Quinol-dependent Nitric Oxide Reductases are dimers in cryoEM structures

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Quinole-dependent nitric oxide reductases (qNORs), that use Nitric oxide (NO) to generate Nitrous oxide (N_2O) as the enzymatic product in agricultural and pathogenic conditions are of major importance to food production, environment and human health. These membrane-bound enzymes strongly contribute to environmental problem at the global level (N_2O is an ozone-depleting and greenhouse gas some 300-fold more potent than CO_2) and play significant roles in survival of pathogens (qNOR from human pathogenic bacterium, *Neisseria meningitidis* is responsible for detoxification of NO produced to combat immune response of the host). We have determined high-resolution cryo-EM structure of active quinol-dependent nitric oxide reductases (qNOR) from *Neisseria meningitidis* (*Nm*) and *Alcaligenes xylosoxidans (Ax)* at 3.06Å and 3.2Å, respectively [1,2]**.** For *Nm*qNOR, we have also determined the crystallographic structure at 3.15Å. All of the crystallographic structures including that of *Nm*qNOR are monomeric [3] while both cryoEM structures showed clear dimeric arrangement. We have identified a number of factors that may trigger destabilisation of helices necessary for preserving the integrity of dimer including the use of zinc in crystallisation. Activity assay of both *Nm*qNOR and *Ax*qNOR in the presence of $ZnCl_2$ or $ZnSO_4$ abolished the activity. A closer examination of the *Nm*qNOR crystallographic and cryoEM structures revealed a significant movement of TMII where one of the Zn (called Zn1) is present in the crystallographic structure and the other was at Glu498 which is pulled away from binding to Fe_B in order to ligate the second Zn (called Zn2). It is unclear if the loss of activity is due to the binding of Zn1 located near TMII or is a consequence of the removal Glu498 from the coordination of Fe_B. The mutation of Glu to Ala led to an inactive enzyme with the size exclusion chromatography indicating the major species to be a monomer. We were able to determine the cryoEM structure of this monomer (~85kD) showing that the mutation, in addition to TMII movement, causes destabilisation of additional helices (TMIX and TMX). These results and their wider implications for structure determination of membrane proteins would be discussed in the context of enzyme mechanism.

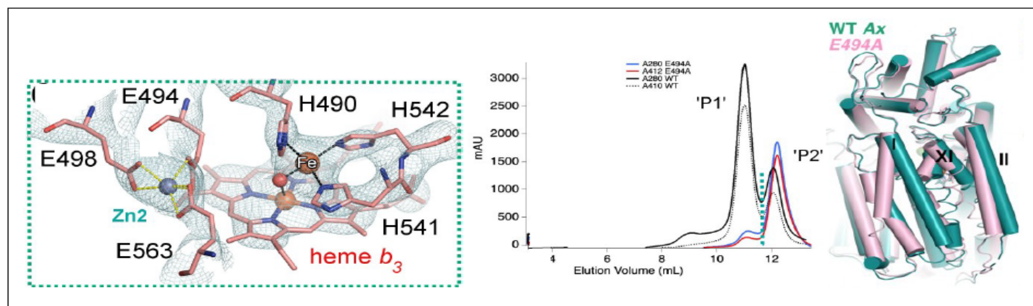

Figure 1. L to R. Zn2 site in crystallographic NmqNOR structure showing Glu 498 is pulled into ligate to Zn. Equivalent mutation in *Ax*NqNOR (Glu494Ala) leads to primarily Peak 2 (associated with monomer) with complete loss of activity. cryoEM structre of this mutant compared to WT *Ax*qNOR monomer from the dimeric cryoEM structure revealing movements of helices including TMII, feature observed in *Nm*qNOR crystallographic structure.

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