**Supplementary Information –Tables and Figures**

**Table S1. Redox tower.** Electrochemical potentials of redox couples relevant to extant phototrophy are in bold. Redox couples in red are possible transitional intermediates between anoxygenic phototrophy and oxygenic phototrophy, i.e. substrates with redox potentials >+500 mV. Redox couples in blue are not considered likely transitional intermediates because they require either complex multi-electron transfer reactions (N2+6 H2O->2 NO3-+**10 e-+**12 H+), produce toxic intermediates (NH3/NH2OH, N2O/NO, Cl-/ClO4-, Cl-/ClO3-, Cl-/ClO2-), or have redox potentials greater than YZ/YZ. (N2/N2O, H2O/H2O2, Mn2+/Mn3+). This leaves Mn compounds as the most likely electron donors to drive the evolution of high potential phototrophy.

|  |  |
| --- | --- |
| **Redox Couple (pH7.0)** | **Redox Potential** |
| **P\*/P+ (PSI)** | -1300mV |
| **P\*/P+ (RCI)** | -1200mV |
| **P\*/P+ (RCII)** | -1000mV |
| **P\*/P+ (PSII)** | -700mV |
| **CH2Oorganic/HCO3-** | -482mV |
| **H2/2H+** | -414mV |
| HS-+HSO3-/S2O32- | -410mV |
| Ferredoxin | -390mV |
| NADH/NAD+ | -324mV |
| **Fe2+/Fe3O4 (Magnetite)** | -314mV |
| **Fe2+/a-FeOOH(Goethite)** | -274mV |
| **HS-/S0** | -270mV |
| CH4/CO2 | -240mV |
| **HS-/SO42-** | -217mV |
| **HS-/HSO32-** | -170mV |
| S2-/SO32- | -116mV |
| **Fe2+/b-FeOOH(Lepidocrocite)** | -088mV |
| MQH2/MQ | -075mV |
| **Fe2+/Fe(OH)3 (pH 7)** | +014mV |
| **S2O32-/S4O62-** | +024mV |
| SO32-/S0 | +050mV |
| NH2OH/NO2- | +010mV |
| UQH2/UQ | +113mV |
| **AsO33-/AsO43-** | +139mV |
| DMS/DMSO | +160mV |
| **P/P+ (RCI-*Firmicutes*)** | +225mV |
| **P/P+ (RCI-*Chlorobi*)** | +240mV |
| H2O2/O2 | +270mV |
| NH4+/NO2- | +340mV |
| **P/P+ (RCII-*Chloroflexi*)** | +390mV |
| NO/NO2- | +360mV |
| **NO2-/NO3-** | +430mV |
| SeO33-/SeO43- | +475mV |
| **P/P+** **(PSI-*Cyanobacteria*)** | +480mV |
| **P/P+ (RCII-*Proteobacteria*)** | +505mV |
| Mn2+(HCO3-)2/ Mn3+(HCO3-)2+ | +520mV |
| Cl-/ClO4- | +560mV |
| Mn2+(OH-)+/Mn3+(OH-)2+ | +600mV |
| Cl-/ClO3- | +630mV |
| 0.5N2/NO3- | +713mV |
| Fe2+/Fe3+ (pH 2) | +760mV |
| Cl-/ClO2- | +780mV |
| Mn2+/Mn4+ | +798mV |
| **YD/YD.** | +800mV |
| **H2O/0.5 O2** | +810mV |
| **Y/Y.** | +850mV |
| Cl-/ClO4- | +873mV |
| NH3/NH2OH | +740mV |
| Mn2+(HCO3-)+/ Mn3+(HCO3-)2+ | +920mV |
| N2O/NO | +1175mV |
| **YZ/YZ.** | +1200mV |
| **P/P+** **(PSII-*Cyanobacteria*)** | +1250mV |
| Mn2+/Mn3+ | +1300mV |
| H2O/H2O2 | +1350mV |
| N2/N2O | +1355mV |

**Table S2.** Identification of genes associated with pathways shown in Fig. S2.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Electron shuttle** | **Thiocapsa marina DSM 5653** | **Thiocapsa KS1** | **Thiocapsa KS1 Genes** |
| **Respiration** |  |  |  |  |
| nuoABCDEFGHIJKLMN - Complex I | NAD,  quinol | 1 | 1 | THIOKS1v1\_1320048,  THIOKS1v1\_1320049,  THIOKS1v1\_1320050,  THIOKS1v1\_1320051,  THIOKS1v1\_1320052,  THIOKS1v1\_1320053,  THIOKS1v1\_1320054,  THIOKS1v1\_1320055,  THIOKS1v1\_1320056,  THIOKS1v1\_1320057,  THIOKS1v1\_1320058,  THIOKS1v1\_1320059,  THIOKS1v1\_1320060,  THIOKS1v1\_1320061 |
| RnfABCDEG – RNF complex | Ferredoxin, NAD | 2 | 2 | THIOKS1v1\_2020007,  THIOKS1v1\_2020008,  THIOKS1v1\_2020009,  THIOKS1v1\_2020010/  THIOKS1v1\_2030001,  THIOKS1v1\_2030002,  THIOKS1v1\_2030003,  THIOKS1v1\_2030004,  THIOKS1v1\_2030005;  THIOKS1v1\_2340046,  THIOKS1v1\_2340047,  THIOKS1v1\_2340048,  THIOKS1v1\_2340049,  THIOKS1v1\_2340050,  THIOKS1v1\_2340051,  THIOKS1v1\_2340052 |
| POR containing RNF complex | Ferredoxin, NADH | 1 | 1 | THIOKS1v1\_570037,  THIOKS1v1\_570038/  THIOKS1v1\_580001,  THIOKS1v1\_580002,  THIOKS1v1\_580003,  THIOKS1v1\_580004,  THIOKS1v1\_580005,  THIOKS1v1\_580006,  THIOKS1v1\_580007,  THIOKS1v1\_580008 |
| SdhABC(D) – Complex II | quinol | 2 | 2 | THIOKS1v1\_2330078,  THIOKS1v1\_2330079,  THIOKS1v1\_2330080;  THIOKS1v1\_2760004,  THIOKS1v1\_2760005,  THIOKS1v1\_2760006,  THIOKS1v1\_2760007 |
| PetABC - Complex III | Quinol,  cytochrome c | 1 | 1 | THIOKS1v1\_1900015,  THIOKS1v1\_1900016,  THIOKS1v1\_1900017 |
| CcoNOQP - C-family oxygen reductase | cytochrome c | 1 | 1 | THIOKS1v1\_1390007,  THIOKS1v1\_1390008,  THIOKS1v1\_1390009,  THIOKS1v1\_1390010 |
| CcoNO - C-family oxygen reductase | cytochrome c | 0 | 1 | THIOKS1v1\_2000071,  THIOKS1v1\_2000072 |
| cydAB - bd oxidase | quinol | 1 | 1 | THIOKS1v1\_2540052/  THIOKS1v1\_2550001,  THIOKS1v1\_2550002 |
| F1Fo-type ATP synthase – Complex V |  | 1 | 1 | THIOKS1v1\_2240016,  THIOKS1v1\_2240017,  THIOKS1v1\_2240018,  THIOKS1v1\_2240019,  THIOKS1v1\_2240020,  THIOKS1v1\_2240022,  THIOKS1v1\_2240023,  THIOKS1v1\_2240024,  THIOKS1v1\_2240025 |
| V1Vo-type ATP synthase |  | 3 | 3 | THIOKS1v1\_1240012,  THIOKS1v1\_1240013,  THIOKS1v1\_1240014,  THIOKS1v1\_1240015,  THIOKS1v1\_1240016,  THIOKS1v1\_1240017,  THIOKS1v1\_1240018;  THIOKS1v1\_1320029;  THIOKS1v1\_2960011,  THIOKS1v1\_2960012,  THIOKS1v1\_2960013,  THIOKS1v1\_2960014,  THIOKS1v1\_2960015,  THIOKS1v1\_2960016,  THIOKS1v1\_2960017,  THIOKS1v1\_2960019,  THIOKS1v1\_2960020;  THIOKS1v1\_2970002,  THIOKS1v1\_2970003,  THIOKS1v1\_2970004,  THIOKS1v1\_2970005,  THIOKS1v1\_2970006,  THIOKS1v1\_2970007,  THIOKS1v1\_2970008,  THIOKS1v1\_2970009 |
|  |  |  |  |  |
| **Phototrophy** |  |  |  |  |
| PufLMC - RCII with tetraheme cytochrome c | cytochrome c | 1 | 1 | THIOKS1v1\_2370041,  THIOKS1v1\_2370042,  THIOKS1v1\_2370043 |
| PufC2 | cytochrome c | 1 | 1 | THIOKS1v1\_40013,  THIOKS1v1\_50001 |
| PuhA |  | 1 | 1 | THIOKS1v1\_2370019 |
| PufBA - Light-harvesting complex |  | 3 | 3 | THIOKS1v1\_2370039,  THIOKS1v1\_2370040;  THIOKS1v1\_2370044,  THIOKS1v1\_2370046;  THIOKS1v1\_2370047,  THIOKS1v1\_2370048 |
| PucBA - Light-harvesting complex |  | 2 | 2 | THIOKS1v1\_2880001,  THIOKS1v1\_2880002;  THIOKS1v1\_2880003,  THIOKS1v1\_2880004 |
| cyt. c4-like diheme protein |  | 8 | 9 | THIOKS1v1\_910004,  THIOKS1v1\_1320036,  THIOKS1v1\_1320037,  THIOKS1v1\_2100054,  THIOKS1v1\_2320016,  THIOKS1v1\_2320017,  THIOKS1v1\_2340026,  THIOKS1v1\_2540031,  THIOKS1v1\_2610004 |
| HiPIP |  | 2 | 2 | THIOKS1v1\_40012;  THIOKS1v1\_2530035 |
|  |  |  |  |  |
| **Sulfur** |  |  |  |  |
| FccAB - Hydrogen sulfide oxidation to sulfur | cytochrome c | 2 | 2 | THIOKS1v1\_1060007,  THIOKS1v1\_1060009;  THIOKS1v1\_2860008,  THIOKS1v1\_2860009 |
| SQR - Hydrogen sulfide oxidation to sulfur | quinol | 1 | 1 | THIOKS1v1\_2830013 |
| DsrABEFHCMKLJOPNRS - Hydrogen sulfide reduction to sulfite | quinol | 1 | 1 | THIOKS1v1\_2910007,  THIOKS1v1\_2910008,  THIOKS1v1\_2910009,  THIOKS1v1\_2910010,  THIOKS1v1\_2910011,  THIOKS1v1\_2910012,  THIOKS1v1\_2910013,  THIOKS1v1\_2910014,  THIOKS1v1\_2910016,  THIOKS1v1\_2910017,  THIOKS1v1\_2910018, THIOKS1v1\_2910019,  THIOKS1v1\_2910020,  THIOKS1v1\_2910021,  THIOKS1v1\_2910022,  THIOKS1v1\_2910023 |
| rhd-tusA-dsrE2 |  | 1 | 1 | THIOKS1v1\_1130017,  THIOKS1v1\_1130018,  THIOKS1v1\_1130019 |
| AprABM/ AprAB-QmoAB-HDRBC - Sulfite oxidation to sulfate | quinol | 1 (AprABM) | 1 (AprAB-QmoAB-HdrBC) | THIOKS1v1\_1970002,  THIOKS1v1\_1970003,  THIOKS1v1\_1840023,  THIOKS1v1\_1840024,  THIOKS1v1\_1840026,  THIOKS1v1\_1840027 |
| SoeABC – Sulfite oxidation to sulfate | quinol | 1 | 1 | THIOKS1v1\_2550011,  THIOKS1v1\_2550012,  THIOKS1v1\_2550013 |
| SoxBXAKL - Thiosulfate reduction to sulfate | cytochrome c | 1 | 1 | THIOKS1v1\_620009,  THIOKS1v1\_620011,  THIOKS1v1\_620012,  THIOKS1v1\_620013,  THIOKS1v1\_620014 |
| SoxYZ - Thiosulfate reduction to sulfur | cytochrome c | 1 | 1 | THIOKS1v1\_1770012,  THIOKS1v1\_1770013 |
| Sat - ATP sulphurylase |  | 1 | 1 | THIOKS1v1\_630011 |
|  |  |  |  |  |
| **Nitrogen** |  |  |  |  |
| NxrABDC - Nitrite oxidation to nitrate | cytochrome c | 0 | 1 | THIOKS1v1\_910010,  THIOKS1v1\_910011,  THIOKS1v1\_910012,  THIOKS1v1\_910013 |
| NifHDK – Nitrogen fixation |  | 1 | 1 | THIOKS1v1\_1660002,  THIOKS1v1\_1660003,  THIOKS1v1\_1660004 |
| NapDAGHB - Nitrate reduction to nitrite | quinol | 1 | 1 | THIOKS1v1\_1600004,  THIOKS1v1\_1600005,  THIOKS1v1\_1600006,  THIOKS1v1\_1600007,  THIOKS1v1\_1600008 |
| NarB – Nitrate reduction to nitrite |  | 1 | 1 | THIOKS1v1\_770003 |
| OTR-like octaheme cytochrome c | quinol | 2 | 2 | THIOKS1v1\_2130021;  THIOKS1v1\_2840006 |
| HAO-like octaheme cyt. *c* | cytochrome c | 2 | 2 | THIOKS1v1\_1350025;  THIOKS1v1\_2860012/  THIOKS1v1\_2860013 |
| NorB - Nitric oxide reduction to nitrous oxide | cytochrome c | 1 | 1 | THIOKS1v1\_1610004 |
| NosZ - Nitrous oxide reduction to nitrogen | cytochrome c | 1 | 1 | THIOKS1v1\_2340029 |
|  |  |  |  |  |
| **Hydrogenases** |  |  |  |  |
| HynSL – membrane bound Ni-Fe Hydrogenase | quinol | 1 | 1 | THIOKS1v1\_1330012,  THIOKS1v1\_1330013,  THIOKS1v1\_1330014,  THIOKS1v1\_1330015 |
| HupSLC – membrane bound Ni-Fe Hydrogenase | quinol | 1 | 1 | THIOKS1v1\_2830021,  THIOKS1v1\_2830022,  THIOKS1v1\_2830023 |
| HyqBCEFGI – membrane bound Ni-Fe Hydrogenase | quinol | 1 | 1 | THIOKS1v1\_2480030/  THIOKS1v1\_2490001,  THIOKS1v1\_2490002,  THIOKS1v1\_2490003,  THIOKS1v1\_2490005,  THIOKS1v1\_2490004/  THIOKS1v1\_2500001,  THIOKS1v1\_2500003 |
| HoxEFUYHI - Ni-Fe Hydrogenase | NAD | 1 | 1 | THIOKS1v1\_1730040/  THIOKS1v1\_1740001,  THIOKS1v1\_1740002,  THIOKS1v1\_1740003,  THIOKS1v1\_1740004 |
| HoxFUYH - Ni-Fe Hydrogenase | NAD | 1 | 1 | THIOKS1v1\_2350023,  THIOKS1v1\_2350025,  THIOKS1v1\_2350026,  THIOKS1v1\_2350027,  THIOKS1v1\_2350028,  THIOKS1v1\_2350029 |
| HydBGDA - Ni-Fe Sulfhydrogenase | NAD | 0 | 1 | THIOKS1v1\_1570008,  THIOKS1v1\_1570009,  THIOKS1v1\_1570010,  THIOKS1v1\_1570011 |

**Table S3.** **Nitrate-reducing enzyme acitivities in different cell fractions.** *Thiocapsa*KS1 cultures were analyzed after growth on different combinations of electron donors and nitrogen sources.

|  |  |  |  |
| --- | --- | --- | --- |
| **Electron donor** | **N-sourcea** | **Cell fraction** | **Specific activityb**  **(mU∙(mg protein)-1)** |
| NO2- | 5 mM NH4+ | Cell-free extract | 200 to 1,400 |
| NO2- | 5 mM NH4+ | Cytosolic | 300 to 700 |
| NO2- | 5 mM NH4+ | Membrane | 700 to 1,700 |
| H2 | 5 mM NH4+ | Cell-free extract | 0c |
| H2 | 5 mM NH4+ | Cytosolic | 0c |
| H2 | 5 mM NH4+ | Membrane | 0c |
| Fructose | 2 mM NO3- | Cell-free extract | 0c to 300 |
| Fructose | 2 mM NO3- | Cytosolic | 0c to 200 |
| Fructose | 2 mM NO3- | Membrane | 0c |

a Ammonium was added for assimilation to support cell growth. In the fructose incubations nitrate was added to test if the nitrite-oxidizing enzyme system is also expressed under conditions requiring assimilatory nitrate reduction to ammonium.

b Even when grown under nitrite-oxidizing conditions only nitrate-reducing activity was measured in cell-free extracts. For details see main text.

c Since the enzyme test system had a high background reaction of up to 300 mU, measured values below 100 mU were regarded as no enzyme activity.

**Table S4.** **Conservation of residues interacting with the special pair in RCII.** The table shows the conservation of residues that interact with the special pair in RCII. Residue number (based on the *Rhodobacter sphaeroides* sequence), function, and conservation are shown. Residues with point mutations with different chemical properties are highlighted in blue. Strains with measured mid-point potentials are shown. *Thiocapsa* KS1 and *Thiocapsa marina* DSM 5653 share the exact same residues interacting with the special pair as *Thermochromatium tepidum*, which has a mid-point potential of 500 mV. In *Chloroflexi* L168H is replaced with a tyrosine or phenylalanine, mutations known to decrease the redox potential of the special pair by ~95 mV(Lin et al., 1994), in agreement with their measured values.

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**Table S5. Sequenced genomes with RCII.** All *Proteobacteria* and *Gemmatimonadetes* RCII complexes contain a subunit H (PuhA) that is missing in *Chloroflexi*. The majority of organisms with a RCII utilize a tetra-heme cytochrome *c* protein (PufC) that acts as an electron wire between the soluble electron carrier and the special pair in RCII. A subset of *Alphaproteobacteria* are missing PufC and instead receive electrons directly from the soluble electron carrier.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Genome** | **Phylum** | **PufL** | **PufM** | **PufC** | **PuhA** |
| Acidiphilium angustum ATCC 35903 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Acidiphilium multivorum AIU301 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Acidiphilium sp. JA12-A1 (ACIDI) | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Acidiphilium sp. PM, DSM 24941 | Alphaproteobacteria | 1 | 1 | 1 |  |
| Afifella pfennigii DSM 17143 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Agrobacterium albertimagni AOL15 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Ahrensia sp. R2A130 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Alphaproteobacterium SCGC AAA298-K06 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Bacteroidales bacterium PSC KfJoeBact2-2 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Bacteroidales bacterium PSC KfJoeBact2-3 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Belnapia moabensis DSM 16746 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Blastomonas sp. AAP53 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Bradyrhizobium sp. BTAi1 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Bradyrhizobium sp. ORS 375 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Bradyrhizobium sp. ORS278 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Bradyrhizobium sp. ORS285 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Bradyrhizobium sp. S23321 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Brevundimonas bacteroides DSM 4726 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Brevundimonas subvibrioides ATCC 15264 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Caenispirillum salinarum AK4 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Caulobacteraceae sp. PMMR1 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Citromicrobium bathyomarinum JL354 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Citromicrobium bathyomarinum JL354 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Dinoroseobacter shibae DFL-12, DSM 16493 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Elioraea tepidiphila DSM 17972 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Erythrobacter litoralis DSM 8509 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Erythrobacter longus DSM 6997 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Erythrobacter sp. JL475 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Erythrobacter sp. NAP1 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Fulvimarina pelagi HTCC2506 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Hoeflea phototrophica DFL-43 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Hyphomicrobium zavarzinii ATCC 27496 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Jannaschia sp. CCS1 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Labrenzia alexandrii DFL-11 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Loktanella sp. SE62 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Loktanella vestfoldensis DSM 16212 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Loktanella vestfoldensis SKA53 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Mesorhizobium loti R88b | Alphaproteobacteria | 1 | 1 |  | 1 |
| Methylobacterium brachiatum 111MFTsu3.1M4 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium chloromethanicum CM4 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium extorquens AM1 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium extorquens DM4 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium extorquens DSM 13060 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium extorquens PA1 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium mesophilicum SR1.6/6 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium populi BJ001 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium radiotolerans JCM 2831 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. 10 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. 13MFTsu3.1M2 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. 174MFSha1.1 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. 275MFSha3.1 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. 285MFTsu5.1 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. 4-46 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. 77 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. 88A | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. B1 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. B34 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. EUR3 AL-11 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. GXF4 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. L2-4 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. MB200 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. UNC300MFChir4.1 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. UNC300MFChir4.1 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. UNC300MFChir4.1 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. UNC378MF | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. UNCCL110 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylobacterium sp. WSM2598 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylocella silvestris BL2, DSM 15510 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylocystis rosea SV97T | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Methylocystis sp. SB2 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Novosphingobium acidiphilum DSM 19966 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Novosphingobium sp. B-7 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Oceanibaculum indicum P24 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Oceanicola sp. HL-35 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Phaeospirillum fulvum MGU-K5 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Phaeospirillum molischianum DSM 120 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Planktomarina temperata RCA23, DSM 22400 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Porphyrobacter cryptus DSM 12079 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Porphyrobacter sp. AAP82 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Porphyrobacter sp. HL-46 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Prosthecomicrobium hirschii ATCC 27832 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Rhodobacter capsulatus B6 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter capsulatus DE442 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter capsulatus R121 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter capsulatus SB1003 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter capsulatus Y262 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter capsulatus YW1 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter capsulatus YW2 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter sp. AKP1 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter sp. SW2 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter sphaeroides 2.4.1 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter sphaeroides 2.4.1, ATCC BAA-808 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter sphaeroides ATCC 17025 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter sphaeroides ATCC 17029 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter sphaeroides KD131 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacter sphaeroides WS8N | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodobacteraceae sp. HIMB11 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Rhodobacterales sp. HTCC2083 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodocista centenaria SW | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Rhodomicrobium udaipurense JA643 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Rhodomicrobium vannielii ATCC 17100 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Rhodopseudomonas palustris 0001L | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris 1a1 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris 420L | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris 7850, DSM 127 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris AP1 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris ATH 2.1.37, ATCC 17007 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris ATH 2.1.6, ATCC 17001 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris ATH 2.1.6, NCIB 8288 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris BIS3 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris BisA53 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris BisB18 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris BisB5 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris CEA001 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris CGA009 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris DCP3 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris DX-1 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris HaA2 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris JA1, ATCC BAA-37 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris JSC-3b | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris KD1 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris No7 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris O.U.11, DSM 7375 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris P4 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris Pfennig 1850, DSM 126 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris R1, DSM 8283 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris RCH350 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris RCH500 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris RSP24 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris S-1, DSM 131 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris S55 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris TIE-1 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas palustris WS17 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodopseudomonas sp. B29 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodospirillum photometricum DSM 122 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Rhodospirillum rubrum F11 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodospirillum rubrum S1, ATCC 11170 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Rhodovibrio salinarum DSM 9154 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Rhodovulum sp. PH10 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Roseivivax halodurans JCM 10272 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Roseivivax sp. 22II-s10s | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Roseobacter denitrificans OCh 114 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Roseobacter litoralis Och 149 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Roseobacter sp. AzwK-3b | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Roseobacter sp. CCS2 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Roseobacter sp. LE17 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Roseovarius sp. 217 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Roseovarius sp. TM1035 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Rubritepida flocculans DSM 14296 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Rubrivivax benzoatilyticus ATCC BAA-35 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Salinarimonas rosea DSM 21201 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Salipiger mucosus DSM 16094 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Sandarakinorhabdus limnophila DSM 17366 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Sandarakinorhabdus sp. AAP62 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Skermanella stibiiresistens SB22 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Sphingomonas astaxanthinifaciens DSM 22298 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Sphingomonas echinoides ATCC 14820 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Sphingomonas jaspsi DSM 18422 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Sphingomonas sanxanigenens NX02, DSM 19645 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Sphingomonas sp. PAMC 26605 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Sphingomonas sp. PAMC 26617 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Sphingomonas sp. PAMC 26621 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Stappia stellulata DSM 5886 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Sulfitobacter guttiformis KCTC 32187 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Sulfitobacter sp. NB-77 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Thalassiobium sp. R2A62 | Alphaproteobacteria | 1 | 1 |  | 1 |
| Thalassobaculum salexigens DSM 19539 | Alphaproteobacteria | 1 | 1 | 1 | 1 |
| Limnohabitans sp. Rim28 | Betaproteobacteria | 1 | 1 | 1 | 1 |
| Limnohabitans sp. Rim47 | Betaproteobacteria | 1 | 1 | 1 | 1 |
| Methyloversatilis sp. FAM1 | Betaproteobacteria | 1 | 1 | 1 | 1 |
| Methyloversatilis sp. NVD | Betaproteobacteria | 1 | 1 | 1 | 1 |
| Methyloversatilis sp. RZ18-153 | Betaproteobacteria | 1 | 1 | 1 | 1 |
| Methyloversatilis universalis FAM5 | Betaproteobacteria | 1 | 1 | 1 | 1 |
| Methyloversatilis universalis Fam500 | Betaproteobacteria | 1 | 1 | 1 | 1 |
| Polynucleobacter necessarius asymbioticus MWH-MoK4 | Betaproteobacteria | 1 | 1 | 1 | 1 |
| Rhodocyclaceae bacterium RZ94 | Betaproteobacteria | 1 | 1 | 1 | 1 |
| Rubrivivax benzoatilyticus JA2 | Betaproteobacteria | 1 | 1 | 1 | 1 |
| Rubrivivax gelatinosus CBS | Betaproteobacteria | 1 | 1 | 1 | 1 |
| Rubrivivax gelatinosus IL144 | Betaproteobacteria | 1 | 1 | 1 | 1 |
| Rubrivivax gelatinosus S1 | Betaproteobacteria | 1 | 1 | 1 | 1 |
| Allochromatium vinosum DSM 180 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Congregibacter litoralis KT71 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Ectothiorhodospira haloalkaliphila ATCC 51935 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Ectothiorhodospira sp. PHS-1 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Gammaproteobacterium sp. NOR5-3 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Gammaproteobacterium sp. NOR51-B | Gammaproteobacteria | 1 | 1 | 1 |  |
| Gammaproteobacterium sp. HIMB55 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Haliea rubra CM41\_15a, DSM 19751 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Halorhodospira halochloris A | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Halorhodospira halophila SL1 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Lamprocystis purpurea DSM 4197 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Marichromatium purpuratum 984 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Marine gammaproteobacterium sp. HTCC2080 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Nevskia ramosa DSM 11499 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| **Thiocapsa sp. KS1** | Gammaproteobacteria | 1 | 1 | 2 | 1 |
| Thiocapsa marina 5811, DSM 5653 | Gammaproteobacteria | 1 | 1 | 2 | 1 |
| Thiocystis violascens 611, DSM 198 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Thioflavicoccus mobilis 8321 | Gammaproteobacteria | 1 | 1 | 1 | 2 |
| Thiorhodococcus drewsii AZ1 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Thiorhodococcus sp. AK35 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Thiorhodospira sibirica A12, ATCC 700588 | Gammaproteobacteria | 1 | 1 | 1 | 1 |
| Thiorhodovibrio sp. 970 | Gammaproteobacteria | 1 | 1 | 2 | 1 |
| Anaerolinea YNP | Chloroflexi | 1 | 1 | 1 |  |
| Candidatus Chlorothrix halophila | Chloroflexi | 1 | 1 | 1 |  |
| Chloroflexus aggregans DSM 9485 | Chloroflexi | 1 | 1 | 1 |  |
| Chloroflexus aurantiacus J-10-fl | Chloroflexi | 1 | 1 | 1 |  |
| Chloroflexus sp. MS-G | Chloroflexi | 1 | 1 | 1 |  |
| Chloroflexus sp. Y-396-1 | Chloroflexi | 1 | 1 | 1 |  |
| Chloroflexus sp. Y-400-fl | Chloroflexi | 1 | 1 | 1 |  |
| Kouleothrix aurantiaa JCM 19913 | Chloroflexi | 1 | 1 | 1 |  |
| Oscillochloris trichoides DG6 | Chloroflexi | 1 | 1 | 1 |  |
| Roseiflexus castenholzii HLO8, DSM 13941 | Chloroflexi | 1 | 1 | 1 |  |
| Roseiflexus sp. RS-1 | Chloroflexi | 1 | 1 | 1 |  |
| Gemmatimonas sp. AP64 | Gemmatimonadetes | 1 | 1 | 2 | 1 |

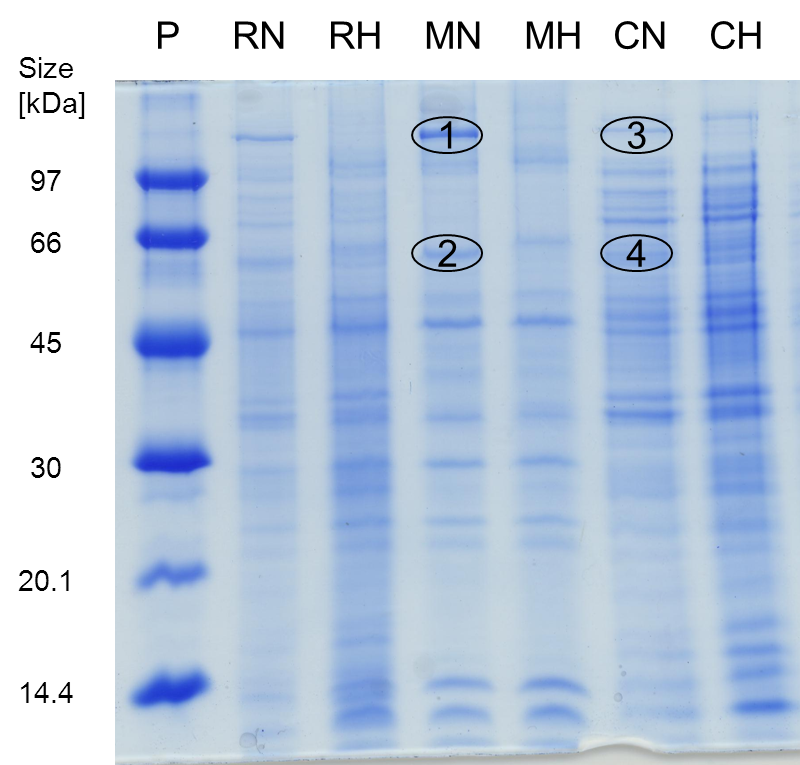
**Figure S1. Redox tower.** The electrochemical potential difference of the ground state (P) vs excited state (P\*) of different reaction centers are shown as arrows (RCI-green, RCII-pink, PSI and PSII-yellow). The lengths of the arrows are proportional to the energies of the excitons absorbed by the reaction centers. Anoxygenic reaction centers utilizing bacteriochlorophylls (RCI, RCII) exhibit smaller potential differences than the chlorophyll containing reaction centers (PI and PSII) of *Cyanobacteria*. The known inorganic electron donors for phototrophy are shown color-coded with their reaction center types. Most reaction centers (RCI, RCII, and PSI) have special pair redox potentials (P/P+) that are <+500 mV and would be unable to oxidize high-potential electron donors such as H2O. The highest potential electron donor for extant, known anoxygenic phototrophs is nitrite (+430 mV).



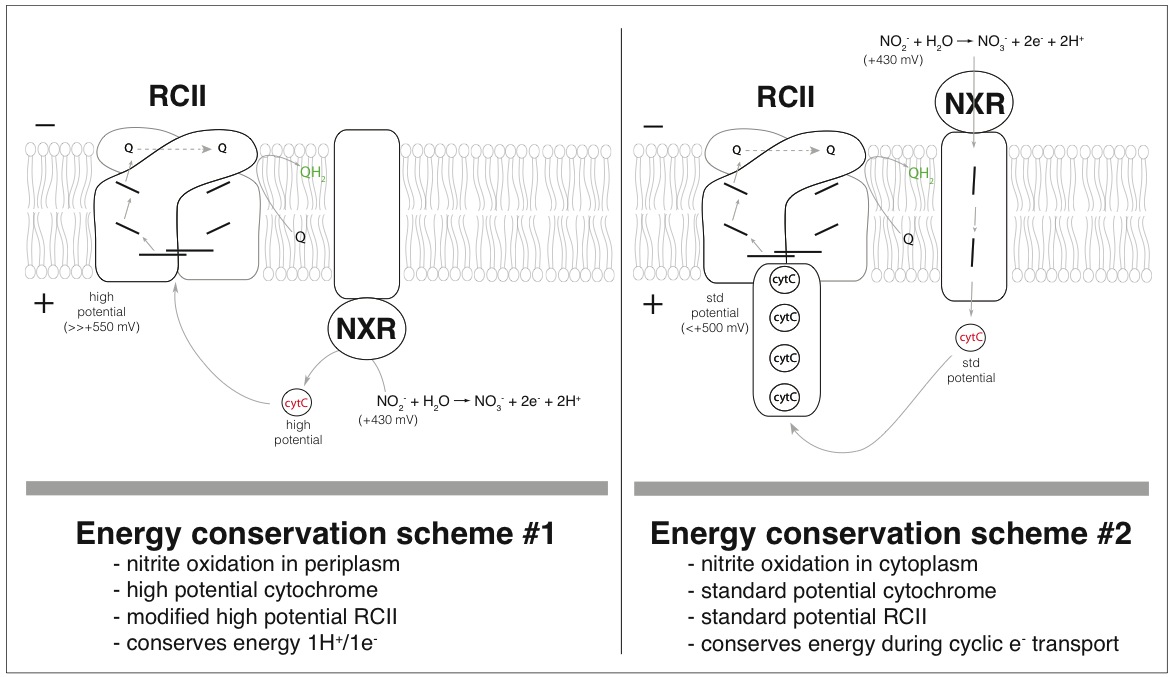
**Fig S2. Energy costs of reverse electron transport in *Thiocapsa*KS1.** *Thiocapsa*KS1 can grow photolithoautotrophically using the Calvin cycle. To make the NADH required for carbon fixation it uses reverse electron transport to transfer electrons from higher potential electron donors (H2S, S0, S2O32−, and NO2-) to the lower potential NAD+ (-320 mV). This is accomplished by reversing complex I (red pathway), utilizing ubiquinols reduced by RCII, and energy from the membrane electrochemical gradient (NAD+ + QH2 + 4H+out → NADH + H+ + Q + 4H+in). In contrast, reverse electron transport in chemolithoautotrophic metabolisms (ex. nitrite oxidation and acidophilic iron oxidation) must also run complex III in reverse to generate NADH (black pathway).



**Fig. S3.** One-dimensional SDS-PAGE analysis of proteins in cell fractions from *Thiocapsa*KS1 grown phototrophically on nitrite or hydrogen as sole electron donor. 2 mM ammonia served as nitrogen source. Each line was loaded with ~5.5 µg of protein. P = peptide marker, R = cell-free extract, M = membrane fraction, C = cytosolic fraction, N = nitrite-grown cells, H = hydrogen-grown cells. Peptide mass fingerprints were obtained for bands 1 to 4. Bands 1 and 3: NxrA (nitrite oxidoreductase α subunit); bands 2 and 4: NxrB (nitrite oxidoreductase β subunit).



**Figure S4. Bioenergetics of nitrite oxidation in *Thiocapsa*KS1.** There are two possible ways to oxidize nitrite in *Thiocapsa*KS1: 1) use a high redox potential reaction center capable of oxidizing nitrite *via* a high-potential electron carrier, or 2) transfer electrons from nitrite into the shared low-potential electron carrier pool, possibly with consumption of energy from the membrane electrochemical gradient. Genomic and biochemical results show that *Thiocapsa* KS1 employs the latter strategy.



**Fig. S5.** **Phylogenetic analyses of *Thiocapsa* KS1 NXR and related enzymes of the DMSO reductase type II family.** (A) Bayesian inference tree (SD=0.009998) of the large (NxrA) subunit. In total, 1,745 alignment positions were considered. (B) Bayesian inference tree (SD=0.009885) of the small (NxrB) subunit, obtained by using 697 alignment positions. Names of validated enzymes are indicated (Clr, chlorate reductase; Ddh, dimethylsulfide dehydrogenase; Ebd, ethylbenzene dehydrogenase; NAR, Nitrate reductase; NXR, nitrite oxidoreductase; Pcr, perchlorate reductase; Ser, selenatereductase). Filled and open circles indicate Bayesian inference statistical support larger 90 and 70%, respectively. The arrow indicates the outgroup; the scale bar represents 10% estimated sequence divergence. NXRs with the active center located on the cytoplasmic side of the membrane are indicated in red (*Nitrobacter*-type) and blue (*Thiocapsa* KS1), NXRs oriented towards the periplasm in green. Note the three independent origins of NXR.





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