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Inorganic Chemistry Approaches to Activity-Based Sensing: From Metal Sensors to Bioorthogonal Metal Chemistry

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Abstract

The complex network of chemical processes that sustain life motivates the development of new synthetic tools to decipher biological mechanisms of action at a molecular level. In this context, fluorescent and related optical probes have emerged as useful chemical reagents for monitoring small-molecule and metal signals in biological systems, enabling visualization of dynamic cellular events with spatial and temporal resolution. In particular, metals occupy a central role in this field as analytes in their own right, while also being leveraged for their unique biocompatible reactivity with small-molecule substrates. This Viewpoint highlights the use of inorganic chemistry principles to develop activity-based sensing platforms mediated by metal reactivity, spanning indicators for metal detection to metal-based reagents for bioorthogonal tracking, and manipulation of small and large biomolecules, illustrating the privileged roles of metals at the interface of chemistry and biology.

Graphical Abstract

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Author Contributions

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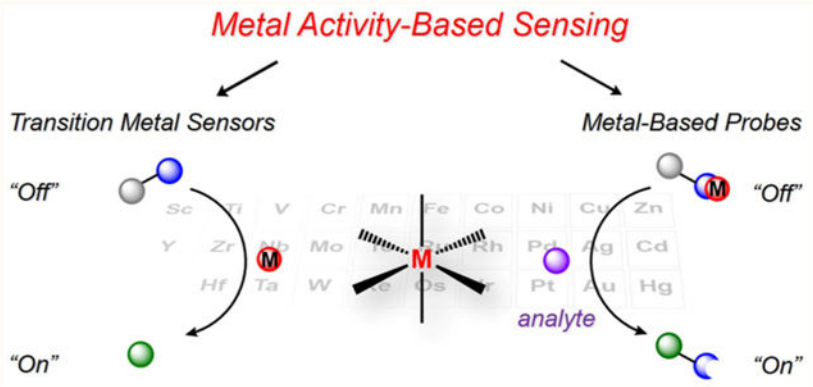

INTRODUCTION

Metals are essential nutrients in living systems, exhibiting a diversity of chemistry with biomolecules spanning catalysis to electron transfer to static and dynamic structural stabilization.[1] However, this same chemical diversity of metal reactivity can, when dysregulated, contribute to numerous diseases, including cancer[2] and neurodegenerative,[3] metabolic,[4,5] and cardiovascular disorders.[6] Indeed, depending on properties such as Lewis acidity, polarizability, oxidation state, and spin state, metals can engage in both ionic and covalent bonding interactions, giving rise to coordination complexes with varying degrees of metal association. As a result, the total metal pool in a given biological context is divided into two stores: a static pool, comprising tightly bound metal centers (e.g., metal cofactors), and a labile pool, which encompasses loosely associated metals (e.g., metal signals).[7,8]

The traditional view of this biological inorganic chemistry is that redox-inactive metals such as sodium, potassium, and zinc are well-recognized for their roles in signaling processes,[1,9] whereas redox-active transition metals like copper and iron are often associated with catalytic centers buried within the active sites of enzymes for metabolic purposes. Indeed, transition metals can access a range of oxidation and spin states while accommodating diverse coordination geometries and thus can promote challenging transformations, but can also participate in detrimental reactivity often associated with reactive oxygen species (ROS) generation (e.g., Fenton chemistry).[1] A broader, more inclusive view of metals in biology has recently emerged with evidence for redox-active metals acting as cellular signals[10–12] to alter enzyme function, highlighted by two examples establishing that reversible copper binding beyond the active site can enhance (e.g., mitogen-activated protein kinase, MEK1)[13,14] or inhibit (e.g., phosphodiesterase 3B, PDE3B)[15] the catalytic activity of proteins. The unique chemistry of metals emphasizes their diverse reactivity at a molecular scale as well as the importance of dynamic metal regulation in higher-order biological contexts spanning cells, tissues, organs, and organisms.

In contrast to their organic counterparts in biology, metals can be neither created nor destroyed and are instead carefully regulated through acquisition, trafficking, and efflux pathways.[16] As such, dynamic communication between the static and labile metal pools is necessary to maintain proper homeostasis of metal stores, requiring a detailed understanding

of the cellular mechanisms employed to control metal trafficking and reactivity as well as techniques to identify both types of pools.[17–19] To meet this goal, mass spectrometry techniques, such as laser-ablation inductively coupled plasma mass spectrometry[20–22] and secondary-ion mass spectrometry,[23] along with X-ray-based methods that include X-ray absorbance spectroscopy,[24] X-ray fluorescence microscopy,[25] particle-induced X-ray emission,[26] and electron microscopy[27,28] have been commonly utilized to study total metal pools.[19] However, such technologies are limited to providing relative metal distribution statistics within fixed biological samples as opposed to furnishing dynamic, spatially resolved information regarding metal speciation in living specimens.[17,19] Indeed, molecular imaging has been extensively used to trace the activity of labile metal pools in real time and space, illuminating intricate metal-dependent biological pathways,[18,29,30] and in this regard, a variety of fluorescent and other optical probes designed for the selective detection of essential transition metals and their toxic heavy-metal counterparts in living systems have been developed.[29,31–37] While the diverse chemical properties of metals offer various features to target when designing metal-responsive sensors, they also present challenges for achieving high selectivity in complex biological environments.[17] In particular, the ability to distinguish between different oxidation states of the same metal (e.g., $Fe^{II/III}$, $Cu^{I/II}$), achieve specificity for transition metals over more abundant alkali and alkaline-earth congeners, and prevent adventitious fluorescence quenching via electron transfer from paramagnetic centers[38] is critical for the development of effective tools for imaging labile metal pools.[8] In this regard, activity-based sensing (ABS) approaches that exploit unique metal reactivity profiles have enabled the detection of metals such as iron,[39] cobalt,[40] nickel,[41] and copper[42] with high metal and oxidation state specificity.

In a separate but related avenue of research, transition-metal complexes have also found utility as bioorthogonal molecular probes owing to their broad and tunable reactivity toward small-molecule substrates.[43,44] Such activity-based strategies are attracting growing interest because they have the potential to improve selectivity toward molecular analytes in complex biological settings beyond conventional lock-and-key molecular recognition. Representative examples include carbon monoxide (CO) monitoring through a palladium-mediated carbonylation,[45] ethylene detection via ruthenium-catalyzed meta-thesis,[46] and nitric oxide (NO),[47] hydrogen sulfide (H_2S),[48] and superoxide (O_2^-)[49] sensing platforms that exploit the versatile redox and Lewis acid reactivity of copper complexes.

The topic of this Viewpoint is the use of fundamental inorganic chemistry strategies to design highly specific ABS probes. In particular, we summarize types of inorganic reactivity exploited, as well as commonly employed metal-based triggers for mapping labile metal pools and associated biorelevant small molecules. With a focus on inorganic principles applied to molecular probe design to encourage more activity in the field, we highlight representative examples to illustrate concepts rather than provide a comprehensive list of probes, and readers can refer to other literature reports for further consideration.[29–35,50] Along these lines, we restrict our discussion to ABS probes that have been reported to operate in cells and more complex biological environments with requisite selectivity and signal-to-noise responses. We then close by considering potential future avenues to explore in the area of activity-based metal chemistry, particularly beyond cellular imaging.

FROM BINDING-BASED SENSING (BBS) TO ABS

The design of fluorescent probes for metal sensing has widely explored the versatile coordination chemistry and reactivity of metals, encompassing two general mechanisms for achieving specificity: (1) BBS (Figure 1a) and (2) ABS (Figure 1b).[37] In both cases, a site that recognizes the metal center is appended to a fluorophore to effect a change in fluorescence upon interaction with the metal analyte of interest.

The binding-based approach relies on a chelating ligand site that reversibly binds the metal of interest and induces a fluorescence response via mechanisms such as internal charge transfer (ICT), photoinduced electron transfer (PET), or Förster resonance energy transfer (FRET). In this context, probes that elicit a “turn-on” increase or ratiometric color change response are more useful than “turn-off” probes, where spatial information for the latter is more challenging to obtain. Metal selectivity is often conferred by considering preferences in coordination numbers and geometries, hard–soft acid–base theory, and/or ligand-binding thermodynamics as dictated by the Irving–Williams series.[51] Using these fundamental coordination chemistry principles, fluorescent sensors have been successfully developed for the detection of many endogenous metals[29,30,35,37] in biological systems, most commonly Ca^{II} [52] and Zn^{II},[30,35] as well as Cu^{I} [53–55] and Ni^{II}.[56] However, applying this binding-based strategy becomes challenging when dealing with metals that are more weakly binding on the Irving–Williams series and/or can be paramagnetic in many coordination environments, such as Mn^{II} and Fe^{II}. To overcome such limitations, the ABS approach makes use of metal-specific transformations such that, upon metal sensing, a chemoselective reaction is induced to modify the optical properties of the fluorophore and results in an irreversible turn-on or change in the fluorescence response. Because ABS relies on both metal binding and a coupled chemical reaction event, this method can potentially offer a higher degree of metal specificity over binding-based detection alone, and if the activity-based reaction results in a cleavage product with fluorophore separation and release from the metal, this approach can also limit potential quenching from paramagnetic metal analytes. On the other hand, unlike BBS probes, typical ABS reagents do not allow for reversible metal sensing. Nevertheless, ABS has enabled the detection of redox-active transition metals including Fe^{II}, Co^{II}, or Cu^{I} with high metal and oxidation state selectivity.[37] More broadly, this ABS approach is not limited to metals; indeed, transient and reactive small molecules that would be difficult to track owing to their similar shape and size to other biological metabolites are also capable of engaging in highly specific and bioorthogonal chemical reactions.[32,43,57,58] Representative examples from our laboratory include redox-neutral 2-aza-Cope-type rearrangements for formaldehyde sensing,[59,60] oxidative conversion of aryl boronates to phenols for hydrogen peroxide (H_2O_2) detection,[61–65] and reductive organic azide to aniline transformations for H_2S sensing.[66–68] Here, we highlight select examples that incorporate transition-metal reactivity trends into new chemical tools for biological imaging using ABS approaches, spanning metal detection to metal-directed small-molecule detection and bioorthogonal metal catalysis (Figure 2).

ABS PROBES FOR METALS

The unique chemical properties of metals, including tunable Lewis acidity and redox activity, have been exploited toward the development of reactivity triggers for imaging bioavailable metal pools. The following section highlights a selection of fluorescent ABS probes for biologically and/or environmentally important metals, focusing on triggers that can be generally applied to multiple imaging modalities.

ABS of Iron and Cobalt.

ABS approaches have proven particularly useful for detecting iron and cobalt, two essential redox-active metal nutrients for living organisms. Given the reducing environment inside the cell, labile iron pools are predominantly ferrous and in high-spin configurations. As such, along with the preferred divalent oxidation state for cobalt, these paramagnetic metal centers often induce fluorescence quenching and manifest low metal–ligand stabilities on the Irving–Williams series,[51] limiting the possibilities for a selective recognition- and binding-based approach to fluorescence detection. To overcome these challenges, the rich oxidative chemistry of both metalloenzymes[69] and their synthetic counterparts stands out as a viable mechanism for the design of selective Fe^{II} and Co^{II} fluorescent indicators. In particular, oxidants such as O_2, 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO),[70] or trimethylamine *N*-oxide[71] provide co-reagents for iron-mediated oxidative chemistry. For example, inspired by the O_2 chemistry of Fe^{II} and, in particular, the 2-His-1-carboxylate triad motif found in mononuclear non-heme Fe^{II} enzymes, our laboratory developed Iron Probe 1 (**IP1**), a hydroxymethyl fluorescein derivative bearing a tris(pyridine) carboxylate-type ligand cage as an iron recognition and activity site.[72] Upon Fe^{II} coordination, O_2 activation triggers intra-molecular C–O bond cleavage and oxidation to release the fluorophore, resulting in an increase in the fluorescence intensity. The successful application of these inorganic design principles in living systems was demonstrated using the corresponding acetoxymethyl ester derivative (Figure 3a) **IP1-AM**, a more cell-permeable analogue, which enabled the detection of changes in cellular labile iron pools upon various stimulation conditions. A related compound, Cobalt Probe 1 (**CP1**),[40] featuring a tetradentate N3O-based ligand, was shown to undergo a similar O_2-dependent C–O bond scission to uncage the Tokyo Green fluorophore, permitting selective fluorescent Co^{II} sensing in aqueous solution and in living cells (Figure 3a). The versatility of this cobalt-specific ABS ligand was further demonstrated by its attachment to a 2-(2′-hydroxyphenyl)benzothiazole excited-state intramolecular-proton-transfer-based chromophore[73] and luciferin[74] to measure the Co^{II} levels in cells and animals, respectively.

In parallel, Hirayama, Nagasawa, and colleagues designed **RhoNox-1**, featuring an *N*-oxide ABS trigger that is selectively reduced to the corresponding amine by Fe^{II}, resulting in a turn-on fluorescence signal (Figure 3b).[75] This strategy has been extended to other fluorophores for multicolor imaging, including coumarin, rhodol, and silicon rhodamine (Figure 3f),[76–78] and applied to organelle-specific iron imaging by introducing targeting units.[79–82] A related Fe^{II}-dependent nitroxide reduction enables a turn-on fluorescence response following Fe^{II} reduction of the TEMPO radical that otherwise quenches the fluorophore emission (Figure 3c).[83]

Inspired by iron-mediated endoperoxide activation observed in antimalarial drugs (e.g., Artemisinin), Renslo and co-workers designed **Trx-puro**, a histochemical stain for labile Fe^{II} comprised of a trioxalane-caged puromycin (Figure 3d). Upon reaction of the probe with Fe^{II}, free puromycin is released, which can be translated onto nascent peptides and detected by a puromycin antibody after fixation.[84] Our laboratory synthesized FRET Iron Probe 1 (**FIP-1**), a fluorescein/cyanine 3 conjugate using an analogous endoperoxide linker (Figure 3d). Cleavage of the peroxide moiety in **FIP-1** by reaction with Fe^{II} separates the fluorophores and minimizes FRET, thus enabling ratiometric detection of cellular labile iron pools.[85] **FIP-1** provides the first direct imaging evidence of changes in labile iron pools upon induction of ferroptosis (Figure 3g),[85] a form of cell death that is gaining increasing attention.[86] More recently, we adapted the iron-selective endoperoxide trigger to develop Iron-Caged Luciferin 1 (**ICL-1**), a luciferin-based bioluminescent probe for monitoring labile iron in live animals, observing with the Skaar laboratory that bacterial infection can lead to an increase in labile iron pools (Figure 3h).[87] PET imaging of labile iron pools has also been achieved using endoperoxide tracers (Figure 3i).[88] Finally, by exploiting the Lewis acidity of Fe^{II}, a recent report highlighted the use of an iron-mediated cyclization of a phenolic unit adjacent to a C═N bond appended to a coumarin derivative (Figure 3e).[89] Upon cyclization, generation of a benzoxazole ring prevents C═N bond isomerization and rotation, enabling fluorescence turn-on in aqueous solution and in living cells.

ABS of Copper.

Unlike divalent iron and cobalt, monovalent copper is the dominant oxidation state of this metal in reducing cellular environments.[90] As a diamagnetic ion, Cu^{I} exhibits a preference for sulfur-based ligands or nitrogen-rich environments, and fluorescence quenching can be circumvented in certain cases; indeed, numerous binding-based probes have been developed for this analyte.[29,30,37,53–55] Nevertheless, the dynamic fluctuations of labile copper pools observed across many cell types have motivated the advancement of ABS strategies applied to biological copper detection. In early work in this area, Taki and colleagues drew inspiration from the vast literature on the O_2 activation by bioinorganic model complexes for copper-dependent enzymes[91–93] and reported **FluTPA2**,[94] a probe featuring a Tokyo Green derivative caged by the Cu^{I} chelator tris(2-pyridylmethyl)amine (TPA). This compound undergoes a Cu^{I}- and O_2-dependent oxidative cleavage of the TPA benzyl ether linkage with subsequent oxidation and release to generate the highly fluorescent Tokyo Green fluorophore product (Figure 4a). **FluTPA2** selectively detects Cu^{I} over Cu^{II} in the presence of glutathione (GSH) with a >100-fold turn-on and can report changes in cellular copper levels. This ABS trigger was further adapted for mitochondria targeting (Figure 4c)[95] and separately coupled with coumarin-based fluorophores.[96] Our laboratory employed this motif to cage luciferin and create Copper-Caged Luciferin 1 (**CCL-1**; Figure 4a) for in vivo bioluminescent copper imaging, showing in a diet-induced mouse model of non-alcoholic fatty liver disease (NAFLD) that copper deficiency occurs as early as 2 weeks before metabolic symptoms of the disease manifest at 2 months (Figure 4d).[4]

In contrast to the growing use of chemical probes for bioimaging of Cu^{I}, detection of Cu^{II} in biological environments is rarer. An early approach for Cu^{II} sensing was developed by Czarnik and colleagues,[42] employing a hydrazide-substituted spirorhodamine fluorophore

locked in a non-fluorescent ring-closed form. Binding of Cu^{II} promotes hydrazide hydrolysis with concomitant ring-opening of the spirorhodamine, thus turning-on the fluorophore (Figure 4b). Depending on the fluorophore/trigger combination, hydrazide-based Cu^{II} detection has been reported in many solvents, and two-dye systems with a hydrazide motif have been reported to enable ratiometric imaging of Cu^{II} in living cells.[97–99]

ABS of Heavy Metals.

In addition to optical detection of endogenous metals in biological settings, ABS can also provide entry to monitor the accumulation of heavy-metal counterparts. Mercury, one of the more prevalent toxic elements in the environment, has been an important target for both in vivo and ex vivo detection. Owing to the highly thiophilic nature of Hg^{II}, mercury-promoted desulfurization reactions provide a versatile strategy for designing fluorescent indicators.[100] Akin to the spirorhodamine sensors for Cu^{II}, Tae and co-workers reported a thiosemicarbazide-substituted spirolactam–rhodamine as a highly sensitive and selective Hg^{II} sensor (Figure 5a), where mercury coordination facilitates 1,3,4-oxadiazole formation along with HgS precipitation.[101] Of note, the fast kinetics of this probe in aqueous conditions enabled its application toward imaging Hg^{II} in both living cells and zebrafish models (Figure 5f).[102] This ring-opening scaffold has been tailored for other fluorophores,[103–105] including a FRET-based sensor for ratiometric measurements[103] and a near-IR silicon–rhodamine fluorophore.[105]

Analogous selenium–mercury interactions provide a related pathway for Hg^{II} sensing. For example, selenophosphinyl-protected fluorescein **FSe-1** can be selectively hydrolyzed upon interaction with Hg^{II} (Figure 5b).[106] Deprotection of the hydroxyl groups induces ring opening of the fluorescein lactone unit, affording a 156-fold increase in fluorescence. A similar strategy has also been described using thiophosphinyl units as triggers[107] or naphthalimide as the fluorophore,[108] the latter of which facilitated ratiometric imaging of mercury in cells. In addition, Hg^{II}-promoted ring opening of the selenolactone **RBSe** results in fluorophore turn-on and HgSe release (Figure 5b).[109,110] Although the probe does respond to Ag^{I} as well, its ability to react with Hg^{II} along with organomercury species was shown to be viable in cells and zebrafish organs upon treatment with $HgCl_2$ or CH_3HgCl (Figure 5e).

Beyond the principles of hard–soft acid–base chemistry, Koide and colleagues introduced organometallic ABS, and, in particular, the oxymercuration reaction for oxidation-resistant sensing of Hg^{II}, where vinyl and terminal butyne were used as caging units on the phenolic groups of fluorescein-derived fluorophores (Figure 5c).[111,112] These caging groups are readily removed upon reaction with divalent mercury, releasing the fluorescent moiety required for signal quantification. The oxymercuration-based sensing mechanism is amenable to monitoring both Hg^{II} as well as methylmercury in cells and zebrafish tissues.[113,114]

Other heavy metals are also amenable to ABS detection. In this regard, palladium and platinum metals are major industrial contributors for the synthesis of pharmaceuticals and fine chemicals given the prevalence of cross-coupling reactions in building complex molecules.[115] Owing to their potential hazardous health effects, sensing of residual

palladium and platinum is of interest for quality control of active pharmaceuticals and validation of heavy-metal thresholds. In innovative applications of classic Tsuji-Trost and Claisen chemistry, fluorophore derivatives bearing palladium/platinum-cleavable *O*-allyl or propargyl caging moieties on phenolic hydroxyl groups have been applied for the detection of exogenous palladium or platinum in cells, fish models, and solution.[116–119] Formation of an electrophilic palladium or platinum allyl intermediate followed by hydrolysis affords the fluorescent dye. Moreover, this ABS strategy can be tuned for the detection of palladium in various oxidation states (Pd^0, Pd^{II}, or Pd^{IV}) when using a propargyl ether unit.[116] Incorporation of an allyl ether engenders oxidation state specific metal sensing, where a Tsuji-Trost deprotection is facilitated by Pd^0, whereas a Claisen rearrangement is promoted by Pd^{II} or Pt^{IV} only (Figure 5d).[119]

As an abundant first-row transition metal, nickel has gained attention for its use as a potential substitute for its heavy metal congeners, Pd and Pt.[120] As such, developing tools for trace nickel detection for process control becomes a necessity. In this context, altering the ligands and reaction conditions typically employed for Pd-catalyzed deallylation reactions, Lee and co-workers recently reported the first Ni^{II} reactivity-based sensor.[41] Using a conformationally twisted *N*-arylbenzotriazole scaffold, Ni-catalyzed deallylation of the allyl carbamate substituent unveils the unprotected amino group that enables a turn-on fluorescent response.

ABS PROBES UTILIZING BIOORTHOGONAL METAL REACTIVITY

In addition to providing a versatile platform for biological and environmental metal detection, metal-directed ABS chemistry offers a modular approach for monitoring and manipulating other small molecules in their native environments owing to the diverse binding affinities, Lewis acidities, redox activities, hard–soft acid–base preferences, and geometries that metals uniquely possess. In particular, transition metals can often participate as fluorescence quenchers via electron- or energy-transfer pathways, thus providing a versatile strategy for designing turn-on responsive indicators using ABS reactions to selectively separate the metal from the fluorophore or alter the oxidation state of the metal to inhibit excited-state electron/energy transfer. The following sections briefly summarize a selection of metal-based methods for small-molecule sensing, again focusing on ABS reactions that are biocompatible. Of note is the growing success of bioorthogonal metal chemistry to probe transient small molecules, such as reactive oxygen, sulfur, and carbon species, for which it would be challenging to develop traditional lock-and-key binding receptors with sufficient selectivity in complex living systems.

ABS Using Bioorthogonal Metal–Ligand Substitution Reactions.

As one of the fundamental reactions of inorganic chemistry, metal–ligand exchange provides a distinct mechanism for sensing, which has largely been exploited in the context of analyte-induced displacement of a metal quencher, leading to a fluorescence turn-on response. A representative set of examples involves detection of H_2S via displacement and subsequent precipitation of copper(II) sulfide from copper(II) dye complexes. Chang and colleagues reported a copper(II) dipicolylamine fluorescein complex that permitted in

vitro H_2S detection.[121] In the absence of H_2S, the paramagnetic Cu^{II} center quenches the fluorescence of the probe. Upon reaction with H_2S, removal of the quenching copper center driven by the formation and precipitation of copper sulfide triggers a turn-on response from the fluorophore. Along these same lines, Nagano and co-workers reported **HSip-1**, a copper(II) azamacrocycle fluorescein complex (Figure 6a).[48] **HSip-1** was successfully used for H_2S imaging in cells with reduced interference from thiol-containing GSH and cysteine residues (Figure 6c). From these starting points, many metal-directed ABS probes for H_2S detection have been reported, emphasizing both the generality and practicality of such simple transition-metal-based reactivity trends.[122–125]

In a separate application of bioorthogonal metal–ligand substitution reaction chemistry, CO detection has been accomplished by exploiting its binding affinity toward certain transition metals to facilitate displacement from a fluorophore to provide a spectral readout. Complementing the organometallic methods utilizing CO as a substrate (vide infra), many metal-based chromogenic and fluorogenic probes have been developed for CO detection in the gas phase and in solution.[126] As a leading example for imaging CO in tissues, Sancenón and colleagues incorporated a hydrophilic vinyl ligand onto a Ru^{II} complex to overcome previous aqueous solubility issues of related metal–CO complexes. Coordination of the fluorophore 5-(3-thienyl)-2,1,3-benzothiadiazole to the Ru^{II} center via a nitrogen atom quenched its emission, and substitution of this fluorophore ligand with the CO analyte triggered an increase in fluorescence resulting from free fluorophore generation as a function of the CO concentration (Figure 6b).[127] The sensitivity, selectivity, and aqueous solubility of the ruthenium-based sensor enabled its successful application to cellular CO imaging (Figure 6d).[127]

ABS Using Bioorthogonal Metal-Mediated Redox Chemistry.

In addition to non-redox ligand substitution processes, the inherent redox activity of metals can also be utilized as a triggering mechanism for monitoring transient and reactive analytes. In an innovative early application of this strategy, Lippard and co-workers described an NO detection system via a copper-promoted reductive nitrosylation using a copper(II) aminoquinoline–fluorescein complex (**CuFL**; Figure 7a).[47] Upon reaction of the copper complex with NO, demetalation and subsequent ligand nitrosylation at the secondary amine site establish the turn-on signal required for NO monitoring. Notably, this sensor demonstrates exquisite specificity for NO over other ROS species, including HNO, under cellularly relevant conditions (Figure 7d),[47,128] where the metal-based reaction offers a potential advantage over traditional phenyldiamine-substituted dyes employed as general proxies for NO detection because other reactive nitrogen species can trigger the diamine-to-triazole transformation. This probe has been utilized for NO detection in vivo, showing the key defensive role of NO for survival of the *Bacillus anthracis* pathogen.[129] Subsequent synthetic modifications to incorporate two NO sensing units along with an ester moiety provided access to a cell-permeable probe that enabled NO visualization in the mouse main olfactory bulb (Figure 7e).[130] Following upon this work, Ali and colleagues reported a copper(II) dansyl-based turn-on NO sensor (Figure 7b).[131]

HNO detection can be achieved by its interactions with Cu^{II} complexes to effect a one-electron reduction of the metal center. This feature was exploited by Lippard and co-workers to design the HNO-responsive fluorescent probe **Cu^{II}[BOT1]** (Figure 7b).[132,133] In the absence of HNO, the paramagnetic Cu^{II} center coordinated to *N*-(triazolylmethyl)-*N*,*N*-dipicolyl quenches emission of the boron–dipyrromethene (BODIPY) fluorophore. HNO-induced reduction to the corresponding diamagnetic Cu^{I} complex triggers a fluorescence turn-on response by relieving this free-radical quenching. Building on this initial report, the Cu^{II}/HNO reactivity has been more broadly harnessed to develop other fluorescent indicators for imaging cellular HNO.[134–136]

Drawing inspiration from heme and non-heme iron enzymes that utilize H_2O_2 as an oxidant, Kodera and colleagues developed a fluorescent H_2O_2 indicator combining a reduced resorufin analogue and an iron(III) polypyridine complex to generate **MBFh1**. Oxidation of the dye-bound ferric center with H_2O_2 is proposed to initiate subsequent two-electron ligand oxidation, resulting in C–O bond cleavage to liberate the fluorescent resorufin dye.[137] Subsequent modifications of the fluorophore scaffold to incorporate an *O*-alkylresorufin derivative (**MBFh2**; Figure 7c) afforded a stable probe for the intracellular detection of H_2O_2 (Figure 7f).[138] In a similar approach, Nam and co-workers employed a known zinc sensor and made its corresponding Fe^{II} complex (**ZP1Fe_2**),[139] which is activated by H_2O_2 to promote oxidative *N*-dealkylation and release the bis(carboxylate) fluorescein (Figure 7c). Unlike the ferric-based sensor, this diiron probe selectively reacts with H_2O_2 over other ROS such as $O_2^{\bullet-}$ or ^{t}BuOOH and was found to localize at the lysosome, enabling organelle-specific H_2O_2 detection.[139]

ABS Using Bioorthogonal Organometallic Chemistry.

Organometallic chemistry offers a unique platform for sensing small-molecule analytes that are otherwise poorly reactive for detection in neutral aqueous conditions with high salt and thiol contents. In an early example of incorporating such principles to detect intracellular non-metal analytes, our laboratory made use of the palladium-mediated carbonylation reaction to design the first-generation fluorescent CO probe **COP-1** (Figure 8a).[45] The sensor features a BODIPY dimer ligated to a Pd^{II} center, which then selectively reacts with CO to effect carbonylation of the aromatic backbone ligand and release the fluorophore. Owing to the need for oxidative addition, reductive elimination, and C–C bond formation to release the quenching palladium center, **COP-1** displays high specificity for CO, particularly over other biologically relevant diatomics, including O_2, O_2^-, and NO, serving as a suitable reagent for use in cellular studies (Figure 8d). On the basis of this study, many other fluorescent CO sensors using a similar Pd^{II}-mediated fluorophore release scheme have been subsequently reported for imaging CO in cells, tissues, and animal models, demonstrating the utility and versatility of this bioorthogonal organometallic ABS strategy.[140–142]

In a complementary approach, ABS probes combining the facile CO-induced reduction of Pd^{II} complexes implicated in palladium-mediated carbonylations[143] with the Pd^0-catalyzed Tsuji–Trost reaction have also emerged. Specifically, fluorophores containing allyl cages are selectively turned-on upon deprotection by Pd^0, which, in turn, is generated only upon the reduction of a Pd^{II} source in the presence of CO. Of note, this method makes use of the same

reactivity trends that enabled palladium sensing, emphasizing the versatile role of metals and their chemical properties. Such probes have proven to be sensitive and selective for CO detection in both cells[144–147] and mouse models (Figure 8b).[148]

In another example of bioorthogonal organometallic chemistry for ABS, Michel and co-workers elegantly demonstrated that transition-metal-catalyzed olefin metathesis can be applied to achieve ethylene detection both in aqueous solution and in living cells. The **BEP-4** probe (Figure 8c) integrates a second-generation Hoveyda–Grubbs catalyst onto a BODIPY scaffold, where the reaction of ethylene displaces the BODIPY dye from the heavy-metal ruthenium quencher and thus induces a turn-on response to ethylene in a dose-dependent manner (Figure 8e).[46]

CONCLUDING REMARKS AND FUTURE PROSPECTS

ABS represents a rapidly growing area of research in chemical biology, and ample opportunities are available to pursue metal-directed chemistry in this open field. Indeed, fundamental concepts of inorganic chemistry, from geometric and hard–soft acid–base preferences to kinetics of ligand exchange to tunable Lewis acidity, oxidation state, and spin state, can all be brought to bear in a diverse array of new directions. Coupled with the ability to draw inspiration from the chemistry of bioinorganic and organometallic systems, particularly metalloenzyme active sites, ABS probes can provide unique access to selective and robust approaches toward metal-specific sensing in complex environments as well as pathways for the detection of biologically relevant small molecules through metal-directed reactivity. In this Viewpoint article, we have highlighted several classes of metal-mediated reactions that have been incorporated into triggers for fluorescence and bioluminescence imaging in cells, tissues, or animals. Given the ever-expanding scope of metal-based chemistry, we envision that more sensitive, selective, and robust metal-mediated ABS platforms will be developed to facilitate the detection and manipulation of chemical analytes more broadly and help decipher their contributions to living systems.

Many avenues in metal-directed ABS merit further investigation. Deeper applications of existing fluorescence and bioluminescence probes for metals and metal-interacting small molecules will continue to illuminate new basic biology. Examples from our own laboratory at the cellular and whole-organism levels include the identification of elevations in the labile iron pool upon induction of ferroptosis[85] and liver-localized copper deficiency through the upregulation of copper export proteins during the progression of NAFLD,[4] respectively. For optical imaging, moving excitation and/or detection wavelengths into the red and near-IR regions will enable multicolor monitoring and deep-tissue and/or whole-animal imaging to monitor biomolecules in their true native environments. Likewise, spatial resolution can be additionally improved by attaching chemical and/or biological targeting groups to control probe localization for organelle-specific or tissue detection. Also, the application of metal-directed ABS concepts to other imaging modalities, particularly bioimaging techniques that are amenable to humans, such as MRI, PET, and ultrasonic imaging, will help in the translation of these platforms to diagnostic and therapeutic technologies.

Beyond imaging, bioorthogonal, metal-dependent activation of fluorophores, prodrugs, and larger biomolecules/biopolymers such as proteins and nucleic acids represents another research area that will surely benefit from more contributions that incorporate principles of inorganic coordination chemistry. For example, allyl groups have already been widely exploited as caging units on various chemical cargoes, including fluorophores,[149–151] luciferin,[152] nutrients,[153] and DNA binders,[154] and can be unmasked in cells by bioorthogonal ruthenium catalysts. Additionally, palladium-catalyzed deprotection of the propargyl or allyl groups has afforded new ways to activate prodrugs[155] and proteins.[156] Endogenous metals can also serve as triggers for ABS reagents, as illustrated by the use of elevated levels of iron in cancerous cells compared to their healthy counterparts as stimulants for site-directed prodrug activation as an emerging chemotherapeutic strategy.[157–159]

Finally, a key chemical challenge in the field of metal-directed ABS is the greater incorporation of catalytic reactions into reagent design to increase sensitivity, potency, and signal-to-noise responses. Indeed, despite the fact that metals are broadly employed as catalysts across homogeneous/molecular, heterogeneous, and biological fields, the vast majority of metal-directed ABS probes to date rely on stoichiometric or single-turnover reactions. Innovations in the development of bioorthogonal, synthetic inorganic, and organometallic catalysts are a prime growth area in metal-directed ABS chemistry.[160–165] Taken together, the periodic table not only represents the chemical blueprint for life but also organizes the tools to help decipher the interactions between and build new chemical matter with these elements at a molecular level. ABS that exploits both of these fundamental facets of the metals is a powerful platform for their use in biology and medicine, of which metal and metal-mediated detection and bioorthogonal metal chemistry are select examples of many opportunities for further investigation.

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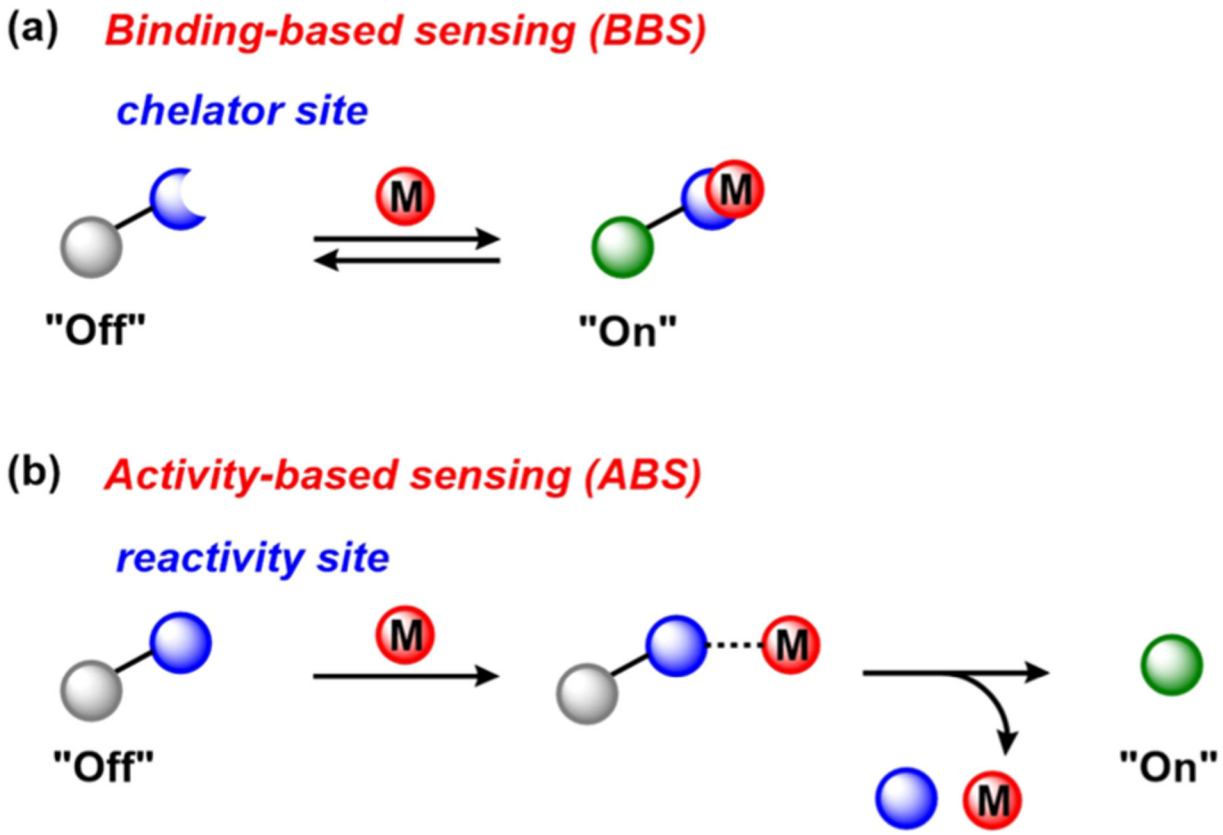

Figure 1.
Approaches for designing probes for metal detection: (a) BBS; (b) ABS. Examples are shown in a “turn-on” fluorescence response mode.

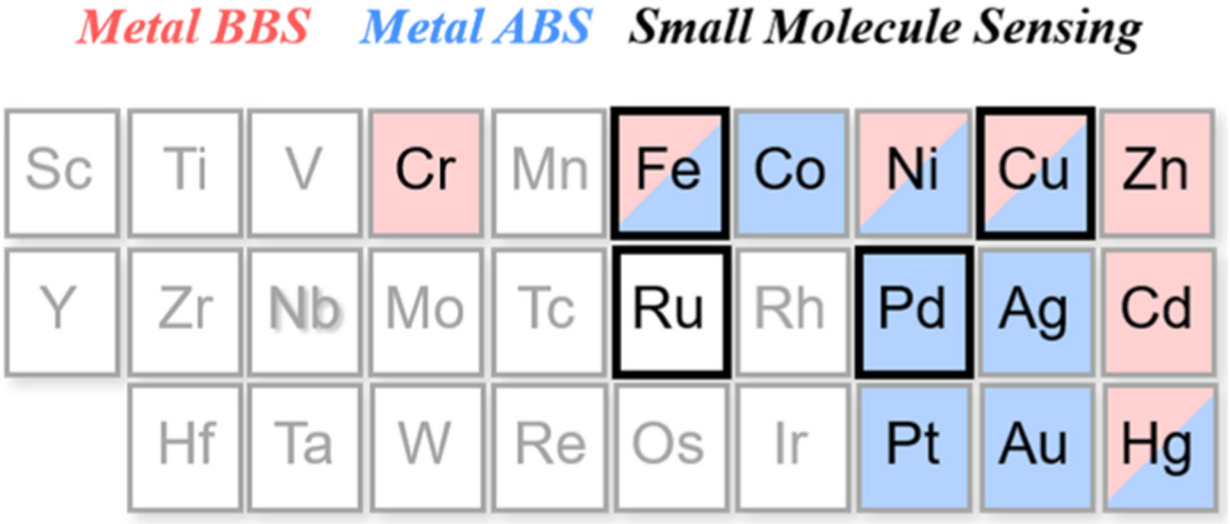

Figure 2.
Summary of the transition metals and their role as analytes (BBS, light-red shading; ABS, blue shading) or indicators for small-molecule detection (black outline).

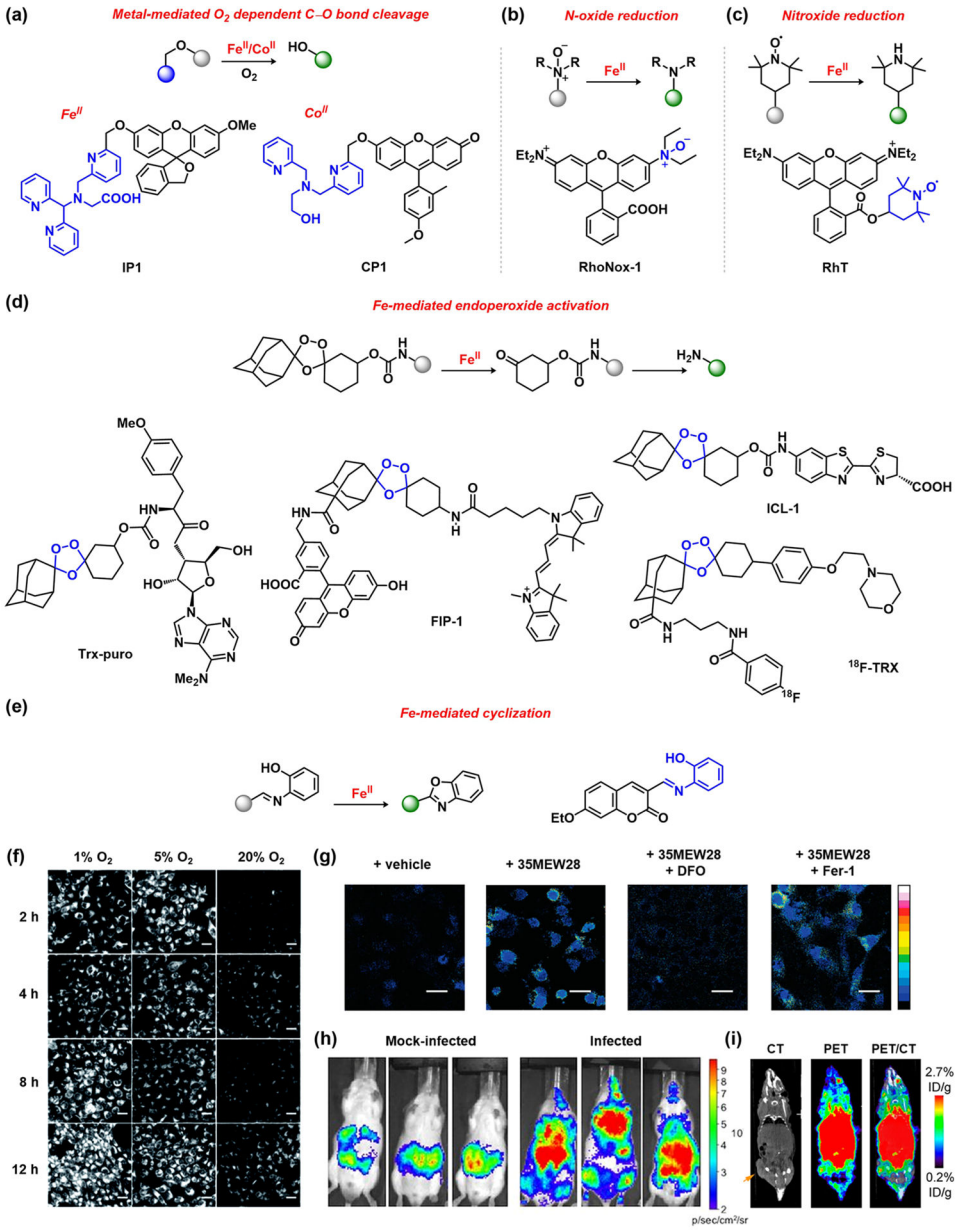

Figure 3.

ABS probes for Fe^{II} and Co^{II} detection: (a) iron[72] and cobalt[40] O_2 activation, followed by C–O cleavage to release fluorophore; iron-mediated (b) *N*-oxide[75] and (c) nitroxide reduction;[83] (d) Fe^{II}-induced endoperoxide activation;[84,85,87,88] (e) Fe^{II}-induced cyclization;[89] (f) monitoring Fe^{II} levels in HepG2 cells under hypoxic conditions (**SiRhoNox-1**;[77] adapted from ref 77. Copyright 2017 Royal Society of Chemistry); (g) detection of changes in labile Fe^{II} pools upon induction of ferroptosis (**FIP-1**;[85] adapted from ref 85. Copyright 2016 American Chemical Society); (h) monitoring Fe^{II} status in mice infected with *Acinetobacter baumannii* (**ICL-1**;[87] adapted from ref 87. Copyright 2017 PNAS); (i) PET/CT to monitor Fe^{II} levels in tumor and normal tissues for mice with

subcutaneous U251 tumors (**^{18}F-TRX**;[88] adapted from ref 88. Copyright 2019 American Chemical Society).

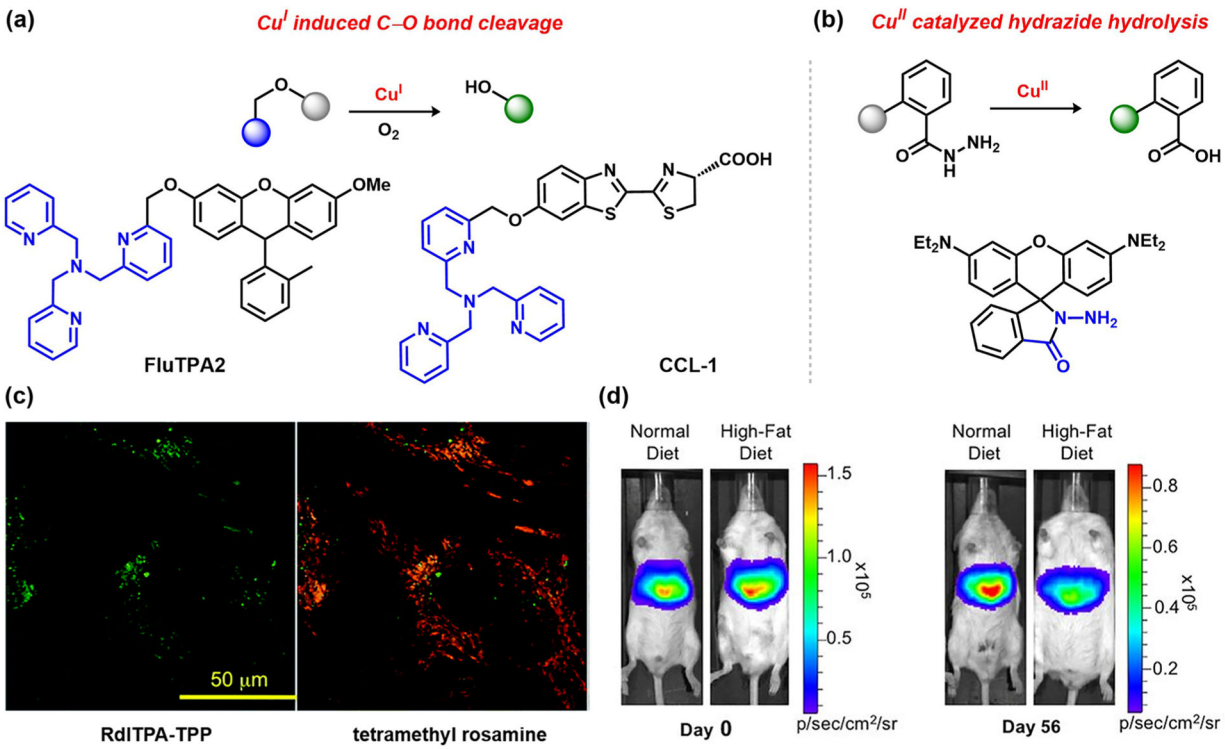

Figure 4.

ABS probes for biological copper detection: (a) biomimetic Cu^{I}–O_2 activation, followed by C–O cleavage to release fluorophore;[4,94] (b) Cu^{II}-catalyzed hydrazide hydrolysis;[42] (c) monitoring copper levels in the mitochondria (**RdlTPA-TPP**;[95] adapted from ref 95. Copyright 2014 Royal Society of Chemistry); (d) monitoring labile Cu^{I} levels in a diet-induced mouse model of NAFLD (**CCL-1**;[4] adapted from ref 4. Copyright 2016 PNAS).

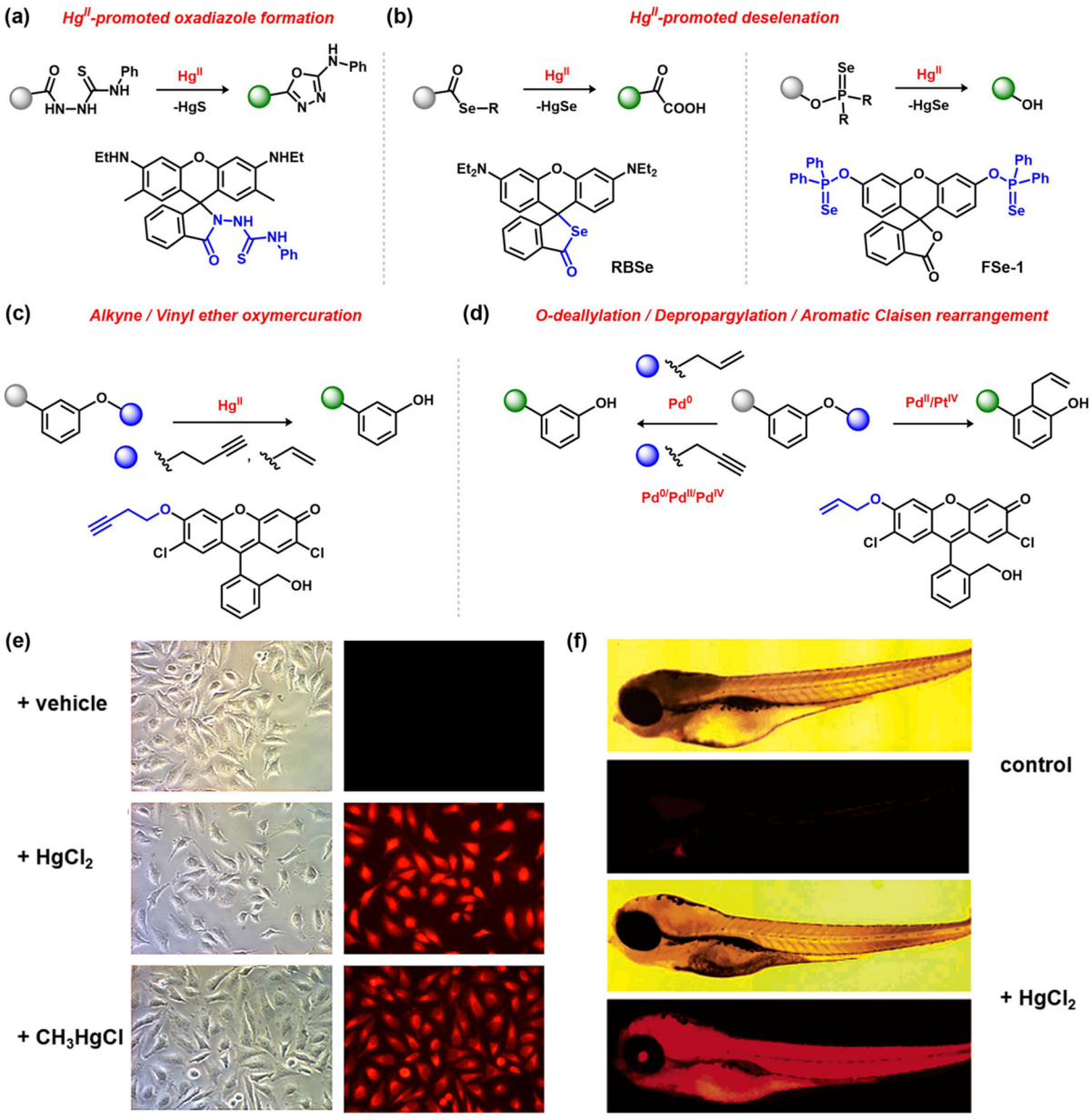

Figure 5.

ABS probes for heavy-metal detection: (a) Hg^{II}-promoted cyclization of thiosemicarbazide;[101] (b) Hg^{II}-mediated deselenation;[106,110] (c) oxymercuration of terminal alkynes or vinyl ethers;[111] (d) $Pd^{0/II/IV}/Pt^{IV}$ detection via *O*-deallylation, depropargylation, or aromatic Claisen rearrangement reactions;[116,119](e) sensing Hg^{II} and CH_3HgCl in HeLa cells (**RBSe**;[110] adapted from ref 110. Copyright 2010 Elsevier); (f) sensing Hg^{II} in zebrafish (top, microscopic image; bottom, fluorescence microscopic image;[102] adapted from ref 102. Copyright 2006 American Chemical Society).

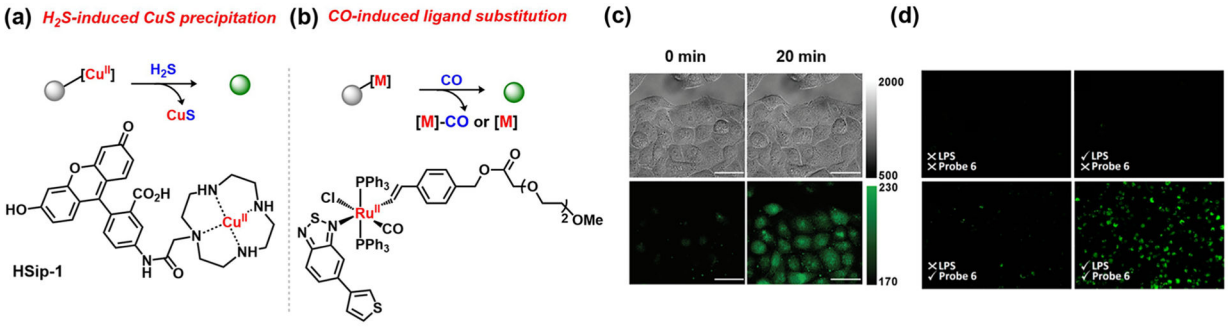

Figure 6.

ABS probes using bioorthogonal metal–ligand substitution reactions: (a) H_2S-induced CuS precipitation to release fluorophore;[48] (b) CO-induced ligand substitution;[127] (c) detection of H_2S in HeLa cells following addition of Na_2S (top, differential interference contrast; bottom, fluorescence; **HSip-1**;[48] adapted from ref 48. Copyright 2011 American Chemical Society); (d) detection of CO in cells from mice treated with lipopolysaccharides (LPS;[127] adapted from ref 127. Copyright 2011 American Chemical Society).

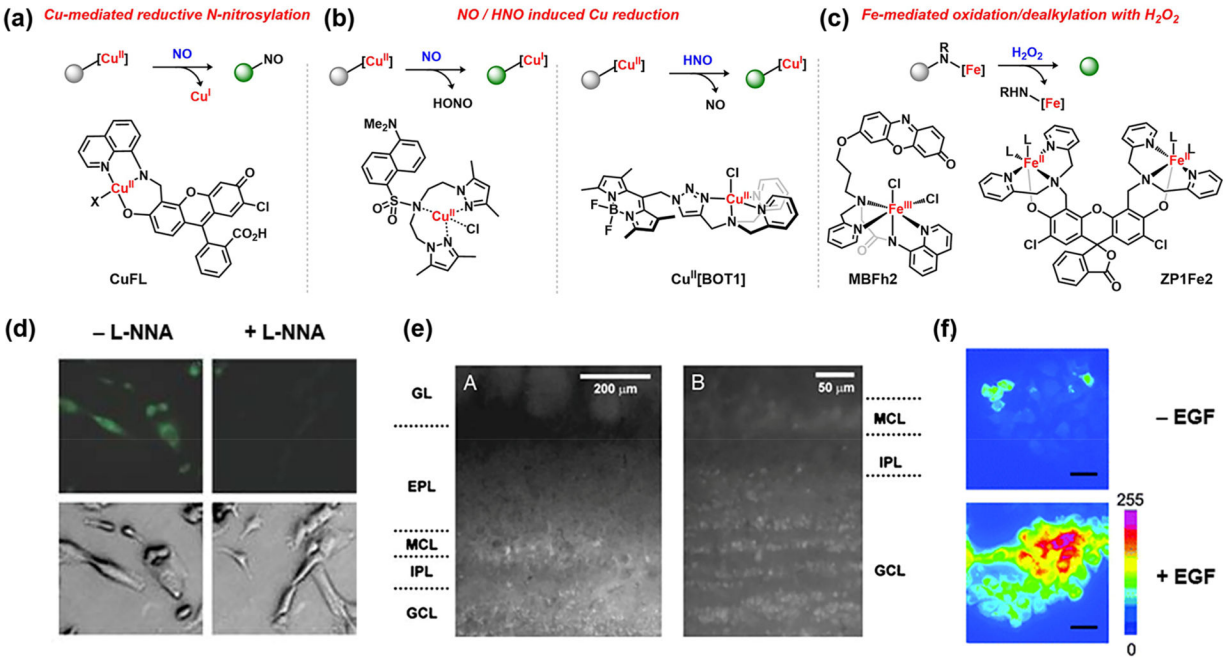

Figure 7.

ABS using bioorthogonal redox reactivity: (a) Cu^{II}-mediated reductive *N*-nitrosylation;[47] (b) NO[131] and HNO[132] reduction of Cu^{II}; (c) biomimetic Fe^{II} and Fe^{III} H_2O_2 activation followed by ligand oxidation to release fluorophore;[138,139] (d) NO detection in SK-N-SH cells treated with 17β-estradiol and with or without N^G-nitro-L-arginine (L-NNA) (**CuFL**;[47] adapted from ref 47. Copyright 2006 Springer Nature); (e) NO detection in the main olfactory bulb (low magnification, left; high magnification, right; **Cu_2(FL2E)**;[130] adapted from ref 130. Copyright 2010 PNAS); (f) monitoring H_2O_2 levels upon EGF stimulation of A431 cells (**MBFh2**;[138] adapted from ref 138. Copyright 2013 Royal Society of Chemistry).

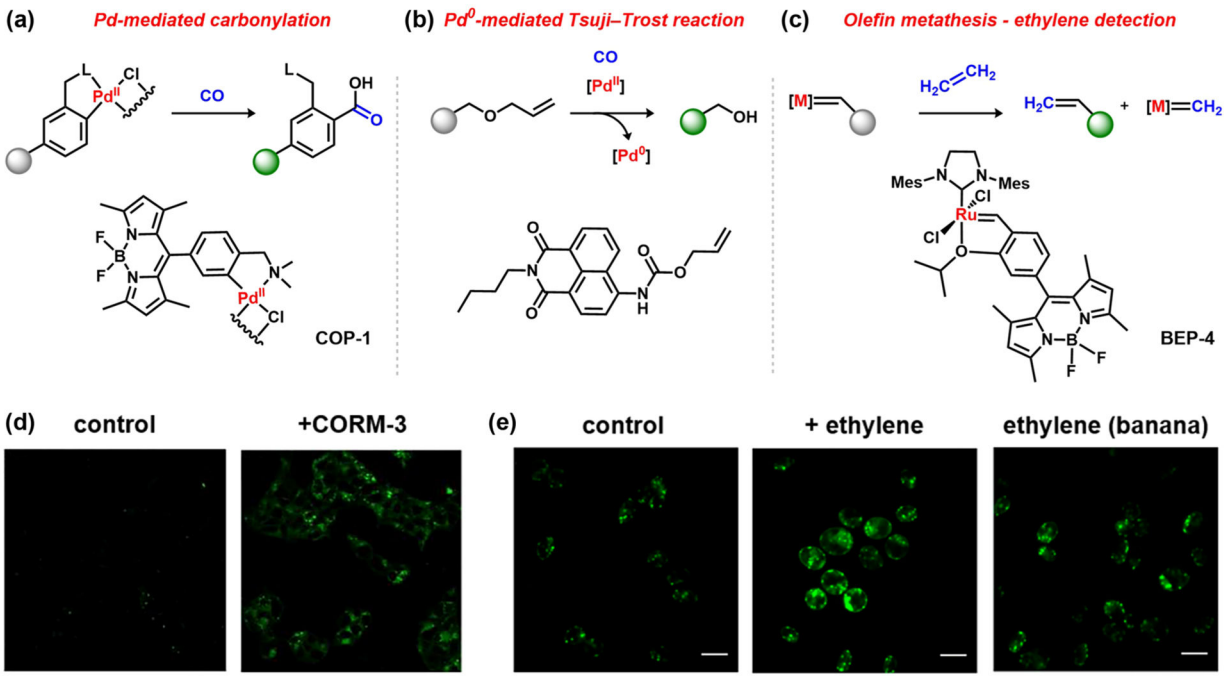

Figure 8.

ABS probes using bioorthogonal organometallic chemistry: (a) CO sensing via palladium-mediated carbonylation;[45] (b) CO-induced Pd^{II} reduction followed by the Pd^{0}-mediated Tsuji–Trost reaction;[146] (c) olefin metathesis ruthenium complex for ethylene sensing;[46] (d) detection of CO in HEK293T cells following treatment with the CO-releasing molecule $[Ru(CO)_3Cl(glycinate)]$ (CORM-3, **COP-1**;[45] adapted from ref 45. Copyright 2012 Amercian Chemical Society); (e) ethylene detection in *Chlamydomonas reinhardtii* cells and banana (**BEP-4**;[46] adapted from ref 46. Copyright 2018 Amercian Chemical Society).