

# Minor Actinides Can Replace Essential Lanthanides in Bacterial Life\*\*

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**Abstract:** Certain f-block elements—the lanthanides—have biological relevance in the context of methylotrophic bacteria. The respective strains incorporate these 4f elements into the active site of one of their key metabolic enzymes, a lanthanide-dependent methanol dehydrogenase. In this study, we investigated whether actinides, the radioactive 5f elements, can replace the essential 4f elements in lanthanide-dependent bacterial metabolism. Growth studies with *Methylacidiphilum fumariolicum* SolV and the *Methylobacterium extorquens* AM1  $\Delta$ *mxaF* mutant demonstrate that americium and curium support growth in the absence of lanthanides. Moreover, strain SolV favors these actinides over late lanthanides when presented with a mixture of equal amounts of lanthanides together with americium and curium. Our combined *in vivo* and *in vitro* results establish that methylotrophic bacteria can utilize actinides instead of lanthanides to sustain their one-carbon metabolism if they possess the correct size and a +III oxidation state.

the exception of promethium) are widespread in the environment and the most abundant ones can be found in similar concentrations as copper and zinc in the earth's crust. They are essential for our daily life and find application in strong magnets, high-tech devices, materials for energy-efficient transport and medicine. Lns exhibit stable +III oxidation states and neighboring elements display very similar ionic radii, however, these gradually decrease along the series (=lanthanide contraction).<sup>[1]</sup> They exhibit highly similar chemical properties and coordination chemistry, making it difficult to separate these elements, which always occur as mixtures in ores and minerals. Since 2011 it is known that methylotrophic bacteria, such as *Methylobacterium radiotolerans*, can use Lns in their one-carbon (C<sub>1</sub>) metabolism, making these elements play an essential biological role. Nanomolar lanthanide (Ln) concentrations in the growth environment of these bacteria, such as *Methylobacterium extorquens* AM1 (Airborne Methylotroph #1, AM1), trigger a 'lanthanide switch' that results in the use of a Ln-dependent methanol dehydrogenase (XoxF-MDH) as the key metabolic enzyme.<sup>[2]</sup> In the absence of Lns, a calcium-dependent variant (MxaF-MDH) catalyzes the methanol oxidation.<sup>[3]</sup> Some bacteria have only a XoxF-type MDH and, consequently, Lns become essential for their C<sub>1</sub> metabolism. MDHs are quinoproteins bearing the redox cofactor pyrroloquinoline quinone (PQQ) and a Lewis acidic metal ion (Ln<sup>3+</sup> or Ca<sup>2+</sup>) in the active site. Bacteria that use Lns are now known to be widespread in the environment and can be found in many different ecosystems:

## Introduction

The 15 elements in the periodic table from lanthanum to lutetium are commonly referred to as lanthanides (Lns), where the 4f shell is gradually filled. These elements (with

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aquatic, the phyllosphere of plants, soil and extreme environments such as volcanic mudpots.<sup>[4]</sup> However, it is still not completely understood why these bacteria evolved to use Lns. Even though light (La–Eu) and heavy (Gd–Lu) Lns can be mobilized by bacteria, light Lns (those with larger ionic radii) better stimulate growth and support high methanol oxidation activity in the volcanic methanotroph *Methylacidiphilum fumariolicum* SolV (SolV).<sup>[1a,5]</sup> However, strains such as AM1 can be modified to more effectively use also heavy (smaller) Lns, such as gadolinium.<sup>[6]</sup>

The heavier 5f elements—the actinides (Ans)—share a number of key properties with their lighter Ln counterparts in the f-block series. Therefore, it is conceivable that associated biological functions of the Lns as 4f elements can be extended to the series of Ans, providing a general insight into the potential role of radioactive f-block elements in biological processes. The interest in the interaction between Ans and living systems is multifaceted and an emerging topic. It ranges from elucidating toxicity pathways to developing remedies for radiometal poisoning and bioinspired separation strategies. Studying microbe–actinide interactions has thus become increasingly important.<sup>[7]</sup> It has been shown that Ans can bind to a variety of biomolecules (e.g. those usually binding also Ca or Fe).<sup>[8]</sup> Still, since the discovery of the biological relevance of 4f elements a decade ago, it remains elusive whether 5f elements can also play an active role in metabolism, carrying out catalytic processes, or maybe even function better than the native metal ions. Previously, for the Ln-binding protein Lanmodulin (LanM) isolated from strain AM1, an even higher affinity of the actinides Ac, Am and Cm than for some Lns has been demonstrated.<sup>[9]</sup>

In this study, three Ans were chosen intentionally as they display similar properties as the light Lns: americium and curium are stable in the +III oxidation state, and their size matches Nd<sup>3+</sup> (ionic radius=98.3 pm, with Am<sup>3+</sup> 98.0 pm and Cm<sup>3+</sup> 97.0 pm). Plutonium is similar to Pr<sup>3+</sup> in the +III oxidation state (99.0 pm, with Pu<sup>3+</sup> 99.5 pm), but possesses a richer redox chemistry.<sup>[10]</sup> Replacing the Lns with these Ans allowed us to probe the impact of size in combination with the oxidation state of metal ions in Ln-dependent biological systems. We thus investigated the effect of americium, curium and plutonium on the microbial life cycle of Ln-using and Ln-dependent bacteria as well as on the activity of Ln-dependent MDH.

## Results and Discussion

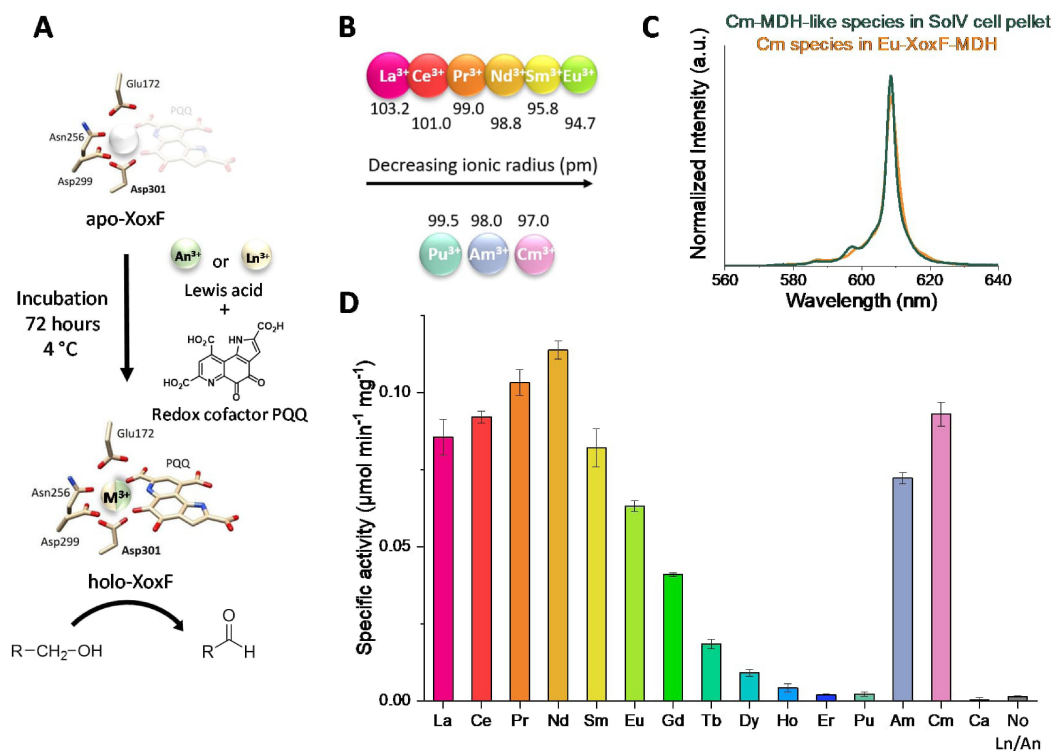
### Actinide reconstitution yields catalytically competent MDH *in vitro*

To test the capability of Ans to participate in the C<sub>1</sub> metabolism of Ln-dependent bacteria, we measured the enzymatic activity of Ln-dependent XoxF-MDH in the presence of various metal ions. We expressed the *xoxF*-gene of strain SolV recombinantly in *Escherichia coli* (*E. coli*) and studied the impact of the metal ion by *in vitro* reconstitution of purified metal-free apo-protein. Production

of soluble and functional apo-XoxF-MDH required adjustment of codon usage for *E. coli* and expression as a fusion protein with a maltose-binding protein (MBP) solubility tag, which was removed later on during purification (Supporting Information Material and Methods: Recombinant protein production of apo-XoxF-MDH in *Escherichia coli*, Figures S1–S3, Tables S2–S5). After incubating this apo-MDH with equimolar concentrations of the redox cofactor PQQ and the Ln or actinide (An) of interest (Figures 1B, S7 and S8), we obtained a fully functioning MDH (Figure 1A) which was able to oxidize methanol to formaldehyde, as verified by a dye-coupled assay (Figures S4 and S5). From La to Nd an increase in enzymatic activity was observed, followed by a sharp decrease or complete loss of methanol oxidation activity along the further series (Figure 1D). A similar trend was found with native Eu-MDH isolated from strain SolV (with 30 % of active sites not occupied by Eu<sup>3+</sup> and thus available for additional metal ions to bind).<sup>[5b,c]</sup> This rise and fall of activity within the series was previously speculated to be due to the higher Lewis acidity, and thus better activation of PQQ and substrate, as well as possibly better binding in the active site, among other factors.

Using the radioactive 5f elements Am<sup>3+</sup> and Cm<sup>3+</sup> as Lewis acids for reconstitution of apo-XoxF-MDH yielded a similarly high enzymatic activity as for the light Lns (Figure 1D), while no substrate conversion could be detected for Pu-MDH, likely due to its versatile redox chemistry and deviation from the +III oxidation state under the chosen assay conditions.<sup>[11]</sup> Under the low concentrations used in the assay, spectroscopic characterization of the Pu species is not possible. Whether Pu is fully oxidized and thus unable to bind to MDH or whether Pu (in any oxidation state) does not yield a fully functioning MDH can not be discriminated from our experiment. Adding a 50-fold excess Am<sup>3+</sup> and Cm<sup>3+</sup> to partial-apo Eu-MDH isolated from SolV (only 70 % active sites occupied by Eu, PQQ already bound) also showed an increase of specific activity with these two An similar to when La<sup>3+</sup> was added (Figure S6), supporting our observations with recombinantly expressed enzyme, that Am<sup>3+</sup> and Cm<sup>3+</sup> can reconstitute MDH and yield a catalytically competent enzyme. As the long reconstitution times with recombinantly expressed enzyme prevented us to investigate the metal binding affinity of MDH using isothermal titration calorimetry (ITC), the results shown in Figure 1 present only a qualitative assessment of the activity with different metal ions. As previously discussed, the amount of metallated active sites may vary for each element due to differences in Lewis acidity, size and ligand exchange rates.<sup>[5c]</sup> Whether factors such as higher covalency in bonding, differences in polarizability and coordination number for Ans also play a role in substrate and cofactor activation, remain elusive from our experiments but will be subject of future studies.<sup>[12]</sup>

To verify that trivalent Ans are binding to the same site in the enzyme than Lns, we performed time-resolved laser-induced fluorescence spectroscopy (TRLFS). The excellent luminescence properties of Cm<sup>3+</sup> were used as a luminescence probe to identify the chemical environments of Eu-MDH incubated with Cm<sup>3+</sup> as well as of SolV cells



**Figure 1.** Evaluation of the impact of Ln and selected An on XoxF-MDH from SolV. A) Workflow of reconstitution of apo-XoxF-MDH purified from *E. coli* with metal and PQQ. B) The ionic radii of the selected Lns and Ans (of the six-coordinate ions) are based on Seaborg and Hobart.<sup>[10]</sup> C) Extracted and deconvoluted spectra of time-resolved laser-induced fluorescence spectroscopy (TRLFS) and subsequent parallel factor analysis (PARAFAC) of Eu-XoxF-MDH incubated with Cm<sup>3+</sup> (in orange) and cell pellet of strain SolV grown with Cm<sup>3+</sup> (in green); for both datasets an excitation wavelength of 396 nm was used. 1 μM of Eu-XoxF-MDH was incubated with 2 μM Cm<sup>3+</sup> in 20 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES) buffer pH 7.2 for 2 h. For the displayed spectra of Cm-MDH-like species in the SolV cell pellet, spectra of the growth studies were recorded during the cultivation. A more detailed description of the speciation of the Cm-MDH-like species in the SolV growth studies with Cm<sup>3+</sup> can be found in ST. 1 and Figure S9. D) Enzymatic activity of reconstituted XoxF-MDH. Each bar shows the average of three replicates.

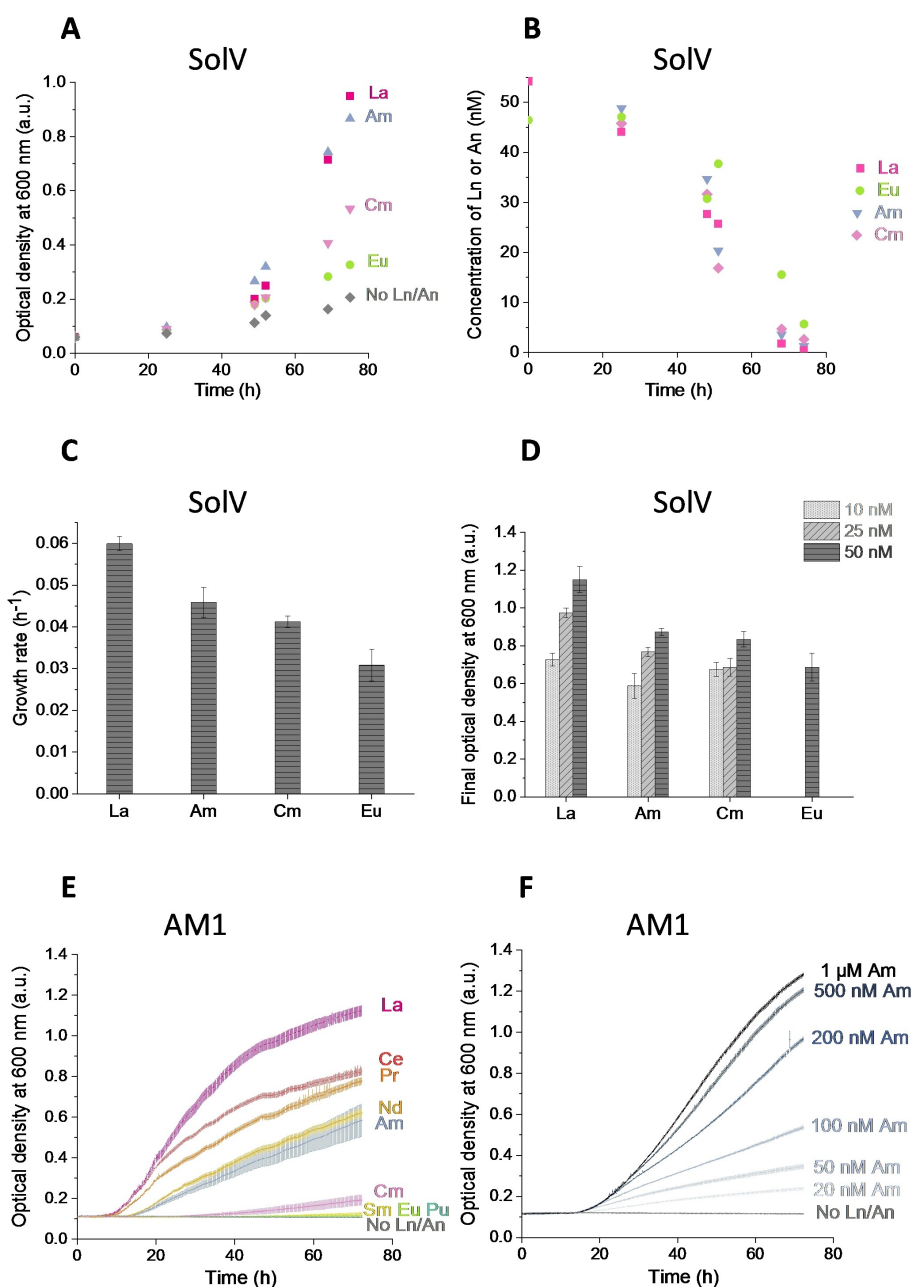
cultivated with Cm<sup>3+</sup> (Figures 1C and S9). In comparison to the free Cm<sup>3+</sup> aquo ion (593.5 nm/68 μs), the emission maximum in both systems shifts strongly to 608.5 nm with two additional, smaller bands at lower wavelengths. Overall, the luminescence characteristics of Eu-MDH incubated with Cm<sup>3+</sup> (608.5 nm/529 μs) and the Cm-MDH-like species observed in SolV cells (608.5 nm/341 μs) after cultivation with Cm<sup>3+</sup> match (Figure 1C). The long lifetimes represent 0 or 1 remaining water molecules in the first hydration sphere (ST. 1), which means Cm<sup>3+</sup> is similarly coordinated as Eu<sup>3+</sup> (8- to 9-fold coordinated in MDH).<sup>[4b,14]</sup> The difference in lifetime might be a result of quenching by the complex environment of the cell itself in comparison to the purified enzyme in solely buffer environment.

Ans support the catalytic turnover of methanol by acting as Lewis acids in the MDH active site similar to the Lns. Our *in vitro* results thus indicate that the right size and charge of the metal ion are crucial to yield an active enzyme. Since MDH plays a key role in the C<sub>1</sub> metabolism of methylotrophic bacteria, we hypothesized that it should be feasible to replace Lns with Ans *in vivo*, assuming that cellular uptake, intracellular transport, and insertion into MDH are not discriminating between 4f and 5f elements.

### Two bacterial strains grow on actinides

We conducted growth experiments with two different Ln-dependent strains: *Methylacidiphilum fumariolicum* SolV and *Methylobacterium extorquens* AM1 Δ*mxoF* (a knock-out mutant with no calcium-dependent Mxof-MDH). The methanotrophic strain SolV requires an atmosphere of air supplemented with CH<sub>4</sub> and CO<sub>2</sub>, 55 °C and a pH of 2–3 for optimal growth.<sup>[15]</sup> Sealed growth vessels were thus necessary (Figure S10). Glass bottles were not suitable for these experiments, as SolV can leach Lns in sufficient amounts to hamper the unambiguous assignment of An-dependent growth.<sup>[15,16]</sup> Inductively coupled plasma mass spectrometry (ICP-MS) measurements of the growth medium supplemented with Am<sup>3+</sup> and Cm<sup>3+</sup> ruled out Ln impurities that could have otherwise stimulated growth (Table S1). Figure 2A shows the growth curves of strain SolV with the La<sup>3+</sup> and Eu<sup>3+</sup> compared to Am<sup>3+</sup> and Cm<sup>3+</sup> at a concentration of 50 nM. La<sup>3+</sup> and Eu<sup>3+</sup> were chosen as representative Lns, since they differ in growth rates due to their ionic radii (Growth studies with all Lns except Pm are shown in Figure S12).<sup>[5b,15]</sup>

After addition of Am<sup>3+</sup> and Cm<sup>3+</sup> to a strain SolV culture we observed a clear boost in growth similar to the



**Figure 2.** Ln- and An-dependent growth of strain SolV and strain AM1  $\Delta mxaF$  with selected Ln and An. A) Optical densities at 600 nm of strain SolV grown with 50 nM of Ln or An shown over time. Samples were incubated at 55 °C and 200 rpm for 5 days under a gas atmosphere of 85 % air, 10 % CH<sub>4</sub> and 5 % CO<sub>2</sub>. B) Determined supernatant concentrations of La<sup>3+</sup>, Eu<sup>3+</sup>, Am<sup>3+</sup> and Cm<sup>3+</sup> in the centrifuged biomass samples of the growth studies shown in A (for Ln with ICP-MS and An LSC analysis was used). C) Fitted growth rates of strain SolV based on the data shown in A. D) Optical density at 600 nm of strain SolV cultures after 96 h with 10, 25 and 50 nM of La<sup>3+</sup>, Am<sup>3+</sup> and Cm<sup>3+</sup> and Eu<sup>3+</sup>. For all strain SolV growth studies, data points show the average of three individual growth experiments (experimental replicates); for clarity, error bars were omitted at A but individual growth curves of all replicates are available in Figure S6. E) Ln- and An-dependent growth of AM1  $\Delta mxaF$  mutant strain with 100 nM Ln or An. F) An-dependent growth of AM1  $\Delta mxaF$  mutant strain with different concentrations from 20 nM to 1  $\mu$ M of Am<sup>3+</sup> in MP + M (125 mM MeOH) medium. For D, four independent replicates and for E duplicates were prepared.

light Lns. The concentration of Lns and Ans in the medium during growth decreased as liquid scintillation counting (LSC, for Am<sup>3+</sup> and Cm<sup>3+</sup>) and inductively coupled plasma mass spectrometry (ICP-MS, for La<sup>3+</sup> and Eu<sup>3+</sup>, Figure 2B) demonstrate. The strongest decrease in metal concentration in the medium was observed after approximately 50–70 h,

matching perfectly with the exponential growth phase in this time frame (Figure 2A). Plutonium did not stimulate the growth of strain SolV above background levels (Figure S13) which was expected as this An did not yield an active MDH. The determined growth rates are shown in Figure 2C and doubling times in Figure S14. Maximum growth rate of

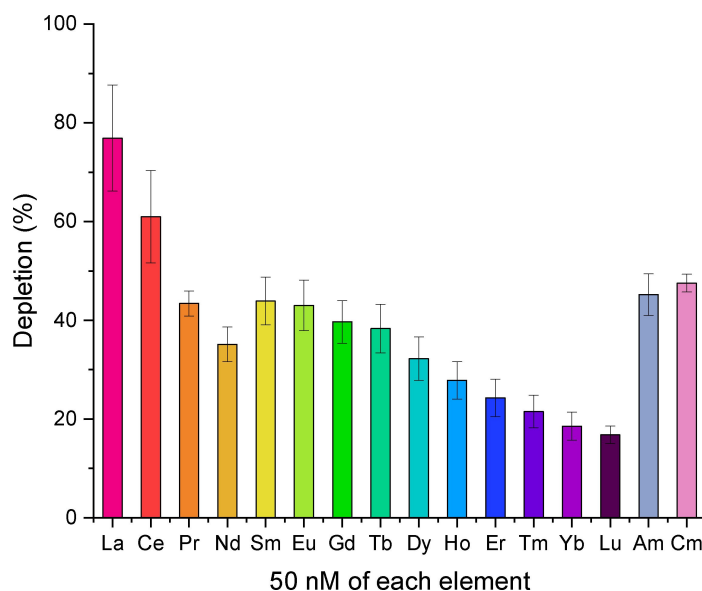
strain SolV was obtained with  $\text{La}^{3+}$  ( $0.0600 \text{ h}^{-1}$ ), followed by  $\text{Am}^{3+}$  ( $0.0459 \text{ h}^{-1}$ ) and  $\text{Cm}^{3+}$  ( $0.0412 \text{ h}^{-1}$ ), while the growth rate with the smaller  $\text{Eu}^{3+}$  ion ( $0.0308 \text{ h}^{-1}$ ) was lower, in line with previous studies.<sup>[5b,15]</sup> Background growth without Ln/An addition showed a linear increase without a clear exponential phase (Figure 2A). The data shown in Figure 2D and Figure S16 confirm that growth is proportional to Ln and An concentrations added (10 nM, 25 nM, 50 nM) and such a concentration dependence has been described previously by Pol *et al.* for the Lns.<sup>[15]</sup> When SolV is presented with a mixture of equal amounts (50 nM) of all Ln together with  $\text{Am}^{3+}$  and  $\text{Cm}^{3+}$ , the strain will deplete the f-block elements based on their size, even favoring the Ans over late Lns (Figure 3).

In addition to the native methanotrophic strain SolV, which is solely Ln-dependent, we used the Ln-utilizing AM1 strain. The methylotroph AM1 grows at neutral pH and  $29^\circ\text{C}$  in the presence and absence of Lns due to an additional Ca-MDH for the oxidation of methanol. Thus, we chose the mutant strain AM1  $\Delta\text{mxaF}$ , where the Ca-MDH pathway (MxaF) is knocked out to ensure sole dependence on f-block elements for MeOH oxidation.<sup>[17]</sup> This mutant strain is known to be able to grow, albeit poorly, on  $\text{Sm}^{3+}$ , but in contrast to strain SolV, not with  $\text{Eu}^{3+}$  (Figure S18).<sup>[2b]</sup> Growth studies using La–Eu, and the actinides Am, Cm and Pu were conducted using a plate reader, allowing for a continuous determination of optical densities at 600 nm ( $\text{OD}_{600}$ , Figure 2E,F). Again, the preference for larger f-block elements is reflected in the An growth experiments by clearly distinguishable trends in the increase of  $\text{OD}_{600}$ . The ions of almost identical size (Figure 2F),  $\text{Am}^{3+}$  and  $\text{Nd}^{3+}$ , display very similar growth behavior.  $\text{Cm}^{3+}$  still triggers some growth, slightly better than  $\text{Sm}^{3+}$ , but for both ions

only minimal growth is monitored, in line with previous observations for  $\text{Sm}^{3+}$  and strain AM1.<sup>[2b]</sup> Plutonium addition to AM1  $\Delta\text{mxaF}$  shows as in strain SolV, again no increase of  $\text{OD}_{600}$  above background. Adding different concentrations of  $\text{Am}^{3+}$  (Figure 2F) to AM1  $\Delta\text{mxaF}$  also revealed a similar proportional growth response as  $\text{Nd}^{3+}$  (Figure S19), firmly demonstrating the dependence on Ans for growth. For the Ln and An dependent growth of AM1  $\Delta\text{mxaF}$ , the cut-off ionic size seems to be around 98 pm. This highlights that the ionic size in combination with a stable +III oxidation state is necessary to support bacterial growth.

## Conclusion

Selected Ans replace essential Lns in living organisms. Our *in vivo* studies with bacterial strains *Methylophilum fumariolicum* SolV and *Methylobacterium extorquens* AM1  $\Delta\text{mxaF}$  as well as the *in vitro* results on Ln-dependent MDH from the latter strain give a coherent picture how the size and charge of the f-block elements ions influence the biological systems. We demonstrate that  $\text{Am}^{3+}$  and  $\text{Cm}^{3+}$  that exhibit ionic radii comparable to the light Lns, such as  $\text{Nd}^{3+}$ , lead to a similar growth response as well as comparable enzymatic activity. LSC, ICP-MS and TRLFS data confirmed an uptake of Ln and An by strain SolV over time, which is in direct relation to the observed increase of biomass. Furthermore, spectroscopic results verified the formation of an Cm-MDH-like species in SolV biomass (ST. 1), which is identical to the Cm-signature in native MDH. Our results establish that  $\text{An}^{\text{III}}$  ions of the right size can indeed be used by bacteria as essential elements for  $\text{C}_1$



**Figure 3.** ICP-MS analysis of SolV supernatant grown in the presence of equimolar concentrations of Ln and An. Graph shows the depletion of the individual Ln and An from growth medium by SolV after the stationary phase was reached. SolV was grown with 50 nM each of Ln and An. The samples were incubated at  $55^\circ\text{C}$  and 200 rpm for 5 days under a gas atmosphere of 85% air, 10%  $\text{CH}_4$  and 5%  $\text{CO}_2$ . Data points show the average of three individual growth experiments.

metabolism. Whether other Ans, such as plutonium, are taken up into AM1 and SolV and are incorporated in MDH (*in vivo* and *in vitro*) can not be directly determined by our experiments; however, the lack of MDH activity after reconstitution attempts and lack of growth with Pu (under the aerobic conditions used) hints towards the importance of a stable +III oxidation state for the enzymes and strains tested in this study.

Given the relevance of f-block elements for mankind, developing new strategies to separate, recycle, and bind these elements with high specificity is very important. These results have implications for future applications of the strains; AM1  $\Delta mxaF$  might be a potential candidate for cleaning up spills of radioactive material in the environment. It has been previously demonstrated that one of the Ln-dependent proteins of AM1, LanM, can tightly bind Ans, in some cases even with higher affinities than for Lns.<sup>[9]</sup> Further, methylotrophic bacteria, such as strain AM1, possess dedicated uptake mechanisms for the poorly bioavailable Lns. A corresponding gene cluster has been recently characterized, suggesting the production of chelators similar to siderophores.<sup>[1b,18]</sup> It is well documented that Ans can bind to siderophores and siderophore-like molecules and that these are recognized by binding partners, such as siderocalin.<sup>[8d]</sup> Isolation of such chelators from Ln-dependent bacteria could thus advance chelating therapies for radioactive An poisoning and after accidental An spills.<sup>[19]</sup>

Extremophilic organisms thriving at low pH values, such as strain SolV, may be implemented in recycling of Lns and Ans from, for example, end-of-lifetime products, industrial feedstocks, or metal leachates. In the latter case, harsh chemical conditions, such as low pH values in combination with high temperatures, are required for a good solubility of the metals, which is tolerable for the extremophilic bacteria. In conclusion, the results presented herein pave the way for a future use of extremophilic bacteria in recycling and separation of Ln and An and provide insight into possible pathways of microbially driven mobility of radioactive elements in the environment.

### Associated Content

Supporting Information is available for this paper. All data has been included in the manuscript or the Supporting Information. Correspondence and requests for all other materials should be addressed to Lena Daumann (lena.dau-[mann@lmu.de](mailto:mann@lmu.de))

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### Author Contributions

LJD and HS conceptualized the idea and wrote the initial draft of this manuscript, LJD acquired funding for this project, all authors developed the methodology and were involved in review and editing of this manuscript. Strain SolV was provided by AP and HOdC, the  $\Delta mxaF$  mutant strain by NCMG. CZ, ASK and CR expressed and purified the XoxF-MDH from strain SolV in *E. coli*, HS and AP purified Eu-MDH from SolV. Growth studies were conducted by HS and RS. HS and RS acquired the TRLFS data. RS and BD wrote the code and fitted the TRLFS data. RS conducted the LSC and ICP-MS measurements. HS conducted the enzymatic activity determination. LJD and RS provided the necessary resources and infrastructure for this work.

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### Conflict of Interest

Authors declare that they have no competing interests.

### Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

**Keywords:** Actinides · Lanthanide-Binding Proteins · Lanthanide-Dependent Bacteria · Lanthanides · Methylotrophy

- [1] a) T. Cheisson, E. J. Schelter, *Science* **2019**, *363*, 489–493; b) K. N. Raymond, B. E. Allred, A. K. Sia, *Acc. Chem. Res.* **2015**, *48*, 2496–2505.
- [2] a) M. Farhan Ul Haque, B. Kalidass, N. Bandow, E. A. Turpin, A. A. DiSpirito, J. D. Semrau, F. E. Löffler, *Appl. Environ. Microbiol.* **2015**, *81*, 7546–7552; b) H. N. Vu, G. A. Subuyuj, S. Vijayakumar, N. M. Good, N. C. Martinez-Gomez, E. Skovran, *J. Bacteriol.* **2016**, *198*, 1250–1259; c) N. M. Good, R. S. Moore, C. J. Suriano, N. C. Martinez-Gomez, *Sci. Rep.* **2019**, *9*, 4248; d) N. C. Martinez-Gomez, E. Skovran, in *Methane Biocatalysis: Paving the Way to Sustainability* (Eds.: M. G. Kalyuzhnaya, X.-H. Xing), Springer International Publishing, Cham, **2018**, pp. 67–82; e) P. Roszczenko-Jasińska, H. N. Vu, G. A. Subuyuj, R. V. Crisostomo, J. Cai, N. F. Lien, E. J. Clippard, E. M. Ayala, R. T. Ngo, F. Yarza, J. P. Wingett, C. Raghuraman, C. A. Hoerber, N. C. Martinez-Gomez, E. Skovran, *Sci. Rep.* **2020**, *10*, 12663–12663.
- [3] Y. Hibi, K. Asai, H. Arafuka, M. Hamajima, T. Iwama, K. Kawai, *J. Biosci. Bioeng.* **2011**, *111*, 547–549.

- [4] a) J. B. Glass, E. T. Cowan, K. H. Johannesson, *FEMS Microbiol. Lett.* **2020**, *367*, fnaa165; b) L. J. Daumann, *Angew. Chem. Int. Ed.* **2019**, *58*, 12795–12802; c) C.-E. Wegner, L. Gorniak, S. Riedel, M. Westermann, K. Küsel, *Appl. Environ. Microbiol.* **2019**, *86*, e01830-01819.
- [5] a) A. M. Shiller, E. W. Chan, D. J. Joung, M. C. Redmond, J. D. Kessler, *Sci. Rep.* **2017**, *7*, 10389; b) B. Jahn, A. Pol, H. Lumpe, T. R. M. Barends, A. Dietl, C. Hogendoorn, H. J. M. Op den Camp, L. J. Daumann, *ChemBioChem* **2018**, *19*, 1147–1153; c) H. Lumpe, A. Pol, H. J. M. Op den Camp, L. J. Daumann, *Dalton Trans.* **2018**, *47*, 10463–10472.
- [6] N. M. Good, H. D. Lee, E. R. Hawker, M. Z. Su, A. A. Gilad, N. C. Martinez-Gomez, *Front. Microbiol.* **2022**, *13*, 820327.
- [7] a) J. R. Lloyd, J. C. Renshaw, *Curr. Opin. Biotechnol.* **2005**, *16*, 254–260; b) J. C. Renshaw, J. R. Lloyd, F. R. Livens, *C. R. Chim.* **2007**, *10*, 1067–1077.
- [8] a) F. Brulfert, S. Safi, A. Jeanson, H. Foerstendorf, S. Weiss, C. Berthomieu, S. Sauge-Merle, É. Simoni, *J. Inorg. Biochem.* **2017**, *172*, 46–54; b) G. J.-P. Deblonde, M. Sturzbecher-Hoehne, A. B. Mason, R. J. Abergel, *Metallomics* **2013**, *5*, 619–626; c) B. Drobot, M. Schmidt, Y. Mochizuki, T. Abe, K. Okuwaki, F. Brulfert, S. Falke, S. A. Samsonov, Y. Komeiji, C. Betzel, T. Stumpf, J. Raff, S. Tsushima, *Phys. Chem. Chem. Phys.* **2019**, *21*, 21213–21222; d) B. E. Allred, P. B. Rupert, S. S. Gauny, D. D. An, C. Y. Ralston, M. Sturzbecher-Hoehne, R. K. Strong, R. J. Abergel, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 10342–10347.
- [9] a) H. Singer, B. Drobot, C. Zeymer, R. Steudtner, L. J. Daumann, *Chem. Sci.* **2021**, *12*, 15581–15587; b) G. J.-P. Deblonde, J. A. Mattocks, H. Wang, E. M. Gale, A. B. Kersting, M. Zavarin, J. A. Cotruvo, *J. Am. Chem. Soc.* **2021**, *143*, 15769–15783; c) G. J.-P. Deblonde, A. Mattocks Joseph, Z. Dong, T. Wooddy Paul, A. Cotruvo Joseph, M. Zavarin, *Sci. Adv.* **2021**, *7*, eabk0273.
- [10] G. T. Seaborg, D. E. Hobart, *Summary of the properties of the lanthanide and actinide elements*, Indian Association of Nuclear Chemists and Allied Scientists, India, **1996**, p. 26.
- [11] D. L. Clark, S. S. Hecker, G. D. Jarvinen, M. P. Neu, in *The Chemistry of the Actinide and Transactinide Elements* (Eds.: L. R. Morss, N. M. Edelstein, J. Fuger), Springer Netherlands, Dordrecht, **2006**, pp. 813–1264.
- [12] a) A. N. Gaiser, C. Celis-Barros, F. D. White, M. J. Beltran-Leiva, J. M. Sperling, S. R. Salpage, T. N. Poe, D. Gomez Martinez, T. Jian, N. J. Wolford, N. J. Jones, A. J. Ritz, R. A. Lazenby, J. K. Gibson, R. E. Baumbach, D. Páez-Hernández, M. L. Neidig, T. E. Albrecht-Schönzart, *Nat. Commun.* **2021**, *12*, 7230; b) K. M. S. Saxena, S. Fraga, *J. Chem. Phys.* **1972**, *57*, 1800–1801.
- [13] a) J. I. Kim, R. Klenze, H. Wimmer, W. Runde, W. Hauser, *J. Alloys Compd.* **1994**, *213–214*, 333–340; b) C. Wilke, A. Barkleit, T. Stumpf, A. Ikeda-Ohno, *J. Inorg. Biochem.* **2017**, *175*, 248–258.
- [14] N. A. Danaf, J. Kretzschmar, B. Jahn, H. Singer, A. Pol, H. J. M. Op den Camp, R. Steudtner, D. C. Lamb, B. Drobot, L. J. Daumann, *Phys. Chem. Chem. Phys.* **2022**, *24*, 15397–15405.
- [15] A. Pol, T. R. M. Barends, A. Dietl, A. F. Khadem, J. Eygensteyn, M. S. M. Jetten, H. J. M. Op den Camp, *Environ. Microbiol.* **2014**, *16*, 255–264.
- [16] C. H. Evans, *Biochemistry of the Lanthanides*, Vol. 8, Springer Science & Business Media, **2013**.
- [17] C. J. Marx, L. Chistoserdova, M. E. Lidstrom, *J. Bacteriol.* **2003**, *185*, 7160–7168.
- [18] a) A. M. Zytznick, N. M. Good, C. C. Barber, M. T. Phi, S. M. Gutenthaler, W. Zhang, L. J. Daumann, N. C. Martinez-Gomez, *bioRxiv* **2022**, <https://doi.org/10.1101/2022.01.19.476857>; b) A. M. Ochsner, L. Hemmerle, T. Vonderach, R. Nüssli, M. Bortfeld-Miller, B. Hattendorf, J. A. Vorholt, *Mol. Microbiol.* **2019**, *111*, 1152–1166.
- [19] a) R. J. Abergel, in *Metal Chelation in Medicine*, The Royal Society of Chemistry, Cambridge, **2017**, pp. 183–212; b) É. Ansoborlo, B. Amekraz, C. Moulin, V. Moulin, F. Taran, T. Bailly, R. Burgada, M.-H. Hengé-Napoli, A. Jeanson, C. Den Auwer, L. Bonin, P. Moisy, *C. R. Chim.* **2007**, *10*, 1010–1019.

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