

Fig. S1 Representative NirS alignment. Yellow boxes represent >70% conserved residues and red boxes represent identical residues. Numbered red boxes mark characteristic conserved motifs (I, III, IV, V, VII and IV distinguish NirS from NirN and NirF) and blue boxes mark functionally important residues, and asterisks beneath alignment mark specific residues where nirS can be differentiated from NirF and NirN. Notably, motif I on the *cyt_c* domain was generally characterised by -Ca**G**CHg- in NirS, motif VI by -LHD- (vs. -PyD- in NirF and -LDD- in NirN), and motif VII at the *cyt_{d1}* domain is identified by the universal absence of a proline in the third position in NirN and NirF (i.e. -PHpGpG- vs. PH!PgeG). Clade 1c: *Hydrogenophilales sp.*; clade 3: *Sulfurimonas paralvinellae*; clade 4: *Calidifontibacillus erzurumensis*; clade 1d: *Brocadia sp. WS118*; clade 1e: *c. Magnetaquicoccus inordinatus*; clade 1a: *Stutzerimonas stutzeri*; clade 1f: *Deltaproteobacteria bact. GWA2_43_19*; clade 5: *Levilinea saccharolytica*; clade 1h: *Scalindua brodae*; clade 1j: *Thermus oshimai*; clade 1b: *Thauera linaloolentis*; clade 2: *Methylomicrobium album*; clade 6: *candidatus Methylomirabilis oxyfera*; clade 1g: *Roseiflexus castenholzii*; clade 1k: *Colwellia psychrerythraea*; halophilic archaea NirS-like: *Natrinema pellirubrum*. The Fig. was prepared in ESPscript/ENDscript.