Outlook

# The Chemistry of Lanthanides in Biology: Recent Discoveries, Emerging Principles, and Technological Applications

Joseph A. Cotruvo, Jr.*

Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802, United States

ABSTRACT: The essential biological role of rare earth elements lay hidden until the discovery in 2011 that lanthanides are specifically incorporated into a bacterial methanol dehydrogenase. Only recently has this observation gone from a curiosity to a major research area, with the appreciation for the widespread nature of lanthanide-utilizing organisms in the environment and the discovery of other lanthanide-binding proteins and systems for selective uptake. While seemingly exotic at first glance, biological utilization of lanthanides is very logical from a chemical perspective. The early lanthanides (La, Ce, Pr, Nd) primarily used by biology are abundant in the environment, perform similar chemistry to other biologically useful metals and do so more efficiently due to higher Lewis acidity, and possess sufficiently distinct coordination chemistry to allow for selective uptake, trafficking, and incorporation into enzymes. Indeed, recent advances in the field illustrate clear analogies with the biological coordination chemistry of other metals, particularly Ca^{II} and Fe^{III}, but with unique twists—including cooperative metal binding to magnify the effects of small ionic radius differences—enabling selectivity. This Outlook summarizes the recent developments in this young but rapidly expanding field and looks forward to potential future discoveries, emphasizing continuity with principles of bioinorganic chemistry established by studies of other metals. We also highlight how a more thorough understanding of the central chemical question—selective lanthanide recognition in biology—may impact the challenging problems of sensing, capture, recycling, and separations of rare earths.

Biological lanthanide coordination chemistry ➡ Technology
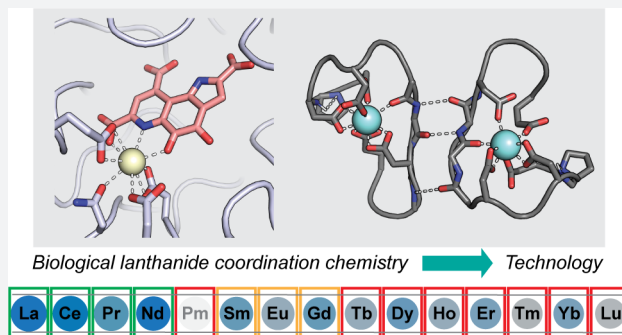
INTRODUCTION: EXPANDING THE BIOINORGANIC PERIODIC TABLE

Rare earth (RE) elements—the lanthanides, yttrium, and scandium—are critical components of numerous modern technologies, from permanent magnets in wind turbines and electric car batteries, to lasers and phosphors, to medical imaging agents.[1,2] However, the combination of the insolubility[3] and similarity in physical properties[4] of REs presents myriad challenges in basic science, environmental sustainability,[5,6] and economics[7] for their separations in mining or recycling applications.[8] The primary sources of REs are phosphate or carbonate minerals, such as bastnäsite, monazite, and xenotime,[9,10] in which multiple REs often co-occur. REs are also present at significant concentrations in coal ash and acid mine drainage, although these lower-grade sources present further challenges for separation from more abundant metal ions, especially Fe^{III}, Mn^{II}, Cu^{II}, and Al^{III}.[11] These challenges have motivated investigations into diverse new approaches[8]—from small molecules,[12−14] supramolecular assemblies such as metal−organic frameworks,[15] novel extraction and chromatographic methods, and even bacterial cells[16,17]—to separate REs from other metals and from each other. The discovery in 2011[18−20] that methylotrophic bacteria specifically incorporate certain REs (the early lanthanides, La−Nd) into pyrroloquinoline quinone (PQQ)-dependent alcohol dehydrogenases (ADHs) has opened new possibilities for more sustainable

and efficient aqueous extraction and separations of these elements.[8,21] The pace of discovery in this field has accelerated rapidly in the last year; here, we review these discoveries from a chemical perspective, highlighting potential, yet to be uncovered biological roles of lanthanides as well as broader applications of these findings to aid in RE mining, recycling, and separations.

The aqueous chemistry of the REs is dominated by the +III oxidation state. Although other oxidation states are accessible, most notably Ce^{IV} and Eu^{II}, under normal physiological conditions it is generally believed that lanthanides would be limited to +III (Ln^{III}).[4,22−24] Important properties of Ln^{III} ions have been extensively reviewed;[4,25] the key characteristics relevant to lanthanide biochemistry (Figure 1) derive from their 4f electrons. The radial probability distribution of 4f orbitals is closer to the nucleus than for 6s and 5p; as a result, the 4f electrons poorly shield outer shells from increasing nuclear charge. Consequently, ionic radius decreases across the series (the lanthanide contraction): for a coordination number (CN) of 8, ionic radii span from 1.16 Å (La^{III}) to 0.98 Å (Lu^{III}).[26,27] The ionic radius of Pr^{III} is similar to that of Ca^{II}, 1.12 Å (for CN = 8). For this reason and the unique spectroscopic features of the 4f electrons, lanthanides have
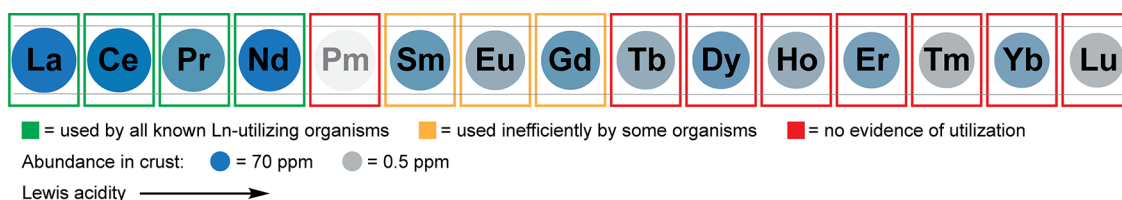
Figure 1. Properties of the lanthanide series. The elements are scaled by ionic radius (Ln^{III}, CN = 8);[26] Lewis acidity increases from La to Lu. Elemental abundances in the crust[31] range from blue (most abundant) to gray (least abundant). Pm has no stable isotopes and is not found in Nature. Boxes are colored according to currently known biological utilization.[32] The other two REs, Y and Sc, are not shown. The ionic radius of Y is similar to that of Ho, and abundance is similar to that of La. Sc is not discussed in this Outlook.

been used extensively in biochemistry as probes of calcium binding sites in proteins.[4,28−30] Furthermore, because the 4f electrons have little impact on bonding, Ln^{III} complexes are largely ionic in character—favoring hard, carboxylate ligands in biology—and flexible, sterically driven coordination geometries. These traits, combined with large ionic radii, allow Ln^{III} (and especially the early lanthanides) to accommodate high CNs (8−12), rarely encountered with other common metal ions. The high charge of the Ln^{III} ions makes them good Lewis acids and results in poor solubility of their hydroxide and phosphate salts, with K_{sp}'s of $\sim10^{-30}$, translating to solubilities of $\sim$0.1−1 pM at pH 7.[3]

Although once thought unlikely to be biologically relevant,[33] there are good reasons why biology has utilized lanthanides.

> There are good reasons why biology has utilized lanthanides.

First, they are fairly abundant; crustal elemental abundances for La−Nd are $\sim$10−70 ppm, similar to other metals like Cu

and Zn, whereas late lanthanides are 10−100 times rarer.[31] Second, their higher charge-to-radius ratio versus other common biological Lewis acids such as Ca^{II}, Mg^{II}, and divalent first-row transition metal ions makes them equally or even more robust Lewis acid catalysts,[34] and they would be more desirable when higher CNs, flexible coordination geometry, and/or redox-inertness are required for catalysis. Third, their functional and/or size similarities with Ca^{II}, Fe^{III}, and Mg^{II} facilitate evolutionary connection to analogous pathways involving these metals, while their coordination chemistry is sufficiently distinct, facilitating selective recognition.

A central theme of this Outlook (relative to other recent reviews[21,32,35−39]) is that the above basic principles, in the context of existing knowledge about how biology handles other metal ions,[40] anticipate the key aspects of lanthanide utilization. Results so far have revealed direct analogies between Ln^{III} acquisition, transport, utilization, and storage to the extensively described bioinorganic chemistry of Ca^{II} and Fe^{III} (Figure 2), and the analogies are likely to continue as more is learned. Cells must resolve the same challenge faced by
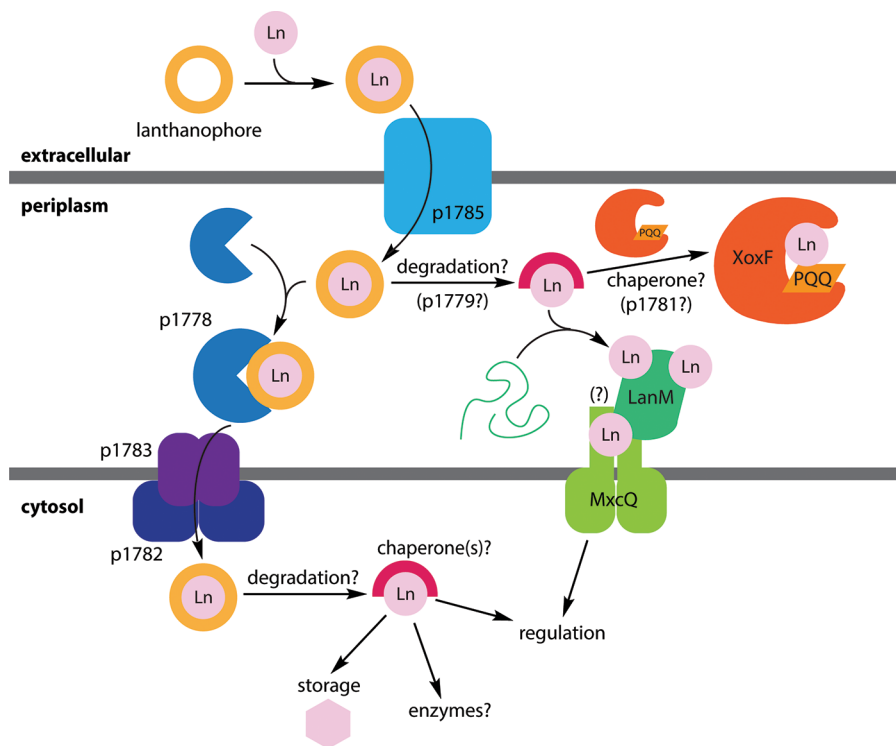

Figure 2. Model for lanthanide uptake and utilization in *M. extorquens* based on the work of the Cotruvo,[41−43] Vorholt,[44] and Martinez-Gomez and Skovran[45] laboratories. Unknown/postulated functions (the exact ligand for p1778, functions of p1779 and p1781, and LanM−MxcQ interaction) are indicated with parentheses and question marks.

the technological utilization of REs—the difficulty of separations. The differences in ionic radius and therefore ligand affinities between adjacent Ln[III] are small, and biology cannot afford dozens of chromatographic steps[8,10] to filter one RE from another. At the same time, it is unable to utilize all REs indiscriminately. Instead, it has devised ways to accommodate a small swath of the periodic table roughly equally well, La—Nd, which are both the most abundant REs and those (due to their size) whose coordination chemistry is most distinct from that of other metals used by biology. This review examines how biology accomplishes this feat through its "lanthanome"[41]—the suite of proteins and other biomolecules involved in lanthanide utilization—and the potential broader applications thereof.

> Cells must resolve the same challenge faced by the technological utilization of REs—the difficulty of separations.

■ LANTHANOENZYMES

A specific biological role of lanthanides was first demonstrated in 2011 with genetic and biochemical characterization of XoxF as a lanthanide- and PQQ-dependent methanol dehydrogenase (Ln-MDH) in several methylotrophs, including *Methylorubrum* (formerly *Methylobacterium*) *extorquens* AM1 (*Me*).[18−20] Methylotrophs are found in soil, water, and plants, where they play an important role in the carbon cycle because of their ability to utilize C_1 compounds such as methanol or even methane (in the case of methanotrophs) as their sole carbon source.[37,46,47] MDHs are critical enzymes for methylotrophs, catalyzing the oxidation of methanol to formaldehyde (Figure 3a).[47,48] This reaction is a formal hydride transfer from the substrate to the metal-ligated C5 carbonyl of the PQQ cofactor, facilitated by the Lewis acidity of the metal ion.[49,50] Single electrons are then transferred from the reduced PQQ cofactor to a *c*-type cytochrome (XoxG[43,51,52] in the case of Ln-MDH). A Ca- and PQQ-dependent MDH (Ca-MDH, MxaFI) had been known for decades,[53] but the function of XoxF had been cryptic.[54,55] Whereas lanthanides were

preferred but not required for growth of the methylotrophs in which XoxFs were first characterized, elegant work by Jetten, Op den Camp, and co-workers identified the first organism with an absolute requirement for lanthanides for growth—a novel thermoacidophilic methanotroph, *Methylacidiphilum fumariolicum* SolV (*Mf*), isolated from a volcanic mudpot.[56] The mudpot water from which this organism was initially isolated had a pH of 1−2 and contained 2−3 μM REs, primarily La, Ce, and Nd.[56] This result demonstrates both the high concentrations of soluble, bioavailable REs possible in acidic environments and the importance of REs for life in such an extreme niche. The fascinating environmental and ecological implications of this finding have been extensively reviewed;[36,37,57] here, the discussion is restricted to chemical aspects. Structural characterization of the native XoxF lanthanoenzyme purified from *Mf* revealed an active site very similar to that of a Ca-MDH, with a Ce[III] ion coordinated to the PQQ and Asp, Glu, and Asn residues (Figure 3b). The only significant difference was an additional carboxylate ligand in XoxF, increasing CN from the 7 observed for Ca[II] in Ca-MDH to the expected 9 for a Ln[III] ion.[56,58]

XoxF-type ADHs are widespread in Nature, more so than the Ca-dependent MxaFI type, and they are proposed to have arisen prior to the Ca-MDHs.[57,59,60] (Most XoxF-type ADHs are likely to be lanthanide-dependent enzymes; however, one XoxF has been found to copurify with Ca.[61]) Biological utilization of lanthanides, like that of iron, may have evolved in an environment in which lanthanides were more soluble and bioavailable than currently, and biology found them useful enough to retain them. Following the initial discovery of XoxF as a Ln[III]-dependent enzyme, several more lanthanide- and PQQ-dependent ADHs using methanol or other alcohols as substrates were found in methylotrophs[51,58,62−65] and in a nonmethylotroph.[66,67] These studies have shown that bacteria differ in lanthanide tolerances for growth, but all characterized to date prefer the early REs. For example, *Mf* grows most efficiently with La—Nd, but Sm—Gd also support growth;[56] by contrast, *Me* will only use La—Nd, and Sm very poorly[68] (Figure 1). These tolerances appear to be driven not only by RE bioavailability but also by coordination chemistry and reactivity considerations, as discussed below.

An intriguing and unique aspect of XoxF is that at least four different metals, La—Nd, are each able to confer activity when
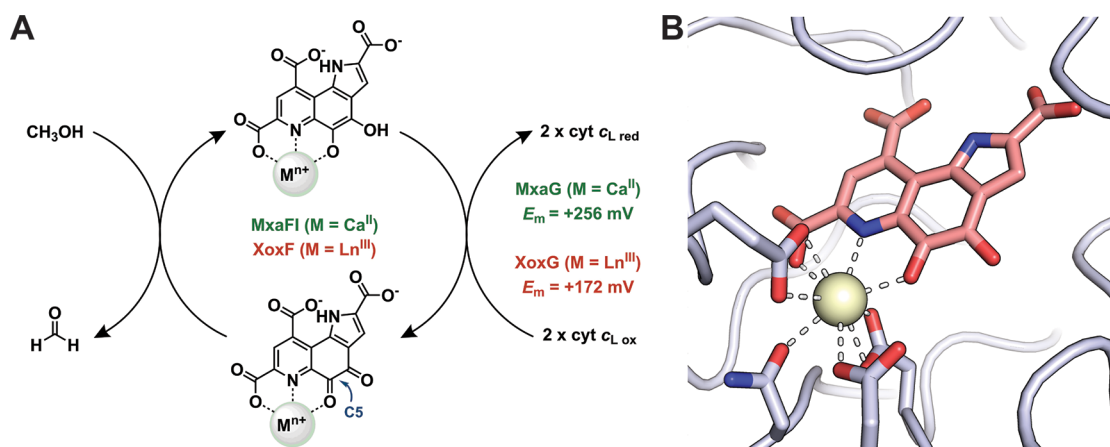

Figure 3. (A) Reaction catalyzed by MDHs, with E_m of the *c*-type cytochrome redox partners of *Me* Ca-MDH (MxaG) and Ln-MDH (XoxG) shown. The reactive C5 position of PQQ is denoted. Modified from ref 43. (B) Active site of *Mf* Ln-MDH (PDB code 4MAE), modeled with Ce.[56] Ce[III] is a cream sphere; protein ligands are in gray sticks, and PQQ is in salmon. A ligand from the crystallization solution has been omitted.
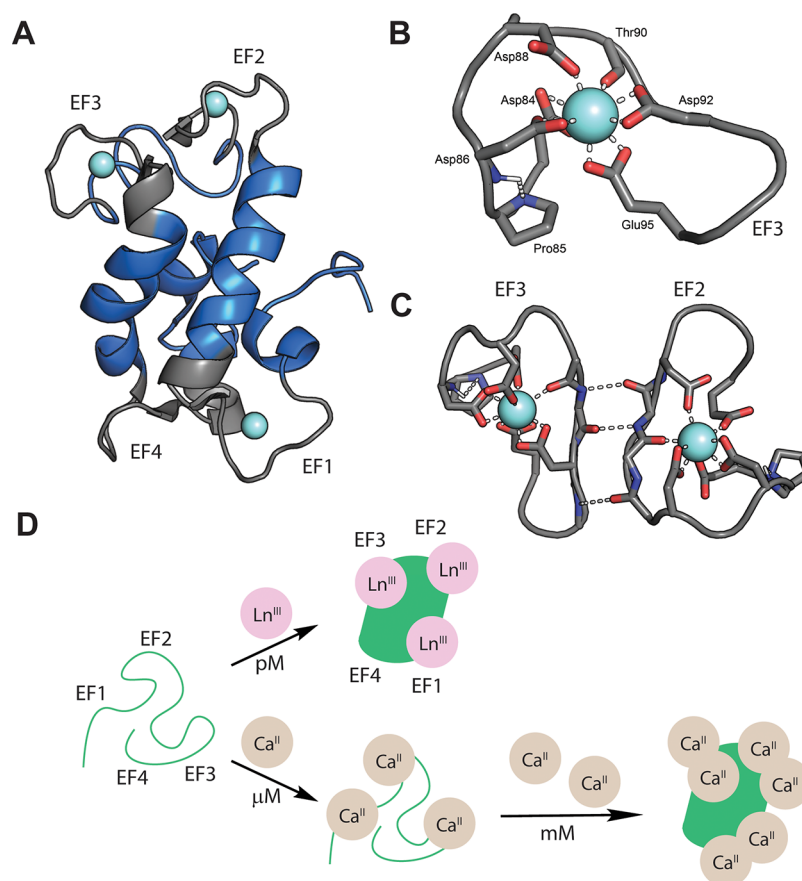
Figure 4. Lanmodulin as a model system to study biological principles of selective RE recognition. (A) Solution structure of Y^{III}−LanM (PDB code 6MI5).[43] Y^{III} ions are in cyan, and EF loops are shown in gray. Metal coordination by EF4 is weak and likely not physiologically relevant.[42] (B) Detail of Y^{III} coordination in LanM (EF3), with coordinating residues and the N_{i+1}−H···N_i hydrogen bond involving the proline residue shown. (C) The hydrogen bonding connectivity of the EF2/3 pair illustrates the importance of cooperativity in RE recognition. (D) Working model for selectivity for Ln^{III} (and Y^{III}) over Ca^{II}.

incorporated into the enzyme active site. Few enzymes, and particularly redox enzymes, are active with more than one or two different metal cofactors.[69,70] Design of an enzyme that can tolerate several lanthanides in the same protein framework is a challenge because of differences in Ln^{III} ionic radius and CN preference as well as interactions with substrate and intermediates, which may affect the methanol oxidation chemistry.[24] Furthermore, in vivo, the electrons extracted from methanol to the Ln^{III}-PQQ cofactor must then be transferred to XoxG,[43,51,71] and the efficiency of this process would be expected to depend on the identity of the Ln^{III} ion coordinated by PQQ.[43]

The chemical similarities of the lanthanide series make the Ln-MDH an excellent system to probe metal-dependent structure−function relationships in a single protein framework. Using *Mf* XoxF, which can be demetalated and reconstituted with all lanthanides in vitro, Daumann reported maximal activity with Pr, using a redox dye-based assay.[24] Similar experiments cannot be carried out at present using *Me* XoxF because metal removal irreversibly inactivates the protein.[43] Instead, the *Me* enzyme can be loaded with early REs in vivo, exhibiting highest activity with La.[43,72] However, assays using the physiological electron acceptor, XoxG, tell a different story:[43] *Me* La-, Ce-, and Nd-XoxFs all exhibit the same maximum velocity, but the K_m for XoxG increases from La to Nd, inversely proportional to ionic radius. We interpreted this result as reflecting an increasing reduction potential (E_m) of

the Ln^{III}-PQQ cofactor with increasing Ln^{III} Lewis acidity, suggesting that redox matching of XoxG and the Ln^{III}-PQQ cofactor is an important constraint for lanthanide utilization in vivo. This proposal is supported by the observation that the E_m of *Mf* XoxG[52] is ∼80 mV higher than that of *Me* XoxG,[43] as *Mf* is tolerant of heavier lanthanides, up to Gd, in vivo. Furthermore, *Me* grows more slowly with Nd than La,[68] suggesting that later REs, even if they could be acquired, likely would not support efficient growth. Thus, lanthanide utilization in MDH seems to be a compromise between lanthanide availability, radius, Lewis acidity, and E_m of its redox partner.[24,43,64]

Further studies of XoxFs[24,43] and functional model complexes[73] metalated with different REs, native or otherwise, will enable spectroscopic analysis of the MDH reaction mechanism, about which significant questions still exist (reviewed recently[32]). Such studies are challenging with the spectroscopically inert Ca^{II}. Substitution of different REs into XoxF also may be useful in trapping different intermediates by changing the rate constants for various steps in the reaction. These studies will be facilitated by a more complete understanding of the mechanism of activation of XoxF, which may involve XoxJ (speculated to bind XoxF to aid in its activation[43]) as well as potential chaperone proteins for the PQQ and Ln^{III} cofactors[41,45,74] (Figure 2). Understanding of this cofactor assembly pathway might allow for expression in a simpler system (e.g., *Escherichia coli*) to facilitate bioengineer-

ing applications such as a methanol biofuel cell.[75] Because, unlike Ca-MDH, XoxF can efficiently oxidize not only methanol to formaldehyde but also (in vitro but likely not in vivo)[56,72] formaldehyde to formate, it may simplify a methanol to CO_2 cell to just two enzymes, XoxF and a formate dehydrogenase. Finally, the proposed effect of Ln^{III} coordination on the E_m of PQQ in XoxF[43] suggests the feasibility of performing electrochemical separations of REs using RE-PQQ complexes, as has been accomplished recently with other redox-active ligands.[13] The speciation of aqueous Ln^{III}-PQQ complexes is heterogeneous,[76,77] but such an approach may be possible with the help of PQQ-binding proteins.

Another open question is whether lanthanoenzymes that are not PQQ-dependent exist. The known lanthanoenzymes are all periplasmic, but discovery of lanthanide import to the cytosol[41] suggests that lanthanoproteins may occur throughout the cell. However, studies so far have not identified putative enzymes other than ADHs that are obviously upregulated by lanthanides,[44,72] so Ln^{III} ions may substitute for other metals[36] such as Ca^{II}, Mg^{II}, or Fe^{III} in certain enzymes or cofactors. Ln^{III} ions will likely bind tightly to any enzymes, facilitating their proteomic identification.

■ LANTHANIDE RECOGNITION IN CELLS

From 2011 to 2018, the only lanthanide-binding proteins characterized were various PQQ-dependent ADHs.[18−20,56,58,62,64,66] Biological utilization of other metal ions involves proteins responsible for selective import/export, trafficking, and regulation.[78−80] Because these proteins are tasked with ensuring correct metalation of enzymes, their metal selectivity is especially critical. We reasoned that identification and characterization of lanthanoproteins with these functions may be more informative than the lanthanoenzymes about the central question of how biology selectively recognizes lanthanides.

The first insights into biological recognition of lanthanides were provided by discovery and characterization of lanmodulin (LanM), reported in late 2018.[42,81] LanM was immediately intriguing to us as its amino acid sequence contained 4 EF-hand motifs (Figure 4a). EF hands are 12-residue Ca^{II}-binding motifs, widespread in biology and usually found in pairs for cooperative binding, such as in the Ca^{II} sensor, calmodulin.[82,83] As a testament to the coordination similarities of Ca^{II} and Ln^{III} ions, typical EF hands bind Ln^{III} with slightly higher (~100-fold) affinity than Ca^{II}, although Ln^{III} coordination is not physiologically relevant in these cases.[84−86] However, these similarities have also been exploited in the development of more selective "lanthanide-binding tags" with nanomolar lanthanide affinity, derived by engineering of natural EF hands.[87,88] LanM exhibits several remarkable properties for an EF-hand protein.[42] First, it undergoes a large, cooperative conformational change in response to binding of all trivalent REs. Second, the NMR solution structure of LanM in the presence of Y^{III} (a diamagnetic RE used as a Ln^{III} surrogate) revealed metal coordination by 5 carboxylates and a backbone carbonyl (CN = 8−9, depending on metal site), whereas Ca^{II} sites in typical EF hands are 7-coordinate (Figure 4b).[81] Third, each EF hand possesses a Pro residue at the second position, a feature present in no previously characterized EF hands.[83] The Pro N is engaged in an unusual N_{i+1}−H···N_i hydrogen bond with the backbone NH of the subsequent residue, a coordinating Asp. Finally, and perhaps most remarkably, LanM responds to picomolar free concentrations of REs,

whereas millimolar Ca^{II} is required for a conformational change (10^8-fold selectivity). This selectivity is much greater than would be expected on the basis of charge and radius alone (~10^4).[4,86,89] Furthermore, LanM binds early REs with higher affinity than late REs, whereas chelators typically prefer the more Lewis-acidic, late REs.[8] These intriguing and unusual properties have motivated us to study LanM as a model system for determining the rules of highly selective biological lanthanide coordination. While much work remains, our studies have suggested that the prolines contribute ~100-fold to RE selectivity, apparently by uncoupling initial Ca^{II} binding from a conformational change.[42]

> These intriguing and unusual properties have motivated us to study LanM as a model system for determining the rules of highly selective biological lanthanide coordination.

The proposed importance of the prolines and their N_{i+1}−H···N_i hydrogen bonds is 2-fold: (1) to constrain the structure of the EF loop such that metals that prefer fewer ligands (e.g., Ca^{II}) force the carboxylates into an arrangement incompetent to produce a conformational change until superphysiological concentrations (Figure 4d) and (2) to buttress against loop constriction as Ln^{III} ionic radius decreases from La to Nd, thereby retaining communication with the adjacent metal site for optimal affinity and cooperativity (Figure 4c). Further structural and spectroscopic work is needed to draw more specific conclusions about metal coordination.

LanM's large, highly RE-selective conformational change suggests a role in lanthanide sensing, perhaps as the substrate of a two-component system (Figure 2) that may be involved in switching between Ca- and RE-dependent physiologies (the "lanthanide switch"[38,44,67,68,90]). Although it is one of very few proteins upregulated in response to early REs in several organisms,[44,72,91] LanM is not essential for lanthanide-dependent growth, pointing to probable redundancies in lanthanide-handling machinery. Furthermore, it is only found in certain methylotrophs, suggesting that other organisms encode unrelated proteins with a similar function.[38]

We also have identified a second RE-binding protein (META1p1781).[41] It shares LanM's unusual property of preferentially binding early over late REs, but it does not possess an EF hand. Although not yet as thoroughly characterized as LanM, this protein is dimeric and binds 2 Ln^{III} ions per dimer, suggesting that cooperative metal binding may contribute to metal selectivity, as in LanM. It seems virtually guaranteed that more RE-binding proteins will be discovered, and their characterization will provide a compendium of biological lanthanide recognition strategies that may be useful for broader applications.

LanM's high selectivity for REs suggests that it may be useful for green, aqueous separation of total REs from other metals. Large-scale separation methods generally involve repeated, acid- and organic solvent-intensive extraction steps using ligands with low selectivities.[8,10] LanM may not be optimal as is for separations of adjacent REs, as Nature has engineered it to respond similarly to La−Nd. However, it may be a privileged scaffold for evolution of selective peptide-based

ligands for REs and other metal ions. These applications may include metal harvesting and recycling, sensing (e.g., rapid detection of REs in the field using LaMP1, a LanM-derived fluorescent protein-based sensor we reported[41]), and metal-lotherapeutic and imaging agents.[2] A particular advantage of LanM is the cooperative metal binding exhibited by its EF hand pairs, which could allow for greater selectivities for adjacent REs than is possible at a single metal-binding site (as in currently used ligands), a general principle highlighted by recent synthetic ligands for size-based separations.[12,14,92]

■ LANTHANIDE UPTAKE

While the solubilities of Ln^{III} are picomolar at pH 7, they are still 8−9 orders of magnitude higher than that of Fe^{III}, which bacteria acquire using secreted small molecules called siderophores.[93−95] As a result, several investigators speculated that lanthanides might be acquired via a lanthanophore.[32,37,39,42,57,96] In Gram-negative bacteria, Fe^{III}−siderophore complexes are typically taken up using TonB-dependent systems comprising specific outer- and inner-membrane transporters, a periplasmic binding protein, and an enzyme for siderophore degradation for iron release.[97−99]

When we first reported LanM in *Me* AM1,[42] we noted that the gene encoding it was adjacent to a putative TonB-dependent transporter, suggesting that the *lanM* gene cluster encodes a lanthanophore uptake system. Genetic studies by Vorholt[44] in a closely related strain showed that the putative transporters of this cluster were essential for lanthanide-mediated growth. Contemporaneously, we used the LaMP1 sensor to reveal several details of lanthanide uptake. First, we showed for the first time that La−Nd are selectively taken up into the *Me* cytosol (Figure 5, confirming the importance of
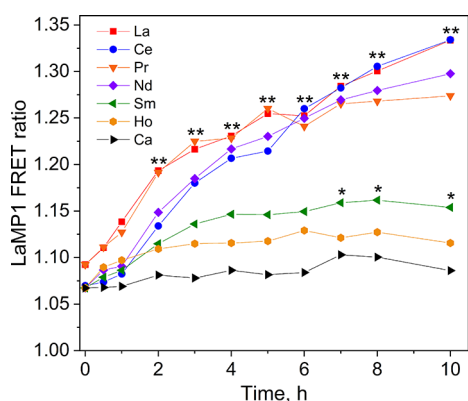

Figure 5. *M. extorquens* selectively uptakes early lanthanides into its cytosol. Cells expressing the LaMP1 sensor were grown without lanthanides, and at $t = 0$, 2 μM Ln^{III} was added to each culture. Increased FRET ratio indicates increased sensor-bound Ln^{III} ions. Error bars omitted for clarity. * $p < 0.05$ (Sm vs Ca), ** $p < 0.01$ (La−Nd vs Ca). Modified from ref 41.

the transporters). Second, we showed that the periplasmic binding protein of the putative uptake system does not bind free Ln^{III} ions (implying that it may bind a Ln^{III} complex instead). Finally, we demonstrated an activity in the spent medium from cells grown without REs that is capable of selectively outcompeting LaMP1 for binding early REs (physical evidence for a lanthanophore).[41] These data all but confirmed the secretion of a molecule required for selective

uptake of early REs (Figure 2) and laid out an approach for its isolation and characterization.

These observations also provided key information about the mechanism by which the lanthanophore achieves its selectivity. The strong ionic radius dependence of lanthanide-dependent regulation[68] and cytosolic lanthanide uptake[41] argued that the lanthanophore does not simply rely on the rather small affinity differences between REs for a single site.[8] Instead, it likely selects for early REs based on (1) differences in overall RE-complex shape, perhaps exploiting the ability of early REs to accommodate higher CNs, with only one conformation recognized by the importer, or (2) cooperative binding of two or more metal ions. Both potential approaches have been anticipated by elegant small-molecule work focused on RE separations, for example, that of Schelter using ionic radius-dependent dimerization equilibrium of an RE complex[12,92] and that of Sun using supramolecular tetrahedral cages templated by metal binding,[14] as well as by LanM's RE recognition strategy. Determination of the lanthanophore's structure will resolve these questions.

> In biology, metal selectivity relies on both affinity differences and conformational changes … in RE harvesting and separation applications, selectivity likely must rely on affinity differences alone.

It is not known whether all lanthanide-utilizing organisms have lanthanophores, and whether the diversity of their architectures matches that of siderophores.[93,94,100] For example, *Mf* may not require a dedicated lanthanophore because of higher RE solubility in its acidic environmental niche.[32,43] Klebensberger has recently reported that iron interferes with lanthanide uptake in *Pseudomonas putida*,[101] highlighting the complexity of selective metal uptake. Finally, it is possible that some previously characterized siderophores might instead (or in addition) be lanthanophores.

As with LanM, there are clear potential applications of lanthanophores for RE harvesting and separations. In fact, siderophores and simplified derivatives are already being explored in this regard,[102,103] but the lanthanophore's selectivity for early REs makes it an attractive alternative. However, the highest-value REs are the rarer, heavy ones, which the *Me* lanthanophore may not bind effectively; one can envision synthetic derivatives that are selective for different sets of REs. As more lanthanide-utilizing organisms are characterized, lanthanophores with different metal selectivities may also be found. One potential caveat is that, in biology, metal selectivity relies on both affinity differences and conformational changes necessary for specific recognition by a receptor. For example, LanM undergoes a conformational change with 10^8-fold selectivity for REs over Ca^{II}, but only 10^6 derives from affinity differences; the rest appears to derive from a conformational effect. By contrast, in RE harvesting and separation applications, selectivity likely must rely on affinity differences alone. Therefore, engineering of biological ligands may be required prior to their implementation as technologies for RE extraction and separations.

LANTHANIDE STORAGE

Given the selective cytosolic uptake of early REs by Me[41] and Me's ability to deplete cultures of early REs,[104] it was reasonable to suggest that bacteria would take advantage of the low solubility of RE (poly)phosphate complexes for lanthanide storage in "lanthanosomes," similar to Ca, which is stored in organisms from bacteria to humans in organelles called acidocalcisomes.[105] In fact, analogous mechanisms exist for storage of many metals: copper is stored in acidocalcisome-like structures in algae[106] and zinc in similar zincosomes in *C. elegans*,[107] and even iron storage in ferritin as an Fe^{III}-oxyhydroxide with variable phosphate content[108] is related. This lanthanide storage mechanism was recently demonstrated by Skovran and Martinez-Gomez,[45] although details remain to be defined. The bioaccumulation of lanthanides presents possibilities for whole-cell lanthanide extraction methods, although the probable storage of only early REs, slow lanthanide accumulation by Me,[104] and the need to carry out further separations from the rest of the bacterial constituents may hinder such an approach.

LANTHANIDES BEYOND BACTERIA

Specific biological functions of lanthanides are best characterized in bacteria. However, REs have been used for decades as fertilizers, having been found to accumulate in plants and enhance growth, crop yields, and drought resistance (refs 109 and 110 and references therein). The precise mechanisms by which REs have these benefits have yet to be elucidated, but understanding them may enable more thoughtful approaches to improving agricultural yields. The targets may relate to plant photosynthesis or cell wall structure and/or to effects on the methylotrophs such as Me that colonize plants because of their substantial methanol production.[36,111]

Unlike bacteria studied to date, which strongly prefer early REs, plants seem to accumulate REs in proportion to their concentrations in the soil,[44,112] suggesting that much of the accumulation occurs nonspecifically, perhaps through Ca or Fe uptake pathways, such as the broad-spectrum metallophore, nicotianamine.[113,114] It is also conceivable that plants take advantage of the increased bioavailability of REs resulting from production of lanthanophores by bacteria residing in the phyllosphere. For example, much like the mammalian protein siderocalin binds Fe^{III}–siderophore complexes as an immune defense mechanism to interfere with iron acquisition by invading pathogens,[115] it is possible that plants produce proteins that can bind Ln^{III}–lanthanophore complexes—either to limit colonization by plant pathogens or as part of a mutualistic association between plant and lanthanide-utilizing microbes to make REs more accessible to the plant. RE uptake may also be linked to the beneficial effects of microbe-derived PQQ for plant growth;[116] the recent identification of an RE-regulated PQQ acquisition system in Me[74] supports the existence of RE and PQQ signaling networks in plant–microbe systems, the mechanisms and targets of which have yet to be fully elucidated.

The proportion of plant-accumulated REs present within cells versus outside is not known. Extracellular REs may be incorporated in place of Ca^{II} in the cell wall. Methanol is produced by de-esterification of pectins, which exposes carboxylates for metal ligation to facilitate cell–cell adhesion.[117,118] This synergy between cell wall strength and structure, Ca^{II} or RE binding, and methanol production (which could be used by associated methylotrophs) may be relevant to the observed positive effects of REs on plants. Cellular RE uptake has been described to occur via endocytosis,[119−121] but this mechanism may be a result of formation of insoluble RE aggregates under the in vitro assay conditions. However, in the plant, at least some of the accumulated REs are presumably present in a soluble, bioavailable form in cells, as Ce^{III} has been reported to be incorporated into chlorophyll, particularly in Mg-limited soils.[112,122] This substitution, presumably occurring via competition for binding to the Mg chelatase that inserts Mg^{II} into protoporphyrin IX during chlorophyll biosynthesis, was associated with enhanced photosynthetic rate of isolated chloroplasts, although it is not clear if this association is causal.[112,122] (Relatedly, methylotrophs like Me are known to produce bacteriochlorophyll,[123] so it bears investigation whether they can incorporate REs more specifically into porphyrin-derived or other cofactors.) Ca^{II} is also present in the Mn_4Ca oxygen evolving complex (OEC) of photosystem II,[124] and it is tempting to suggest that REs may be inserted into the OEC in place of Ca. However, model studies[125,126] and in vitro substitution experiments[127] suggest that the greater Lewis acidity of REs would make the cluster more difficult to oxidize, decreasing water oxidation activity. Most of the above studies have focused on the cheaper and more abundant early REs, but studies using other REs would help disambiguate effects on associated bacteria versus on the plant itself.

Finally, lanthanides may also affect the organisms that consume plants. Studies have suggested positive impacts of RE provision in animal husbandry, suggesting a role for REs in higher organisms.[128] However, REs are not perfect mimics of Ca,[4] as recent studies of calmodulin[129,130] and cadherin[131] have shown, suggesting that REs may not play a general role as Ca substitutes in Ca-dependent proteins. Instead, the growth-promoting effects of REs in higher organisms might plausibly be linked to the microbiome; for example, both beneficial (*E. coli*) and pathogenic (*Pseudomonas aeruginosa*) bacteria possess PQQ-dependent dehydrogenases, and these or other enzymes might benefit from or be inhibited by REs. However, at the moment, no specific mechanism for the effects of REs in organisms other than bacteria has been firmly established.

CONCLUSIONS AND OUTLOOK

It is fitting that the International Year of the Periodic Table (2019) would be the *annus mirabilis* of biological lanthanides, when a number of the key principles and mechanistic details of lanthanide recognition and utilization have come into view, having long laid hidden (Greek: *lanthánein*, the root of lanthanum). These new findings include discovery and characterization of lanmodulin (late 2018),[42,81] discovery of the lanthanide uptake pathway[41,42,44] and its reliance on a lanthanophore,[41] discovery of cytosolic lanthanide uptake[41] and storage,[45] and (likely) characterization of the lanthanophore. The principles gleaned from these recent discoveries allow us to anticipate many of the key roles that lanthanides may play in biology, but the full scope and many details remain to be determined.

A curious aspect of biological lanthanide utilization, and one that has important implications for the translational impact of the biological ligands, is why the organisms characterized to date strongly prefer early REs, whereas any RE would be a suitable Lewis acid catalyst. We propose that this observation

reflects a compromise between several factors. A single set of biological ligands can only retain specificity within a narrow window of ionic radius. The higher abundance of the early REs makes that window an attractive one, but abundance alone is not a sufficient explanation, as Y is similarly abundant but not used, as far as is currently known. The discovery of organisms that can selectively utilize late REs would be biologically and biotechnologically interesting. However, this possibility might be precluded by biology's requirement for selective acquisition and trafficking mechanisms: a major advantage of the early REs is that their larger ionic radius allows for high CNs and thus more effective discrimination versus abundant metal ions that would compete for the polyanionic ligands most favorable for Ln^{III} coordination. The data so far support LanM, META1p1781, and the lanthanophore being outworkings of the same fundamental principle in protein and small-molecule form: using cooperative metal binding to give exquisite metal selectivity by amplification of subtle differences in ionic radius and coordination preferences. There are clear, broader applications of this principle for binding of other REs and other metals by engineered protein, small-molecule, and cell-based approaches, for applications in RE harvesting and beyond. The interface of biology, biochemistry, coordination chemistry, and engineering will be a rich area of exploration as we learn more about the fundamentals and applications of biological lanthanide utilization.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: juc96@psu.edu.

ORCID ⓘ

Joseph A. Cotruvo, Jr.: 0000-0003-4243-8257

Notes

The author declares the following competing financial interest(s): J.A.C. is an inventor on a patent application submitted by the Pennsylvania State University related to some of the work described here.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Haxel, G. B.; Hedrick, J. B.; Orris, G. J. Rare earth elements - Critical resources for high technology. *U.S.G.S. Fact Sheet* **2002**, 087–02.

(2) Kostelnik, T. I.; Orvig, C. Radioactive main group and rare earth metals for imaging and therapy. *Chem. Rev.* **2019**, *119*, 902–956.

(3) Firsching, F. H.; Brune, S. N. Solubility products of the trivalent rare-earth phosphates. *J. Chem. Eng. Data* **1991**, *36*, 93–95.

(4) Evans, C. H. *Biochemistry of the Lanthanides*; Plenum Press: New York, 1990.

(5) Yang, X. J.; Lin, A.; Li, X.-L.; Wu, Y.; Zhou, W.; Chen, Z. China's ion-adsorption rare earth resources, mining consequences and preservation. *Environ. Dev.* **2013**, *8*, 131–136.

(6) Arshi, P. S.; Vahidi, E.; Zhao, F. Behind the scenes of clean energy: The environmental footprint of rare earth products. *ACS Sustainable Chem. Eng.* **2018**, *6*, 3311–3320.

(7) Bomgardner, M. M. The struggle to mine rare earths. *Chem. Eng. News* **2015**, *93* (30), 36–39.

(8) Cheisson, T.; Schelter, E. J. Rare earth elements: Mendeleev's bane, modern marvels. *Science* **2019**, *363*, 489–493.

(9) Long, K. R.; Van Gosen, B. S.; Foley, N. K.; Cordier, D. The Principal Rare Earth Elements Deposits of the United States: A Summary of Domestic Deposits and a Global Perspective. In *Non-Renewable Resource Issues. International Year of Planet Earth*; Sinding-Larsen, R., Wellmer, F. W., Eds.; Springer: Dordrecht, 2012.

(10) Xie, F.; Zhang, T. A.; Dreisinger, D.; Doyle, F. A critical review on solvent extraction of rare earths from aqueous solutions. *Miner. Eng.* **2014**, *56*, 10–28.

(11) Erickson, B. Rare-earth recovery: U.S. efforts to extract valuable elements from coal waste surge. *Chem. Eng. News* **2018**, *96* (28), 29–33.

(12) Bogart, J. A.; Cole, B. E.; Boreen, M. A.; Lippincott, C. A.; Manor, B. C.; Carroll, P. J.; Schelter, E. J. Accomplishing simple, solubility-based separations of rare earth elements with complexes bearing size-sensitive molecular apertures. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 14887–14892.

(13) Fang, H.; Cole, B. E.; Qiao, Y.; Bogart, J. A.; Cheisson, T.; Manor, B. C.; Carroll, P. J.; Schelter, E. J. Electro-kinetic separation of rare earth elements using a redox-active ligand. *Angew. Chem., Int. Ed.* **2017**, *56*, 13450–13454.

(14) Li, X.-Z.; Zhou, L.-P.; Yan, L.-L.; Dong, Y.-M.; Bai, Z.-L.; Sun, X.-Q.; Diwu, J.; Wang, S.; Bünzli, J.-C.; Sun, Q.-F. A supramolecular lanthanide separation approach based on multivalent cooperative enhancement of metal ion selectivity. *Nat. Commun.* **2018**, *9*, 547.

(15) Gao, H. Y.; Peng, W. L.; Meng, P. P.; Feng, X. F.; Li, J. Q.; Wu, H. Q.; Yan, C. S.; Xiong, Y. Y.; Luo, F. Lanthanide separation using size-selective crystallization of Ln-MOFs. *Chem. Commun.* **2017**, *53*, 5737–5739.

(16) Bonificio, W. D.; Clarke, D. R. Rare-earth separation using bacteria. *Environ. Sci. Technol. Lett.* **2016**, *3*, 180–184.

(17) Park, D. M.; Brewer, A.; Reed, D. W.; Lammers, L. N.; Jiao, Y. Recovery of rare earth elements from low-grade feedstock leachates using engineered bacteria. *Environ. Sci. Technol.* **2017**, *51*, 13471–13480.

(18) Hibi, Y.; Asai, K.; Arafuka, H.; Hamajima, M.; Iwama, T.; Kawai, K. Molecular structure of La^{3+}-induced methanol dehydrogen-ase-like protein in *Methylobacterium radiotolerans*. *J. Biosci. Bioeng.* **2011**, *111*, 547–549.

(19) Fitriyanto, N. A.; Fushimi, M.; Matsunaga, M.; Pertiwiningrum, A.; Iwama, T.; Kawai, K. Molecular structure and gene analysis of Ce^{3+}-induced methanol dehydrogenase of *Bradyrhizobium* sp. MAFF211645. *J. Biosci. Bioeng.* **2011**, *111*, 613–617.

(20) Nakagawa, T.; Mitsui, R.; Tani, A.; Sasa, K.; Tashiro, S.; Iwama, T.; Hayakawa, T.; Kawai, K. A catalytic role of XoxF1 as La^{3+}-dependent methanol dehydrogenase in *Methylobacterium extorquens* strain AM1. *PLoS One* **2012**, *7*, No. e50480.

(21) Martinez-Gomez, N. C.; Vu, H. N.; Skovran, E. Lanthanide chemistry: From coordination in chemical complexes shaping our technology to coordination in enzymes shaping bacterial metabolism. *Inorg. Chem.* **2016**, *55*, 10083–10089.

(22) Bogart, J. A.; Lewis, A. J.; Schelter, E. J. DFT study of the active site of the XoxF-type natural, cerium-dependent methanol dehydro-genase enzyme. *Chem. - Eur. J.* **2015**, *21*, 1743–1748.

(23) Prejanò, M.; Marino, T.; Russo, N. How can methanol dehydrogenase from *Methylacidiphilum fumariolicum* work with the alien Ce^{III} ion in the active center? A theoretical study. *Chem. - Eur. J.* **2017**, *23*, 8652–8657.

(24) Lumpe, H.; Pol, A.; Op den Camp, H. J. M.; Daumann, L. J. Impact of the lanthanide contraction on the activity of a lanthanide-dependent methanol dehydrogenase - a kinetic and DFT study. *Dalton Trans.* **2018**, *47*, 10463–10472.

(25) Cotton, S. A.; Raithby, P. R. Systematics and surprises in lanthanide coordination chemistry. *Coord. Chem. Rev.* **2017**, *340*, 220–231.

(26) Shannon, R. D. Revised effective ionic radii and systematic studies of interatomic distances in halides and chalcogenides. *Acta Crystallogr., Sect. A: Cryst. Phys., Diffr., Theor. Gen. Crystallogr.* **1976**, *32*, 751−767.

(27) Seitz, M.; Oliver, A. G.; Raymond, K. N. The lanthanide contraction revisited. *J. Am. Chem. Soc.* **2007**, *129*, 11153−11160.

(28) Horrocks, W. D.; Sudnick, D. R. Lanthanide ion probes of structure in biology. Laser-induced luminescence decay constants provide a direct measure of the number of metal-coordinated water molecules. *J. Am. Chem. Soc.* **1979**, *101*, 334−340.

(29) Mulqueen, P.; Tingey, J. M.; Horrocks, W. D. J. Characterization of lanthanide(III) ion binding to calmodulin using luminescence spectroscopy. *Biochemistry* **1985**, *24*, 6639−6645.

(30) Bentrop, D.; Bertini, I.; Cremonini, M. A.; Forsen, S.; Luchinat, C.; Malmendal, A. Solution structure of the paramagnetic complex of the N-terminal domain of calmodulin with two Ce³⁺ ions by ¹H NMR. *Biochemistry* **1997**, *36*, 11605−11618.

(31) Rumble, J. R. *CRC Handbook of Chemistry and Physics, 100th ed. (Internet Version 2019)*; CRC Press/Taylor & Francis: Boca Raton, FL, 2019.

(32) Daumann, L. Essential and ubiquitous: The emergence of lanthanide metallobiochemistry. *Angew. Chem.* **2019**, in press. DOI: 10.1002/ange.201904090.

(33) Lim, S.; Franklin, S. J. Lanthanide-binding peptides and the enzymes that might have been. *Cell. Mol. Life Sci.* **2004**, *61*, 2184−2188.

(34) Perrin, D. D. *Ionisation Constants of Inorganic Acids and Bases in Aqueous Solution*, 2nd ed.; Pergamon Press: New York, 1982.

(35) Skovran, E.; Martinez-Gomez, N. C. Just add lanthanides. *Science* **2015**, *348*, 862−863.

(36) Chistoserdova, L. Lanthanides: New life metals? *World J. Microbiol. Biotechnol.* **2016**, *32*, 138.

(37) Semrau, J. D.; DiSpirito, A. A.; Gu, W.; Yoon, S. Metals and methanotrophy. *Appl. Environ. Microbiol.* **2018**, *84*, e02289−17.

(38) Chistoserdova, L. New pieces to the lanthanide puzzle. *Mol. Microbiol.* **2019**, *111*, 1127−1131.

(39) Picone, N.; Op den Camp, H. J. M. Role of rare earth elements in methanol oxidation. *Curr. Opin. Chem. Biol.* **2019**, *49*, 39−44.

(40) Lippard, S. J.; Berg, J. M. *Principles of Bioinorganic Chemistry*; University Science Books: Mill Valley, CA, 1994.

(41) Mattocks, J. A.; Ho, J. V.; Cotruvo, J. A., Jr. A selective, protein-based fluorescent sensor with picomolar affinity for rare earth elements. *J. Am. Chem. Soc.* **2019**, *141*, 2857−2861.

(42) Cotruvo, J. A., Jr.; Featherston, E. R.; Mattocks, J. A.; Ho, J. V.; Laremore, T. N. Lanmodulin: A highly selective lanthanide-binding protein from a lanthanide-utilizing bacterium. *J. Am. Chem. Soc.* **2018**, *140*, 15056−15061.

(43) Featherston, E. R.; Rose, H. R.; McBride, M. J.; Taylor, E. M.; Boal, A. K.; Cotruvo, J. A., Jr. Biochemical and structural characterization of XoxG and XoxJ and their roles in lanthanide-dependent methanol dehydrogenase activity. *ChemBioChem* **2019**, in press. DOI: 10.1002/cbic.201900184.

(44) Ochsner, A. M.; Hemmerle, L.; Vonderach, T.; Nüssli, R.; Bortfeld-Miller, M.; Hattendorf, B.; Vorholt, J. A. Use of rare earth elements in the phyllosphere colonizer *Methylobacterium extorquens* PA1. *Mol. Microbiol.* **2019**, *111*, 1152−1166.

(45) Roszczenko-Jasińska, P.; Vu, H. N.; Subuyuj, G. A.; Crisostomo, R. V.; Cai, J.; Raghuraman, C.; Ayala, E. M.; Clippard, E. J.; Lien, N. F.; Ngo, R. T.; Yarza, F.; Hoeber, C. A.; Martinez-Gomez, N. C.; Skovran, E. Lanthanide transport, storage, and beyond: genes and processes contributing to XoxF function in *Methylorubrum extorquens* AM1. 2019, bioRxiv 647677. bioRxiv preprint server. https://www.biorxiv.org/content/10.1101/647677v1.

(46) Anthony, C. *The Biochemistry of Methylotrophs*; Academic: London, 1982.

(47) Chistoserdova, L.; Kalyuzhnaya, M. G.; Lidstrom, M. E. The expanding world of methylotrophic metabolism. *Annu. Rev. Microbiol.* **2009**, *63*, 477−499.

(48) Anthony, C., Methanol Dehydrogenase, a PQQ-Containing Quinoprotein Dehydrogenase. In *Enzyme-Catalyzed Electron and Radical Transfer: Subcellular Biochemistry*; Holzenburg, A., Scrutton, N. S., Eds.; Springer US: Boston, MA, 2000; pp 73−117.

(49) Anthony, C.; Williams, P. The structure and mechanism of methanol dehydrogenase. *Biochim. Biophys. Acta, Proteins Proteomics* **2003**, *1647*, 18−23.

(50) Zhang, X.; Reddy, S. Y.; Bruice, T. C. Mechanism of methanol oxidation by quinoprotein methanol dehydrogenase. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 745−749.

(51) Zheng, Y.; Huang, J.; Zhao, F.; Chistoserdova, L. Physiological effect of XoxG(4) on lanthanide-dependent methanotrophy. *mBio* **2018**, *9*, No. e02430-17.

(52) Versantvoort, W.; Pol, A.; Daumann, L. J.; Larrabee, J. A.; Strayer, A. H.; Jetten, M. S. M.; van Niftrik, L.; Reimann, J.; Op den Camp, H. J. M. Characterization of a novel cytochrome c_{GJ} as the electron acceptor of XoxF-MDH in the thermoacidophilic methanotroph *Methylacidiphilum fumariolicum* SolV. *Biochim. Biophys. Acta, Proteins Proteomics* **2019**, *1867*, 595−603.

(53) Anthony, C.; Zatman, L. J. The microbial oxidation of methanol. 2. The methanol-oxidizing enzyme of *Pseudomonas* sp. M 27. *Biochem. J.* **1964**, *92*, 614−621.

(54) Skovran, E.; Palmer, A. D.; Rountree, A. M.; Good, N. M.; Lidstrom, M. E. XoxF is required for expression of methanol dehydrogenase in *Methylobacterium extorquens* AM1. *J. Bacteriol.* **2011**, *193*, 6032−6038.

(55) Schmidt, S.; Christen, P.; Kiefer, P.; Vorholt, J. A. Functional investigation of methanol dehydrogenase-like protein XoxF in *Methylobacterium extorquens* AM1. *Microbiology* **2010**, *156*, 2575−2586.

(56) Pol, A.; Barends, T. R. M.; Dietl, A.; Khadem, A. F.; Eygensteyn, J.; Jetten, M. S. M.; Op den Camp, H. J. M. Rare earth metals are essential for methanotrophic life in volcanic mudpots. *Environ. Microbiol.* **2014**, *16*, 255−264.

(57) Keltjens, J. T.; Pol, A.; Reimann, J.; Op den Camp, H. J. M. PQQ-dependent methanol dehydrogenases: rare-earth elements make a difference. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 6163−6183.

(58) Deng, Y. W.; Ro, S. Y.; Rosenzweig, A. C. Structure and function of the lanthanide-dependent methanol dehydrogenase XoxF from the methanotroph *Methylomicrobium buryatense* 5GB1C. *J. Biol. Inorg. Chem.* **2018**, *23*, 1037−1047.

(59) Kalyuzhnaya, M. G.; Lapidus, A.; Ivanova, N.; Copeland, A. C.; McHardy, A. C.; Szeto, E.; Salamov, A.; Grigoriev, I. V.; Suciu, D.; Levine, S. R.; Markowitz, V. M.; Rigoutsos, I.; Tringe, S. G.; Bruce, D. C.; Richardson, P. M.; Lidstrom, M. E.; Chistoserdova, L. High-resolution metagenomics targets specific functional types in complex microbial communities. *Nat. Biotechnol.* **2008**, *26*, 1029−1034.

(60) Huang, J.; Yu, Z.; Groom, J.; Cheng, J. F.; Tarver, A.; Yoshikuni, Y.; Chistoserdova, L. Rare earth element alcohol dehydrogenases widely occur among globally distributed, numerically abundant and environmentally important microbes. *ISME J.* **2019**, *13*, 2005−2017.

(61) Wu, M. L.; Wessels, H. J. C. T.; Pol, A.; Op den Camp, H. J. M.; Jetten, M. S. M.; van Niftrik, L.; Keltjens, J. T. XoxF-type methanol dehydrogenase from the anaerobic methanotroph "Candidatus Methylomirabilis oxyfera". *Appl. Environ. Microbiol.* **2015**, *81*, 1442−1451.

(62) Good, N. M.; Vu, H. N.; Suriano, C. J.; Subuyuj, G. A.; Skovran, E.; Martinez-Gomez, N. C. Pyrroloquinoline quinone ethanol dehydrogenase in *Methylobacterium extorquens* AM1 extends lanthanide-dependent metabolism to multicarbon substrates. *J. Bacteriol.* **2016**, *198*, 3109−3118.

(63) Chu, F.; Lidstrom, M. E. XoxF acts as the predominant methanol dehydrogenase in the type I methanotroph *Methylomicrobium buryatense*. *J. Bacteriol.* **2016**, *198*, 1317−1325.

(64) Huang, J.; Yu, Z.; Chistoserdova, L. Lanthanide-dependent methanol dehydrogenases of XoxF4 and XoxF5 clades are differentially distributed among methylotrophic bacteria and they reveal different biochemical properties. *Front. Microbiol.* **2018**, *9*, 1366.

(65) Krause, S. M. B.; Johnson, T.; Samadhi Karunaratne, Y.; Fu, Y.; Beck, D. A. C.; Chistoserdova, L.; Lidstrom, M. E. Lanthanide-dependent cross-feeding of methane-derived carbon is linked by microbial community interactions. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 358−363.

(66) Wehrmann, M.; Billard, P.; Martin-Meriadec, A.; Zegeye, A.; Klebensberger, J. Functional role of lanthanides in enzymatic activity and transcriptional regulation of pyrroloquinoline quinone-dependent alcohol dehydrogenases in *Pseudomonas putida* KT2440. *mBio* **2017**, *8*, No. e00570-17.

(67) Wehrmann, M.; Berthelot, C.; Billard, P.; Klebensberger, J. The PedS2/PedR2 two-component system is crucial for the rare earth element switch in *Pseudomonas putida* KT2440. *mSphere* **2018**, *3*, No. e00376-18.

(68) Vu, H. N.; Subuyuj, G. A.; Vijayakumar, S.; Good, N. M.; Martinez-Gomez, N. C.; Skovran, E. Lanthanide-dependent regulation of methanol oxidation systems in *Methylobacterium extorquens* AM1 and their contribution to methanol growth. *J. Bacteriol.* **2016**, *198*, 1250−1259.

(69) Sobota, J. M.; Imlay, J. A. Iron enzyme ribulose-5-phosphate 3-epimerase in *Escherichia coli* is rapidly damaged by hydrogen peroxide but can be protected by manganese. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 5402−5407.

(70) Cotruvo, J. A., Jr.; Stubbe, J. Metallation and mismetallation of iron and manganese proteins in vitro and in vivo: The class I ribonucleotide reductases as a case study. *Metallomics* **2012**, *4*, 1020−1036.

(71) Kalimuthu, P.; Daumann, L. J.; Pol, A.; Op den Camp, H. J. M.; Bernhardt, P. V. Electrocatalysis of a europium-dependent bacterial methanol dehydrogenase with its physiological electron acceptor cytochrome c_{GJ}. *Chem. - Eur. J.* **2019**, *25*, 8760−8768.

(72) Good, N. M.; Moore, R. S.; Suriano, C. J.; Martinez-Gomez, N. C. Contrasting in vitro and in vivo methanol oxidation activities of lanthanide-dependent alcohol dehydrogenases XoxF1 and ExaF from *Methylobacterium extorquens* AM1. *Sci. Rep.* **2019**, *9*, 4248.

(73) McSkimming, A.; Cheisson, T.; Carroll, P. J.; Schelter, E. J. Functional synthetic model for the lanthanide-dependent quinoid alcohol dehydrogenase active site. *J. Am. Chem. Soc.* **2018**, *140*, 1223−1226.

(74) Ho, J. V.; Cotruvo, J. A., Jr. A periplasmic binding protein for pyrroloquinoline quinone. *Biochemistry* **2019**, *58*, 2665−2669.

(75) Kim, Y. H.; Campbell, E.; Yu, J.; Minteer, S. D.; Banta, S. Complete oxidation of methanol in biobattery devices using a hydrogel created from three modified dehydrogenases. *Angew. Chem., Int. Ed.* **2013**, *52*, 1437−1440.

(76) Dekker, R. H.; Duine, J. A.; Frank, J.; Verweil, P. E. J.; Westerling, J. Covalent addition of H_2O, enzyme substrates and activators to pyrrolo-quinoline quinone, the coenzyme of quinoproteins. *Eur. J. Biochem.* **1982**, *125*, 69−73.

(77) Lumpe, H.; Daumann, L. J. Studies of redox cofactor pyrroloquinoline quinone and its interaction with lanthanides(III) and calcium(II). *Inorg. Chem.* **2019**, *58*, 8432−8441.

(78) Finney, L. A.; O'Halloran, T. V. Transition metal speciation in the cell: insights from the chemistry of metal ion receptors. *Science* **2003**, *300*, 931−936.

(79) Ma, Z.; Jacobsen, F. E.; Giedroc, D. P. Coordination chemistry of bacterial metal transport and sensing. *Chem. Rev.* **2009**, *109*, 4644−4681.

(80) Waldron, K. J.; Robinson, N. J. How do bacterial cells ensure that metalloproteins get the correct metal? *Nat. Rev. Microbiol.* **2009**, *7*, 25−35.

(81) Cook, E. C.; Featherston, E. R.; Showalter, S. A.; Cotruvo, J. A., Jr. Structural basis for rare earth element recognition by *Methylobacterium extorquens* lanmodulin. *Biochemistry* **2019**, *58*, 120−125.

(82) Gifford, J. L.; Walsh, M. P.; Vogel, H. J. Structures and metal-ion-binding properties of the Ca^{2+}-binding helix-loop-helix EF-hand motifs. *Biochem. J.* **2007**, *405*, 199−221.

(83) Halling, D. B.; Liebeskind, B. J.; Hall, A. W.; Aldrich, R. W. Conserved properties of individual Ca^{2+}-binding sites in calmodulin. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, E1216−E1225.

(84) Wang, C.-L.; Aquaron, R. R.; Leavis, P. C.; Gergely, J. Metal-binding properties of calmodulin. *Eur. J. Biochem.* **1982**, *124*, 7−12.

(85) Gilli, R.; Lafitte, D.; Lopez, C.; Kilhoffer, M.-C.; Makarov, A.; Briand, C.; Haiech, J. Thermodynamic analysis of calcium and magnesium binding to calmodulin. *Biochemistry* **1998**, *37*, 5450−5456.

(86) Snyder, E. E.; Buoscio, B. W.; Falke, J. J. Calcium(II) site specificity: Effect of size and charge on metal ion binding to an EF-hand-like site. *Biochemistry* **1990**, *29*, 3937−3943.

(87) Nitz, M.; Sherawat, M.; Franz, K. J.; Peisach, E.; Allen, K. N.; Imperiali, B. Structural origin of the high affinity of a chemically evolved lanthanide-binding peptide. *Angew. Chem., Int. Ed.* **2004**, *43*, 3682−3685.

(88) Allen, K. N.; Imperiali, B. Lanthanide-tagged proteins - an illuminating partnership. *Curr. Opin. Chem. Biol.* **2010**, *14*, 247−254.

(89) Horrocks, W. D. J. Lanthanide ion probes of biomolecular structure. In *Adv. Inorg. Biochem.*; Eichhorn, G. L., Marzilli, L. G., Eds.; Elsevier: New York, 1982; Vol. 4, pp 201−261.

(90) Chu, F.; Beck, D. A. C.; Lidstrom, M. E. MxaY regulates the lanthanide-mediated methanol dehydrogenase switch in Methylomicrobium buryatense. *PeerJ* **2016**, *4*, No. e2435.

(91) Masuda, S.; Suzuki, Y.; Fujitani, Y.; Mitsui, R.; Nakagawa, T.; Shintani, M.; Tani, A. Lanthanide-dependent regulation of methylotrophy in *Methylobacterium aquaticum* strain 22A. *mSphere* **2018**, *3*, No. e00462-17.

(92) Cole, B. E.; Falcones, I. B.; Cheisson, T.; Manor, B. C.; Carroll, P. J.; Schelter, E. J. A molecular basis to rare earth separations for recycling: tuning the TriNOx ligand properties for improved performance. *Chem. Commun.* **2018**, *54*, 10276−10279.

(93) Sandy, M.; Butler, A. Microbial iron acquisition: marine and terrestrial siderophores. *Chem. Rev.* **2009**, *109*, 4580−4595.

(94) Boukhalfa, H.; Crumbliss, A. L. Chemical aspects of siderophore mediated iron transport. *BioMetals* **2002**, *15*, 325−339.

(95) Raymond, K. N.; Dertz, E. A.; Kim, S. S. Enterobactin: An archetype for microbial iron transport. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3584−3588.

(96) Gu, W.; Haque, M. F. U.; Dispirito, A. A.; Semrau, J. D. Uptake and effect of rare earth elements on gene expression in *Methylosinus trichosporium* OB3b. *FEMS Microbiol. Lett.* **2016**, *363*, fnw129.

(97) Moeck, G. S.; Coulton, J. W. TonB-dependent iron acquisition: mechanisms of siderophore-mediated active transport. *Mol. Microbiol.* **1998**, *28*, 675−681.

(98) Noinaj, N.; Guillier, M.; Barnard, T. J.; Buchanan, S. K. TonB-dependent transporters: regulation, structure, and function. *Annu. Rev. Microbiol.* **2010**, *64*, 43−60.

(99) Kenney, G. E.; Rosenzweig, A. C. Methanobactins: maintaining copper homeostasis in methanotrophs and beyond. *J. Biol. Chem.* **2018**, *293*, 4606−4615.

(100) Groom, J. D.; Ford, S. M.; Pesesky, M. W.; Lidstrom, M. E. A mutagenic screen identifies a TonB-dependent receptor required for the lanthanide metal switch in the Type I methanotroph "Methylotuvimicrobium buryatense" 5GB1C. *J. Bacteriol.* **2019**, *201*, e00120−19.

(101) Wehrmann, M.; Berthelot, C.; Billard, P.; Klebensberger, J. Rare earth element (REE)-dependent growth of *Pseudomonas putida* KT2440 depends on the ABC-transporter PedA1A2BC and is influenced by iron availability. 2019, bioRxiv 670216. bioRxiv preprint server. https://www.biorxiv.org/content/10.1101/670216v1.

(102) Deblonde, G. J.-P.; Sturzbecher-Hoehne, M.; Rupert, P. B.; An, D. D.; Illy, M.-C.; Ralston, C. Y.; Brabec, J.; de Jong, W. A.; Strong, R. K.; Abergel, R. J. Chelation and stablization of berkelium in oxidation state +IV. *Nat. Chem.* **2017**, *9*, 843−849.

(103) Allred, B. E.; Rupert, P. B.; Gauny, S. S.; An, D. D.; Ralston, C. Y.; Sturzbecher-Hoehne, M.; Strong, R. K.; Abergel, R. J. Siderocalin-mediated recognition, sensitization, and cellular uptake of actinides. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 10342−10347.

(104) Hogendoorn, C.; Roszczenko-Jasińska, P.; Martinez-Gomez, N. C.; de Graaff, J.; Grassl, P.; Pol, A.; Op den Camp, H. J. M.; Daumann, L. J. Facile arsenazo III-based assay for monitoring rare earth element depletion from cultivation media for methanotrophic and methylotrophic bacteria. *Appl. Environ. Microbiol.* **2018**, *84*, e02887−17.

(105) Docampo, R.; de Souza, W.; Miranda, K.; Rohloff, P.; Moreno, S. N. J. Acidocalcisomes - conserved from bacteria to man. *Nat. Rev. Microbiol.* **2005**, *3*, 251−261.

(106) Hong-Hermesdorf, A.; Miethke, M.; Gallaher, S. D.; Kropat, J.; Dodani, S. C.; Chan, J.; Barupala, D.; Domaille, D. W.; Shirasaki, D. I.; Loo, J. A.; Weber, P. K.; Pett-Ridge, J.; Stemmler, T. L.; Chang, C. J.; Merchant, S. S. Subcellular metal imaging identifies dynamic sites of Cu accumulation in *Chlamydomonas*. *Nat. Chem. Biol.* **2014**, *10*, 1034−1042.

(107) Roh, H. C.; Collier, S.; Guthrie, J.; Robertson, J. D.; Kornfeld, K. Lysosome-related organelles in intestinal cells are a zinc storage site in *C. elegans*. *Cell Metab.* **2012**, *15*, 88−99.

(108) García-Prieto, A.; Alonso, J.; Muñoz, D.; Marcano, L.; Abad Díaz de Cerio, A.; Fernández de Luis, R.; Orue, I.; Mathon, O.; Muela, A.; Fdez-Gubieda, M. L. On the mineral core of ferritin-like proteins: structural and magnetic characterization. *Nanoscale* **2016**, *8*, 1088−1099.

(109) Tyler, G. Rare earth elements in soil and plant systems - A review. *Plant Soil* **2004**, *267*, 191−206.

(110) Ramírez-Olvera, S. M.; Trejo-Téllez, L. I.; García-Morales, S.; Pérez-Sato, J. A.; Gómez-Merino, F. C. Cerium enhances germination and shoot growth, and alters mineral nutrient concentration in rice. *PLoS One* **2018**, *13*, No. e0194691.

(111) Fall, R.; Benson, A. A. Leaf methanol - the simplest natural product from plants. *Trends Plant Sci.* **1996**, *1*, 296−301.

(112) Fashui, H.; Ling, W.; Xiangxuan, M.; Zheng, W.; Guiwen, Z. The effect of cerium(III) on the chlorophyll formation in spinach. *Biol. Trace Elem. Res.* **2002**, *89*, 263−276.

(113) White, P. J.; Broadley, M. R. Calcium in Plants. *Ann. Bot.* **2003**, *92*, 487−511.

(114) Morrissey, J.; Guerinot, M. L. Iron uptake and transport in plants: The good, the bad, and the ionome. *Chem. Rev.* **2009**, *109*, 4553−4567.

(115) Abergel, R. J.; Clifton, M. C.; Pizarro, J. C.; Warner, J. A.; Shuh, D. K.; Strong, R. K.; Raymond, K. N. The siderocalin/enterobactin interaction: a link between mammalian immunity and bacterial iron transport. *J. Am. Chem. Soc.* **2008**, *130*, 11524−11534.

(116) Choi, O.; Kim, J.; Kim, J.-G.; Jeong, Y.; Moon, J. S.; Park, C. S.; Hwang, I. Pyrroloquinoline quinone is a plant growth promotion factor produced by *Pseudomonas fluorescens* B16. *Plant Physiol.* **2008**, *146*, 657−668.

(117) Hepler, P. K. Calcium: a central regulator of plant growth and development. *Plant Cell* **2005**, *17*, 2142−2155.

(118) Voiniciuc, C.; Dean, G. H.; Griffiths, J. S.; Kirchsteiger, K.; Hwang, Y. T.; Gillett, A.; Dow, G.; Western, T. L.; Estelle, M.; Haughn, G. W. FLYING SAUCER1 Is a transmembrane RING E3 ubiquitin ligase that regulates the degree of pectin methylesterification in *Arabidopsis* seed mucilage. *Plant Cell* **2013**, *25*, 944−959.

(119) Wang, L.; Li, J.; Zhou, Q.; Yang, G.; Ding, X. L.; Li, X.; Cai, C. X.; Zhang, Z.; Wei, H. Y.; Lu, T. H.; Deng, X. W.; Huang, X. H. Rare earth elements activate endocytosis in plant cells. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 12936−12941.

(120) Yang, Q.; Wang, L.; He, J.; Yang, Z.; Huang, X. Direct imaging of how lanthanides break the normal evolution of plants. *J. Inorg. Biochem.* **2018**, *182*, 158−169.

(121) Yang, Q.; Wang, L.; He, J.; Wei, H.; Yang, Z.; Huang, X. Arabinogalactan proteins are the possible extracellular molecules for binding exogenous cerium(III) in the acidic environment outside plant cells. *Front. Plant Sci.* **2019**, *10*, 153.

(122) Zhou, M.; Gong, X.; Ying, W.; Chao, L.; Hong, M.; Wang, L.; Fashui, H. Cerium relieves the inhibition of chlorophyll biosynthesis of maize caused by magnesium deficiency. *Biol. Trace Elem. Res.* **2011**, *143*, 468−477.

(123) Sato, K. Bacteriochlorophyll formation by facultative methylotrophs, *Protaminobacter ruber* and *Pseudomonas* AM 1. *FEBS Lett.* **1978**, *85*, 207−210.

(124) Yachandra, V. K.; Yano, J. Calcium in the oxygen-evolving complex: structural and mechanistic role determined by X-ray spectroscopy. *J. Photochem. Photobiol., B* **2011**, *104*, 51−59.

(125) Tsui, E. Y.; Tran, R.; Yano, J.; Agapie, T. Redox-inactive metals modulate the reduction potential in heterometallic manganese−oxido clusters. *Nat. Chem.* **2013**, *5*, 293−299.

(126) Tsui, E. Y.; Agapie, T. Reduction potentials of heterometallic manganese−oxido cubane complexes modulated by redox-inactive metals. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 10084−10088.

(127) Bakou, A.; Ghanotakis, D. F. Substitution of lanthanides at the calcium site(s) in Photosystem II affects electron transport from tyrosine Z to P680$^+$. *Biochim. Biophys. Acta, Bioenerg.* **1993**, *1141*, 303−308.

(128) Redling, K. *Rare earth elements in agriculture with emphasis on animal husbandry*. Ludwig-Maximilians-Universitat München, 2006.

(129) Edington, S. C.; Gonzalez, A.; Middendorf, T. R.; Halling, D. B.; Aldrich, R. W.; Baiz, C. R. Coordination to lanthanide ions distorts binding site conformation in calmodulin. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, E3126−E3134.

(130) Edington, S. C.; Halling, D. B.; Bennett, S. M.; Middendorf, T. R.; Aldrich, R. W.; Baiz, C. R. Non-additive effects of binding site mutations in calmodulin. *Biochemistry* **2019**, *58*, 2730−2739.

(131) Brayshaw, L. L.; Smith, R. C. G.; Badaoui, M.; Irving, J. A.; Price, S. R. Lanthanides compete with calcium for binding to cadherins and inhibit cadherin-mediated cell adhesion. *Metallomics* **2019**, *11*, 914−924.