**Visualization of the Forward and Reverse Reactions Catalyzed by Nitrite Reductase**

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The process of denitrification involves the sequential reduction of nitrate ($NO_3^-$) and nitrite ($NO_2^-$) to dinitrogen ($N_2$). The nitrite reductase from *A. faecalis* (NiR) is a green 110 kDa homotrimer with each monomer containing one type I and one type II copper (Cu) sites. The type I is the site of electron transfer from pseudoazurin. Electrons are then donated internally to the type II Cu site, where $NO_2^-$ is reduced to NO. Crystals of NiR are orthorhombic with a trimer in the asymmetric unit.

To visualize the product bound at the active site, ascorbate *reduced* crystals of NiR were exposed to a NO saturated solution and frozen in liquid $N_2$ in the absence of oxygen. Data were collected at SSRL to 1.3 Å resolution. After refinement at full occupancy, the average B factor for NO is 29 $Å^2$, similar to that observed for water bound to the resting state of the enzyme. The N and O atoms of NO are equidistant from the Cu, thus the Cu-nitrosyl of NiR is characterized with side-on coordination of a diatomic molecule [1].

To examine the ability of the enzyme to catalyze the reverse reaction, *oxidized* crystals of NiR were exposed to a saturated NO solution. Refinement of the structure to 1.4 Å revealed nitrite bound to the copper via its oxygens, indicating completion of the reverse reaction in crystal. Spectroscopic studies [2] further support the conclusion that Cu-NiR can catalyze the reverse reaction.

[1] Tocheva E. I., Rosell F. I., Mauk G. A., Murphy M. E. P., *Science*, 2004, **304**, 867-870. [2] Wijma H. J., Canters G. W., De Vries S., VerbeetM. P., *Biochemistry*, 2004, **43**, 10467.

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