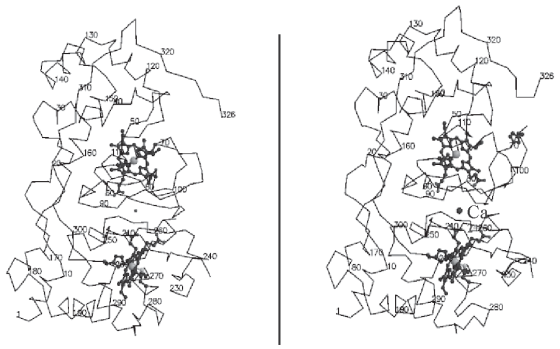
s1.m7.p25 **Structural basis for the mechanism of Ca^{2+} activation of the di-heme cytochrome c peroxidase from Pseudomonas nautica 617.** Maria J Romao,[a] Joao Miguel Dias[a], José Trincao[a], Teresa Alves[a], Cristina Timóteo[a], Cecília Bonifácio[a] , Alice S Pereira[a], Dominique Bourgeois[b,c] and Isabel Moura[a], [a]*REQUIMTE/CQFB, Departamento de Química, FCT, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal, Portugal,* [b]*LCCP, UMR 5075, IBS, 41 avenue Jules Horowitz, 38027 Grenoble Cedex 1, France, and* [c]*ESRF, 6 rue Jules Horowitz, BP 220, 38043 Grenoble Cedex, France. E-mail: mromao@dq.fct.unl.pt*

Keywords: Peroxidases; Calcium activation; Cytochrome c

Cytochrome c peroxidase (CCP) catalyses the reduction of H_2O_2 to H_2O, an important step in the cellular detoxification process. The crystal structure of the di-heme CCP from *Pseudomonas nautica* 617 was obtained in two different conformations in a redox state with the electron transfer heme reduced [1]. Form IN, obtained at pH 4.0, does not contain Ca^{2+}



and was refined at 2.2 Å resolution. This inactive form presents a closed conformation where the peroxidatic heme adopts a six ligand coordination, hindering the peroxidatic reaction from taking place. Form OUT is Ca^{2+} dependent and was crystallized at pH 5.3 and refined at 2.4 Å resolution. This active form shows an open conformation, with release of the distal histidine (His71) ligand, providing peroxide access to the active site. This is the first time that the active and inactive states are reported for a di-heme peroxidase. The crystal structure of the CCP from Ps. *stutzeri* [2] was refined to 1.6 Å. It reveals a very similar conformation to the form IN of the *Ps. nautica* CCP. Together, these structures provide some more clues about the role of the calcium ion in the activation of cytochrome *c* peroxidases.

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[2] Bonifácio C., Cunha C.A., Müller A., Timóteo C.G., Dias J. M., Moura I. and Romao M.J. . (2003). "Crystallization and preliminary X-ray diffraction analysis of the di-haem cytochrome c peroxidase from Pseudomonas stutzeri", *Acta Cryst.* D**59**, 345-347.

s1.m7.p26 **Structure Determination of MntC, the solute binding component of a high-affinity Manganese ABC transport system in the *Synechocystis* sp. PCC 6803.** Rukhman, V.[1], Bhattacharyya- Pakrasi, M.[2] Pakrasi, H.B.[2] and Adir, N.[1] , [1]*Department of Chemistry and Institute of Catalysis, Science and Technology, Technion, Technion City, Haifa 32000 Israel. FAX-972-4-8233735. E-mail: nadir@tx.technion.ac.il and* [2]*Department of Biology, Box 1137, Washington University, St. Louis, MO 63130, USA.*

Keywords: Mangenese transport; Cyanobacteria; Photosynthesis

Synechocystis sp. PCC 6803, high affinity manganese import is carried out by an ABC (ATP-binding cassette) transporter. This system contains the periplasmic substrate-binding membrane-anchored lipoprotein protein (MntC), which is important for photosynthesis. An unanchored soluble form of MntC has been shown to be fully active in mangenese transport both *in vivo* and *in vitro* [1]. Our study is aimed to structurally characterize the soluble form of MntC to understand how manganese is bound and delivered into the cell and also elucidate some of the steps necessary for manganese mobilization into cyanobacterial Photosystem II. Crystals of native and Se-Met derivatives of recombinant MntC protein have been obtained. Crystallization requires prior refolding (due to expression as insoluble inclusion bodies) of the protein in the presence of manganese, while a high concentration of zinc was required in the crystallization liquor. The crystals diffract to a maximum resolution of 2.6Å [2], and both native data sets and Se-Met derivative data sets, 100% complete to 3.0Å have been collected at ESRF ID14 and BM30 beamlines. The MntC crystals belong to the trigonal space group $p3_121$ with unit cell dimensions of 129Å x 129Å x 91Å. Phases have been obtained by SHELX. The polypeptide chain has been built and the structure is in the final stages of refinement using CNS. Data showing that the affinity of the crystallized MntC towards mangenese is greater than that towards zinc will be presented and the structural rational for preference towards manganese will be described.

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