**Figure S1** Sequence alignment of three-domain heme-c copper nitrite reductases (Heme-CuNiR). Asp97, His240 and Tyr323 are highly conserved (highlighted in green). RpNiR (*Ralstonia pickettii* Uniprot: B2UHR8); PosNiR (*Pontibacter sp.* Uniprot: J0V342), TpNiR (*Turneriella parva* Uniprot: I4B638), BbNiR (*Bdellovibrio bacteriovorus* Uniprot: A0A024F3V3), KiNiR (*Kangiella koreensis* Uniprot: C7R6X8), CaaNiR (*Catenovolum agarivorans* Uniprot: W7QS8), HfNiR (*Herbaspirillum frisingense* Uniprot: R0GAR4), OsNiR (*Oceanimonas sp.* Uniprot: H2FX5), ClaNiR (*Glaciecola arctica* Uniprot: K6XK57), BtNiR (*Burkholderia thailandensis* Uniprot: W6BZ5), DjNiR (*Dyella jiangningensis* Uniprot: A0A023NMD0), PhNiR (*Pseudoalteromonas haloplanktis* Uniprot: Q3IG7), RdNiR (*Rhodanobacter denitrificans* Uniprot: D5JAK6) and PsysNiR (*Psychrobacter sp.* Uniprot: F5SNG7). Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and ESPript1 (http://escrip1.ibcp.fr) online server were used.

**Figure S2** Superposition of as isolated wt-RpNiR (white), NO bound wt-RpNiR (purple) and PhNiR (PDBID: 2ZOO) (orange) structures. The linker loop of PhNiR (Thr305 - Asn311) is closer to ligand bound wt-RpNiR conformation with Tyr313 (equivalent to Tyr323 of wt-RpNiR) is ready to open the channel without loop rearrangement. The coordination to T2Cu and hydrogen bonds are shown as dashed yellow and black lines, respectively.
Figure S3  Structural comparison of T2Cu site in resting state wt-RpNiR and wt-RpNiR-NO with AxNiR (AxNiR). Superposed structures of (a) resting state wt-RpNiR, (b) wt-RpNiR-NO and (c) NO removed wt-RpNiR-NO on the AxNiR (PDB ID: 5ONY). The structures of RpNiR with T2Cu (blue sphere) and AxNiR with T2Cu (orange sphere) are shown by white and orange sticks, respectively. The coordination to T2Cu and hydrogen bonds are shown as dashed yellow and black lines, respectively. Water molecules bound to Rp structure and Ax structure are shown as red and small orange spheres, respectively.