Supporting Information

Lanpepsy is a novel lanthanide-binding protein involved in the lanthanide response of the obligate methylotroph *Methylobacillus flagellatus*

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Contents:

- Tables S1–S4
- Figures S1–S4

Supporting Tables

Table S1. List of proteins induced in the presence of 20 µM lanthanum. A q-value cutoff of <0.05 and a fold-change cutoff of >4 was applied and at least two unique peptides needed to be detected for each protein.

Locus tag	Description	Fold-change	q-value
Mfla_0344	XoxF (La^{3+}-dependent methanol dehydrogenase)	13.7	1.6E-07
Mfla_2314	XoxF (La^{3+}-dependent methanol dehydrogenase)	9.6	2.3E-05
Mfla_1632	Acriflavin resistance protein	9.0	1.2E-04
Mfla_2312	XoxG (cytochrome *c*)	8.5	8.8E-06
Mfla_1253	TonB-dependent receptor	8.0	8.8E-06
Mfla_0908, Mfla_1052*	LanP (Propeptide, PepSY and peptidase M4)	5.6	2.8E-03
Mfla_1254	Two component, sigma54 specific, transcriptional regulator, Fis family	5.3	1.9E-03
Mfla_0343	GAF modulated sigma54 specific transcriptional regulator, Fis family	4.5	1.6E-03

* The genome of *M. flagellatus* KT has been described to contain a large (potentially phage-related) duplication (22), thus both locus tags Mfla_0908 and Mfla_1052 refer to an identical protein sequence.

Table S2. List of proteins that showed decreased abundance in the presence of 20 µM lanthanum. A q-value cutoff of <0.05 and a fold-change cutoff of >4 was applied and at least two unique peptides needed to be detected for each protein.

Locus tag	Description	Fold-change	q-value
Mfla_1724	DUF3828 domain-containing protein	14.8	8.76E-05
Mfla_2037	MxaC, protein involved in Ca2+ insertion into methanol dehydrogenase	11.7	1.26E-02
Mfla_0817	Signal transduction histidine kinase, glucose-6-phosphate specific	10.8	3.46E-04
Mfla_2035	MxaL protein, putative	10.2	1.82E-03
Mfla_0816	Two component transcriptional regulator, LuxR family	10.0	2.24E-03
Mfla_1185	Thioredoxin	9.5	8.83E-06
Mfla_2039	MxaS, protein involved in methanol oxidation	9.4	4.95E-02
Mfla_2038	MxaA, protein involved in Ca2+ insertion into methanol dehydrogenase	9.1	1.61E-03
Mfla_2041	MxaI (Methanol dehydrogenase [cytochrome c] subunit 2)	8.7	4.78E-02
Mfla_2042	MxaG (cytochrome *c*, class I)	8.7	4.32E-02
Mfla_2043	MxaJ (Amino acid ABC transporter substrate-binding protein, PAAT family)	7.2	3.39E-02
Mfla_2034	MxaD protein, putative	6.1	1.39E-02
Mfla_2040	MxaR (ATPase associated with various cellular activities, AAA_3)	4.8	4.10E-03
Mfla_0898, Mfla_1042*	Uncharacterized protein	4.3	7.25E-04

* The genome of *M. flagellatus* KT has been described to contain a large (potentially phage-related) duplication (22), thus both locus tags Mfla_0898 and Mfla_1042 refer to an identical protein sequence.

Table S3. Characterization of LanP using isothermal titration calorimetry (ITC). The ITC cell contained 50 µM LanP (Mfla_0908) and the syringe 5 mM of the metal ligand; both were dissolved in 25 mM MOPS and 150 mM NaCl at pH 7.2. A control for the heat of dilution (injection of metal into buffer) was performed and subtracted from the measurement with protein. A model with two sets of sites was used to fit the isotherms of La^{3+}, Nd^{3+}, and Eu^{3+}, while a model with one set of sites was fitted for Ca^{2+}. For Fe^{3+}, no fit could be obtained, suggesting no binding. Displayed thermograms are baseline corrected.

Titrated metal	Thermogram / isotherm plots	Thermodynamic parameters
$LaCl_3$		N_1 (sites): 6.1±0.16 K_{D1} (M): 1.1e-6±0.10e-6 ΔH_1 (kJ/mol): 0.47±0.83 $-T\Delta S_1$ (kJ/mol): -34.5 N_2 (sites): 0.21±0.15 K_{D2} (M): 4.9e-6±1.2e-6 ΔH_2 (kJ/mol): 210±150 $-T\Delta S_2$ (kJ/mol): -236 Reduced Chi-Sqr. (kJ^2/mol^2): 0.873
$NdCl_3$		N_1 (sites): 6.1±0.31 K_{D1} (M): 1.1e-6±0.26e-6 ΔH_1 (kJ/mol): -1.5±0.89 $-T\Delta S_1$ (kJ/mol): -32.6 N_2 (sites): 0.22±0.35 K_{D2} (M): 4.5e-6±1.2e-6 ΔH_2 (kJ/mol): 240±380 $-T\Delta S_2$ (kJ/mol): -265 Reduced Chi-Sqr. (kJ^2/mol^2): 0.985
$EuCl_3$		N_1 (sites): 6.4±0.31 K_{D1} (M): 1.1e-6±0.22e-6 ΔH_1 (kJ/mol): -1.8±0.66 $-T\Delta S_1$ (kJ/mol): -32.2 N_2 (sites): 0.15±0.33 K_{D2} (M): 4.8e-6±0.68e-6 ΔH_2 (kJ/mol): 330±770 $-T\Delta S_2$ (kJ/mol): -363 Reduced Chi-Sqr. (kJ^2/mol^2): 0.514
$CaCl_2$		N (sites): 4.3±0.41 K_D (M): 14e-6±11e-6 ΔH (kJ/mol): -2.0±0.41 $-T\Delta S$ (kJ/mol): -25.8 Offset (kJ/mol): 1.4±0.24* Reduced Chi-Sqr. (kJ^2/mol^2): 0.056 *Without the offset as a fitting parameter, no fit could be obtained.

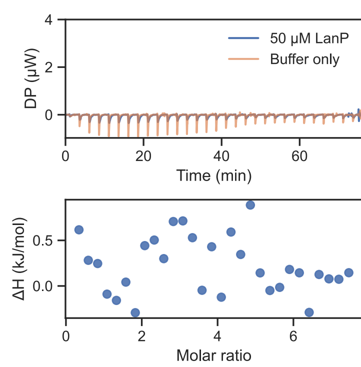
$FeCl_3$		No fit possible (no binding)

Table S4. List of primers used in this work.

Purpose	Sequence
PCR of homologous region upstream of Mfla_0343	Fwd: TCATCTAGAAATCAGGCAGTCATTCCACTGTGGTC Rev: CATGAAGCTCCTAGCCTAAAGACCAGGCACCCCTC
PCR of homologous region downstream of Mfla_0343	Fwd: TTTAGGCTAGGAGCTTCATGGTTGATCGTAGGAAC Rev: AACAAGCTTGCAGCAAAGCAATGCCTGTACG
PCR of homologous region upstream of Mfla_0908	Fwd: CAGTCTAGACTATTGTGCCCAAGTAGCTTTATTC Rev: ATCATTCCTTTCCCACCATTTATCTCCATATAATCA
PCR of homologous region downstream of Mfla_0908	Fwd: AATGGTGGGAAAGGAATGATAGCTTTTTGACTGGAC Rev: CTTCTGCAGAGTGTGGCACTATCATATCGATTC
PCR of homologous region upstream of Mfla_1253	Fwd: TTCTCTAGAGTCAGGAACAGCTCCTCAATCAG Rev: TTTAATTGGTTTTTAACATACAATCCTTCCATAGTTG
PCR of homologous region downstream of Mfla_1253	Fwd: TATGTTAAAAACCAATTAAACTGACTTATTATTGATTAC Rev: GCAAAGCTTCACAATCTACATGCACGAAAGG
PCR of homologous region upstream of Mfla_1632	Fwd: TGTTCTAGATCAACCAGCATGACCGCAATATTTC Rev: TTCAGGCCACCGACATCATGGGGTATCAGCTCCT
PCR of homologous region downstream of Mfla_1632	Fwd: CATGATGTCGGTGGCCTGAAGGTTGGGATTCTCTG Rev: TGGAAGCTTTATTGACGGTGTCACTGTCATCCTTG
PCR of Mfla_0908 for cloning into pET-16b	Fwd: GCGCATATGGATCATCACTTTCCCAAAGGAAAGG Rev: AACGGATCCTCATTCCTTGCCAATCTGGTAAAAC
PCR for removal of His_{10}-tag from Mfla_0908	Fwd: TTGGGAAAGTGATGATCCATGGTATATCTCCTTCTTAAAGTTAAACA Rev: CTTTAAGAAGGAGATATACCATGGATCATCACTTTCCCAA

Supporting Figures

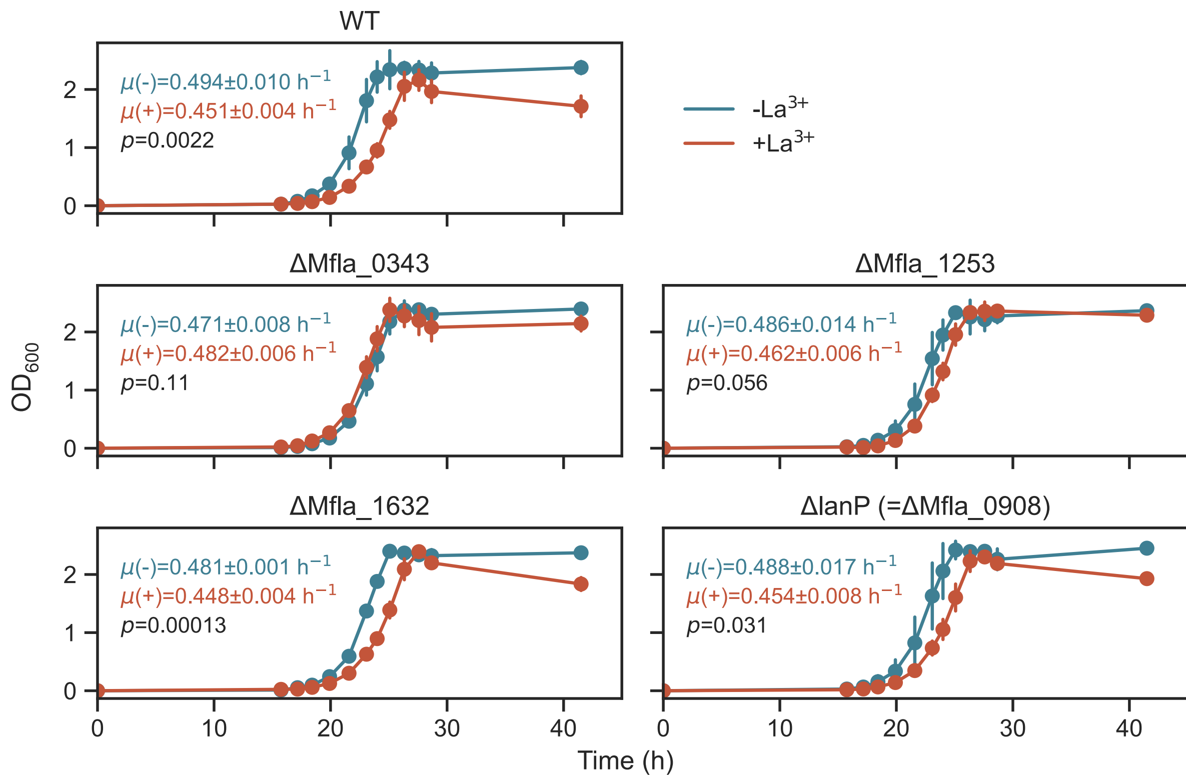


Figure S1. Growth curves of the wild type (WT) and deletion strains with (+) and without (-) La^{3+} addition (20 µM final concentration). The error bars show the standard deviation of three biological replicates. The growth rate μ was calculated from the best fit to the exponential growth of each replicate and reported as the mean value ± standard deviation. The p-value originates from a t-test of the growth rates in presence and absence of La^{3+} for each strain.

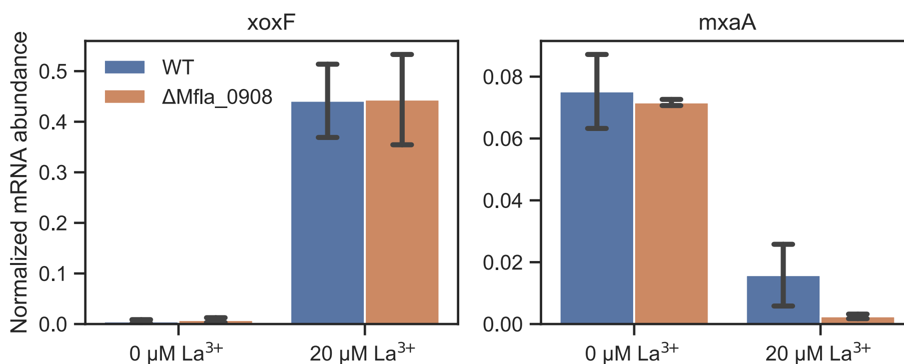


Figure S2. RT-qPCR analysis of *xox* and *mxa* cluster expression in *M. flagellatus* KT wild-type and ΔMfla_0908 strains. The highly induced (judging from the proteomics data) genes *xoxF* (*Mfla_0344*) and *mxaA* (*Mfla_2038*) were used as marker genes for the expression of the corresponding gene clusters. mRNA abundances were normalized to the housekeeping (according to proteomics) gene *Mfla_0284* (50S ribosomal protein L22).

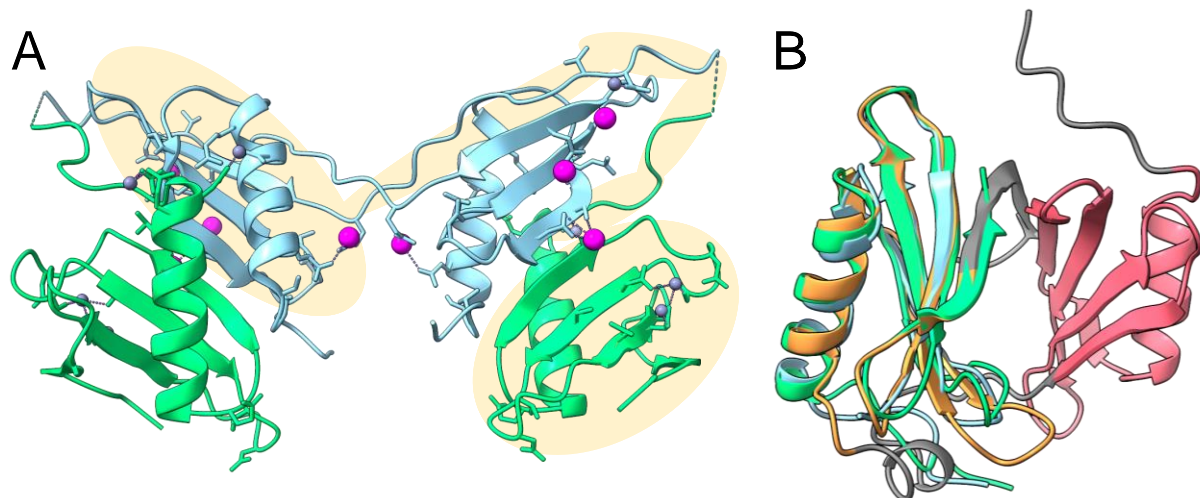


Figure S3. Structural comparison of UipA (PDB ID 7ATK) and LanP (predicted structure by AlphaFold). **(A)** Crystal structure of the UipA dimer. The two PepSY domains are colored green and blue. The yellow shaded regions represent a single monomer of the domain-swapped dimer. The magenta spheres depict uranium ions, while the smaller grey spheres represent zinc ions. **(B)** Predicted structure of LanP aligned and superimposed with the two individual PepSY domains of UipA (green and blue).

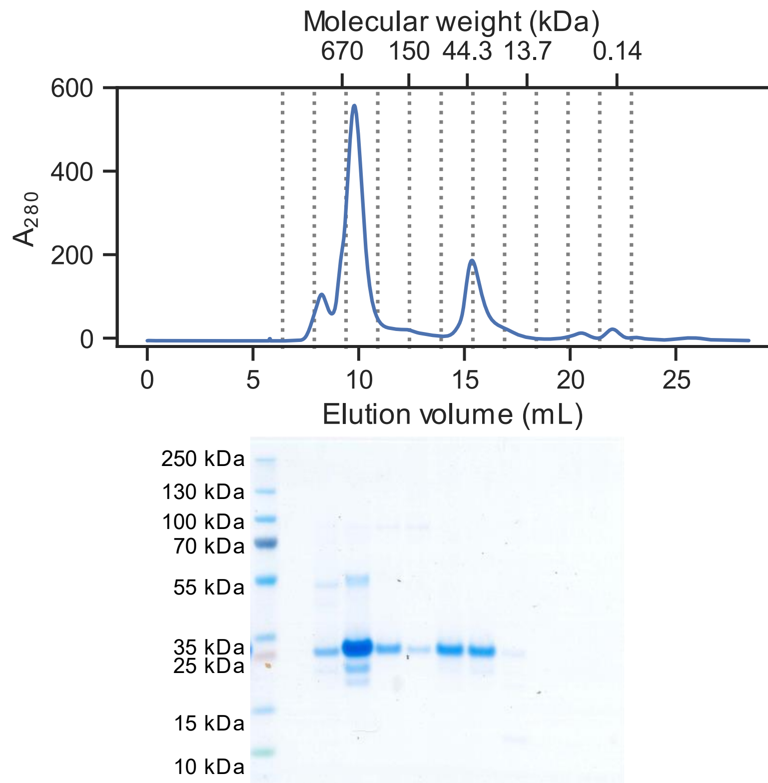


Figure S4. Size-exclusion chromatography of affinity-purified His_{10}-tagged Mfla_0908 and SDS-PAGE analysis of the resulting fractions. A Superdex 200 Increase 10/300 GL column (GE Healthcare) was used. The molecular weight axis on top was determined using standards (thyroglobulin, γ-globulins, albumin, ribonuclease A, p-aminobenzoic acid). The fractions indicated by the grey dotted lines were analyzed by SDS-PAGE as shown on the gel below.

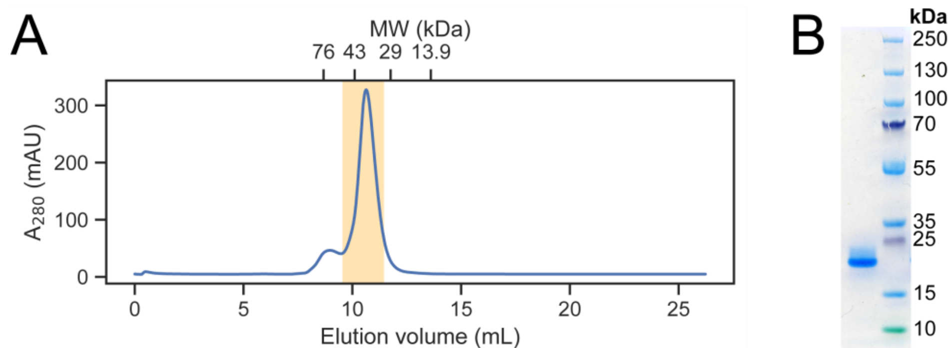


Figure S5. Purification of untagged LanP (Mfla_0908) expressed in *E. coli*. **(A)** Size-exclusion chromatogram (Superdex 75 10/300 GL) after two steps of anion-exchange chromatography. The top axis represents the elution volumes of molecular weight standards. The yellow shaded area depicts the fractions that were combined to yield pure protein. **(B)** SDS-PAGE analysis of 1 µg of size-exclusion purified LanP.

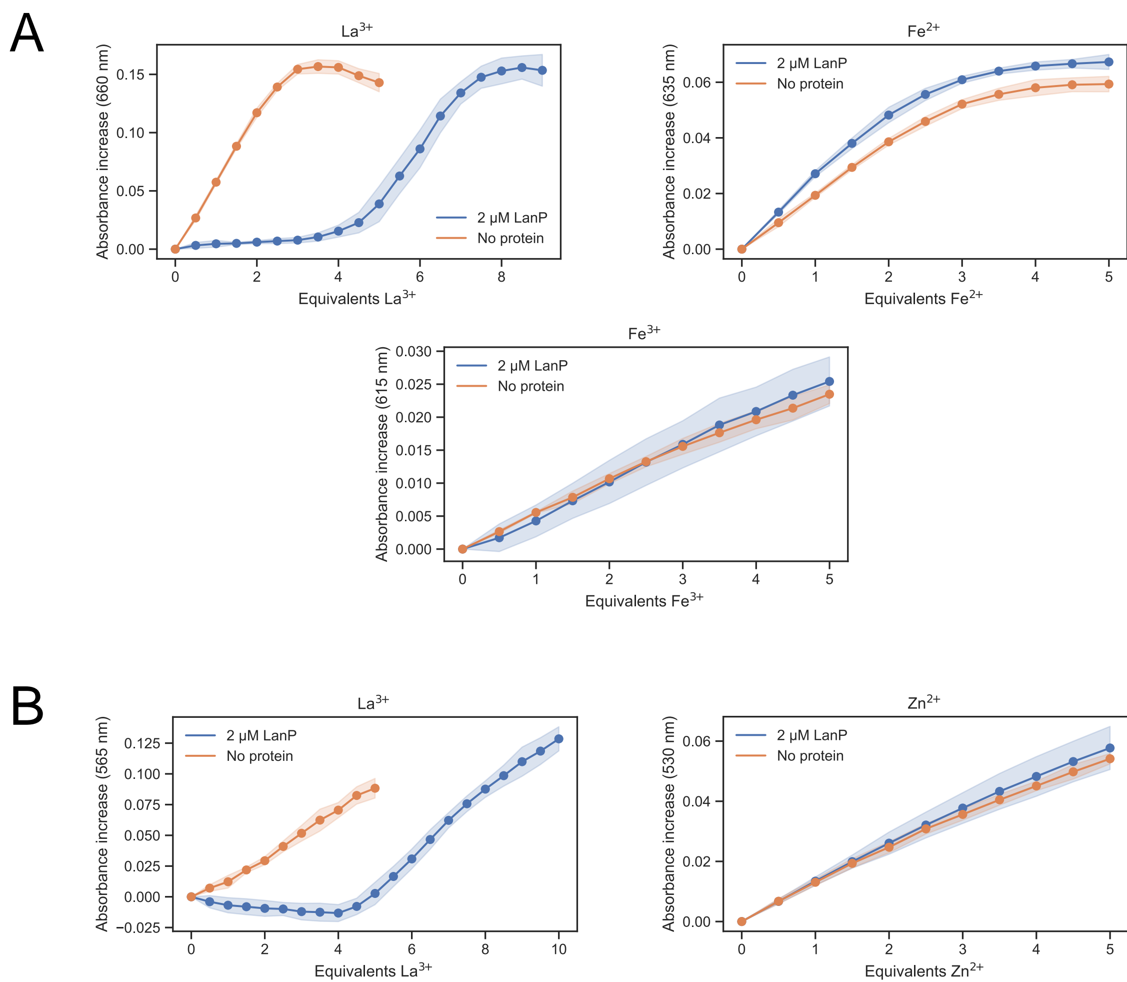


Figure S6. Dye competition assays performed using 2 μM LanP (Mfla_0908) and different metal ions. The non-lanthanide metals required different dyes (10 μM) and wavelengths for the assay to work optimally. **(A)** Competition assays performed using La^{3+} (control), Fe^{2+}, and Fe^{3+} with arsenazo III as dye. **(B)** Competition assays performed using La^{3+} (control) and Zn^{2+} with xylenol orange as dye.