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**Supporting information for article:**

**Identification of a tyrosine switch in copper-haem nitrite reductases**

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*RpN1R* 1 .....MSRLHTVRSLMRAGLISVVLG.....ALLAGTAAARASAKLPDGFPPRGPPIHMLLSPDPVPPVPHRNYPAKVLV  
*RpN1R* 1 .....MVGIFCIIGIASSGKTKTNAELINSLNETATPGEMPIVIELLDAPVAPITRKHAKVIV  
*PosN1R* 1 .....MKTTKHTLHRGAALIALALLAPMFLACGKTKTEKALHAPVPPRIDRSGNAKVLV  
*TpN1R* 1 .....MKDWNKRLKLLTGALACLAMPVLDANIKGEEVAVLTDAPVPPRIDRKHAKVIV  
*BbN1R* 1 .....MLKKNLKKFKLLGALTLSLINSAMAKDLPEQALTLSPVPPPAIREDHNAKVLV  
*KkN1R* 1 .....MKLAMLVGVAAVLAVPLNAKSLPVEKATLTAAPVPPPAIREDHNAKVI  
*CaaN1R* 1 .....MVLGLAVTLPATPATPATPATVAAKPVVGVKSGKTGDFPPQGPPIRAVLLSPVPPPTIRNYPKVLV  
*HfN1R* 1 .....MKPCKLVAAISFALAFSAQASSLPVEEAVLTLSPVPPPTIRNYPKVLV  
*OsN1R* 1 .....MKRSQITVTFWLLLLAGISS.VYAQDKNLVTEPAVLLSPVPPPTIRDYSAKVI  
*PaN1R* 1 .....MKDLQSIKQIRATFLSVLLGAVVAASAQAATTPGKAPGDFGPPQGPPIHMLLSPDPVPPVPHRNYPAKVLV  
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*Dj1N1R* 1 .....MSRSFLILAAAGLALAGCSGKPSAVFVGEADASPAAIAATHGDFGPPGPIHMLLSPDPVPPVPHRNYPAKVLV  
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*RdN1R* 1 .....  
*PsysN1R* 1 .....  
b1

*RpN1R* 73 ELEVVEKEMQISEGVSYFVFFGGTVPGSFIRVRQGDVEFHLNHRPSSKMPHNI LHVVTGGGGAAASSFTAPGHESSDPTFKALNEGLYVY  
*PosN1R* 63 RLEVTEVVKKLDAGVDYDFWFFGGSVVPGKPIRVRQNDVEFHLNHRPSSKMPHNI LHVVTGGGGAAASSFTAPGHESSDPTFKALNEGLYVY  
*TpN1R* 57 ELETKEIKRGLADGVDFWFFGGTVPGPMIRVRGDDVEFHLNHRPSSKMPHNI LHVVTGGGGAAASSFTAPGHESSDPTFKALNEGLYVY  
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*OsN1R* 51 NLETKVEKVRMADGVDFWFFGGTVPGQPIRVRQGDVEFHLNHRPSSKMPHNI LHVVTGGGGAAASSFTAPGHESSDPTFKALNEGLYVY  
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*PhN1R* 57 NLETKVEQVGRADGVDFWFFGGTVPGSFIRVRQGDVEFHLNHRPSSKMPHNI LHVVTGGGGAAASSFTAPGHESSDPTFKALNEGLYVY  
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*PsysN1R* 93 KMEVTEKVMRPLADGVDFWFFDGTVPGSFIRVRQGDVEFHLNHRPSSKMPHNI LHVVTGGGGAAASSFTAPGHESSDPTFKALNEGLYVY  
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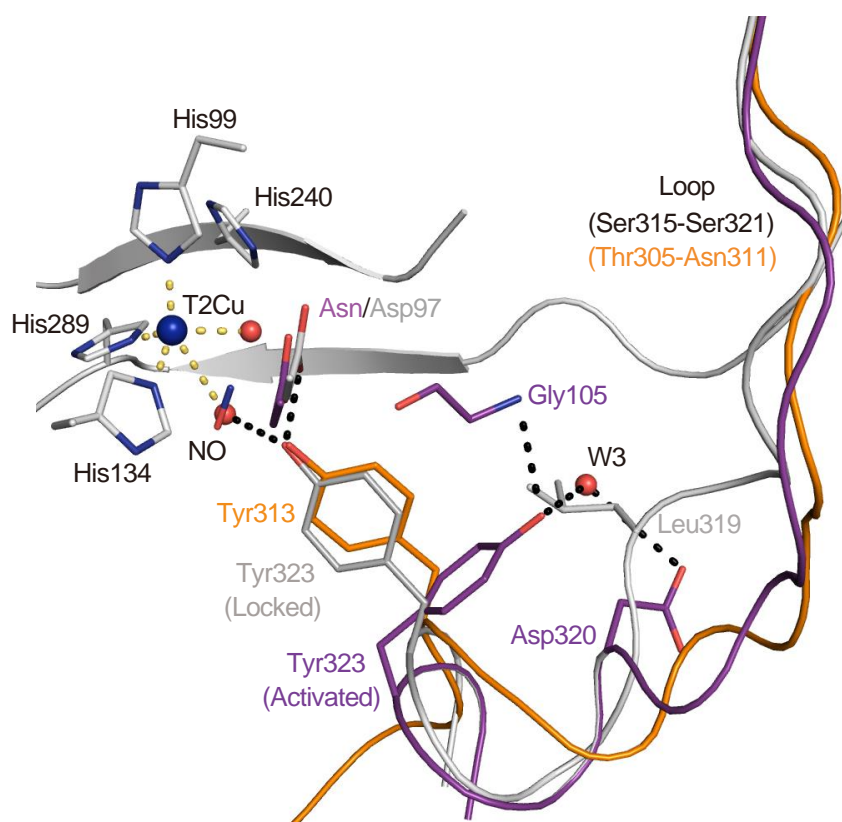
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*PosN1R* 155 HCAAPVGMHANGMYGLIYVEPEGLPKVDHREYVMOGDFYTAGKIRERKGLQDFDMEKADHRPSVVFNGAEALTDGKALHAKVGETVR  
*TpN1R* 149 HCAAPVGMHANGMYGLIYVEPEGLPKVDHREYVMOGDFYTAGKIRERKGLQDFDMEKADHRPSVVFNGAEALTDGKALHAKVGETVR  
*BbN1R* 149 HCAAPVGMHANGMYGLIYVEPEGLPKVDHREYVMOGDFYTAGKIRERKGLQDFDMEKADHRPSVVFNGAEALTDGKALHAKVGETVR  
*KkN1R* 148 HCAAPVGMHANGMYGLIYVEPEGLPKVDHREYVMOGDFYTAGKIRERKGLQDFDMEKADHRPSVVFNGAEALTDGKALHAKVGETVR  
*CaaN1R* 143 HCAAPVGMHANGMYGLIYVEPEGLPKVDHREYVMOGDFYTAGKIRERKGLQDFDMEKADHRPSVVFNGAEALTDGKALHAKVGETVR  
*HfN1R* 164 HCAAPVGMHANGMYGLIYVEPEGLPKVDHREYVMOGDFYTAGKIRERKGLQDFDMEKADHRPSVVFNGAEALTDGKALHAKVGETVR  
*OsN1R* 143 HCAAPVGMHANGMYGLIYVEPEGLPKVDHREYVMOGDFYTAGKIRERKGLQDFDMEKADHRPSVVFNGAEALTDGKALHAKVGETVR  
*PaN1R* 148 HCAAPVGMHANGMYGLIYVEPEGLPKVDHREYVMOGDFYTAGKIRERKGLQDFDMEKADHRPSVVFNGAEALTDGKALHAKVGETVR  
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*PsysN1R* 185 HCAAPVGMHANGMYGLIYVEPEGLPKVDHREYVMOGDFYTAGKIRERKGLQDFDMEKADHRPSVVFNGAEALTDGKALHAKVGETVR  
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*RpN1R* 257 LFVNGGGPNLSSFVIGEDFDKVVYGGGTLNVQKNVQTTLPAGGAAIVVFKFARVFCSVLVLDHISFRFNKGAALILKIDGPEESKLYYSG  
*PosN1R* 247 LFVNGGGPNLSSFVIGEDFDKVVYGGGTLNVQKNVQTTLPAGGAAIVVFKFARVFCSVLVLDHISFRFNKGAALILKIDGPEESKLYYSG  
*TpN1R* 241 LFVNGGGPNLSSFVIGEDFDKVVYGGGTLNVQKNVQTTLPAGGAAIVVFKFARVFCSVLVLDHISFRFNKGAALILKIDGPEESKLYYSG  
*BbN1R* 241 LFVNGGGPNLSSFVIGEDFDKVVYGGGTLNVQKNVQTTLPAGGAAIVVFKFARVFCSVLVLDHISFRFNKGAALILKIDGPEESKLYYSG  
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*CaaN1R* 235 LFVNGGGPNLSSFVIGEDFDKVVYGGGTLNVQKNVQTTLPAGGAAIVVFKFARVFCSVLVLDHISFRFNKGAALILKIDGPEESKLYYSG  
*HfN1R* 256 LFVNGGGPNLSSFVIGEDFDKVVYGGGTLNVQKNVQTTLPAGGAAIVVFKFARVFCSVLVLDHISFRFNKGAALILKIDGPEESKLYYSG  
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*PaN1R* 240 LFVNGGGPNLSSFVIGEDFDKVVYGGGTLNVQKNVQTTLPAGGAAIVVFKFARVFCSVLVLDHISFRFNKGAALILKIDGPEESKLYYSG  
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*Dj1N1R* 264 LFVNGGGPNLSSFVIGEDFDKVVYGGGTLNVQKNVQTTLPAGGAAIVVFKFARVFCSVLVLDHISFRFNKGAALILKIDGPEESKLYYSG  
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*PsysN1R* 277 LFVNGGGPNLSSFVIGEDFDKVVYGGGTLNVQKNVQTTLPAGGAAIVVFKFARVFCSVLVLDHISFRFNKGAALILKIDGPEESKLYYSG  
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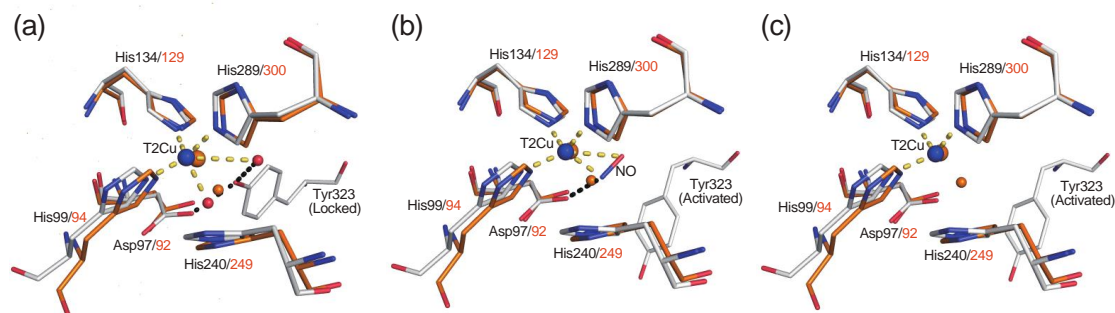
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*PosN1R* 338 QTEQKI LPE.....GSTVQMPKNT.PKAASFANKEMRMELGKTLYAQNCCHCHAEQGGKGFPPPAKSDVFAAD  
*TpN1R* 333 QADTV LGE.....GGATQMP.AEK.ATQAVAKSKEERLAEKRYGTTCCCHCHNEGGVPKFPPPAKSDVFAAD  
*BbN1R* 332 KTKDGI RPE.....GGVIQIIG.AEA.PKQIPAKTLEERLISGKRYESSCFCHCHSNQGGFGFPPPAKSDVFAAD  
*KkN1R* 331 KIDDR RPE.....GSAVQSVDKK...VVEVKATNLEKIKFKRKYDANCMACHCHPNQGGFGFPPPAKSDVFAAD  
*CaaN1R* 326 KQKDSV LGE.....GSAVQSVGDA...APVFTAQNKAEKIRFORIYEANCMACHCHAKGKGFPPPAKSDVFAAD  
*HfN1R* 347 RELDSV LGD.....RAQPNLAAVTTATANAASGKLTLDQVQAARALFAGTCSVCHGNCAGLFGVPPPAKSDVFAAD  
*OsN1R* 326 KQADNV LPE.....GSAVQVAVNT...HFKVTKKEHMYKRYVEANCMACHCHNEGGVPPPAKSDVFAAD  
*PaN1R* 331 KQADNV LPE.....GSAIQTLDE...YIYKANTKEERLFORIYEANCMACHCHSNQGGFGFPPPAKSDVFAAD  
*BtN1R* 349 KELDTLNGDGVTAAGSGPTGSPAAAAAARKGGASLVKTSAAASAPLSLPAQKAGSTVFASCAACHCHSACTGLCPVPPPAKSDVFAAD  
*Dj1N1R* 355 KEVDSV LGD.....RANPNLHVAATAKANAASGLTKDDQIAAKQOLFCTCSVCHCHANGGLFGFPPPAKSDVFAAD  
*PhN1R* 332 KEAVNV LPE.....GSAIQSLDNT...FTRITANNKDEQLRFORIYEANCMACHCHANGGLFGFPPPAKSDVFAAD  
*RdN1R* 355 KEVDSV LGD.....RSEPNLKAIVTATAHAAGTLTKAESVMAAKOLFCTCSVCHCHANGGLFNPPPAKSDVFAAD  
*PsysN1R* 368 KVRQV LPE.....GSAAQSLGNSNTPKPKVITAKNLQRLKMCESVYASNCACHCHPNQGGFGVNAFPPPAKSDVFAAD  
a4 h3 a5 a6 a7 a8

*RpN1R* 423 PK.RAMNIVLHGLNGKIVNQEYDVSMPPTQLNDDEVANITVVLNSWNPGRVSAEDVKKVRAQAPAKVAEAEH.  
*PosN1R* 411 VD.RSIVVHGLGKQIVNQEYVNSMPPKLRSDDEIANVTEVFNNSWNGKSGELTPQVKKAKANYKH.....  
*TpN1R* 405 KV.RAIRVLLHGLGKQIVNQEYDVSMPPTQLNDDEVANITVVLNSWNPGRVSAEDVKKVRAQAPAKVAEAEH.  
*BbN1R* 403 KD.RGIVSAVHGLGKQIVNQEYDVSMPPTQLNDDEVANITVVLNSWNPGRVSAEDVKKVRAQAPAKVAEAEH.  
*KkN1R* 402 PL.RGVHAIHGLGKQIVNQEYDVSMPPTQLNDDEVANITVVLNSWNPGRVSAEDVKKVRAQAPAKVAEAEH.  
*CaaN1R* 427 HD.RAIRVILHGLGKQIVNQEYDVSMPPTQLNDDEVANITVVLNSWNPGRVSAEDVKKVRAQAPAKVAEAEH.  
*HfN1R* 392 .YDRAIKAVLHGLGKQIVNQEYDVSMPPTQLNDDEVANITVVLNSWNPGRVSAEDVKKVRAQAPAKVAEAEH.  
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*BtN1R* 441 PK.RAIGLHGLGKQIVNQEYDVSMPPTQLNDDEVANITVVLNSWNPGRVSAEDVKKVRAQAPAKVAEAEH.  
*Dj1N1R* 430 DKDHLIGLPLHGLGKQIVNQEYDVSMPPTQLNDDEVANITVVLNSWNPGRVSAEDVKKVRAQAPAKVAEAEH.  
*PhN1R* 403 PL.LGVNAIIGLGGKQIVNQEYDVSMPPTQLNDDEVANITVVLNSWNPGRVSAEDVKKVRAQAPAKVAEAEH.  
*RdN1R* 430 PK.RAMTVTHGLGKQIVNQEYDVSMPPTQLNDDEVANITVVLNSWNPGRVSAEDVKKVRAQAPAKVAEAEH.  
*PsysN1R* 442 PN.RAIDAIHNGLSGKQIVNQEYDVSMPPTQLNDDEVANITVVLNSWNPGRVSAEDVKKVRAQAPAKVAEAEH.  
a9 b22 b23 a10 a11

**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), *KkNiR* (*Kangiella koreensis* Uniprot: C7R6X8), *CaaNiR* (*Catenovolum agarivorans* Uniprot: W7QSG8), *HfNiR* (*Herbaspirillum frisingense* Uniprot: R0GAR4), *OsNiR* (*Oceanimonas sp.* Uniprot: H2FXF5), *ClaNiR* (*Glaciacola arctica* Uniprot: K6XK57), *BtNiR* (*Burkholderia thailandensis* Uniprot: W6BZM5), *DjNiR* (*Dyella jiangningensis* Uniprot: A0A023NMD0), *PhNiR* (*Pseudoalteromonas haloplanktis* Uniprot: Q3IGF7), *RdNiR* (*Rhodanobacter denitrificans* Uniprot: D5JAK6) and *PsysNiR* (*Psychrobacter sp.* Uniprot: F5SNG7). Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>) and ESPript1 (<http://esript.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.