P040 Electron Input Pathways in a Bacterial Nitric Oxide Reductase

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Bacterial respiratory nitric oxide reductase (NOR) is a membrane bound cytochrome *bc* complex which catalyses the two electron reduction of two molecules of nitric oxide (NO) to nitrous oxide (N_2O) and water. $2NO+2H^{+}+2e^{-} \rightarrow N_2O+H_2O$. In the model denitrifying bacteria *Paracoccus denitrificans* the two electrons for this reaction are provided by one of two periplasmic electron donors: cytochrome c_{550} or pseudoazurin. Two protons required to complete the reaction are derived from the periplasm.

Published turnover numbers of the purified two-subunit NorCB complex measured *in vitro*, are appear relatively low possibly as a result of the well known inhibition by substrate. The problem of measuring physiologically relevant rates is compounded by the fact that many published assays of NO consumption use a combination of both chemical reductants/mediators and natural electron donors whose site of interaction with NorCB is unclear.

To address this issue we have measured rates of NO consumption in cytoplasmic membrane vesicles using succinate/cyt *c*, ascorbate/TMPD ascorbate/cyt *c* as electron donors. These rates will be compared with the both published rates for NorCB and with those obtained in our experiments either using ascorbate as an electron donor and a range of mediators and those in which the native electron donors cyt *c* and pseudoazurin were the co-substrate.