Title

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Journal

Angewandte Chemie, 62(11)

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Publication Date

2023-03-06

DOI

10.1002/anie.202213644

Peer reviewed

HHS Public Access

Author manuscript

Angew Chem Int Ed Engl. Author manuscript; available in PMC 2023 December 28.

Published in final edited form as:

Angew Chem Int Ed Engl. 2023 March 06; 62(11): e202213644. doi:10.1002/anie.202213644.

Metalloallostery and Transition Metal Signaling: Bioinorganic Copper Chemistry Beyond Active Sites

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Abstract

Transition metal chemistry is essential to life, where metal binding to DNA, RNA, and proteins underpins all facets of the central dogma of biology. In this context, metals in proteins are typically studied as static active site cofactors. However, the emergence of transition metal signaling, where mobile metal pools can transiently bind to biological targets beyond active sites, is expanding this conventional view of bioinorganic chemistry. This Minireview focuses on the concept of metalloallostery, using copper as a canonical example of how metals can regulate protein function by binding to remote allosteric sites (e.g., exosites). We summarize advances in and prospects for the field, including imaging dynamic transition metal signaling pools, allosteric inhibition or activation of protein targets by metal binding, and metal-dependent signaling pathways that underlie nutrient vulnerabilities in diseases spanning obesity, fatty liver disease, cancer, and neurodegeneration.

Graphical Abstract

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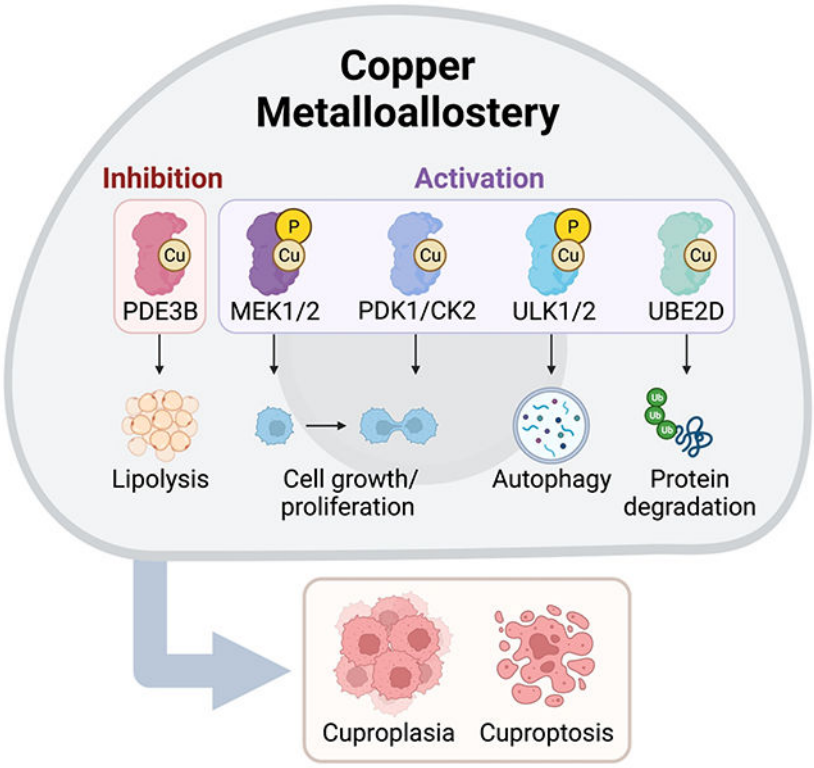
Conflict of Interest

The authors declare no conflict of interest.



Keywords

Copper Fluorescent Sensor; Cuproplasia; Cuproptosis; Metalloallostery; Transition Metal Signaling

1. Introduction

The periodic table organizes the elements of the chemistry of life (Figure 1). Indeed, organic and inorganic elements alike sustain the growth, development, and survival of all organisms across all kingdoms of life and are ubiquitous in basic biology and translational medicine. In this context, metals are essential at every stage of the central dogma. They are required for proper structure and function of all molecules of DNA and RNA and an estimated one-third to one-half of all proteins in the cell.[1] In addition to structural roles, metals in proteins perform key reactivity roles that enable oxygen transport, hydrolytic chemistry, and electron transfer. They are also involved in more complex chemistries including metabolic and epigenetic transformations.[1]

Despite the widespread use of metals in biology and a breadth of protein functions, the conventional view of metallobiochemistry is rather constrained for what defines a metalloprotein. Traditionally, metalloproteins have been identified and classified largely as proteins with metal cofactors tightly bound to active sites to support protein structure and promote catalytic activity (Figure 2a). This type of binding, which represents the static metal pool, is typically used to protect the cell against aberrant reactivity of redox-active

transition metals such as copper and iron, which can cause oxidative stress and damage if left unregulated.[2] Indeed, because cells cannot synthesize their required metals, they have evolved complex mechanisms of metal import, export, trafficking, and redox proteins involved in metal nutrient homeostasis.[3–5] This situation has given rise to the recognition of a second major metal pool, termed the labile metal pool, where metals can rapidly move and exchange between targets in different locations in the cell.[6–9] Metals in the labile pool transfer through a thermodynamic gradient of metal-binding sites to funnel into the tightly-bound static metal pool.[10] Taken together, the total metal pool represents a four-dimensional exchangeable and dynamic equilibrium between tightly-bound static and weakly-bound labile metal pools in space and time.

The focus of this Minireview is to broaden this conventional dogma of metal–protein interactions beyond static metal binding at their active sites. We expand the definition of metalloproteins to be more inclusive and encompass protein targets that have metal binding to secondary binding exosites—remote allosteric binding sites beyond the active site—in a transient manner for signaling purposes (Figure 2b). We term this concept metalloallostery, in which dynamic, labile metal pools can bind to proteins and induce positive or negative allosteric regulation of activity. We highlight advances in and prospects for the field and use copper as a canonical example of metalloallostery in physiology and pathology (Figure 2c). We also refer the reader to more comprehensive reviews on copper and transition metal signaling.[6,11–15]

2. Transition Metal Signaling: Chemical Probes Enable Imaging of Labile Copper Fluxes

Molecular imaging probes have revolutionized the study of chemistry in biological systems. A pioneering example for bioinorganic chemistry is the development of fluorescent sensors for Ca^{II}. Initiated by Tsien's revolutionary work in the 1980s,[18] the design of a BAPTA chelator for selective host–guest recognition enabled selective Ca^{II} imaging. Beyond Ca^{II},[19] we and others have expanded this chemical toolbox for detection of a broader range of metal nutrients across the periodic table, including transition metals.[2,9,20–23] Indeed, metal and oxidation state-specific probes have been designed for a variety of d-block metals, including Zn^{II},[2,21,24] $Fe^{II/III}$,[22,25–30] $Cu^{I/II}$,[2,20,31–34] Co^{II},[35] and Ni^{II}.[36,37] These reagents have established that d-block transition metals can also exist in labile pools and be mobilized in response to cellular stimuli, characteristics that were once thought to be restricted to s-block alkali and alkaline earth metals like sodium, potassium, and calcium.

These fluorescent probes detect the labile metal pools, which can complement multiple analytical techniques that measure mass or electronic structure to detect total metal pools.[7] Due to exchange within total metal pools, design of these probes requires caution of appropriate dissociation constants (K_d) to prevent perturbation of both static and labile metal pools from their biological states. For example, dissociation constants for static, tight-binding Cu^{I}-binding proteins and ligands have been reported to be in the range of 10^{14}–10^{18} M.[10] Thus, probes with varying K_d values in the nanomolar to picomolar range have been developed to probe new biological roles for labile metal sites.[38]

In this context, our laboratory has advanced the field of biological metal imaging by introducing a second type of approach, termed activity-based sensing,[25,38,39] to complement traditional binding-based approaches for metal detection. Binding-based strategies rely on molecular recognition, where selectivity for lock-and-key binding is determined by coordination chemistry principles. Included are hard–soft acid–base reactivity preferences and ligand field theory for selective metal complexation (Figure 3a). However, challenges in using binding-based approaches for metal sensor design often arise when seeking to discriminate weakly-binding metals along the Irving–Williams series of relative stabilities of transition metal complexes. To overcome these inherent limitations, activity-based sensing uses molecular reactivity to achieve high metal- and oxidation state-specificity by taking advantage of the specialized and unique characteristics of a given metal center (Figure 3b). In this approach, metal-specific transformations can induce changes in fluorescence response through tandem binding and reactivity modes and provide enhanced specificity. This approach is particularly effective for weakly-binding metals on the Irving–Williams series and for those that have no crystal field stabilization energy that results in d-orbital stabilization through formation of metal–ligand bonds.

Using copper as an example, our laboratory and others have developed a suite of chemical probes to image labile Cu^{I} pools across a variety of biological length scales. The probes range from cell models with single-cell (CTAP-1, CS1, CS3, CF3, CF4, CSR1, Crisp-17, FCP-1 and others)[8,40–45] and subcellular (Mito-CS1)[46] resolution to in vivo animal (CS790[47] and CCL-1[48]) models (Figure 3c). Moreover, these chemical tools have enabled the discovery and characterization of key foundational principles in the cell biology of metals, including labile transition metal pools that are rapidly mobilized by cellular activity. For example, the first identification of labile intracellular copper pools was established by molecular imaging using Fahrni's Cu^{I}-responsive CTAP-1[49] and our group's Coppersensor 1 (CS1) probes.[40] CS1 was a first-generation reagent that could respond to both expansion and depletion of labile Cu^{I} pools by copper supplementation and chelation, respectively. Subcellular Cu^{I} imaging with Mito-CS1, which utilizes a triphenylphosphonium (TPP) mitochondrial localization moiety, revealed the prioritization of mitochondrial Cu^{I} pools in the hierarchy of cellular labile copper homeostasis.[46]

Our laboratory opened a field of transition metal signaling by observing the first examples of rapid cellular copper mobilization in response to physiological stimuli. Using the Coppersensor 3 (CS3) probe and X-ray fluorescence microscopy, we discovered that Cu^{I} can move from somatic cell bodies to peripheral dendritic processes upon neuronal depolarization.[8] Subsequently, CTR1 was identified as a copper ion channel using Copper Fluor-3 (CF3).[41] CF3 was one of several next-generation probes exhibiting improved hydrophilicity to enable broader live-cell and live-tissue imaging of endogenous labile Cu^{I} pools. For example, in vivo Cu^{I} imaging in developing zebrafish embryos using Copper Fluor-4 (CF4) revealed an essential role for copper in regulating brain behavior. A copper-dependent brain circuit in the locus coeruleus neuron cluster of the brainstem was discovered to regulate rest–activity cycles.[42] We also developed FRET Copper Probe-1 (FCP1) to identify crosstalk between labile Cu^{I} pools and glutathione redox balance.[45]

The next set of copper probes, Coppersensor 790 (CS790)[47] and Copper-Caged Luciferin 1 (CCL-1),[48] opened the door to in vivo imaging in murine models. First-generation near-infrared fluorescent probe CS790 was able to map expected elevations in liver copper in a murine model of Wilson's disease, a genetic disorder in the copper export protein ATP7B that is characterized by excess liver copper accumulation.[50,51] Application of first-generation bioluminescent probe CCL-1 in a diet-induced mouse model of nonalcoholic fatty liver disease (NAFLD) revealed a hepatic copper deficiency. Real-time imaging of labile copper pools with the photostable red Copper Silicon Rhodol 1 (CSR1) probe, which showed fast fluxes of copper released upon induction of lipid signaling and metabolism, contributed to the discovery of copper-dependent fat regulation through lipolysis.[43]

3. Copper as an Allosteric Inhibitor: cAMP Signaling and Lipid Regulation

With chemical probes showing the existence of dynamic and mobile copper pools, our laboratory provided an early example of metalloallostery. Our laboratory identified a protein target with a copper-binding allosteric site (e.g., exosite) during studies of metals in lipid biology and metabolic diseases. Specifically, we found that copper is an endogenous regulator of lipolysis, the central process in burning fat (Figure 4a).[43] Indeed, early observations suggested a correlation between copper and lipid metabolism. For example, cattle fed copper-supplemented diets showed a decrease in subcutaneous backfat adipose tissue and cholesterol, but intramuscular fat that contributes to marbling was not affected.[52–55] In our initial experiments, we used the *Atp7b*$^{-/-}$ mouse model of Wilson's disease as a genetic model of copper misregulation, where mutation of the copper exporter protein ATP7B results in excess copper accumulation in the liver.[43] We identified two reciprocal relationships, one between copper and lipid status and another between copper/lipid ratios in liver and adipose tissue. In the *Atp7b*$^{-/-}$ mouse liver, we observed high copper and low lipid levels compared to wild-type but reciprocal low copper and high lipid levels in white adipose tissue. Upon investigation of 3',5'-cyclic AMP (cAMP)-dependent lipolysis in a 3T3-L1 adipocyte cell model of white adipose tissue, we found that copper depletion decreased lipolysis while copper supplementation increased lipolysis. Further mechanistic biochemical studies showed how isoform-specific phosphodiesterase 3B (PDE3B), at a cysteine-rich motif exosite, is the molecular origin of metalloallosteric regulation by copper (Figure 4a). In particular, copper binding to PDE3B at a key allosteric C768 site (Figure 2c) inhibits enzyme activity responsible for cAMP-dependent lipolysis of triglycerides into fatty acids and glycerol. This work provides a primary example of how metalloallostery can broaden the definition of a metalloprotein beyond active sites. Not only does copper regulate function by binding to a remote allosteric site rather than to the protein active site, but reversible metal binding causes a *decrease* rather than an increase in catalytic activity of the enzyme target.

Translating this copper–lipid connection in vivo, we developed the luciferin-generating Copper-Caged Luciferin 1 (CCL-1), a bioluminescent copper imaging probe, and found a liver-localized copper deficiency in a high-fat diet murine model of non-alcoholic fatty liver disease (NAFLD).[48] NAFLD is a growing epidemic as the most common liver disease,[56] which can progress to more serious diseases including non-alcoholic steatohepatitis (NASH), cirrhosis, and liver cancer. Importantly, this copper nutrient deficiency is both

metal- and tissue-selective.[48] Building on clinical observations linking NAFLD to low copper supply,[57] longitudinal copper imaging using CCL-1 in mice fed a high-fat diet versus normal diet revealed an early liver-localized copper deficiency that preceded weight gain and other metabolic symptoms of NAFLD. Concomitant upregulation in copper export protein markers Copper-transporting ATPase 1 and 2 (ATP7A and ATP7B) in the high-fat diet models suggested that excess copper export was the origin for the observed copper deficiency. Building on this result, we designed a targeted ionophore-based metal supplementation (TIMS) platform for tissue-specific delivery of copper.[58] We illustrate this approach by developing Gal-Cu(gtsm), a liver-selective supplement for this metal nutrient. Specifically, we leveraged the high expression of asialoglycoprotein receptors (ASGPR) on the surface of hepatocytes and associated receptor-mediated endocytosis as a "Trojan Horse" strategy to selectively deliver copper to liver tissue. Because copper uptake involves an active transport mechanism, it prevents unregulated copper release that could result in oxidative stress. Taken together, this body of work provides a bona fide biochemical target of copper metalloallostery and reveals copper deficiency as a metal-dependent disease vulnerability that can be targeted in lipid-associated disorders such as obesity and fatty liver disease.

4. Copper as an Allosteric Activator: Kinase Signaling and Cancer Metabolism

In addition to acting as an allosteric inhibitor of protein activity, copper can also serve as an allosteric protein activator. Kinases, which are central hubs of cell signaling, are emerging targets of copper nutrient regulation (Figure 4a). Connecting observations that copper produces reactive oxygen species (ROS) and that kinase pathways are activated by hydrogen peroxide, it has been hypothesized that copper can activate kinases through ROS. Contrary to this hypothesis, early work by Klotz and co-workers reported a non-ROS-mediated mechanism for phosphoinositide 3-kinase (PI3K) in the PI3K/Akt signaling pathway.[59] For other kinase signal transduction pathways, such as c-Jun N-terminal kinase (JNK) and p38-mitogen-activated protein kinases (MAPKs) responsive to stress stimuli, it is more ambiguous whether ROS are involved in the mechanism of copper-dependent activation.[60–63] Here we provide leading examples of positive allosteric regulation of kinase activity by copper.

A landmark example of allosteric copper activation is its role in the mitogen-activated protein kinase (MAPK) pathway reported by Brady, Thiele, and Counter. Manipulation of copper influx through ablation of high affinity copper transporter 1 (CTR1) reduced ability of MAP kinase kinase (MEK1) to phosphorylate MAP kinases extracellular signal-regulated kinase 1 and 2 (ERK1/2) (Figure 4a).[64] Furthermore, decreasing expression of CTR1 or disrupting copper binding to MEK1 reduced serine/threonine-protein kinase $BRAF^{V600E}$-driven signaling.[65] Conversely, copper addition activates MEK1 phosphorylation of ERK and downstream cell growth and proliferation pathways. Another example of copper-dependent activation of kinases by Brady is Unc51-like kinase 1 and 2 (ULK1/2),[66] a pair of kinases that lie downstream from mechanistic target of rapamycin (mTOR) and drive autophagy (Figure 4a). Disruption of copper binding reduced ULK1/2-dependent

signaling and formation of autophagosome complexes and has potential to be targeted for cancer therapy. Likewise, receptor tyrosine kinase (RTK)-mediated cellular signaling is also activated by copper metalloallostery through tyrosine kinase receptors EGFR and MET, increasing downstream protein kinase B (PKB, also known as Akt) and ERK signal transduction and increasing cancer cell proliferation and migration. Guo, Wei, and colleagues reported that pyruvate dehydrogenase kinase 1 (PDK1) is also a copper-responsive kinase, which activates its downstream substrate Akt to facilitate tumorigenesis.[67] Casein kinase 2 (CK2), a kinase responsible for generating 21% of the phosphoproteome, is similarly modulated by copper (Figure 4a).[68,69] As a final example of cancer-relevant copper metalloallostery beyond kinases, Opazo and Bush showed that copper binds to the E2 ubiquitin-conjugating enzyme D (UBE2D) at a conserved CXXXC motif to promote ubiquitination (Figure 4a). As such, copper plays an important role in protein quality control, which also has implications for neurodegenerative diseases.[70]

5. Copper as a Regulator of Gene Expression: Transcription Factors

Beyond copper's role in allosteric regulation of metabolic enzymes and pathways, it also regulates gene expression via transcription factors. These copper-sensing transcription factors respond to copper deficiency or copper excess by controlling protein–DNA interactions. Here we discuss select examples of copper signaling in transcription factor activity in yeast and bacteria. A more extensive review on transcription factors that respond to and regulate copper, as well as iron and zinc, has been published.[71]

In *S. cerevisiae* yeast, Ace1 and Mac1 both contain cysteine motifs that bind four Cu^{I} ions for copper sensing. Despite their very similar DNA-binding regions (Figure 5a),[72] Cu^{I} binding triggers opposite effects on gene expression. Cu^{I} binding converts Ace1 to an active DNA-binding form (Figure 5b), while Cu^{I} binding deactivates DNA binding of Mac1 (Figure 5c). In response to copper excess, Ace1 activates gene expression of copper metallothioneins CUP1 and CRS5 and the copper-zinc superoxide dismutase SOD1 (Figure 5b).[73] The metallothioneins sequester excess copper, and SOD1 acts as a cellular stress response by breaking down superoxide radicals. In response to copper deficiency, Mac1 activates gene expression of copper uptake transporters CTR1 and CTR3 and metalloreductases FRE1 and FRE7 to increase cellular copper pools (Figure 5c).[74] In *E. coli* bacteria, the Cu efflux regulator (CueR) activates the primary copper efflux ATPase CopA upon elevated copper.[75] Comparison of metal binding domain of CueR with others in the mercury resistance (MerR) family highlights the importance of structure to confer metal specificity for copper versus zinc and mercury. In contrast to copper-sensing transcription factors for copper regulation, the multiple antibiotics resistance regulator (MarR) senses Cu^{II} in order to respond to antibiotic stress.[76] Antibiotics like ampicillin induce copper signaling, and Cu^{II} oxidizes a cysteine in MarR that results in dissociation from its DNA. In *M. tuberculosis*, copper-sensitive operon repressor (CsoR) was identified as a Cu^{I}-sensing transcription factor that is present in eubacteria compared to CueR that is limited to proteobacteria.[77] When Cu^{I} is coordinated to CsoR's subunit bridging site, CsoR is released from the DNA. Together, these metalloregulatory transcription factors demonstrate the rich chemistry of copper signaling to regulate protein–DNA interactions.

6. Conclusions and Future Prospects

In this Minireview, we have summarized advances in the emerging field of metalloallostery. We have focused on copper as a canonical example to showcase the expansion of traditional bioinorganic chemistry and protein metallobiochemistry beyond active site regulation. The development of binding-based and activity-based sensing probes for molecular imaging of copper has enabled the discovery of labile transition metal pools that can be mobilized by external physiological stimuli and contribute to essential cell signaling pathways. Biochemical identification and characterization of copper as either an allosteric inhibitor and activator of protein function depends on the target (e.g., phosphodiesterases, kinases, ubiquitin ligases, and transcription factors). These characterizations presage that the dynamic metalloproteome is likely underestimated and that many more classes of copper-dependent proteins remain to be discovered. Indeed, copper is but one of many metal nutrients that are required for life, and this growing body of work points to adopting a more inclusive and sophisticated view to defining metal–protein interactions. Finally, metalloallostery contributes to the deciphering of key transition metal signaling targets and pathways in health and disease. In obesity and fatty liver disease, copper regulates cAMP-dependent lipolysis and lipid accumulation. In cancer, copper-dependent kinase signaling controls growth and autophagy pathways. These roles and relationships of metals in biology are foundational examples for further study.

Many sources, targets, and physiological/pathological consequences of metalloallostery and transition metal signaling have yet to be discovered. There remains a constant need for development of new molecular imaging probes to identify and monitor labile metal pools with high metal specificity, and in the case of redox-active metals, oxidation state specificity. Increasing the scope of metals that can be monitored, along with improvements in signal-to-noise responses and spatiotemporal specificity, will illuminate new metal biology and medicine. Achieving four-dimensional imaging will span biological length scales of single molecule, subcellular, single cell, tissue, and whole animal. Imaging probes for translational use, particularly as companion diagnostics, are another direction that is ripe for growth.

In addition to measuring sources of metal pools, there is also a need for new omics approaches for metal-dependent biological targets, particularly in metalloproteomics to identify and characterize molecular sites of metalloallosteric regulation. For example, with advances in activity-based probes and proteomics with improved binding and specificity, metal target identification will have greater depth for expanding the pool of known metalloproteins. In addition, improved computational predictions and analyses will aid in the investigation of metalloproteins[80–82] through searching for amino acid metal-binding motifs and other protein trends.[83,84] Various histidine, cysteine, and carboxylate metal-binding motifs have been reported in traditional metalloproteins.[13] In contrast, motifs for metalloallostery sites are not yet well established and are difficult to predict. As more metalloallosteric proteins are discovered, coordination sites and trends will emerge for the labile metal pool. For example, the key copper-binding residue in PDE3B is a single conserved cysteine on a flexible loop unique to PDE3s.[43] Traditional tools of bioinorganic chemistry, including structure and spectroscopy of newly identified metalloproteins, as well

as the potential for synthetic model complexes, will further advance the scope of this metallobiochemistry.

Finally, because the field of metalloallostery is in its relative infancy, it is important to invest efforts to understand the physiology and pathology of transition metal signaling at the molecular, cellular, and systems level. In parallel, exciting opportunities await to translate this basic science knowledge to develop advanced diagnostic and therapeutic platforms to treat metal-dependent disease vulnerabilities. Copper is a prime example of the "goldilocks" effect of metal nutrients, where one needs just the right amount of a metal in the right place at the right time for proper cellular and organismal function. This precise balance is exemplified by two contrasting genetic copper disorders. Menkes disease is characterized by copper deficiency[85] while Wilson's disease is characterized by copper overload (Figure 4b).[86] Indeed, copper deficiency is now correlated with lipid accumulation in obesity and fatty liver disease, with a copper-PDE3B-cAMP pathway providing an example of how negative metalloallostery can contribute to an impactful transition metal signaling pathway. Likewise, the emergence of copper-dependent kinase signaling presages an opportunity for copper depletion as a disease-modifying therapy. This overall metal balance is essential for both cell health and cell death. For example, cuproptosis is a newly discovered form of copper-dependent cell death[87] akin to iron-dependent cell death (e.g., ferroptosis),[88] while cuproplasia is a newly recognized form of copper-dependent cell growth and proliferation (Figure 4c).[15] Taken together, the concept of metalloallostery offers a molecular mechanism that continues to spawn new discoveries and perspectives on contributions of metal nutrients to health, aging, and disease.

Acknowledgements

We thank the NIH (GM 79465, GM 139245, ES 28096), Merck, and Agilent for research support. V.N.P. thanks the National Science Foundation for a graduate research fellowship and was supported by the NIH Chemical Biology Interface Training Grant T32 (GM 066698). C.J.C. is a CIFAR Fellow. All figures were created with BioRender.com.

Biographies

Vanha N. Pham received her B.A. in Chemistry and Chemical Biology in 2017 from Cornell University, where she worked with Prof. Yimon Aye on the development of a spatiotemporal tool for redox signals in bacterial growth. She then joined the University of California, Berkeley Chemical Biology Graduate Program as an NSF Graduate Research Fellow and is completing her Ph.D. in Chemistry with mentor Prof. Chris Chang. She is working on applying multi-omics approaches to decipher the roles of copper and formaldehyde in hepatic signaling and metabolism.

Christopher J. Chang is the Class of 1942 Chair Professor of Chemistry and Molecular and Cell Biology at the University of California, Berkeley, and Faculty Scientist at Lawrence Berkeley National Laboratory. Chris earned B.S./M.S. degrees from Caltech in 1997 and worked with Prof. Harry Gray, spent a year as a Fulbright scholar with Dr. Jean-Pierre Sauvage, and earned his Ph.D. from MIT in 2002 with Prof. Dan Nocera. After postdoctoral studies with Prof. Steve Lippard at MIT, Chris joined the Berkeley faculty in 2004. Research in the Chang laboratory focuses on the study of metals and redox-active molecules in biology and energy. They develop activity-based sensing and proteomics probes and catalysts to address questions in neuroscience, metabolism, and sustainable synthesis.

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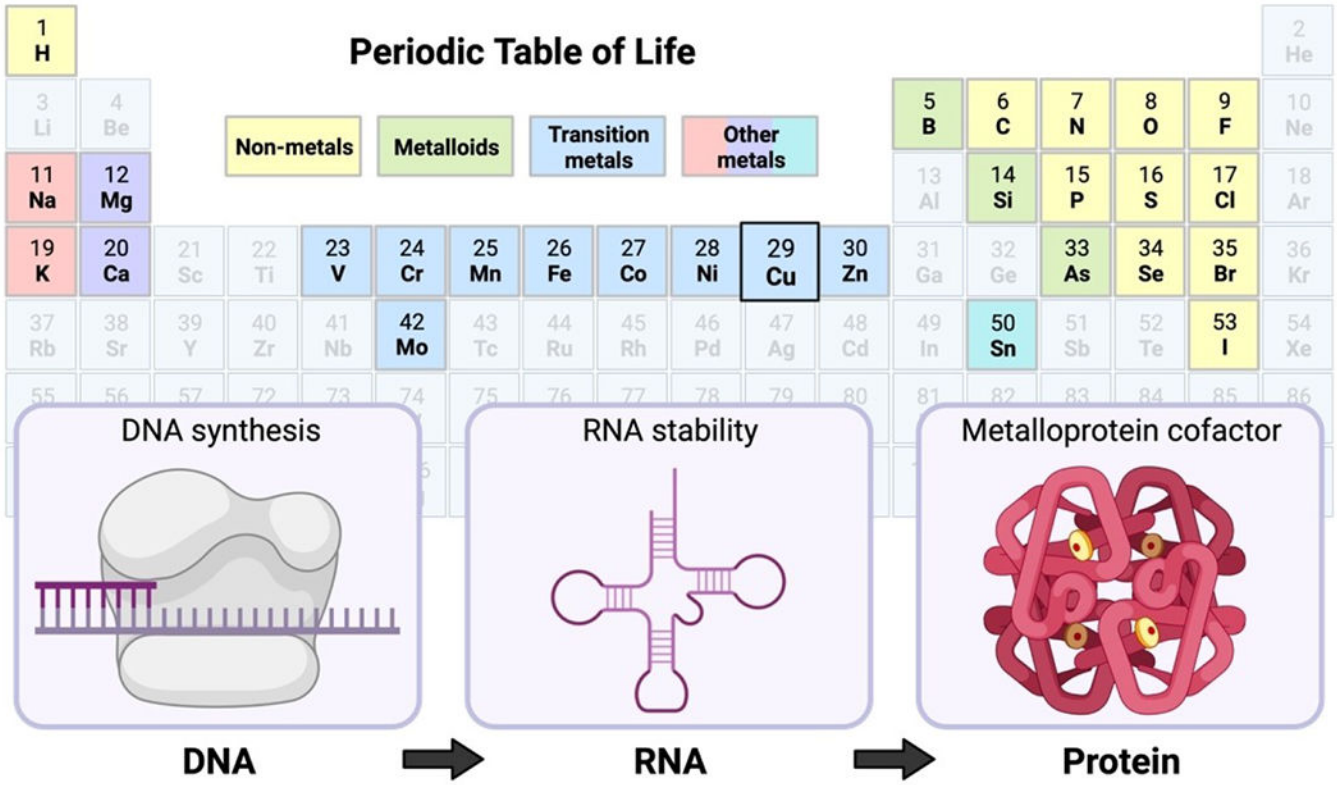

Figure 1.

Metals and the periodic table of life. The periodic table highlighting in color major chemical elements that are essential for life, classified as non-metals (yellow), metalloids (green), transition metals (blue), and other metals (other colors). Metals are prevalent throughout the central dogma of biology and bind widely to DNA, RNA, and proteins. For example, divalent cations are necessary for DNA polymerase to synthesize DNA, tRNA tertiary structure is stabilized by several magnesium ions, and hemoglobin is a metalloprotein that contains iron-dependent heme groups as cofactors. This Minireview focuses on copper as a canonical transition metal nutrient that expands metal–protein interactions beyond active site coordination to include metalloallostery, where copper can activate or inhibit protein function by binding to remote exosites outside the primary active site. Figure was created with BioRender.com.

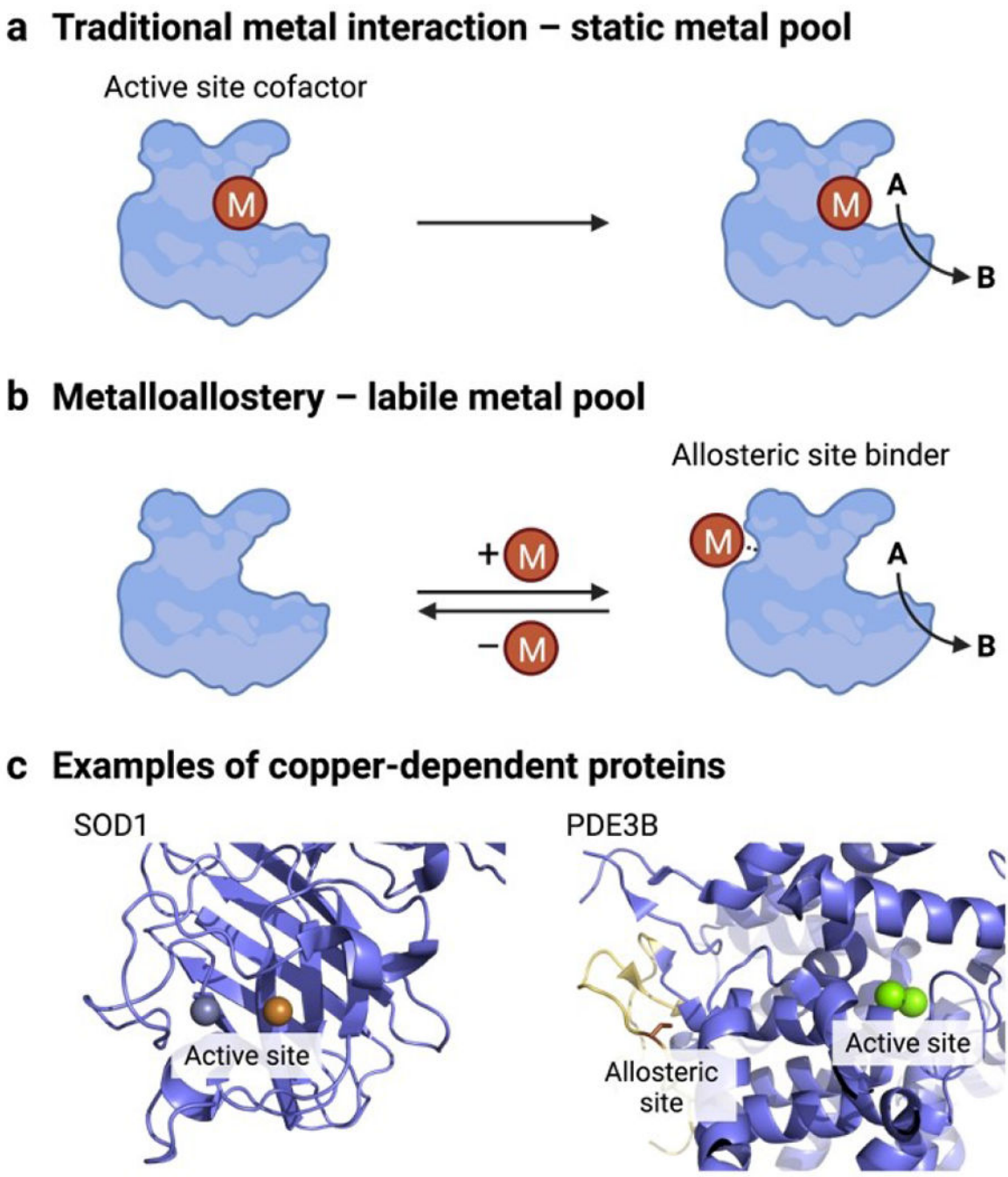

Figure 2.

Metalloproteins classified by the type of metal-binding interaction. a) Traditionally, metalloproteins are defined primarily as proteins with metals bound within active sites to regulate their function. These metals are typically part of the static metal pool because they often bind tightly to protein active sites. b) Metallloallostery represents an emerging class of metal–protein interactions, where dynamic interactions between the metal and protein occur at remote exosites outside the active site to regulate their function. These metals are typically part of the labile metal pool, as the interactions are transient and dynamic. c) Examples

of these different classes of copper-dependent proteins include copper–zinc superoxide dismutase (SOD1, active site) and phosphodiesterase 3B (PDE3B, allosteric site). SOD1: PDB 1PU0,[16] PDE3B: PDB 1SO2.[17] Cu^{I} and PDE3B copper-binding cysteine are shown in brown. Figure was created with BioRender.com.

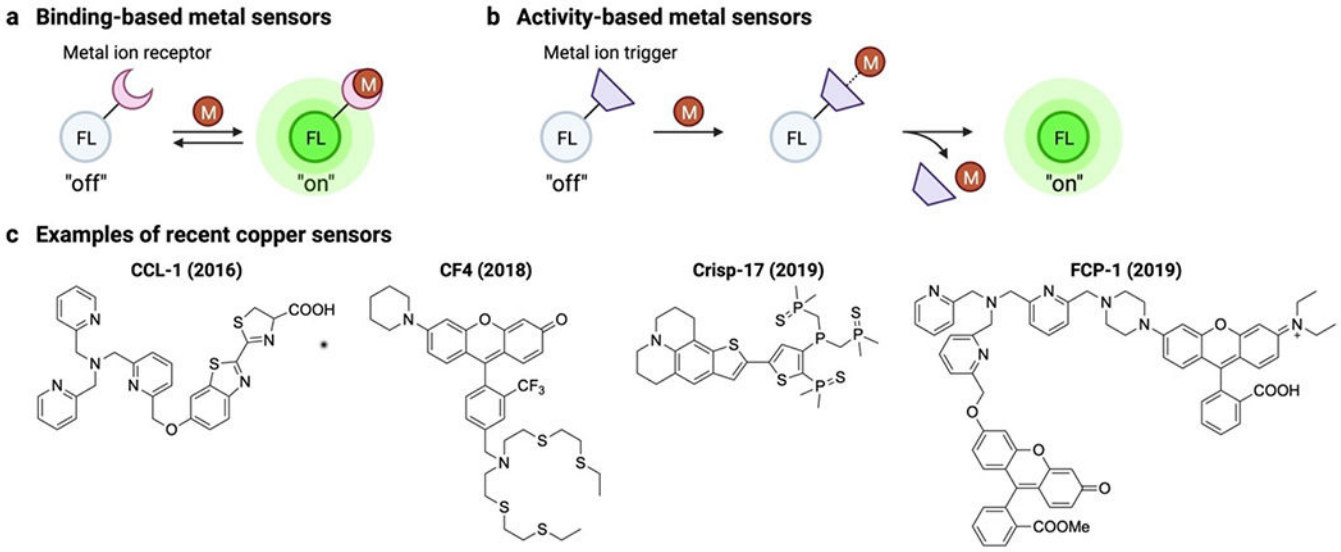

Figure 3.

Two major approaches to designing metal sensors for biological imaging. a) Binding-based sensing utilizes lock-and-key molecular recognition, with a metal ion-specific receptor or chelator for selective detection. Upon metal binding, the fluorophore (FL) is turned “on”, indicating that the metal is sensed. b) Activity-based sensing utilizes a metal ion trigger that promotes a reaction upon metal binding. Upon metal binding with a subsequent tandem reaction, the fluorophore (FL) is turned “on”, indicating that the metal is sensed. c) Representative examples of recent copper sensors with varying copper motifs and applications include Copper-Caged Luciferin-1 (CCL-1),[48] Copper Fluor-4 (CF4),[42] copper ratiometric indicator utilizing stabilized phosphines (crisp-17),[44] and FRET copper probe-1 (FCP-1).[45] Figure was created with BioRender.com.

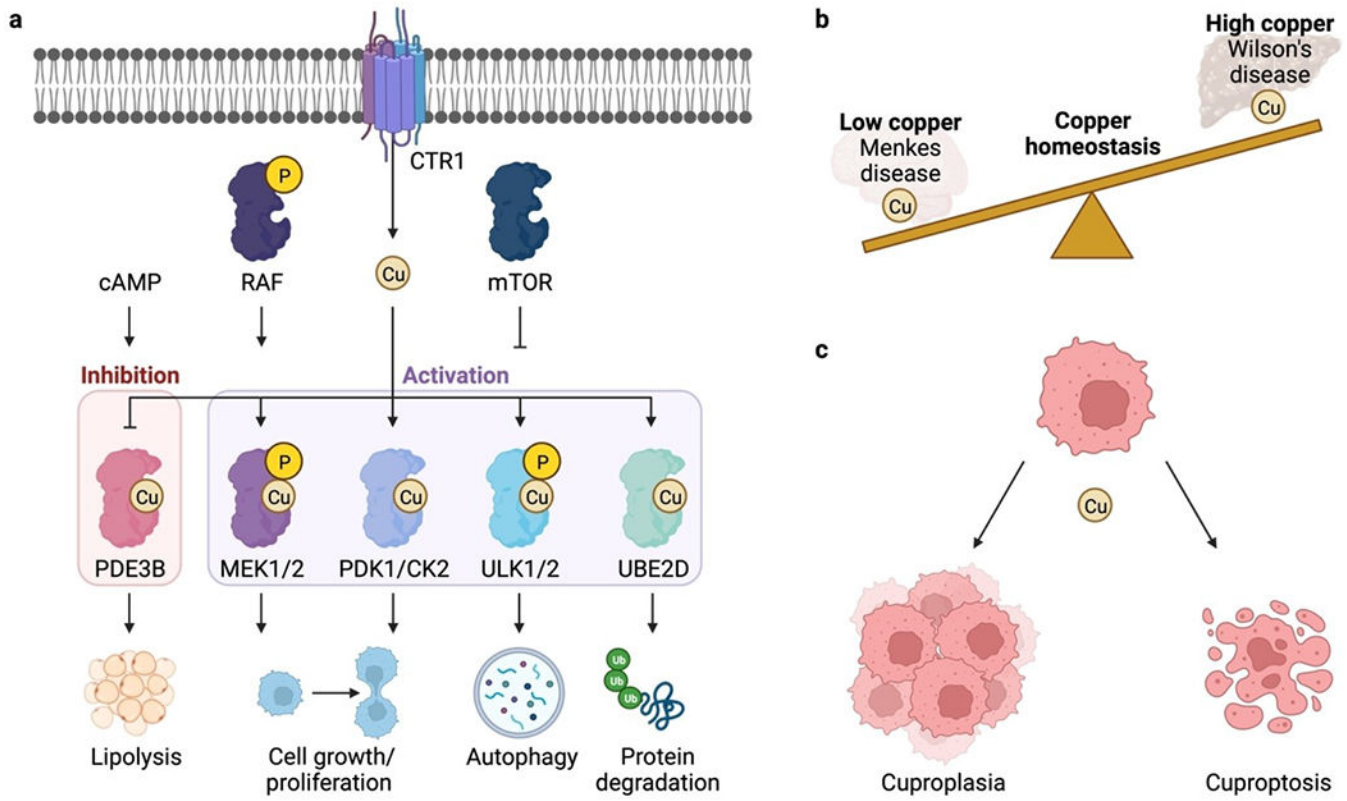

Figure 4.

Copper metalloallostery influences diverse transition metal signaling pathways that drive cell behavior and are connected to diseases spanning obesity and non-alcoholic fatty liver disease (NAFLD) to cancer. a) Targets of copper metalloallostery. As an example of negative allosteric regulation, phosphodiesterase 3B (PDE3B) is inhibited by binding to copper. PDE3B induces cAMP-dependent lipolysis, demonstrating copper regulation of lipid metabolism. Various kinases important to cell health and cancer pathways are activated by copper. In the MAPK/ERK signaling pathway, mitogen-activated protein kinase kinase 1 and 2 (MEK1/2) activity is enhanced by copper and activates extracellular signal-regulated kinase 1 (ERK1). Pyruvate dehydrogenase kinase 1 (PDK1) and casein kinase 2 (CK2) activities are also copper dependent and activate the protein kinase B (PKB, also known as Akt) signaling pathway. Unc51-like kinase 1 and 2 (ULK1/2) drive autophagy and are another example of copper regulation of cell health. The E2 ubiquitin-conjugating enzyme D (UBE2D) family of enzymes are activated by copper to promote ubiquitin-mediated protein degradation, and thus play a role in regulating protein quality and preventing cell death. b) The importance of a balanced copper homeostasis is exemplified by low copper in Menkes disease and high copper in Wilson's disease. c) Cuproplasia and cuproptosis are newly discovered copper-dependent cell proliferation and cell death pathways, respectively, that are metal-dependent disease vulnerabilities that can be exploited for development of new diagnostic and treatment platforms. Figure was created with BioRender.com.

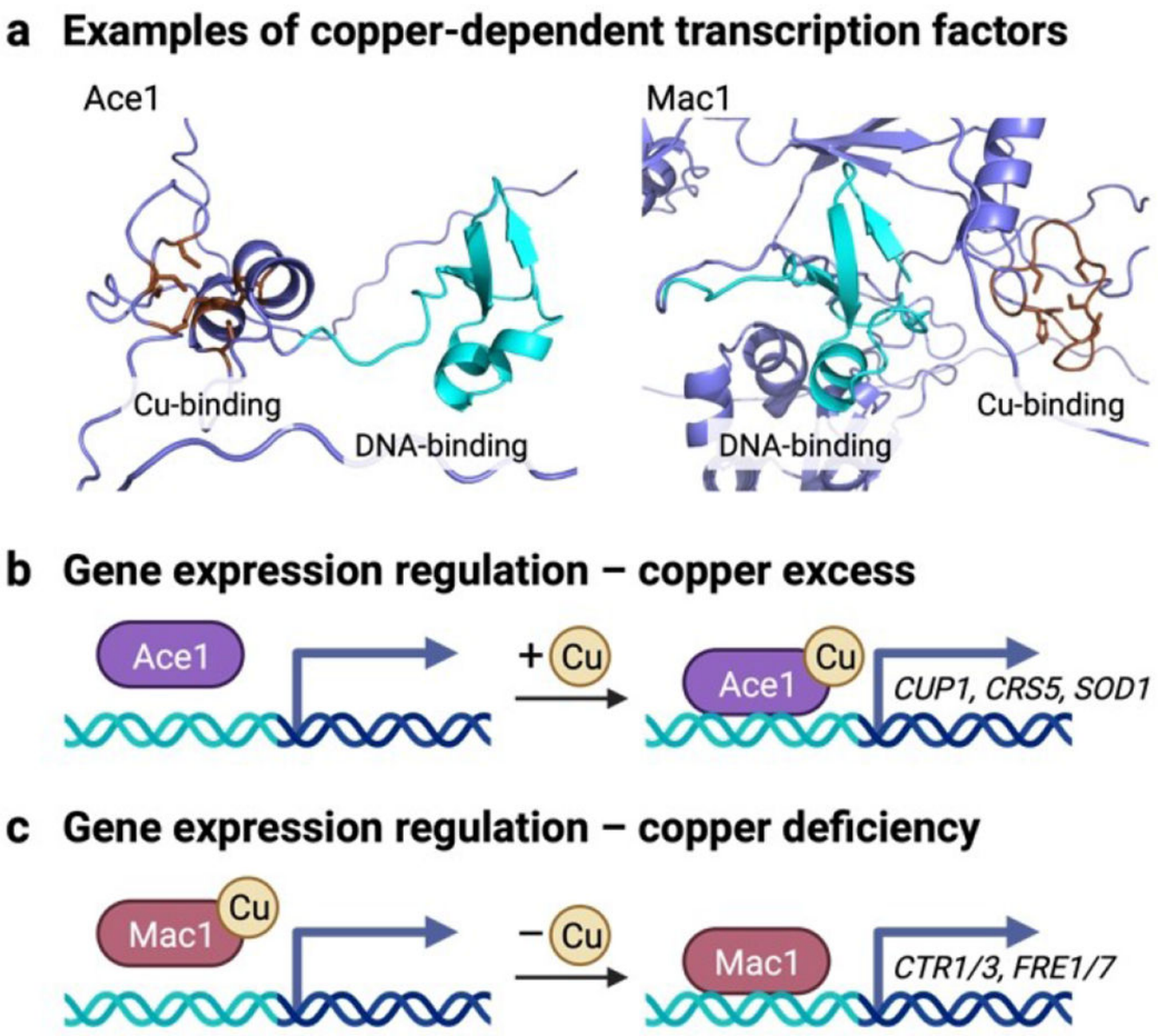

Figure 5.

Copper metalloallostery regulates gene expression through copper-dependent transcription factors. a) AlphaFold[78,79] predicted structures of *S. cerevisiae* transcription factors Ace1 (Uniprot P15315) and Mac1 (Uniprot P35192) with labeled Cu^{I}-binding cysteine motifs (brown) and DNA-binding regions (cyan). b) Ace1 regulation of CUP1, CRS5, and SOD1 expression in response to copper excess. c) Mac1 regulation of CTR1/3 and FRE1/7 expression in response to copper deficiency. Figure was created with BioRender.com.