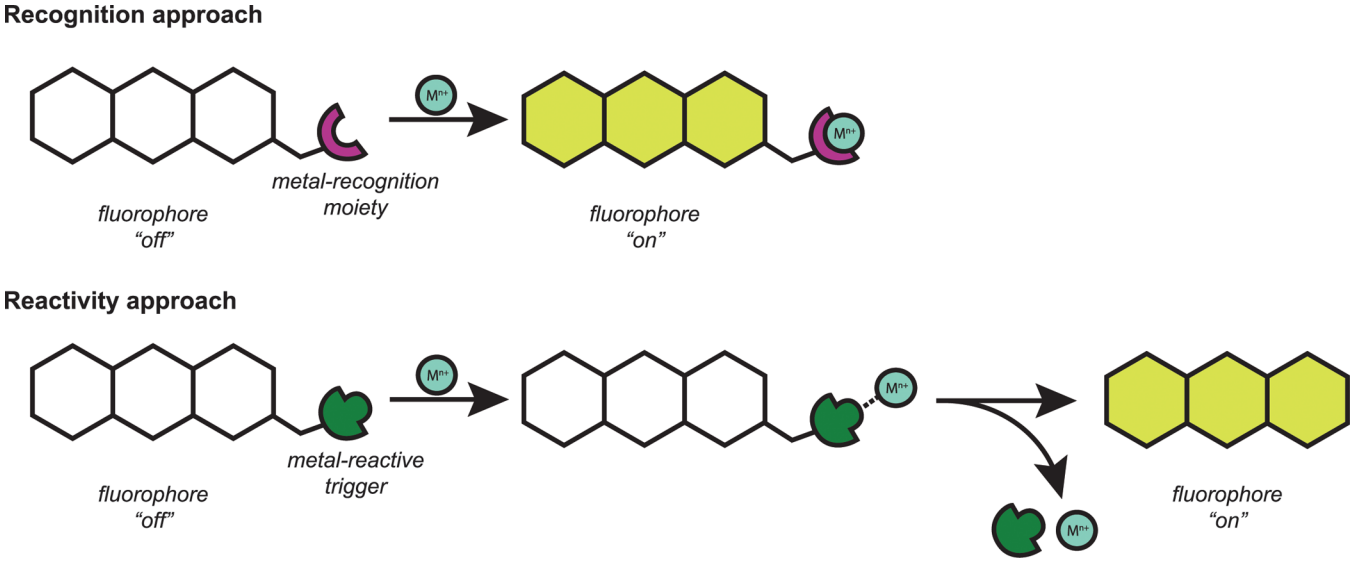

Figure 2. Recognition- and reactivity-based approaches for fluorescent metal probes.

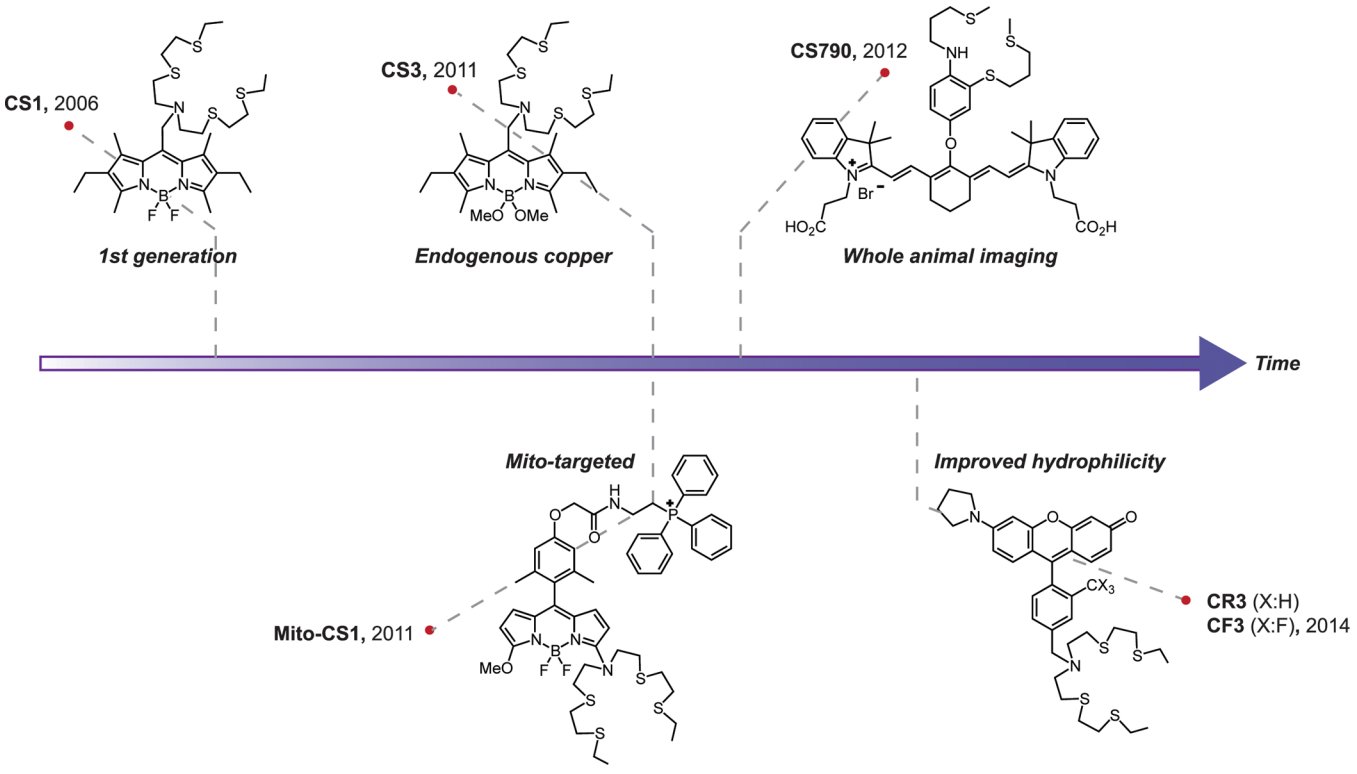

Figure 3. Development of recognition-based fluorescent probes for copper.

We now summarize work from our laboratory on the development and application of recognition- and reactivity-based fluorescent probes to uncover new signaling roles for transition metals.

3. RECOGNITION-BASED FLUORESCENT PROBES FOR COPPER AND NICKEL

Our studies of transition metal signaling have largely focused on copper, particularly in the Cu(I) state favored by the reducing intracellular environment.[17] Copper plays a central role in physiological processes, including respiration,[18] neurotransmitter synthesis and metabolism,[19] pigmentation,[20] antioxidant defense,[21] and epigenetic modification.[22] However, misregulation of copper is also connected to cancer,[23,24] neurodegeneration,[9] and metabolic diseases[25] such as obesity and diabetes. Inspired by classic coordination chemistry by Rorabacher[26] as well as Nature's use of methionine ligands,[27] which offer oxidative resistance (compared to cysteine) and high selectivity for soft Cu(I) over abundant hard alkali/alkaline earth metals and Zn(II), we have developed several generations of recognition-based fluorescent copper probes that make use of thioether receptors (Figure 3).[9,11,12] The closed-shell d^{10} configuration of Cu(I) lends itself to recognition-based sensing by photoinduced electron transfer (PET) and related

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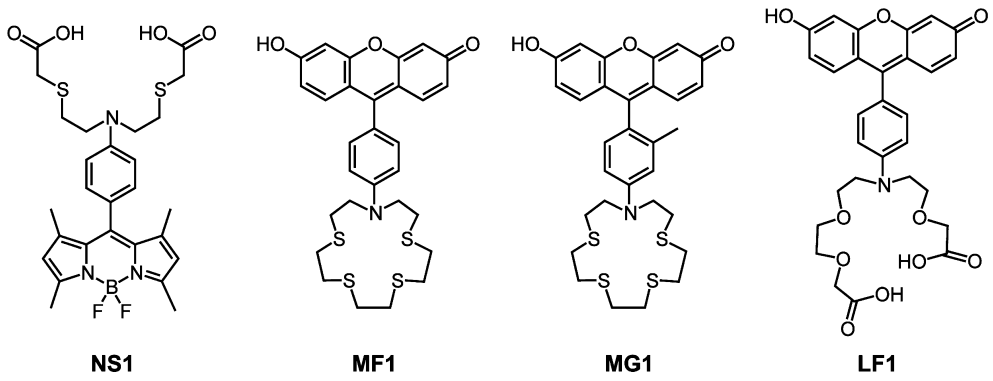

Figure 4. Recognition-based fluorescent metal probes: Nickelsensor 1 (NS1), Mercuryfluor 1 (MF1),[52] Mercury Green 1 (MG1),[53] and Leadfluor 1 (LF1)[54,55]

mechanisms, but Cu(I) sensing remains challenging owing to redox activity.[28]

BODIPY Coppersensor (CS) Probes

Coppersensor 1 (CS1) represents the first example of a visible excitation/emission fluorescent copper probe.[29,30] CS1 combines a boron dipyrromethene (BODIPY)-based fluorophore[31] with an azatetrathia receptor (termed NS_4'), the acyclic form of the Cu(I)-binding tetrathiazacrown ether (NS_4) present in the original fluorescent probe for cellular Cu(I) developed by Fahrni.[32] CS1 features a 10-fold turn-on response to Cu(I) with a K_d of 4×10^{-12} M. Imaging of this probe in HEK 293T cells treated with $CuCl_2$ showed a fluorescence enhancement that is reversed upon addition of a membrane-permeable Cu(I) chelator. These results were validated in an independent study[33] that also showed that CS1 did not respond to copper supplementation in other mammalian cell lines tested, including M17, U87MG, SH-SY5Y, and CHO cell lines. Indeed, each new biological model must be assessed independently, as CS1 has proved valuable alongside complementary direct analysis techniques for assessing copper dynamics in various bacterial,[34] plant,[35] and yeast[36−38] models of copper misregulation and/or hyperaccumulation.

Replacement of the fluorines of CS1 with more electron-rich methoxy substituents yielded the next-generation probe Coppersensor 3 (CS3).[39] The increased electron density on the fluorophore results in improvements in turn-on response (75-fold for CS3 vs 10-fold for CS1) and quantum yield (Φ = 0.40 for CS3, Φ = 0.13 for CS1 in their copper-bound forms) as well as tighter binding to Cu(I) ($K_d = 9 \times 10^{-14}$ M for CS3, $K_d = 4 \times 10^{-12}$ M for CS1). Given the apparent K_d of Cu(I) for glutathione ($K_d = 9 \times 10^{-12}$ M),[5,40] these enhancements enabled CS3 to distinguish between HEK 293T cells grown in normal media and those treated for 20 h with the membrane-impermeable Cu(I) chelator bathocuproine disulfonate (BCS) and to provide evidence for copper translocation in neurons upon depolarization. The live-cell CS3 imaging data were consistent with results from analogous fixed samples analyzed by X-ray fluorescence microscopy (XFM), a synchrotron-based technique that can directly map total copper.[41] Further modifications of the BODIPY scaffold afforded RCS1[42] and Mito-CS1,[43] the latter of which utilizes the triphenylphosphonium targeting moiety for specific mitochondrial localization.[44] Mito-CS1 undergoes a 10-fold fluorescence enhancement upon binding Cu(I) with a $K_d = 7 \times 10^{-12}$ M. Treatments of HEK 293T cells and human patient fibroblasts with $CuCl_2$ and BCS, alongside quantification of total copper by ICP-MS, showed that Mito-CS1 can probe labile mitochondrial Cu(I).

Fluorescent Copper Probes with Improved Hydrophilicity

Despite their effective use in a variety of biological models, the hydrophobicity of BODIPY-based CS probes (log D = 3.46)[45,46] limits their utility for some applications, such as thick-tissue imaging. Therefore, we developed a class of more hydrophilic Cu(I) sensors based on the rhodol fluorophore, a hybrid fluorescein-rhodamine dye[47] that maintains high optical brightness, pH insensitivity, and photostability, while also exhibiting enhanced hydrophilicity (log D = 0.96−1.15).[46] Variations of the xanthone nitrogen substituents afforded the Copper Rhodol (CR) family,[46] and substitution of methyl to trifluoromethyl on pendant aryl ring of the receptor led to Copper Fluor 3 (CF3). The trifluoromethyl group provides steric bulk to prevent aryl-aryl rotations that can quench fluorescence in addition to withdrawing electron density from the aryl ring to increase PET quenching of the apo probe.[48] CF3 features a 40-fold turn-on to Cu(I) and can monitor labile Cu(I) dynamics in dissociated hippocampal neuronal cultures and retinal tissue by one- and two-photon microscopy.

A Near-Infrared Probe for Imaging Copper in Live Animals

To overcome the poor tissue-penetrating ability of visible light, we developed Coppersensor 790 (CS790) based on a cyanine 7 dye scaffold.[49] CS790 exhibits a 15-fold turn-on response to Cu(I) with 760 nm excitation and 790 nm emission. CS790AM, featuring acetoxymethyl esters to assist in probe retention,[50] was capable of monitoring changes upon copper supplementation and chelation in live hairless SKH-1 mice. CS790AM was then applied to $Atp7b^{-/-}$ mice, a model that is both metabolically and phenotypically similar to Wilson's disease due to inactivation of the ATP7B (copper exporter) gene and subsequent accumulation of copper in the liver. Livers of $Atp7b^{-/-}$ mice showed increased fluorescence, which could be reversed upon treatment with the copper chelator ATN-224, relative to those from wild type mice.

Thioether Receptors for Nickel Detection

The NS_4' receptor can be modified to develop fluorescent sensors for other transition or toxic metals. One example is Nickelsensor-1 (NS1), the first fluorescent sensor for cellular imaging of Ni(II).[51] NS1 couples an NS_2O_2 receptor to a BODIPY chromophore to give a 25-fold fluorescence turn-on to Ni(II) with good selectivity over a panel of metals, albeit with a weak K_d of 195 μM. The AM-ester version NS1 can distinguish between control A549 cells, cells exposed to $NiCl_2$, and those treated with a divalent metal chelator. Figure 4 shows further modifications to target Hg(II) and Pb(II).

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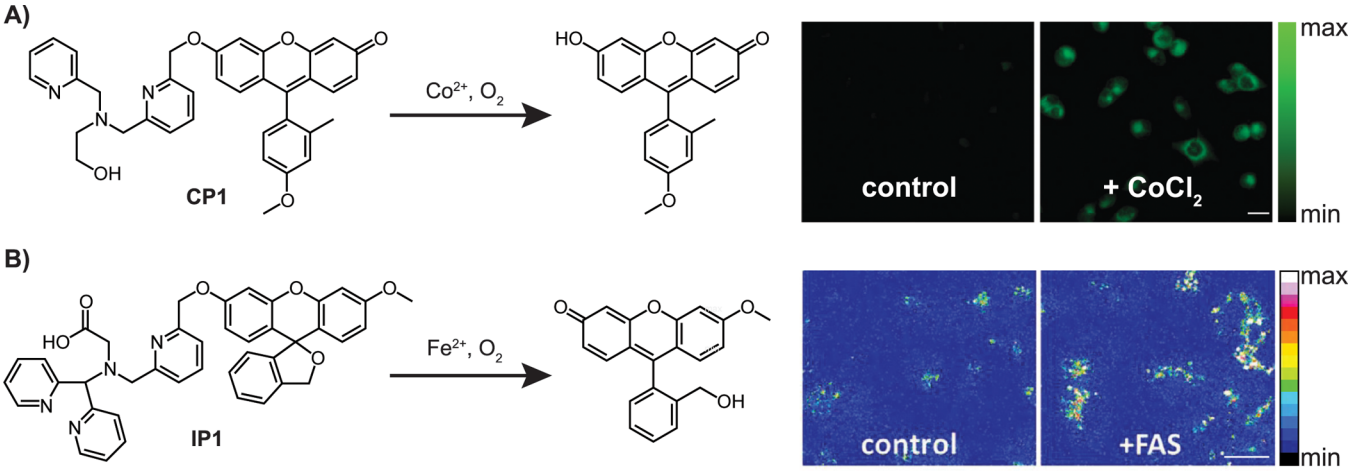

Figure 5. Reactivity-based fluorescent probes that undergo oxidative dealkylation after binding target analyte cobalt (A) or iron (B). (A) Cobalt Probe 1 (CP1) can respond to changes in Co(II) levels in human lung carcinoma A549 cells when growth media is supplemented with $CoCl_2$. (B) Iron Probe 1 (IP1) can detect changes in labile Fe(II) in HepG2/C3A liver cells supplemented with ferrous ammonium sulfate (FAS). Scale bars, 20 μm; fluorescence intensities in green (A) or rainbow (B) scale.

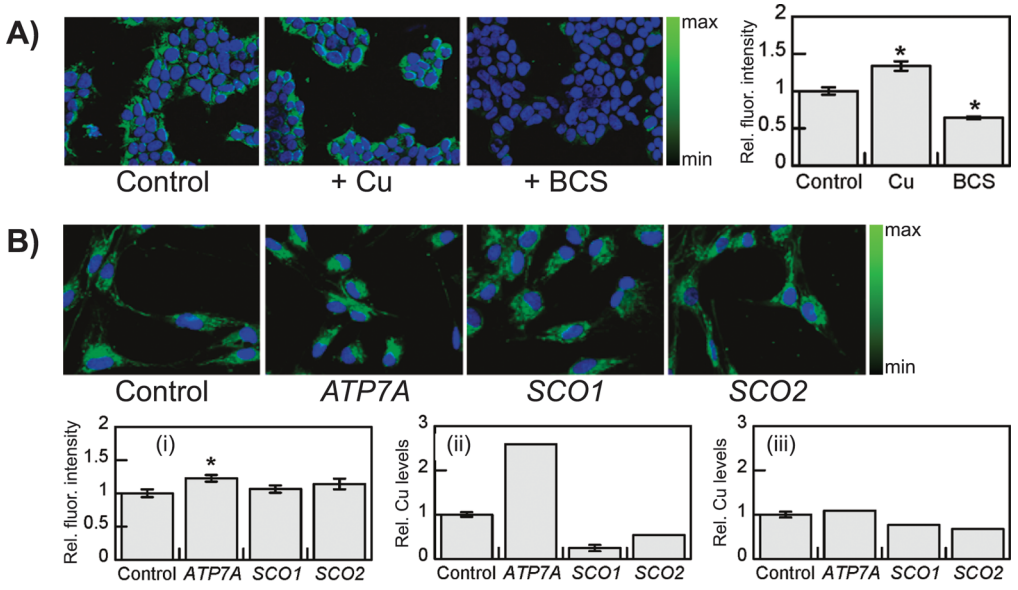

Figure 6. Mito-CS1 imaging and supporting studies suggest that cells prioritize mitochondria for copper homeostasis. Green channel, Mito-CS1; blue channel, nuclear stain. (A) Live cell imaging of copper supplementation and chelation in HEK-293T cells. Mean relative fluorescence intensity from Mito-CS1 signal in the green channel is quantified in the accompanying bar graph. (B) Live cell imaging of patient fibroblasts. Mean relative fluorescence intensity of Mito-CS1 channel quantified in bar graph (i) and ICP-OES measurements of (ii) total and (iii) mitochondrial copper levels show that the cells maintain mitochondrial copper homeostasis even in situations of genetically induced copper deficiency.

4. REACTIVITY-BASED FLUORESCENT PROBES FOR COBALT AND IRON

Although turn-on recognition-based fluorescent probes have been successfully developed for copper and nickel, analogous indicators for transition metals like iron and cobalt have proven more elusive. These analytes, particularly in their divalent oxidation state, are potent fluorescent quenchers by electron[6] and/or energy[7] transfer pathways. Indeed, commercial Fe(II) probes[56] rely on fluorescence quenching, which results in a loss of spatial information. Moreover, Fe(II) and Co(II) are relatively low in the Irving–Williams stability series,[57] rendering selectivity challenging for recognition over biologically relevant and tight ligand-binding divalent metals, such as Zn(II) and Cu(II). We have therefore pursued a reactivity-based approach to the detection of Co(II) and Fe(II) that features metal dissociation after the reaction to liberate a free fluorophore, thereby avoiding quenching.[58] In particular, to circumvent the Irving-Williams series, we sought to exploit selective reactivity of Fe(II) and Co(II) with oxygen, as other transition metal competitors like Ni(II), Cu(II), and Zn(II) have little to no oxygen reactivity and abundant alkali and alkaline earth metals are redox-inactive. This approach has been elegantly demonstrated by Taki and colleagues for Cu(I) detection.[59]

Cobalt Probe 1 (CP1)[60] features a Tokyo Green derivative[48] whose phenol is capped by a tetradentate N_3O ligand. Co(II) binding and air oxidation triggers C–O bond cleavage and fluorescent dye release, resulting in an 18-fold turn-on to 20 equiv Co(II) with excellent selectivity over s- and d-block metals, including Fe(II). CP1 can detect changes in Co(II) levels in human lung carcinoma A549 cells when growth media is supplemented with $CoCl_2$ (Figure 5A).

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Inspired by mononuclear nonheme Fe(II) enzymes containing canonical 2-histidine/1-carboxylate ligand motifs,[61] we developed Iron Probe 1 (IP1), where the fluorescein-derived fluorophore is capped with a pentadentate N_4O ligand set.[62] IP1 responds to 20 equiv Fe(II) with a 6-fold fluorescence enhancement selective over biologically relevant alkali and alkaline earth metals, other transition metals, and 100 equiv various reactive oxygen species. A fluorescence increase is observed upon treatment of IP1 with Co(II), but IP1 shows no fluorescence enhancement to treatment with cyanocobalamin (vitamin B_{12}), a derivative of the most abundant form of cobalt in mammalian systems. This first-generation iron probe can respond to changes in labile Fe(II) pools as demonstrated in HepG2/C3A liver cells with iron supplementation and chelation by ferrous ammonium sulfate and desferroxamine, as well as stimulation with hepicidin and vitamin C (Figure 5B).

5. FLUORESCENT COPPER PROBES REVEAL EXAMPLES OF TRANSITION METAL DYNAMICS

Prior to biological application, fluorescent probes should be characterized with their intended use in mind, as one-size-fits-all probes, metal sources, and chelators do not exist, owing to the diversity of biological systems. In addition to characterization in in vitro models and biological models with pharmacological and genetic manipulation of metal content, we have introduced the concept of metal-unresponsive control dyes and their testing alongside metal-responsive dyes to establish when metal-responsive dyes report on changes in labile metal pools.[45] It is ideal to test metal supplementation and chelation effects on both metal-responsive and control probes to delineate receptor/metal-dependent versus the dye-dependent signal. Because dyes are inherently hydrophobic, most fluorophores will exhibit variations in signal when the hydrophobicity of the solution changes, such as due to addition of lipids or proteins to aqueous buffer. However, the relative responses of changes in metal status in the presence of such in vitro additives and in native biological specimens with both types of probes will set a baseline for any potential background signal and help elucidate the appropriateness of a chemical tool for a given biological situation.[11] With supporting data, fluorescent probes can be useful in studying labile metals in a variety of cell, tissue, and animal models where probe reactivity, solubility, and cellular distribution are nonuniform. Indeed, they have helped to reveal several principles underlying the nascent field of transition metal signaling. We provide three recent examples from our laboratory and collaborations in which labile copper contributes to physiology to illustrate the breadth of dynamic transition metal regulation.[45,63−66]

Mitochondria are Prioritized Organelles for Copper Homeostasis

Compartmentalization, particularly within and between organelles, is an essential aspect of metal homeostasis. The targetable fluorescent probe Mito-CS1 helped reveal prioritization of mitochondrial copper pools over other cellular compartments during copper deficiency.[43] Comparison of Mito-CS1 staining in wild type human patient fibroblasts versus cells containing mutations in the assembly genes of copper-containing cytochrome *c* oxidase (*SCO1* and *SCO2*) showed that labile mitochondrial Cu(I) is unaltered in the *SCO* mutants, which was corroborated by ICP-OES (inductively coupled plasma optical emission spectroscopy) analysis on isolated mitochondria. Interestingly, while total cellular copper levels were higher in *ATP7A* knockout and lower in the *SCO1* and *SCO2* fibroblasts relative to wild type,

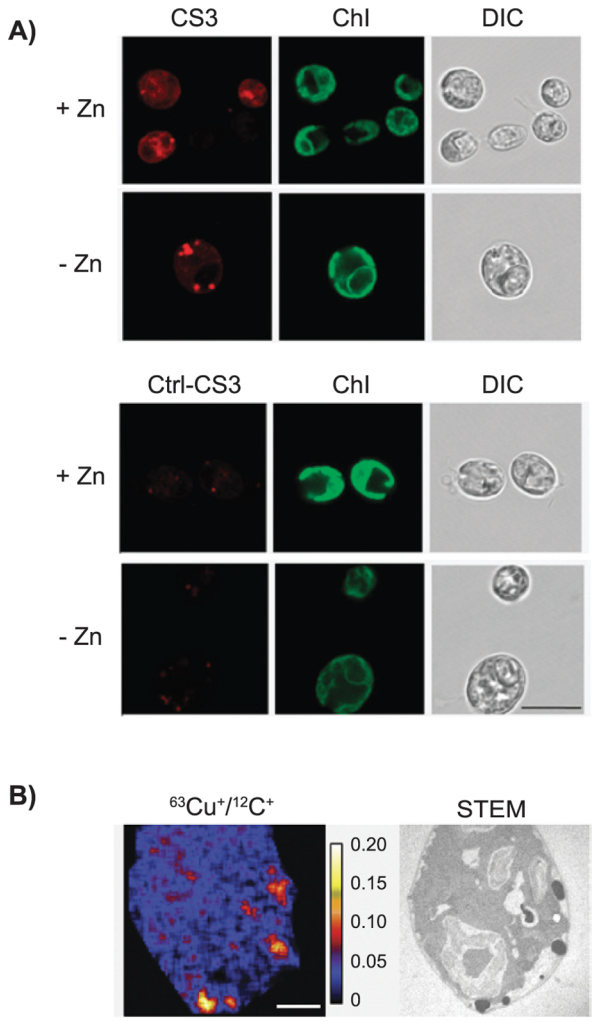

Figure 7. Identifying cuprosomes as copper traps during zinc deficiency. (A) Imaging of zinc-limited and zinc-replete wild-type *C. reinhardtii* with CS3 and Ctrl-CS3 to observe intracellular copper distribution. Chl, chlorophyll autofluorescence; DIC, differential interference contrast. Scale bar, 10 μm. (B) Nano-SIMS shows that copper colocalizes with electron-dense structures in STEM (scanning electron microscopy) images in zinc-limited cells. Legend represents the ratio^{63} $Cu^+/^{12}C^+$. Scale bar, 1 μm.

mitochondrial copper levels in patient livers were strikingly similar between control and *SCO1* or *SCO2* patients (Figure 6). Establishing that mitochondria are prioritized over other compartments for copper homeostasis potentially links dynamic regulation of this transition metal to functions of this organelle.

Cuprosomes as Dynamic Metal Storage Compartments

Metals must be matched to the right protein partners within the cell, and protein mismetalation can have dire pathological consequences. Another principle of transition metal homeostasis uncovered with the help of fluorescent probes is the reversible sequestration of copper in response to zinc deficiency within a novel organelle termed the "cuprosome".[45] CS3 facilitated identification of this copper storage compartment induced under zinc-deficient conditions in *Chlamydomonas reinhardtii* green algae in experiments conducted alongside Ctrl-CS3, a matched control dye for CS3 that does not respond to metal due to replacement of receptor sulfur atoms with methylene units. Pilot fluorescence microscopy experiments with CS3, but not Ctrl-CS3, showed that fluorescent hot spots were more frequently

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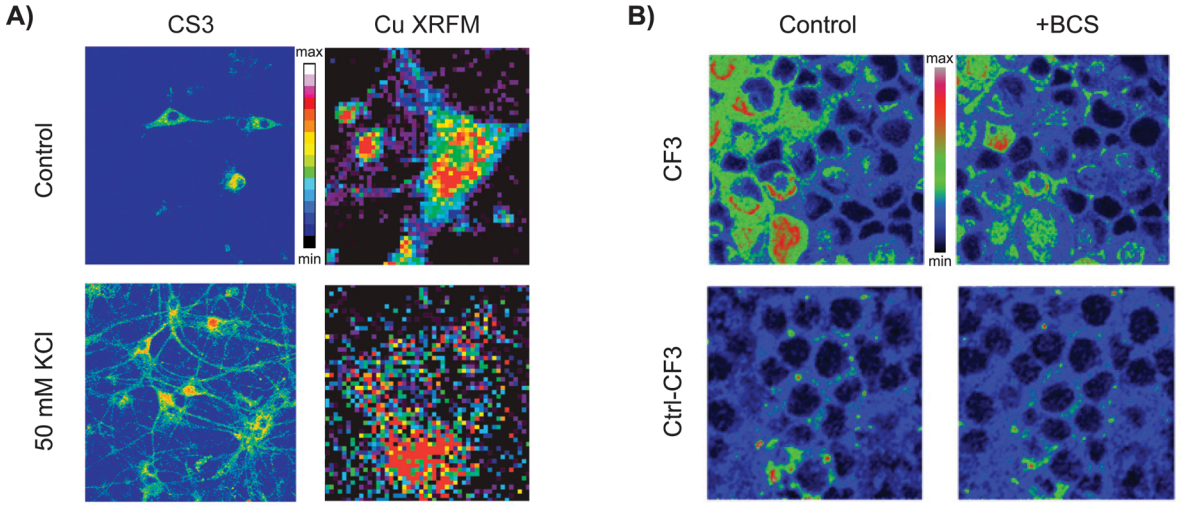

Figure 8. Imaging reveals labile copper pools in neurons and retinal tissue. (A) Copper visualization in resting and depolarized live rat hippocampal neurons with CS3 and fixed rat hippocampal neurons with XFM. (B) Live two-photon imaging on retinal neurons bolus with CF3 and Ctrl-CF3 under control and acute copper chelation conditions.

observed in zinc-deficient cells than in control cells (Figure 7A). Moreover, CS3, but not Ctrl-CS3, fluorescence could be attenuated with the addition of a copper chelator, and algae with mutations resulting in altered lipid handling gave a comparable CS3 signal compared to wild type. Direct copper imaging by Nano-SIMS (Figure 7B) and X-ray absorption spectroscopy, along with additional biochemical experiments, established the existence of these copper traps. This study suggests that, by reversibly sequestering copper in these storage compartments, green algae may minimize protein mismetalation during zinc deficiency without losing copper for future use.

Copper Dynamics in the Brain

Fluorescent probes have also revealed transition metal dynamics that can influence more complex physiological processes. Application of newly developed fluorescent copper sensors has helped identify mobile copper pools in dissociated hippocampal neurons and retinal tissue connected to canonical calcium signaling pathways and excitability during development. We applied CS3 to hippocampal neurons in live-cell imaging experiments corroborated with direct copper imaging by XFM in analogous fixed samples.[39] In resting neurons, labile and total copper were largely localized to the cell soma. Upon activation induced by KCl depolarization, copper pools relocalized from the soma to dendritic processes (Figure 8A). Interestingly, this copper translocation was calcium-dependent, as shown by CS3 and XFM imaging experiments in which Ca(II) release was blocked through the application of the intracellular Ca(II) chelator BAPTA-AM or by treatment with dantrolene, a drug that binds ryanodine receptors to decrease cytosolic Ca(II).

More recently, we developed rhodol dyes CR1−6 and CF3 with improved hydrophilicity and with two-photon cross sections suitable for tissue imaging, which enabled one- and two-photon labile copper imaging in dissociated neuronal cell culture and retinal tissue in conjunction with control dyes Ctrl-CR3 and Ctrl-CF3.[46] Indeed, we observed decreases in CF3 fluorescence upon acute bath application of the copper chelator BCS to dissociated hippocampal neurons as well as isolated mouse retinal tissue, whereas Ctrl-CF3 fluorescence remained unchanged by this treatment (Figure 8B). After establishing the presence of a labile copper pool in both neural models, we investigated the effects of altering this labile pool on spontaneous activity, a basic property of all developing neural circuits. We observed that acute and reversible pharmacological treatment with the copper chelator BCS, as well as the genetic knockout of the copper importer Ctr1, increased event frequency and the percentage of cells involved in spontaneous correlated activity, as shown by calcium imaging. The data provide evidence for a physiological role of copper in neural function.

■ CONCLUDING REMARKS

The concept that transition metals can act as dynamic cell signals expands the roles that these essential elements can contribute to biological systems. Fluorescent probes are unique tools to explore this emerging paradigm with spatial and temporal resolution in cells, tissue, and organisms. We are currently focused on developing indicators for biological iron, improving the available K_d range of copper sensors, and developing additional probes for subcellular and in vivo transition metal imaging. Expanding the synthetic toolbox of metal recognition- and reactivity-based probes will open new avenues for the study of metals in biology.

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Author Contributions

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Notes

The authors declare no competing financial interest.

Biographies

Allegra Aron is an NSF Graduate Research Fellow in the laboratory of Prof. Chris Chang at UC Berkeley. Allegra graduated from Brown University in 2011 with an Sc.B. in Chemistry. Her research interests include the development of molecular probes for the detection of redox-active metals in biological systems.

Karla Ramos-Torres graduated from the University of Puerto Rico, Río Piedras in 2011 with a B.S. in Chemistry. She is currently a Ph.D. candidate at UC Berkeley, where she works in the laboratory of Prof. Chris Chang on the development of molecular probes for the detection of biological copper.

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