## Structure and Function of a Hexameric Copper-containing Nitrite Reductase

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The terrestrial nitrogen cycle sustained by some bacteria plays an important role in all organism kingdoms. Inorganic nitrogen is introduced into the biosphere by the biological fixation of atmospheric nitrogen to produce $NH_3$ and is finally removed from there again through the process of denitrification. Denitrification is the dissimilatory reduction of $NO_3^-$ or $NO_2^-$ to produce $N_2$ via NO and $N_2O$. Copper-containing nitrite reductase (NIR) catalyzes one-electron reduction of $NO_2^-$ to NO. We have determined the X-ray crystal structure of novel hexameric NIR (HdNIR) from a methylotrophic denitrifying bacterium, *Hyphomicrobium denitrificans*. In Fig. 1, the overall structure of HdNIR reveals a trigonal prism-shaped molecule, in which the monomer consisting of 447 residues and three Cu atoms is organized into a hexamer (a dimer of trimers). Each monomer is made up of three structurally similar Greek-key β barrel folding domains (cupredoxin domains I to III); the domains I and II bind one type 1 Cu (domain I, type 1 $Cu_N$; domain II, type 1 $Cu_C$) and are combined with an unusual long loop comprising 31 amino acid residues (Fig. 1c). As shown in Fig. 2, the type 1 $Cu_N$ has five ligands (2His, Cys, Met, and backbone carbonyl group), but the type 1 $Cu_C$ binds four ligands (2His, Cys, and Met). The type 2 Cu having 3His ligands is located at the interface formed by the domain II of one monomer and the domain III of an adjacent monomer. The distance between the type 1 $Cu_C$ and type 2 Cu connected through the sequence segment (-Cys-His-) is 12.6 Å. The enzyme receives one electron at the type 1 $Cu_C$ from an electron donor protein (cytochrome $c_{550}$) and catalyzes one-electron reduction of $NO_2^-$ to NO at the type 2 Cu, which intramolecularly accepts an electron from the reduced type 1 $Cu_C$. Moreover, the type 1 $Cu_N$ is essential for dimerization of the trimers. The hexameric structure of HdNIR is also maintained in a solution and the enzyme containing the six catalytic centers in one molecule behaves as a multi-active site enzyme in the periplasm.

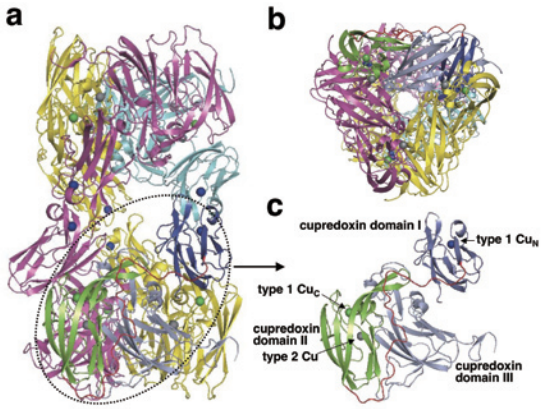


**Fig. 1** (a) Overall structure of hexamer HdNIR, (b) bottom view of the hexamer, and (c) structure of a monomer.

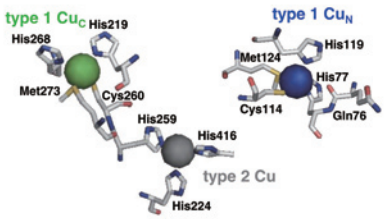


**Fig. 2** The type 1 and type 2 Cu sites of HdNIR.

## Charge Transfer Through DNA Nanoscaled Assembly Programmable with DNA Building Blocks

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DNA has been used extensively to form nanoscale structures that may be used as nanotechnology sensors and devices in the future. Despite recent advances in understanding the charge transfer (CT) through DNA, the CT through DNA nanoscaled assembly has not been clarified. A further understanding not only of the CT through DNA nanoscaled assembly, but also the kinetic mechanisms, which provide information about the rapid CT sequences, are of fundamental importance in order to create functionalized nanometer-scaled DNA wires and arrays. In this study we have reported photoinduced long-range CT of over 140 Å through a programmable DNA nanoscaled assembly based on rapid CT through the guanine cytosine alternating sequence $((GC)_n)$ by using time-resolved transient-absorption measurements. We revealed that the CT rate through the DNA with the $(GC)_n$ sequence actually is rapid. We also demonstrated that the DNA block system makes it possible to achieve the CT over 140 Å through the DNA nanoscaled assembly based on the rapid CT through the $(GC)_n$ sequences. Moreover, the CT through the nanoscaled DNA assembly sequence is programmable by using DNA blocks.