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Stimulus-Responsive Prochelators for Manipulating Cellular Metals

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CONSPECTUS

Metal ions are essential for a wide range of physiological processes, but they can also be toxic if not appropriately regulated by a complex network of metal trafficking proteins. Intervention in cellular metal distribution with small molecule or peptide chelating agents has promising therapeutic potential to harness metals to fight disease. Molecular outcomes associated with forming metal-chelate interactions *in situ* include altering concentration and subcellular metal distribution, inhibiting metalloenzymes, enhancing the reactivity of a metal species to elicit a favorable biological response, or passivating the reactivity of a metal species to prevent deleterious reactivity. The systemic administration of metal chelating agents, however, raises safety concerns due to the potential risks of indiscriminate extraction of metals from critical metalloproteins and inhibition of metalloenzymes. One can estimate that chelators capable of complexing metal ions with dissociation constants in the sub-micromolar range are thermodynamically capable of extracting metal ions from some metalloproteins and disrupting regular function. Such dissociation constants are easily attainable for multidentate chelators interacting with first-row d-block metal cations in relevant +1, +2, and +3 oxidation states. To overcome this challenge of indiscriminate metal chelation, we have pursued a prodrug strategy for chelating agents in which the resulting “prochelator” has negligible metal binding affinity until a specific stimulus generates a favorable metal binding site. The prochelator strategy enables conditional metal chelation to occur preferentially in spatial or temporal location initiated by disease- or therapy-associated stimuli, thereby minimizing off-target metal chelation. Our design of responsive prochelators encompasses three general approaches of activation: the “removal” approach operates by eliminating a masking group that blocks a potential metal chelation site to reveal the complete binding site under the desired conditions; the molecular “switch” approach involves a reversible conformational change between an inactive and active form of a chelator with differential metal binding affinity under specific conditions; while the “addition” approach adds a new ligand donor arm to the prochelator to constitute a complete metal chelation site. Adopting these approaches, we have created four categories of triggerable prochelators that respond to 1) reactive oxygen species, 2) light, 3) specific enzymes, and 4) biological regulatory events. This account highlights progress from our group on building prochelators that showcase these four categories of responsive metal chelating agents for manipulating cellular metals. The creation and chemical understanding of such stimulus-responsive prochelators enables exciting applications for understanding the cell biology of metals and for developing therapies based on metal-dependent processes in a variety of diseases.

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Graphical abstract

Introduction

Metal ions have unique chemical properties that impart indispensable functions to all forms of life, from archaea to mammals.¹ Nature relies on reversible metal-ligand binding events to propagate biochemical signals, uses Lewis acidic metal centers to facilitate hydrolytic reactivity, and takes advantage of redox-active metal centers to mediate chemical transformations and electron transfers.² Yet, despite their essentiality in biology, excessive or otherwise misregulated metals are also implicated in a range of diseases, from infection and cancer to neurodegenerative and metabolic disorders.³ Given the importance of metal homeostasis for optimal health, intervention of metal trafficking pathways with small molecule or peptide chelating agents provides attractive strategies both to understand fundamentals of biological metal regulation, and potentially to develop novel therapies for hijacking cellular metal machinery to fight disease.

A metal chelating agent, or chelator, refers to a ligand that coordinates to a metal center by multiple points of attachment, thereby forming a ring with the metal atom and affording high thermodynamic stability to the resultant metal-ligand complex. The conventional clinical concept of chelation therapy involves administration of a chelating agent to eliminate transgressing metals from the body, sequestering their pathogenic actions by forming and excreting high-affinity metal complexes. Metal-chelating compounds can have broader biological repercussions and therapeutic benefits beyond toxic metal elimination, however. In an earlier review, we categorized recent advances in expanding these other therapeutic possibilities into four general strategies of how chelating agents can modulate metallobiology.⁴ These strategies include altering metal distribution, inhibiting specific metalloenzymes associated with diseases, enhancing the reactivity of a metal complex to elicit a desired cytotoxic or other favorable reactivity, or alternatively, passivating the reactivity of a metal complex to inhibit cytotoxic or otherwise deleterious reactivity (Figure 1).

While chelation therapy aims to manipulate metal concentration, distribution and reactivity, systemic administration of chelating agents raises safety concerns due to the potential risks of indiscriminate metal depletion or withholding from critical metalloproteins.⁵ The ability of a protein ligand P to retain its metal ion cofactor M can be expressed by a dissociation constant, K_d , defined by the following equilibrium expression:

$$MP \rightleftharpoons M+P, K_d = \frac{[M][P]}{[MP]}$$

From this simple 1:1 equilibrium it is easy to see that the concentration of uncomplexed [M] equals the K_d value when P is half-saturated with M. The smaller the K_d , the better the binder, and ultimately the lower the concentration of uncomplexed [M]. Non-specific sites on protein surfaces are estimated to have K_d values 10^{-6} M, whereas metalloproteins have K_d values below 10^{-7} M.⁶ By this analysis, agents that bind metals tight enough such that uncomplexed [M] is in the sub-micromolar range have the thermodynamic potential to extract metal ions from some metalloproteins.

Tailoring “smart” chelators to a particular metal target without adversely disturbing normal metal balance is the impetus for designing agents that alter their metal binding capacity on command. A prochelator strategy, a prodrug counterpart of chelator, provides pharmacological opportunities to realize the aforementioned therapeutic benefits of metal chelation by restricting the site and timing of metal complexation to a desired set of conditions that stimulate prochelator-to-chelator conversion.

To fulfill the requirements for conditional metal chelation, the general framework of a prochelator includes two functional elements: a hobbled chelation site with negligible metal binding propensity, and a reactive moiety for converting a stimulus into a chemical or configurational modification that allows subsequent metal chelation. We have developed three general approaches for incorporating these two elements into prochelator designs (Figure 2). One approach is to block the chelation site with the reactive moiety (Figure 2a). The protective mask is then removed upon activation by a stimulus to expose the chelation site. A second approach is a molecular switch, where the reactive moiety reversibly switches configuration between inactive and active states with differential metal binding properties (Figure 2b). Thirdly, addition of an extra anchor for metal binding can dramatically improve thermodynamic stability of a complex (Figure 2c). In these cases, the prochelator contains an incomplete binding site and the reactive moiety is positioned to generate a new metal-ligand interaction in response to the stimulus.

By adopting these three construction approaches, we have created four categories of conditionally activated prochelators that respond to 1) reactive oxygen species, 2) light, 3) specific enzymes and 4) biological regulatory events. In the following sections, we expand on these categories with representative examples of our general considerations in designing prochelators for manipulating cellular metals.

1. Prochelators responsive to reactive oxygen species (ROS)

Oxidative stress, which manifests from unmitigated reactive oxygen species (ROS), has been implicated in a range of diseases, including cancer, cardiovascular and neurodegenerative diseases, and others.⁷ Metal ions that are capable of redox cycling within the cellular milieu can catalyze ROS formation through Fenton-like reactions in which highly reactive hydroxyl radicals are generated from hydrogen peroxide (H_2O_2). Notably, Fe and Cu can perpetuate oxidative stress if they are not bound by a protective protein or other ligands that dampen $\text{Fe}^{3+/2+}$ or $\text{Cu}^{2+/+}$ redox cycling.

Recognizing that redox-active metals and H_2O_2 contribute to oxidative stress, our strategy for designing ROS-responsive prochelators relies on the peroxide-mediated transformation of aryl boronates to phenols.⁸ This biocompatible reaction has also been developed for fluorescence sensing of H_2O_2 and peroxynitrite,^{9,10} and has been extended to ROS-activated metalloenzyme inhibitors.¹¹ Phenols are prevalent functionalities in multidentate chelators, as phenolate oxygens are attractive donor atoms for metal cations, especially Fe^{3+} . Installation of a boronate mask effectively blocks the chelation moiety, affording a prochelator with negligible or low metal affinity. The selective removal of boronates by peroxide and peroxynitrite therefore directs chelator release and metal binding events preferentially to a local environment with persistent and elevated H_2O_2 concentrations. Figure 3 showcases a series of boronate-masked prochelators we developed based on the “removal” strategy for conditionally passivating Fe and Cu against Fenton reactivity.^{12–16}

1.1 First- and second-generation prochelators for H_2O_2 -triggered Fe binding—

Our first-generation design, BSIH, contains a well-studied aroylhydrazone chelator SIH (salicylaldehyde isonicotinoyl hydrazone)¹⁷ with a boronic acid pinacol ester in place of the phenolic oxygen.¹² As a membrane permeable, tridentate chelator, SIH incapacitates redox-active Fe in cell culture.^{18–21} High doses or prolonged exposure, however, exacerbate SIH cytotoxicity in several cell lines, which may be associated with indiscriminate metal depletion.^{22,23} In contrast, prochelator BSIH, with little metal affinity, has been shown to be non-toxic to retinal pigment epithelial cells and rat cardiomyocytes, while providing significant cytoprotection against cellular oxidative damage induced by hydrogen peroxide.^{22,23} This favorable biological profile of BSIH is likely a benefit from the boronate protecting mask, which restricts active SIH release and redox-active Fe sequestration to oxidatively-stressed sites.

A potential limitation of the SIH scaffold, however, is its short half-life in cell culture media and plasma due to hydrolytic instability of its labile hydrazone bond.²⁴ While BSIH is more stable in vitro compared to SIH, it fails to yield a full complement of SIH upon reaction with H_2O_2 in cellular contexts.²⁵ We therefore developed a later-generation prochelator based on the SIH analogue, HAPI, which demonstrates increased resistance to hydrolysis in plasma.²⁶ To create BHAPI, we introduced a self-immolative linker that undergoes spontaneous 1,6-benzyl elimination upon H_2O_2 activation to release HAPI.¹⁴ BHAPI exhibits superior hydrolytic stability and prochelator-to-chelator conversion rates compared to BSIH. Evidence in retinal pigment epithelial cells indicates that BHAPI does not perturb the Fe regulatory machinery in non-stressed cells, unlike its parent chelator HAPI.¹⁴ Furthermore,

BHAPI was shown to protect cells from damage by paraquat, an herbicide that promotes cellular oxidative stress but is not itself an oxidant.

We applied a similar self-immolative linkage strategy to create TIP as another hydrolytically stable prochelator. TIP is based on a triazole framework of the clinically used agent ICL670A (Exjade[®], deferasirox).¹⁵ The lipophilicity and strong iron affinity of ICL670A contribute to its oral availability and its accessibility to cardiac iron for treating transfusional iron overload.²⁷ Nevertheless, these properties may also increase the risks of undesirable metal binding or extraction from key metalloproteins, which is speculated to cause cytotoxicity in several cell lines.^{5,19,23} By masking one of its phenol groups, the prochelator version TIP indeed shows peroxide-induced iron chelation activity in vitro. Unfortunately, this property did not translate to improved activity in cell culture, and TIP itself was found to be cytotoxic on its own.^{15,23}

Despite our attempts to “improve” the framework of these tridentate iron chelators and prochelators, a direct comparison of BSIH, BHAPI, TIP and their respective chelators to protect rat cardiomyocytes against H₂O₂-induced toxicity revealed the original BSIH as having the most favorable combination of low inherent toxicity with significant protection against oxidative insult (Figure 4).²³ We speculate that the self destruction of the SIH core may in fact be favorable for mitigating local iron-induced damage, while not allowing a high-affinity metal chelator to persist in the absence of labile, redox-active iron.

1.2 ROS-activated prochelators based on 8-hydroxyquinoline—Derivatives of 8-hydroxyquinoline (8HQ) comprise a compelling class of chelating agents with broad pharmacological applicability against cancer, infection, and neurodegeneration.^{28–30} The ability of 8HQ derivatives to form lipophilic complexes with Cu²⁺ and Zn²⁺ that translocate across cell membranes to exert biological activity has been well documented.^{30,31} Cytotoxicity of 8HQ-Cu combinations, however, can be general and is not restricted to cells associated with disease. In an effort to mitigate the off-target effects associated with hydroxyquinolines, we investigated our prochelator strategy as a way to generate the active pharmacophore preferentially under disease-like conditions.

In the realm of neurodegeneration, clioquinol and PBT2 are two 8HQ derivatives that showed early promise in Alzheimer’s disease.³² The mechanism of action of these bidentate ligands is thought to involve their chaperone-like activity to redistribute extracellular Cu²⁺ and Zn²⁺ from the amyloid beta (A β) plaques back into the cell with concomitant activation of neuroprotective cell signaling pathways.³³ The association of ROS formation induced by deviant Cu-A β peptide interactions inspired our design of a prochelator named QBP that converts to 8HQ in response to H₂O₂ activation.¹³ Under conditions that mimic early Alzheimer’s pathology involving Cu²⁺, A β peptides and biological reductants that exacerbate ROS generation, QBP is unmasked to release 8HQ, which subsequently chelates Cu²⁺ away from A β aggregates and quenches Cu-mediated ROS formation (Figure 5).

Besides the capability of QBP to commandeer metal ions in A β pathology, the utility of Cu as an antimicrobial agent inspired us to exploit the novel application of this prochelator against intracellular pathogens.³⁴ While the underlying mechanisms are still being

elucidated, an emerging model of infection posits that activated macrophage cells of the innate immune system concentrate Cu into phagosomes to facilitate microbial killing.³⁵ We reasoned that Cu ionophores like 8HQ could synergize with this inherent Cu response of the immune system. Furthermore, phagocytes initiate a burst of ROS in response to infection. We therefore investigated the ability of QBP to leverage these two aspects of the immune response to defend against the opportunistic fungal pathogen *Cryptococcus neoformans*.³⁶ While QBP remains non-toxic to the host immune cells, its active form 8HQ induces Cu-dependent fungicidal activity *in vitro* and in a mouse pulmonary infection model (Figure 6).³⁶ Notably, the ionophoric activity of 8HQ enables Cu delivery into the fungal cells, which overcomes the Cu-resistance mechanisms of *C. neoformans* to exert antifungal activity. The targeted antimicrobial activity of QBP provides a new approach to harness Cu mobilization in combination with the oxidative burst for microbial killing, which appears promising for the future treatment of fungal infections.³⁷

Given the effectiveness of QBP, we are interested in tracking its presence and transformation within cells or animals. A multifunctional reporter that imparts a fluorescent signal upon 8HQ release would be useful to probe its spatial and temporal localization for further mechanistic investigations. We therefore introduced another 8HQ-based prochelator, BCQ, that contains a *cis*-cinnamate protecting group linked to the self-immolative boronic mask (Figure 7a).³⁸ Upon peroxide stimulation, deprotection of BCQ releases equimolar quantities of 8HQ and coumarin fluorophore, which in principle would enable real-time monitoring of prochelator activation. While improvements to the sensitivity and release kinetics are desirable for such applications, this multifunctional design represents a first attempt to incorporate stimulus response, visual readout, and active compound release in a single scaffold.

2. Prochelators responsive to light

Using light as an external stimulus to produce a biologically active molecule allows transformation of an inactive prodrug to its active form with spatial and temporal control, which has shown considerable potential to treat skin-related diseases and certain types of cancer.³⁹ In efforts to embrace both phototrigger and prospective metal chelation functionalities, we have developed prochelators with potential metal binding sites that can either be irreversibly activated by light or reversibly switched between inactive and active states.

A prochelator “removal” strategy was previously reported for prochelators based on aroylhydrazones, SIH and PIH with (*o*-nitrobenzyl)ethyl masking groups that respond to UVA irradiation to release the active metal chelators, along with potentially cytotoxic nitrosoketone byproducts.⁴⁰ In our search for a favorable unmasking strategy with release of functional yet nontoxic components, we adopted a *trans*-(*o*-hydroxy)cinnamate ester photocleavable masking group that transforms into a naturally occurring coumarin photoproduct (Figure 7b). The resulting multifunctional prochelator PC-HAPI alleviates both direct photodamage and metal-catalyzed oxidative stress in UVA-irradiated cells.⁴¹ It responds readily to UVA exposure, releasing two active components: the aroylhydrazone metal chelator HAPI and the nontoxic coumarin umbelliferone. HAPI sequesters redox-

active Fe to protect retinal pigment epithelial cells from UVA damage, whereas the fluorescent byproduct umbelliferone exhibits a strong absorption profile in the UVA range that further reduces the intensity of damaging radiation in the surrounding biological milieu.

An alternative to the “removal” approach is to put chelating molecules under the control of a bi-stable photochromic “switch” such that only one photoisomer presents an optimal metal binding site.⁴² Hydrazones are known to display reversible photoisomerization, and we identified HAPI as a dual-wavelength photoswitching molecule.⁴³ In its resting equilibrium, HAPI exists predominantly as the *E* isomer, which is preorganized for chelation of di- and trivalent metal ions. Irradiation with UVA light, however, favors the *Z* photoisomer, which has reduced metal affinity. UVC light or thermal relaxation triggers the reversion to the *E* configuration (Figure 8). Furthermore, binding of the *E* isomer to Fe³⁺ or Cu²⁺ prevents photoisomerization, thereby providing tripartite control over the system. HAPI therefore represents a unique example of a dual-wavelength, reversible photoswitching metal chelator, which may provide desirable properties for phototriggered applications.

3. Prochelators responsive to enzymatic activity

Enzymatic activation provides an intriguing strategy for inducing chelator release in response to an enzyme that is uniquely upregulated in a target disease. Our first creation focused on the enzymatic formation of the insoluble amyloid beta (A β)-rich plaques that are hallmarks of Alzheimer’s disease. The A β peptide derives from the transmembrane amyloid precursor protein (APP) after sequential cleavage by β - and γ -secretases. Genetic studies correlating β -secretase (BACE) activity to increased plaque loads and reduced cognitive abilities implicated BACE as a therapeutic target and potential biomarker of the disease.⁴⁴ As we saw earlier in the 8HQ example, appropriate Cu²⁺ chelators can inhibit the ROS-generating reactivity of Cu²⁺-A β complexes. In order to merge a Cu²⁺ chelating motif with a BACE recognition substrate, we modified the known Swedish mutant sequence of APP with a key histidine to create a prochelator peptide, nicknamed “SWH” with the sequence EVNLDHFWADR (Figure 9a).⁴⁵ SWH is cleaved between the leucine and aspartic acid residues upon reaction with BACE to yield a chelating peptide (“CP”) fragment known as an ATCUN motif (amino terminal copper and nickel binder).⁴⁵ In principle, this enzyme-triggered design allows site-specific passivation of Cu redox reactivity as A β is being processed from APP, and therefore prior to plaque formation. By elaborating the SWH sequence with a cholesterol cell membrane anchor and a FRET (Förster resonance energy transfer) donor/acceptor pair, we further developed a β -secretase membrane-anchored probe (β -MAP) that monitors real-time BACE activity in living cells (Figure 9b and c).⁴⁶

Another promising application of enzyme-responsive prochelators arises from exploiting the unique enzymatic reactivity and metallobiology associated with antibiotic resistant organisms. Bacterial production of β -lactamase enzymes is a major mechanism of drug resistance that deactivates the broad class of β -lactam antibiotics that includes the extensively used cephalosporins and carbapenems. Cephalosporin derivatives can be used as reactive moieties susceptible to β -lactamase activity for release of cytotoxic agents, including the O, S bidentate chelator pyrithione.⁴⁷ Pyrithione has been found to synergize with copper to exert broad-spectrum antibacterial activity, however, its toxicity is not

restricted to pathogenic microbes.⁴⁸ We recently showed that cephalosporin prochelator DB4-51 provides a strategy to activate the copper-binding and cytotoxic activity of pyrrhione preferentially to drug-resistant bacteria that produce β -lactamases (Figure 10).⁴⁹

4. Peptide prochelators activated by biological regulatory events

Protein phosphorylation is a post-translational modification important for cellular signaling and regulatory processes. By covalent and enzymatically reversible addition of phosphoryl groups to particular amino acid residues, phosphorylation enables regulation of protein activity, localization and protein-protein interactions.⁵⁰ Importantly for our purposes, phosphorylation may also change the metal binding propensity of a protein, especially if the reaction sites are appropriately positioned among other metal-binding residues. By using Tb^{3+} as a luminescent probe, we identified that the phosphorylation status of a 14-residue peptide fragment of α -synuclein, a protein implicated in Parkinson's disease, dramatically alters its metal binding affinity (Figure 11).⁵¹ While the prochelator peptide of α -syn (residues 119–132) and its phosphoserine congener pS129 show negligible metal affinity, phosphorylation at tyrosine residue pY125 provides a critical anchor for coordination with trivalent metal ions like Tb^{3+} , Al^{3+} and Fe^{3+} , providing our first illustration of a prochelator activated by the “addition” approach. This phosphorylation-dependent metal binding further prompts conformational change and dimerization of the peptide fragment, which intimates the potential for post-translational modifications and metal ion binding to affect the pathogenic aggregation of full-length α -synuclein or other phosphorylated proteins. Moreover, principles elucidated in the α -syn peptide study have helped inform subsequent optimization of constructs intentionally designed to take advantage of phosphorylation-dependent switching in metal chelation and luminescence sensitization to create fluorescent reporters of kinase and phosphatase activity.⁵²

Allostery is another exquisite regulatory mechanism in biology. In these cases, the binding of one species to a protein influences the binding or reactivity of another species to a distinct receptor site elsewhere on the protein. This principle of allosteric receptor modulation inspired our effort to develop a conditionally switchable prochelator peptide that alters its propensity to bind one metal in response to dynamic changes in the concentration of a second metal. Composed of 25 native amino acids, the prochelator peptide (PCP) harbors two distinct binding motifs for two distinct metal ions: Tb^{3+} and Zn^{2+} . The Tb^{3+} in this case is used as a higher valent and luminescent surrogate to the more biologically relevant Ca^{2+} . To create PCP, we flanked a lanthanide binding tag sequence (which was itself evolved from classic calcium-binding loops) by two separated halves of a Cys_2His_2 motif reminiscent of classic zinc fingers.⁵³ The cooperative, allosteric metal-peptide interaction enables the resulting PCP to self assemble into a heterometallic species in which the chelation of one metal ion induces a conformational change and enhances the affinity for another, and *vice versa* (Figure 12). PCP forms a 1:2 complex with Zn^{2+} at pH 7.4 in which the addition of Tb^{3+} increases its affinity for Zn^{2+} by 3-fold ($\log \beta_2$ from 13.8 to 14.3), whereas the 1:1 luminescent complex with Tb^{3+} is brighter, longer lived and 20-fold tighter in the presence of Zn^{2+} ($\log K$ from 6.2 to 7.5 and luminescence lifetime from 1.3 to 2.4 ms). PCP represents another construct of molecular switches for metal chelation. This unique example of positive, heterometallic allostery implements the design of an artificial receptor system in

a biologically compatible framework where both the effector and substrate species are metal ions. Even though our initial concept has been exemplified with Tb^{3+} and Zn^{2+} as allosteric partners, the modular peptide template can be customized for targeting other metals. Such models will be valuable in future investigation of heterometallic allostery for controlling free metal ion concentrations in complex biological environments, where dynamic fluctuations in various metal concentrations influence function.

Conclusion and outlook

In summary, the prochelator strategy has been developed to overcome undesirable metal localization, depletion and toxicity of chelating agents by incapacitating metal binding until specific stimuli activate the chelation site. While studies to date have shown many successes in vitro and in cell culture studies, challenges for the future include rigorously determining mechanism of action of these agents in complex cell, tissue and whole animal models to test hypotheses about the roles of metals and chelating agents in influencing cellular processes. Additional challenges include discovering unique interactions between stimuli and novel reactive moieties for developing new classes of prochelators. Recent advances in cell biology offer direct accessibility to critical enzymes and carrier proteins, with detailed molecular and functional characteristics readily available for identifying unique biomarkers as new disease- or therapy-related stimuli. The creation and chemical understanding of these stimulus-responsive metal manipulators may illuminate how small molecules can influence the complex interactions between the genome, the proteome and the metallome, and may enable exciting future applications in targeting a variety of diseases by targeting their metal-dependent processes.

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References

1. Haas KL, Franz KJ. Application of Metal Coordination Chemistry to Explore and Manipulate Cell Biology. *Chem Rev.* 2009; 109:4921–4960. [PubMed: 19715312]
2. Bertini, I.Gray, HB.Stiefel, EI., Valentine, JS., editors. *Biological Inorganic Chemistry: Structure and Reactivity.* University Science Books; Sausalito, CA: 2007.
3. Sigel, A.Sigel, H., Sigel, RKO., editors. *Interrelations between Essential Metal Ions and Human Diseases.* Vol. 13. Springer Netherlands; Dordrecht: 2013.
4. Franz KJ. Clawing Back: Broadening the Notion of Metal Chelators in Medicine. *Curr Opin Chem Biol.* 2013; 17:143–149. [PubMed: 23332666]
5. Hasinoff BB, Patel D, Wu X. The Oral Iron Chelator ICL670A (Deferasirox) Does Not Protect Myocytes against Doxorubicin. *Free Radic Biol Med.* 2003; 35:1469–1479. [PubMed: 14642395]
6. Xiao ZG, Wedd AG. The Challenges of Determining Metal-Protein Affinities. *Nat Prod Rep.* 2010; 27:768–789. [PubMed: 20379570]
7. Halliwell B. Oxidative Stress and Neurodegeneration: Where Are We Now? *J Neurochem.* 2006; 97:1634–1658. [PubMed: 16805774]
8. Kuivila HG. Electrophilic Displacement Reactions 3: Kinetics of the Reaction between Hydrogen Peroxide and Benzeneboronic Acid. *J Am Chem Soc.* 1954; 76:870–874.

9. Miller EW, Albers AE, Pralle A, Isacoff EY, Chang CJ. Boronate-Based Fluorescent Probes for Imaging Cellular Hydrogen Peroxide. *J Am Chem Soc.* 2005; 127:16652–16659. [PubMed: 16305254]
10. Zielonka J, Sikora A, Hardy M, Joseph J, Dranka BP, Kalyanaraman B. Boronate Probes as Diagnostic Tools for Real Time Monitoring of Peroxynitrite and Hydroperoxides. *Chem Res Toxicol.* 2012; 25:1793–1799. [PubMed: 22731669]
11. Jourden JLM, Cohen SM. Hydrogen Peroxide Activated Matrix Metalloproteinase Inhibitors: A Prodrug Approach. *Angew Chem Int Ed.* 2010; 49:6795–6797.
12. Charkoudian LK, Pham DM, Franz KJ. A Pro-Chelator Triggered by Hydrogen Peroxide Inhibits Iron-Promoted Hydroxyl Radical Formation. *J Am Chem Soc.* 2006; 128:12424–12425. [PubMed: 16984186]
13. Dickens MG, Franz KJ. A Prochelator Activated by Hydrogen Peroxide Prevents Metal-Induced Amyloid β Aggregation. *ChemBioChem.* 2010; 11:59–62. [PubMed: 19937900]
14. Kielar F, Helsel ME, Wang Q, Franz KJ. Prochelator BHAPI Protects Cells against Paraquat-Induced Damage by ROS-Triggered Iron Chelation. *Metallomics.* 2012; 4:899–909. [PubMed: 22700084]
15. Kielar F, Wang Q, Boyle PD, Franz KJ. A Boronate Prochelator Built on a Triazole Framework for Peroxide-Triggered Tridentate Metal Binding. *Inorg Chim Acta.* 2012; 393:294–303.
16. Leed MG, Wolkow N, Pham DM, Daniel CL, Dunaief JL, Franz KJ. Prochelators Triggered by Hydrogen Peroxide Provide Hexadentate Iron Coordination to Impede Oxidative Stress. *J Inorg Biochem.* 2011; 105:1161–1172. [PubMed: 21708101]
17. Ponka P, Borova J, Neuwirt J, Fuchs O. Mobilization of Iron from Reticulocytes - Identification of Pyridoxal Isonicotinoyl Hydrazone as a New Iron Chelating Agent. *FEBS Lett.* 1979; 97:317–321. [PubMed: 761636]
18. Horackova M, Ponka P, Byczko Z. The Antioxidant Effects of a Novel Iron Chelator Salicylaldehyde Isonicotinoyl Hydrazone in the Prevention of H_2O_2 Injury in Adult Cardiomyocytes. *Cardiovasc Res.* 2000; 47:529–536. [PubMed: 10963725]
19. Bendova P, Mackova E, Haskova P, Vavrova A, Jirkovsky E, Sterba M, Popelova O, Kalinowski DS, Kovarikova P, Vavrova K, Richardson DR, Simunek T. Comparison of Clinically Used and Experimental Iron Chelators for Protection against Oxidative Stress-Induced Cellular Injury. *Chem Res Toxicol.* 2010; 23:1105–1114. [PubMed: 20521781]
20. Kurz T, Gustafsson B, Brunk UT. Intralysosomal Iron Chelation Protects against Oxidative Stress-Induced Cellular Damage. *FEBS J.* 2006; 273:3106–3117. [PubMed: 16762036]
21. Simunek T, Boer C, Bouwman RA, Vlasblom R, Versteilen AM, Sterba M, Gersl V, Hrdina R, Ponka P, de Lange JJ, Paulus WJ, Musters RJ. SIH-a Novel Lipophilic Iron Chelator--Protects H9c2 Cardiomyoblasts from Oxidative Stress-Induced Mitochondrial Injury and Cell Death. *J Mol Cell Cardiol.* 2005; 39:345–354. [PubMed: 15978614]
22. Charkoudian LK, Dentchev T, Lukinova N, Wolkow N, Dunaief JL, Franz KJ. Iron Prochelator BSIH Protects Retinal Pigment Epithelial Cells against Cell Death Induced by Hydrogen Peroxide. *J Inorg Biochem.* 2008; 102:2130–2135. [PubMed: 18835041]
23. Jansova H, Machacek M, Wang Q, Haskova P, Jirkovska A, Potuckova E, Kielar F, Franz KJ, Simunek T. Comparison of Various Iron Chelators and Prochelators as Protective Agents against Cardiomyocyte Oxidative Injury. *Free Radic Biol Med.* 2014; 74:210–221. [PubMed: 24992833]
24. Buss JL, Ponka P. Hydrolysis of Pyridoxal Isonicotinoyl Hydrazone and Its Analogs. *Biochim Biophys Acta.* 2003; 1619:177–186. [PubMed: 12527114]
25. Jansova H, Bures J, Machacek M, Haskova P, Jirkovska A, Roh J, Wang Q, Franz KJ, Kovarikova P, Simunek T. Characterization of Cytoprotective and Toxic Properties of Iron Chelator SIH, Prochelator BSIH and Their Degradation Products. *Toxicology.* 2016; 350:15–24. [PubMed: 27046792]
26. Hruskova K, Kovarikova P, Bendova P, Haskova P, Mackova E, Stariat J, Vavrova A, Vavrova K, Simunek T. Synthesis and Initial in Vitro Evaluations of Novel Antioxidant Aroylhydrazone Iron Chelators with Increased Stability against Plasma Hydrolysis. *Chem Res Toxicol.* 2011; 24:290–302. [PubMed: 21214215]

27. Al-Rousan RM, Paturi S, Laurino JP, Kakarla SK, Gutta AK, Walker EM, Blough ER. Deferasirox Removes Cardiac Iron and Attenuates Oxidative Stress in the Iron-Overloaded Gerbil. *Am J Hematol*. 2009; 84:565–570. [PubMed: 19650117]
28. Song, Yn, Xu, H., Chen, W., Zhan, P., Liu, X. 8-Hydroxyquinoline: A Privileged Structure with a Broad-Ranging Pharmacological Potential. *MedChemComm*. 2015; 6:61–74.
29. Block SS. Fungitoxicity of 8-Quinolinols. *J Agric Food Chem*. 1955; 3:229–234.
30. Tardito S, Bassanetti I, Bignardi C, Elviri L, Tegoni M, Mucchino C, Bussolati O, Franchi-Gazzola R, Marchio L. Copper Binding Agents Acting as Copper Ionophores Lead to Caspase Inhibition and Paraptotic Cell Death in Human Cancer Cells. *J Am Chem Soc*. 2011; 133:6235–6242. [PubMed: 21452832]
31. Li CH, Wang JA, Zhou B. The Metal Chelating and Chaperoning Effects of Clioquinol: Insights from Yeast Studies. *J Alzheimers Dis*. 2010; 21:1249–1262. [PubMed: 21504115]
32. Bush AI. Drug Development Based on the Metals Hypothesis of Alzheimer's Disease. *J Alzheimers Dis*. 2008; 15:223–240. [PubMed: 18953111]
33. Crouch PJ, Barnham KJ. Therapeutic Redistribution of Metal Ions to Treat Alzheimer's Disease. *Acc Chem Res*. 2012; 45:1604–1611. [PubMed: 22747493]
34. Borkow G, Gabbay J. Copper as a Biocidal Tool. *Curr Med Chem*. 2005; 12:2163–2175. [PubMed: 16101497]
35. Hodgkinson V, Petris MJ. Copper Homeostasis at the Host-Pathogen Interface. *J Biol Chem*. 2012; 287:13549–13555. [PubMed: 22389498]
36. Festa RA, Helsel ME, Franz KJ, Thiele DJ. Exploiting Innate Immune Cell Activation of a Copper-Dependent Antimicrobial Agent During Infection. *Chem Biol*. 2014; 21:977–987. [PubMed: 25088681]
37. Cavet, Jennifer S. Copper as a Magic Bullet for Targeted Microbial Killing. *Chem Biol*. 2014; 21:921–922. [PubMed: 25126988]
38. Franks AT, Franz KJ. A Prochelator with a Modular Masking Group Featuring Hydrogen Peroxide Activation with Concurrent Fluorescent Reporting. *Chem Commun*. 2014; 50:11317–11320.
39. Spring BQ, Rizvi I, Xu N, Hasan T. The Role of Photodynamic Therapy in Overcoming Cancer Drug Resistance. *Photochem Photobiol Sci*. 2015; 14:1476–1491. [PubMed: 25856800]
40. Yiakouvaki A, Savovic J, Al-Qenaei A, Dowden J, Pourzand C. Caged-Iron Chelators a Novel Approach Towards Protecting Skin Cells against UVA-Induced Necrotic Cell Death. *J Invest Dermatol*. 2006; 126:2287–2295. [PubMed: 16710308]
41. Franks AT, Wang Q, Franz KJ. A Multifunctional, Light-Activated Prochelator Inhibits UVA-Induced Oxidative Stress. *Bioorg Med Chem Lett*. 2015; 25:4843–4847. [PubMed: 26152427]
42. Shinkai S, Shigematsu K, Sato M, Manabe O. Photoresponsive Crown Ethers.6. Ion-Transport Mediated by Photoinduced *cis*—*trans* Interconversion of Azobis(Benzocrown Ethers). *J Chem Soc, Perkin Trans*. 1982; 1:2735–2739.
43. Franks AT, Peng D, Yang W, Franz KJ. Characterization of a Photoswitching Chelator with Light-Modulated Geometric, Electronic, and Metal-Binding Properties. *Inorg Chem*. 2014; 53:1397–1405. [PubMed: 24428136]
44. Evin G, Barakat A, Masters CL. Bace: Therapeutic Target and Potential Biomarker for Alzheimer's Disease. *Int J Biochem Cell Biol*. 2010; 42:1923–1926. [PubMed: 20817005]
45. Folk DS, Franz KJ. A Prochelator Activated by β -Secretase Inhibits A β Aggregation and Suppresses Copper-Induced Reactive Oxygen Species Formation. *J Am Chem Soc*. 2010; 132:4994–4995. [PubMed: 20297791]
46. Folk DS, Torosian JC, Hwang S, McCafferty DG, Franz KJ. Monitoring β -Secretase Activity in Living Cells with a Membrane-Anchored FRET Probe. *Angew Chem Int Ed*. 2012; 51:10795–10799.
47. O'Callaghan CH, Sykes RB, Staniforth SE. New Cephalosporin with a Dual Mode of Action. *Antimicrob Agents Chemother*. 1976; 10:245–248. [PubMed: 984765]
48. Chandler CJ, Segel IH. Mechanism of the Antimicrobial Action of Pyrrithione: Effects on Membrane Transport, ATP Levels, and Protein Synthesis. *Antimicrob Agents Chemother*. 1978; 14:60–68. [PubMed: 28693]

49. Besse DM, Arshad M, Seed PC, Franz KJ. Development of an Antibacterial Prochelator to Target Drug-Resistant Bacteria. 2016 manuscript under review.
50. Hunter T. Signaling-2000 and Beyond. *Cell*. 2000; 100:113–127. [PubMed: 10647936]
51. Liu LL, Franz KJ. Phosphorylation of an α -Synuclein Peptide Fragment Enhances Metal Binding. *J Am Chem Soc*. 2005; 127:9662–9663. [PubMed: 15998051]
52. Pazos E, Vázquez ME. Advances in Lanthanide-Based Luminescent Peptide Probes for Monitoring the Activity of Kinase and Phosphatase. *Biotechnol J*. 2014; 9:241–252. [PubMed: 24323746]
53. Alies B, Wiener JD, Franz KJ. A Prochelator Peptide Designed to Use Heterometallic Cooperativity to Enhance Metal Ion Affinity. *Chem Sci*. 2015; 6:3606–3610.

Biographies

Qin Wang was born in Guangzhou, China. She received both her B.S. and M.S. degrees from Peking University, where she conducted research on the selectivity of antineoplastic vanadium compounds in human tumor and normal cells with Prof. Xiaogai Yang and Prof. Kui Wang. She then joined the lab of Prof. Katherine J. Franz at Duke University in 2011, where part of her graduate work focuses on development and characterization of boronate-masked prochelators for metal chelation therapy.

Katherine J. Franz is the Alexander F. Hehmeyer Professor of Chemistry at Duke University. After attending Wellesley College for her undergraduate degree, she obtained her PhD in inorganic chemistry with Prof. Stephen J. Lippard at MIT, and then completed an NIH postdoctoral fellowship with Prof. Barbara Imperiali, also at MIT. Since 2003, Kathy and her research group at Duke have been developing and exploring molecules that manipulate the coordination chemistry of metal ions in complex and dynamic environments like those found in biological systems.

Figure 1.

Modulating metallobiology with chelating agents: **(a)** traditional notion of chelation therapy as reducing the total body burden of heavy metals; **(b)** metal complexation enabling passive diffusion of metals across membranes; **(c)** inactivating an enzyme by protein–metal–chelator ternary complex formation; **(d)** forming a chelate complex that promotes cytotoxic (or other) reactivity; **(e)** forming a chelate complex that inhibits redox cycling (or other) activity. Each example shows a generic metal, as these approaches can in principle apply across the periodic table. Reproduced with permission from ref. 4. Copyright (2013) Elsevier.

Figure 2.

Three general approaches of prochelator activation in response to a stimulus. Blue shapes (star, triangle and dented rectangle) represent the reactive moieties of prochelators and their color change to orange indicates a chemical change initiated by the stimulus.

Figure 3.

Boronate-based prochelators for ROS-triggered metal chelation and inhibition of $\text{Fe}^{3+}/\text{Fe}^{2+}$ or $\text{Cu}^{2+}/\text{Cu}^+$ redox cycling.

Figure 4.

Epifluorescence microscopy images of mitochondrial health of H9c2 rat cardiomyoblast cells treated with 100 μ M chelators or prochelators with (**top**) or without (**bottom**) H₂O₂ for 24 h prior to staining with the JC-1 probe of mitochondrial membrane potential (Ψ_m). Red fluorescence of the Control and BSIH panels indicates healthy mitochondria, whereas green fluorescence for TIP and H₂O₂ signals pronounced membrane depolarization (loss of Ψ_m). Slight shifts in red to green intensity ratio of SIH, HAPI, BHAPI and ICL670A suggest moderate effects on membrane integrity by these agents. All of the chelators and prochelators examined here, with the exception of TIP, were able to partially prevent the loss of Ψ_m induced by H₂O₂. Scale bars represent 100 μ m. Reproduced with slight modification with permission from ref. [23]. Copyright (2014) Elsevier.

Figure 5. H_2O_2 generated from $\text{Cu-A}\beta$, O_2 and ascorbic acid unmasks pro-chelator QBP to release 8-hydroxyquinoline, which extracts Cu^{2+} from $\text{A}\beta$ and prevents further redox cycling and $\text{A}\beta$ aggregation.¹³

Figure 6.

Conditional activation of QBP allows selective microbial killing of *C. neoformans*. (a) ROS generated within macrophages converts QBP to 8HQ. Combined with the influx of copper during infection, the resultant lipophilic $\text{Cu}(8\text{HQ})_2$ complexes induces pathogen killing. (b) Comparison of *C. neoformans* survival in the absence or presence of activated mouse macrophage-like RAW 264.7 cells. (c) Cell viability of the macrophages described in (b). Whereas 8HQ was toxic, QBP was not toxic at any concentration tested, even at 200 μM , which was shown in (b) to reduce *C. neoformans* survival. Reproduced with slight modification with permission from refs. [36,37]. Copyright (2014) Elsevier.

Figure 7.

Activation of multifunctional fluorogenic prochelators. **(a)** The self-immolative boronate mask of BCQ is removed upon peroxide stimulation to release active metal chelator 8HQ and fluorophore umbelliferone.³⁸ **(b)** UVA irradiation transforms prochelator PC-HAPI into HAPI and umbelliferone for dual cytoprotection from UVA damage.⁴¹

Figure 8.

UVA light photoisomerizes (*E*)-HAPI to metastable (*Z*)-HAPI*, which relaxes back either rapidly upon exposure to UVC light, or thermally over the course of hours. HAPI binds Cu^{2+} and Fe^{3+} with high affinity, whereas HAPI* does not. Formation of Cu-HAPI or Fe-HAPI complexes inhibits photoconversion and “locks” in the conformation.⁴³

Figure 9.

Enzymatic activation of prochelators by β -secretase (BACE). **(a)** Cleavage of prochelator peptide SWH by BACE releases chelator CP that sequesters Cu^{2+} from Cu-A β . **(b)** Key design features of β -secretase membrane-anchored probe (β -MAP). **(c)** β -MAP monitors real-time BACE activity in living cells by fluorescence increase (**top row**), which diminishes upon co-treatment with a BACE inhibitor (**bottom 2 rows**). Reproduced with permission from ref. [46]. Copyright (2012) John Wiley & Sons, Inc.

Figure 10. Enzymatic activation of DB4-51 by β -lactamase releases bidentate metal chelator pyrithione, which binds Cu^{2+} to exacerbate killing of drug resistant bacteria.

Figure 11.

(a) Tyrosine phosphorylation of prochelator peptide α -Syn(119–132) provides an additional anchor for tight Tb^{3+} binding with significant increase in luminescence (not all metal-binding residues are displayed in cartoon). (b) Luminescence emission plots of 2 μM α -syn peptide fragments in the presence of 40 μM Tb^{3+} in 10 mM HEPES buffer with 100 mM NaCl, pH 7.0, $\lambda_{\text{ex}} = 270$ nm. Reproduced with permission from ref. [51]. Copyright (2005) American Chemical Society.

Figure 12.

(a) Model of cooperative metal binding of two different metal ions to prochelator peptide PCP (flexible black cylinder). The higher Tb³⁺ emission observed in the presence of Zn²⁺ is emphasized by a glow. (b) Titrations of PCP with TbCl₃ monitored by sensitized Tb³⁺ emission at 545 nm in the absence (blue, circles) or presence (red, squares) of ZnSO₄ that is either present initially (**left**) or added subsequently to Tb³⁺ (**right**). Insets show full emission spectra from 300–650 nm. Conditions: 0.5 μM PCP, 0–2.5 μM TbCl₃, 0 or 0.5 μM ZnCl₂ in 5 mM HEPES buffer with 50 μM DTT, pH 7.4; λ_{ex} = 280 nm. Reproduced with permission from ref. [53]. Copyright (2015) Royal Society of Chemistry.