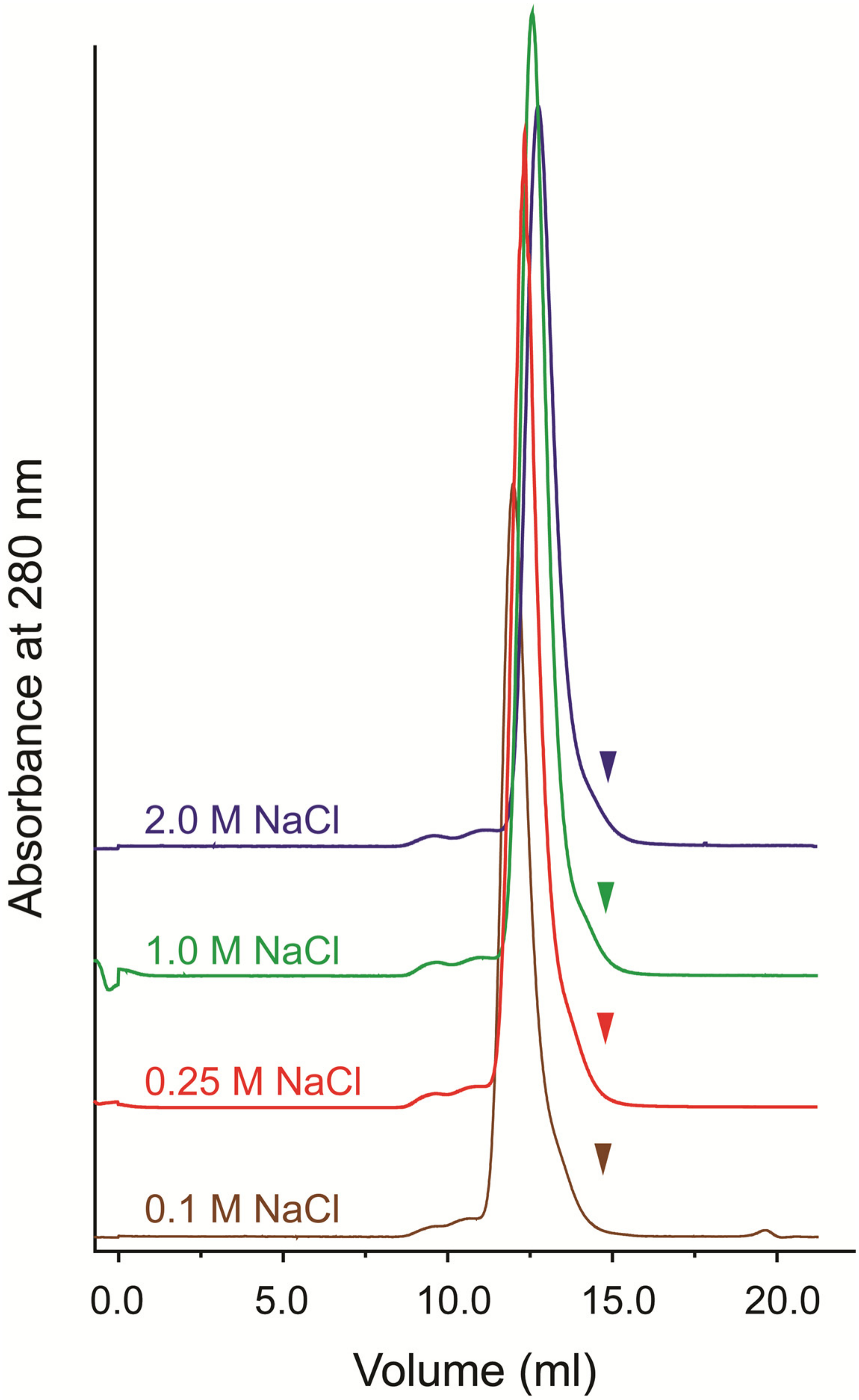
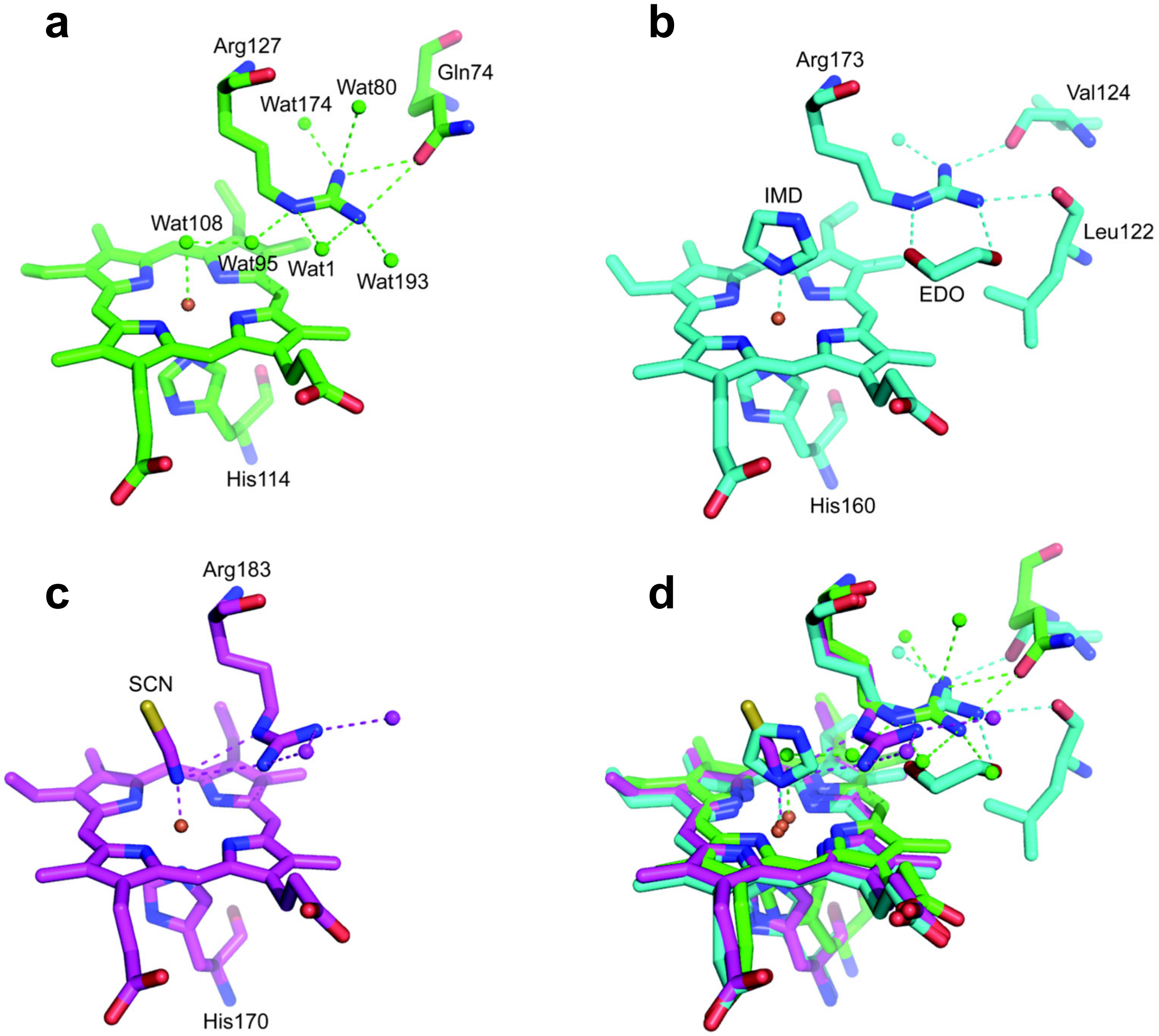
Supplemental Figure S1 (Mlynek *et al.*)



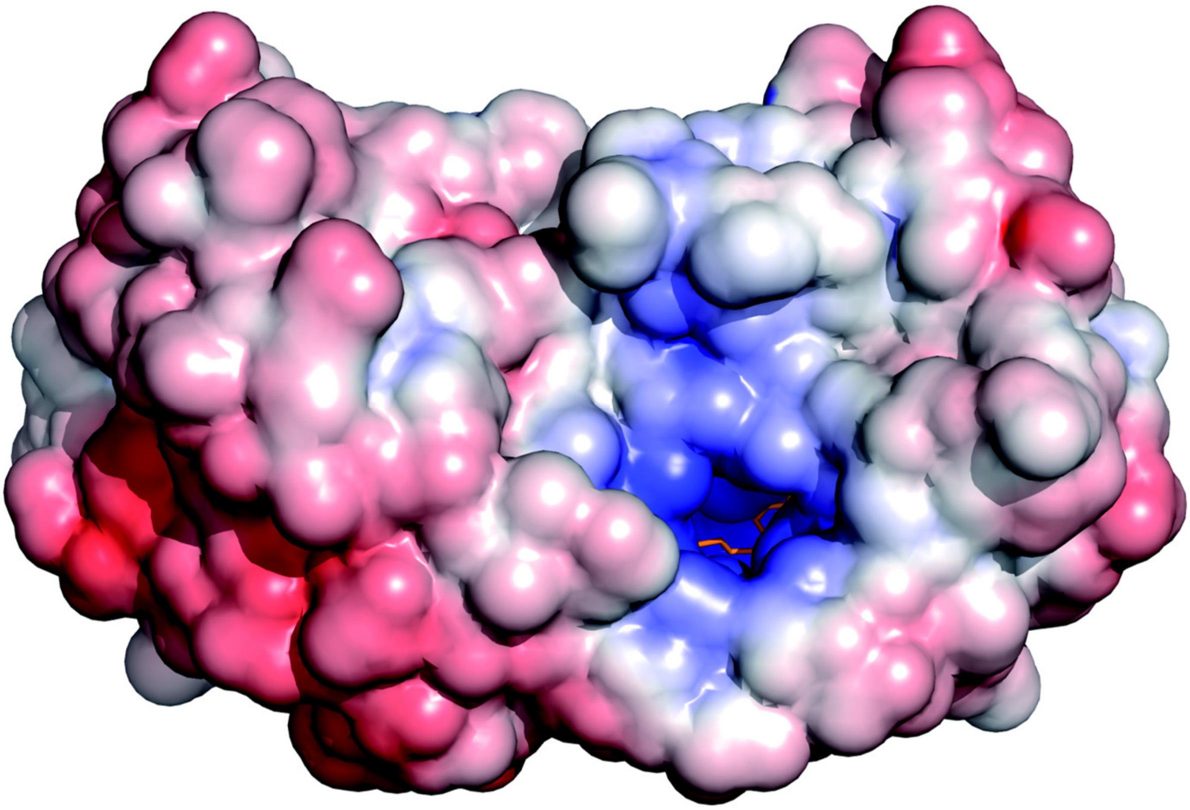
Supplemental Figure S1 Stability of the NwCld dimer at different salt concentrations. The curves depict the absorbance (280 nm) trace of NwCld eluting from a Superdex 75 10/300 column at different salt concentrations. Arrowheads indicate the expected elution volume of an NwCld monomer. This elution volume was calculated based on the column calibration at the respective salt concentration.

Supplemental Figure S2 (Mlynek *et al.*)



Supplemental Figure S2 Environment of the catalytically important arginine residue as found in the structures of (**a**) dimeric NwCld, (**b**) pentameric NdCld, and (**c**) hexameric AoCld. The NwCld structure represents the enzyme in its native state, as a water molecule (Wat108) was found to coordinate with the heme iron (panel a). Instead of water, imidazole (IMD) and thiocyanate (SCN) are bound to the heme iron of NdCld (panel b) and AoCld (panel c), respectively. In the case of NdCld, water molecules that stabilize the arginine residue in the native enzyme were replaced by ethylene glycol (EDO) from the cryo solution. Carbon atoms are depicted in green, cyan, and magenta. Oxygen and nitrogen atoms are shown in red and blue, respectively. Water molecules are shown as spheres in green (panel a), cyan (panel b), and magenta (panel c). Heme irons are shown as orange spheres. (**d**) Overlay of the three structures.

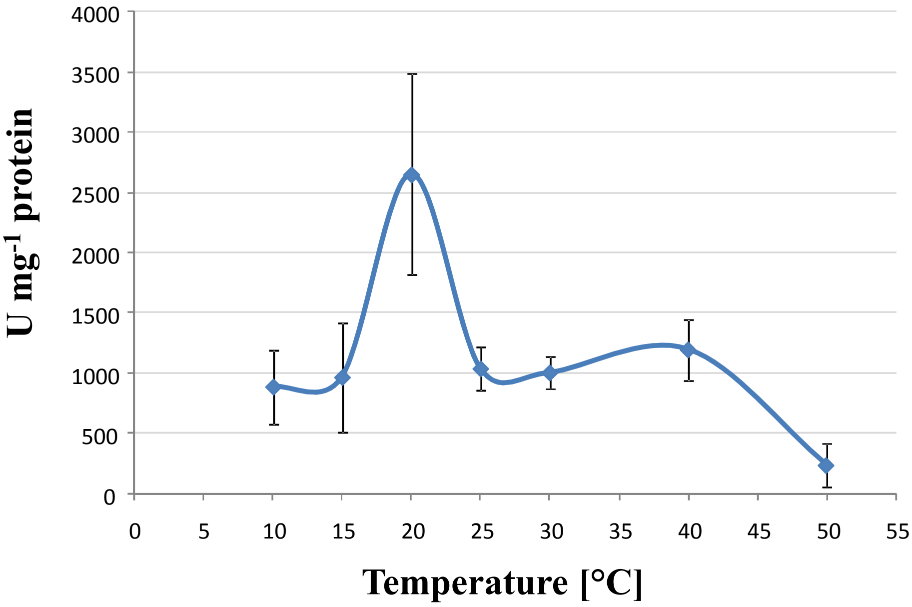
Supplemental Figure S3 (Mlynek *et al.*)



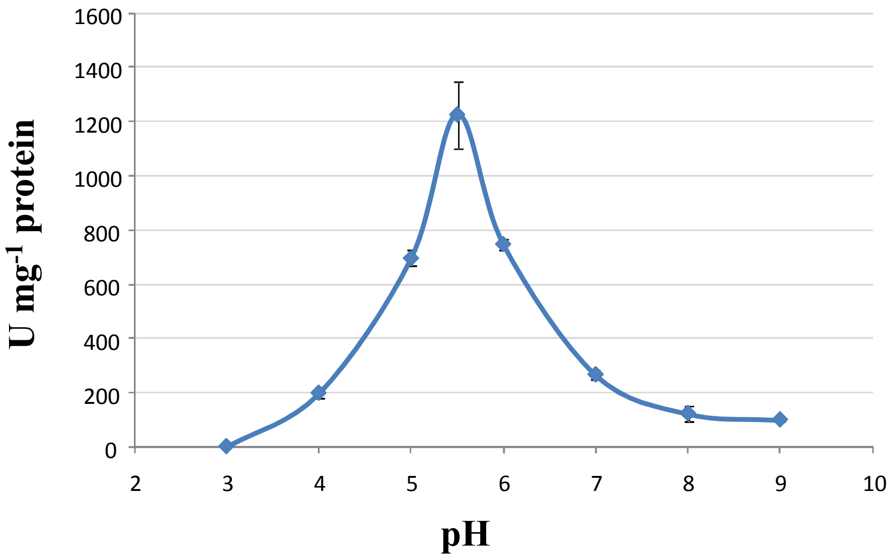
Supplemental Figure S3 Electrostatic surface representation of the NwCld holoenzyme. The solvent-accessible surface of the NwCld dimer is colored according to its electrostatic potential (blue for positive, red for negative). Hemes are presented as orange stick models. The orientation of the structure is the same as in Fig. 4a in the main text.

Supplemental Figure S4 (Mlynek *et al.*)

a



b



Supplemental Figure S4 Specific activities of NwCld at different temperatures (a) or pH values (b). Experiments were carried out with starting substrate concentrations of 1 mM $NaClO_2$. One unit (U) is defined as the amount of Cl^- produced per minute (μmol/min). Data points represent mean values from triplicate experiments. Error bars depict standard deviations.

Supplemental Figure S5 (Mlynek *et al.*)

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Nitrobacter winogradskyi             3  -------------FTVFTGGDS-------------------------------------G-AWSI--LSVAPVIGESLMAASHLAIAPSLSLGDTSAT--   47
Nitrobacter sp. Nb-311A              3  -------------FTIFTGGDT-------------------------------------G-AWNI--LSMASVIGDSLMPASHLAIAPSASLGDAAV---   46
Bradyrhizobium japonicum             2  -------------FRTFRGGHS-------------------------------------G-GWRI--TSISPVTGEPLPFMPALSVTDSEAVSLPLVPSR   48
Limnobacter sp. MED105               4  -------------HYSFIGGQE-------------------------------------G-QWRV--TRCDTVVGAPIEAVPRLNVVNT-AASQLSQR--   47
Pseudomonas aeruginosa               4  -------------HYSFIGGSE-------------------------------------G-SWRV--TSCETLIGIPLEIVERVNVVNM-PSTNLIER--   47
Gloeobacter violaceus                4  -------------RYSFLGGKR-------------------------------------G-PWRV--ARLDGLRGAGLEAVERLQIVQG-EWAESASE--   47
Cyanothece sp. PCC 7425             14  -------------RYSFIGGRT-------------------------------------G-QWQV--VKIRNVLGPGLQLVEKVNILNG-AVAEIPLD--   57
Nitrococcus mobilis                  5  -------------LFAFVGGEI-------------------------------------G-SWRV--IETKTVAGEGLAEVKRLNVVNA-AVPLLPDD--   48
Pseudomonas stutzeri                 3  -----------------------------------------------------------------------AVAGAPLPGIPRLNVAAG-SVSPQPPG--   28
Klebsiella pneumoniae                5  -------------LFTFAGGET-------------------------------------G-VWRV--VRMDAVAGAPLPGIPRLDVAAG-SVSPQPLG--   48
Cupriavidus metallidurans            5  -------------LFAFVGADI-------------------------------------G-PWRI--VRAETRVGEPLPEAKRLNVVSA-SELQSETN--   48
Ralstonia pickettii                  5  -------------LFAFVGADI-------------------------------------G-PWRI--VRAETRVGEPLPEAKRLNVVSA-SELQSETN--   48
plasmid pAKD4                        0  ....................................................................................................    0
Janthinobacterium sp. Marseille      5  -------------LFGFVGGDS-------------------------------------G-AWEV--TQMRAVKGAPLPEVKTIAILNGFSIQGHHAH--   49
Sorangium cellulosum                 7  -------------RVSFVAGSA-------------------------------------G-AWRV--ERTVLVRGEELPAAPRLQRVEGGSFVAPPEA--   51

Ca. Nitrospira defluvii              1  .ADREKLLTESGVYGTFATFQMDHDWWDLPGESRVIS------VAEVKGLVEQWSGKILVESYLLRGLSD---------HADLMFRVHARTLSDTQQFLS   84
Azospira oryzae                     11  KIERGTILTQPGVFGVFTMFKLRPDWNKVPAMERKGA------AEEVKKLIEKHKDNVLVDLYLTRGLET---------NSDFFFRINAYDLAKAQTFMR   95
Dechloromonas aromatica             42  KIERGTILTQPGVFGVFTMFKLRPDWNKVPAMERKGA------AEEVKKLIEKHKDNVLVDLYLTRGLET---------NSDFFFRINAYDLAKAQTFMR  126
Dechlorosoma sp. KJ                 42  KIERGTILTQPGVFGVFTMFKLRPDWNKVPAMERKGA------AEEVKKLIEKHKDNVLVDLYLTRGLET---------NSDFFFRINAYDLAKAQTFMR  126
Dechloromarinus chlorophilus         1  ...............................................KKLIEKHKDNVLVDLYLTRGLET---------NSDFFFRINAYDLAKAQTFMR   44
Pseudomonas chloritidismutans        1  ..........................................................LVDLYLTRGLET---------NSDFFFRIHAYDLAKAQTFMR   33
Pseudomonas sp. PK                   1  ...............................................KKLIEKHKDNVLVDLYLTRGLET---------NSDFFFRIHAYDLAKAQTFMR   44
Dechloromonas agitata               40  KI-----LTAPGVFGNFSTYKVRPDYYKLSMAERKGA------AAEVVAVVEKYKDKVKAEAYLTRGFEA---------QSDFFLRIHSYDMAATQAFLV  119
Dechloromonas sp. LT-1               5  ---------------------------------------------------DKHKDKVIVDAYLTRGFEA---------NSDYFLRVHSYDMAATQAFLI   44
Ideonella dechloratans              48  KI-----LSAPGVFVAFSTYKIRPDYFKVALAERKGA------ADEVMAVLEKHKEKVIVDAYLTRGYEA---------KSDYFLRVHAYDAVAAQAFLV  127
Dechlorospirillum sp. WD             6  ----------------------------------------------------KHKDKVIVDTYLTRGLGA---------GSDYLLRVHSTDMAATQAFLV   44
Magnetospirillum magnetotacticum    51  KL-----LTSPGVFGNFSTYKLRSDYYKLSAAERKGA------AAEVMAVVEKHKANIIADAYLTRGFEA---------QSDYFLRVHSMDMAATQAFLV  130

Geobacillus stearothermophilus       6  -------QTLDGWYCLHDFRTIDWSAWKTLPNEEREAAISEFLALVDQWETTESEKQGSHAVYTI--VGQ---------KADILFMILRPTLDELHEIET   87
Listeria monocytogenes               6  -------KTLDGWFCLHDFRSIDWAAWRELNPGNQELMLNELSHFLSDMEITKNIGEGEHTIYSI--LGQ---------KADLVFFTLRDSLEALNEVEN   87
Staphylococcus aureus                6  -------ETLDGWYSLHLFYAVDWASLRIVPKDERDALVTEFQSFLENTATVRSSKSGDQAIYNI--TGQ---------KADLLLWFLRPEMKSLNHIEN   87

Thermus thermophilus                 3  RHVPEPTHTLEGWHVLHDFRLLDFARWFSAPLEAREDAWEELKGLVREWRELEEAGQGSYGIYQV--VGH---------KADLLFLNLRPGLDPLLEAEA   91

Thermoplasma acidophilum             2  ----------TEIYTSVLSYRLLEGKAYSDADTRSLDR----MMRSIDEFFSANPGYINFHIYRS--YRT---------DSDVIFWYSSRNPDLMILAKE   76
Sulfolobus acidocaldarius            2  ----------ANGVYMYVIQAKFNNEWWSTSLQTRRNI----LNRIEELEARSKNDLVALKRFIS--LRY---------DGHLLYWVSDFDTSKLNNLRY   76


Nitrobacter winogradskyi            48  ------------TPWQLRGVASHARYVERAE---KIALT----SVQAGLGRNEATRAALIPIRKSA----AWWEMTQDERRAIFEDKSHHIAASLKYLP-  123
Nitrobacter sp. Nb-311A             47  ------------TPWRLRGVTSHLRYVERAE---KIALT----EVQAGLGRAEATRAALIPIRKSA----AWWELTQDERRKVFEDKSHHIAASLKYLP-  122
Bradyrhizobium japonicum            49  ------------NAWRLAGVPSSLRYTERAE---KQQLV----AVQAGLGRLEATSAALIPIRKSQ----AWWELTQEERRRIFEDRSHHIASSLRFLP-  124
Limnobacter sp. MED105              48  ------------GTWMLQGFTSNVRYAERHE---INQLR----AKQEGLSRPASTCAALIPIKKNA----QWWALSQDERRAIFEAQSHHTEIGLAYLP-  123
Pseudomonas aeruginosa              48  ------------GTWVLQGFTSNVRYAERHE---INQLR----AKQEELNRPTSSCAALIPIKKSP----EWWAMSQEERREIFEAKSHHTEIGLAYLP-  123
Gloeobacter violaceus               48  ------------AAWVLRGLTSNVRYATRPE---VDALR----ERQPALARPEARCAALIPIKKSA----RWWELAQDERRAIFEETSHHTAIGMEFLP-  123
Cyanothece sp. PCC 7425            58  ------------SAWRLQGFASNIRYAIRTE---LEALQ----AVQPMLNRAEAILAVLIPIKKSA----QWWEMAQDERRDIFERESHHTAVGLEYLP-  133
Nitrococcus mobilis                 49  ------------AQWLLRGVTSNERYVTRSE---RAQLT----AKQPVLGRRQATCAAFIPIRKTA----SWWNLAQDERRMILEESSNHIKTGLKYLP-  124
Pseudomonas stutzeri                29  ------------TKWLLRGITSNERYVVREE---KDRLV----AKQPSLGRAEATCAALIPIRKNP----SWWGLSQDERRKIFEEQSRHIHIGLQYLP-  104
Klebsiella pneumoniae               49  ------------TKWLLRGITSNERYVVREE---KDRLV----AKQPSLGRAEATCAALIPIRKNP----SWWGLAQDERRKIFEEQSRHIHIGLQYLP-  124
Cupriavidus metallidurans           49  ------------APWILRGITSNERYVMRAE---KNEIV----AKQQGLARPEATCGALIPIRKNA----AWWELTQDERRSVFE-QSKHVQIGLQYLP-  123
Ralstonia pickettii                 49  ------------APWILRGITSNERYVMRAE---KNEIV----AKQQGLARPEATCGALIPIRKNA----AWWELTQDERRSVFE-QSKHVQIGLQYLP-  123
plasmid pAKD4                        1  ...........................MRAE---KNEIV----AKQQGLARPEATCGALIPIRKNA----AWWELTQDERRSVFE-QSKHVQIGLQYLP-   60
Janthinobacterium sp. Marseille     50  --------------WVLRGVTSNERYVTKEE---KSRLI----ATQEGLGRTESTLAALIPIRKNA----SWWLLTQEERREILEERSHHIQIGMAYLP-  123
Sorangium cellulosum                52  -------------TWVLGGVRSNERYVELEE---RRRLV----AVQEDLGRASSTQAALIPLRKSK----AWWALAQDERRAIFEARSRHIEIGLEYLP-  126

Ca. Nitrospira defluvii             85  AFMGTRLGRHLTSGGLLHGVSKKPTYVAGFP---ESMKT----ELQVNGESGSRPYAIVIPIKKDA----EWWALDQEARTALMQ---EHTQAALPYLK-  169
Azospira oryzae                     96  EFRSTTIGKNADVFETLVGVTKPLNYISKDK---SPGLNA--GLSSATYSGPAPRYVIVIPVKKNA----EWWNMSPEERLKEME---VHTTPTLAYLV-  182
Dechloromonas aromatica            127  EFRSTTVGKNADVFETLVGVTKPLNYISKDK---SPGLNA--GLSSATYSGPAPRYVIVIPVKKNA----EWWNMSPEERLKEME---VHTTPTLAYLV-  213
Dechlorosoma sp. KJ                127  EFRSTTIGKNADVFETLVGVTKPLNYISKDK---SPGLNA--GLSSATYSGPAPRYVIVIPVKKNA----EWWNMSPEERLKEME---VHTTPTLAYLV-  213
Dechloromarinus chlorophilus        45  EFRSTTVGKNADVFETLVGVTKPLNYISKDK---SPELNA--GLSSATYSGPAPRYVIVIPVKKNA----EWWNMSPEERLKEME---VHTTPTLAYLA-  131
Pseudomonas chloritidismutans       34  EFRSTTVGKNADVFETLVGVTKPLNYISKDK---SPELNA--GLTSATYSGPAPRYVIVIPVKKNA----EWWNMSPEERLKEME---VHTTPTLAYLV-  120
Pseudomonas sp. PK                  45  EFRSTTVGKNADVFETLVGVTKPLNYISKDK---SPELNA--GLSSATYSGPAPRYVIVIPVKKNA----EWWNMSPEERLKEME---VHTTPTLAYLV-  131
Dechloromonas agitata              120  DFRATRFGMNAEVTENLVGMTKDLNYITKDK---SPNLNA--GLTGATYRDATPRYAFVIPVKKNA----DWWNLTDEQRLKEME---THTLPTLANLV-  206
Dechloromonas sp. LT-1              45  DFRATRFGMYSDVTEQLLGMTKPLNYITKEK---SPDLNA--GLSSATYSAEAPPYAFVIPVKKSA----EWWNLTDAQRLKEME---THTLPTLGNLV-  131
Ideonella dechloratans             128  DFRATRFGMYSDVTESLVGITKALNYISKDK---SPDLNK--GLSGATYAGDAPRFAFMIPVKKNA----DWWNLTDEQRLKEME---THTLPTLPFLV-  214
Dechlorospirillum sp. WD            45  DWRATKLGMYSDVTENLVGITKALNYISKDK---SPELNA--GLSSATYSDSAPRYVIVIPVKKDA----AWWNMSDEQRLKEIE---VHTHGTLQYLV-  131
Magnetospirillum magnetotacticum   131  DFRATRFGMYSDVTENLVGITKALNYISKDK---SPDLNS--GLSSASYTGDAPRYAIMIPVKKDA----AWWNRSDEERLKEME---VHTQPTLQYLV-  217

Geobacillus stearothermophilus      88  ALNKTKLADYLLPAYSYVSVVELSNYLASGS---EDPYQIPEVRRRLYPILPKTNYICFYPMDKRRQGNDNWYMLSMEQRELMR---AHGMTGRKYAG-  180
Listeria monocytogenes              88  RFNKLAIADYLLPTYSYISVVELSNYLASHMAGGDDPYQNKGVRARLYPALPPKKHICFYPMSKKRDGADNWYMLPMEERQQLIR---DHGLIGRSYAG-  183
Staphylococcus aureus               88  EFNKLRIADFLIPTYSYVSVIELSNYLAGKSD--EDPYENPHIKARLYPELPHSDYICFYPMNKRRNETYNWYMLTMEERQKLMY---DHGMIGRKYAG-  181

Thermus thermophilus                92  RLSRSAFARYLGRSYSFYSVVELGSQEKPLD---PESPY---VKPRLTPRVPKSGYVCFYPMNKRRQGQDNWYMLPAKERASLMK---AHGETGRKYQG-  181

Thermoplasma acidophilum            77  R-VQASMRPIAVSSFSSISIYDESPYNAMNK---KL---------EDSLRLPPLRYFVAYPMSKTP----DWYLLDFDTRKEIMH---EHIKMALNHPDE  156
Sulfolobus acidocaldarius           77  S-LISSGEGFLEEKLTLFSYFKPSPYIGGSAD--KL---------ASYLRLEPLRYFIAYPMKKSP----EWYLLPFEERKEIMD---EHIEIAKTHPDN  157


Nitrobacter winogradskyi           124  -AIARQLYHCRDIG---EPFDFLTWFEYAPEHATMFEDLVGVLRATEEWTY--VEREVDIRLAR-AI..........  183
Nitrobacter sp. Nb-311A            123  -AIARQLYHCRDLG---EPFDFLTWFEYAPEHATMFEDLVGVLRATEEWTY--VEREVDIRLAR-AV..........  182
Bradyrhizobium japonicum           125  -AIARQLYHCRDLG---GPFDFLTWFEFAPAHASLFEELVAMLRRTEEWTY--VEREVDVRVVK-EVLSA.......  187
Limnobacter sp. MED105             124  -EIARQLHHSRDLG---EPFDFLTWFEFAPEHTDAFDELLVKLRTSEEWKY--VEREVDIRLVK-DSL.........  184
Pseudomonas aeruginosa             124  -EIARQLHHSRDLG---EPFDFLTWFEFAPEHTAIFNKLLAQLRSSKEWEY--VEREIDIRLVK-NV..........  183
Gloeobacter violaceus              124  -AVARRLHHCRDIG---EPFDFLTWFEYAPEHTHAFEDLLDRLRVTREWDF--VEREVDIRLEH-IDARI.......  186
Cyanothece sp. PCC 7425            134  -GVARRLLHCRDLG---EEFDFLTWFEFAPEHSSAFNELLLRMRASKEWEY--VEREVEVWLKR-L...........  192
Nitrococcus mobilis                125  -AVARRLHHCRDLGGDAEPFDFLTWFEYAPSDSAAFDELVAELRASQEWTY--VDREIDMRLAR-DE..........  187
Pseudomonas stutzeri               105  -AVARRLHHCRDLG-ENEPFDFLTWFEYSPSDETAFNRLLAELRASVEWQY--VDREIDIRLVH-EPA.........  168
Klebsiella pneumoniae              125  -AVARRLHHCRDLG-ESEPFDFLTWFEYSPSDEPGFNRLLAELRASVEWKY--VDREIDIRLVH-EPA.........  187
Cupriavidus metallidurans          124  -AVARKLHHCRDLS-ENEPFDFLNWFEYAPIHEVEFNRLLSELRASEEWKY--VDREVDIRLTQ-AQV.........  186
Ralstonia pickettii                124  -AVARKLHHCRDLS-ENEPFDFLNWFEYAPIHEVEFNRLLSELRASEEWKY--VDREVDIRLTQ-AQV.........  186
plasmid pAKD4                       61  -AVARKLHHCRDLS-ENEPFDFLNWFEYAPIHEVEFNRLLSELRASEEWKY--VDREVDIRLTQ-AQV.........  123
Janthinobacterium sp. Marseille    124  -AIARRLYHCRDIG-TPEPFDFLTWFEFSPSDASQFDDLVAQLRTTHEWSY--IDHEIDIRLAR-KNG.........  186
Sorangium cellulosum               127  -AVARRLYHSRDLG---GPFDFLTWFEFAEGDAGAFDELVGRLRETEEWSY--VEREIEVRLSR-A...........  185

Ca. Nitrospira defluvii            170  -TVKRKLYHST-GL---DDVDFITYFETE--RLEDFHNLVRALQQVKEFRHNRRFGHPTLLGTM-SPLDEILEKFAQ  238
Azospira oryzae                    183  -NVKRKLYHST-GL---DDTDFITYFETD--DLTAFNNLMLSLAQVKENKFHVRWGSPTTLGTI-HSPEDVIKALAD  251
Dechloromonas aromatica            214  -NVKRKLYHST-GL---DDTDFITYFETD--DLTAFNNLMLSLAQVKENKFHVRWGSPTTLGTI-HSPEDVIKALAD  282
Dechlorosoma sp. KJ                214  -NVKRKLYHST-GL---DDTDFITYFETD--DLTAFNNLMLSLAQVKENKFHVRWGSPTTLGTI-HSPEDVIKALAD  282
Dechloromarinus chlorophilus       132  -NVKRKLYHST-GL---DDT.........................................................  146
Pseudomonas chloritidismutans      121  -NV..........................................................................  122
Pseudomonas sp. PK                 132  -NVKRKLYHST-GL---DDT.........................................................  146
Dechloromonas agitata              207  -NVKRKLYHST-GL---DDTDFITYFETA--DLGAFNNLMLALAKVPENKYHVRWGSPTVLGTI-QSFDSVVNTLSM  275
Dechloromonas sp. LT-1             132  -NVKRKLYHST-GL---DDT.........................................................  146
Ideonella dechloratans             215  -NVKRKLYHST-GL---DDTDFITYFETN--DLGAFNNLMLSLAKVPENKYHVRWGNPTVLGTI-QPIENLVKTLSM  283
Dechlorospirillum sp. WD           132  -NVKRKLYHST-GL---ADA.........................................................  146
Magnetospirillum magnetotacticum   218  -NVKRKLYHST-GL---DDTDFITYFETN--DLGAFNNLLISLAKVPENKHHVRWGSPTVLGTI-QTFETVVKTLSM  286

Geobacillus stearothermophilus     181  -KVTQIITGSV-GL---DDFEWGVTLFSD--DALQFKKLVYEMRFDEVSAR-FGEFGSFFVGTRLPMENVSSFFHV.  248
Listeria monocytogenes             184  -KVQQIIGGSI-GF---DDYEWGVTLFSD--DALEFKRIVTEMRFDEASAR-YAEFGSFFIGNLLLSEQLSKLFTI.  251
Staphylococcus aureus              182  -KIKQFITGSV-GF---DDFEWGVTLFSD--DVLQFKKIVYEMRFDETTAR-YGEFGSFFVGHLINTNEFDQFFAIS  250

Thermus thermophilus               182  -EVMQVISGAQ-GL---DDWEWGVDLFSE--DPVQFKKIVYEMRFDEVSAR-YGEFGPFFVGKY-LDEEALRAFLGL  249

Thermoplasma acidophilum           157  KGIRSYTTYSF-GI---GDQEFVVLYEIP--DIAAWSRVTEKLREARARKW-IIKETPILLGRL-VDAGDIAGFLL.  224
Sulfolobus acidocaldarius          158  QGIRSYTTYSF-GI---ADYEFVVIYEAP--DLSKWINVVERLREAKARKW-VVSEEPILVGE----IGSLDIFLK.  222
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Supplemental Figure S5 Structure-based amino acid sequence alignment of lineage II Clds (first block of 15 sequences), lineage I Clds (next block of 12 sequences), and Cld-like proteins (remaining six sequences). Names printed in red represent proteins whose structures have been determined and which were used to define the shown alignment. Residues that are conserved in all Clds and Cld-like proteins are colored red. The signature residues, which are conserved only in lineage I and II Clds, are shown in green or cyan (the catalytically important arginine). The published lineage I Cld sequences from *D. chlorophilus*, *P*. sp. PK, *D*. sp. LT-1, *D*. sp. WD, and *P. chloritidismutans* are short due to the PCR primers that were used for *cld* gene amplification (1, 2). Therefore, the conservation of residue Glu167 and Arg127 (only *P. chloritidismutans*) (NwCld numbering) cannot be verified for these enzymes. Residues marked in yellow are conserved in most lineage II Clds and are involved in the formation of salt bridges at the dimer interface. Note that one of these residues (Asp134) lacks a homolog in lineage I Clds and the other Cld-like proteins, and probably is vital for dimer formation in lineage II Clds. Please refer to Fig. 2 in the main text for sequence accession numbers.

References

1. **Bender, K. S., M. R. Rice, W. H. Fugate, J. D. Coates, and L. A. Achenbach.** 2004. Metabolic primers for detection of (Per)chlorate-reducing bacteria in the environment and phylogenetic analysis of *cld* gene sequences. Appl. Environ. Microbiol. **70:**5651-5658.
2. **Cladera, A. M., E. Garcia-Valdes, and J. Lalucat.** 2006. Genotype versus phenotype in the circumscription of bacterial species: the case of *Pseudomonas stutzeri* and *Pseudomonas chloritidismutans*. Arch. Microbiol. **184:**353-361.