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Solution Conformations of Peroxiredoxins Visualised by Volta Phase Plates.

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Peroxiredoxins (Prxs) are a large family of peroxidases that are found in all kingdoms of life and play a critical role in removal of reactive oxygen species (ROI), particularly hydrogen peroxide (H_2O_2) [1]. Six isoforms exist in humans, with their up-regulation observed as an antioxidant defense response to treatment with toxic chemotherapeutic agents or radiation therapy [2]. Prx3 is a mitochondrial isoform that is highly abundant in the matrix and belongs to a Prx sub-family that forms rings comprising anti-parallel dimers, known as typical 2-Cys Prxs [3]. In the case of Prx3, the ring is a dodecamer, featuring six dimers that can further assemble to form filaments [4]. It has been shown that only dimer formation is required for H_2O_2 detoxification; with the exact structure/function correlates of rings and filaments remaining unclear [5]. While the catalytic cycle has been extensively studied by X-ray crystallography, it does only provide conformation snapshots. Due to crystal contacts that restrict internal protein motions, the significance and important dynamic aspects of higher quaternary organisations cannot be adequately addressed by crystallographic methods.

Single particle cryo-EM enables three-dimensional structure determination of protein complexes without the need for crystals. Using this technique allows particles to move freely in solution until sudden immobilisation by vitrification. Even though each individual molecule is trapped in a specific conformation, over a large dataset a continuum of conformations can be obtained. We have recently shown that Volta phase plates (VPP) extend the capabilities of cryo-EM and allow time-efficient structure determination of small protein complexes such as Prx3 dodecamers at significantly higher resolution than has previously been achievable [6]. Averaging over all conformations gives rise to an anisotropic electron density map that contains dynamic information.

To unlock this information, Nanoscale Molecular Dynamics (NAMD) simulation software was used and an atomic map of Prx3 solution conformations were build, guided by the VPP cryo-EM structure obtained (Figure 1). In addition to producing a representation of dodecameric Prx3, this approach also reveals structural flexibilities of Prx3 that underpin its function (Figure 1). In contrast, the active sites and inter-dimeric regions appear to be flexible and undergo conformational changes.

Our representation of the Prx3 dodecamer suggests that conformational changes in the active sites are concomitant with movements at the inter-dimeric domains, thereby enabling cross-talk between dimers. Besides revealing structural information on the significance of Prx3 oligomeric states, this study sets the stage for routine use of VPP single particle analysis for dynamic structure determination as a high-resolution technique that is complementary to X-ray crystallography and Small Angle X-ray Scattering. [7]

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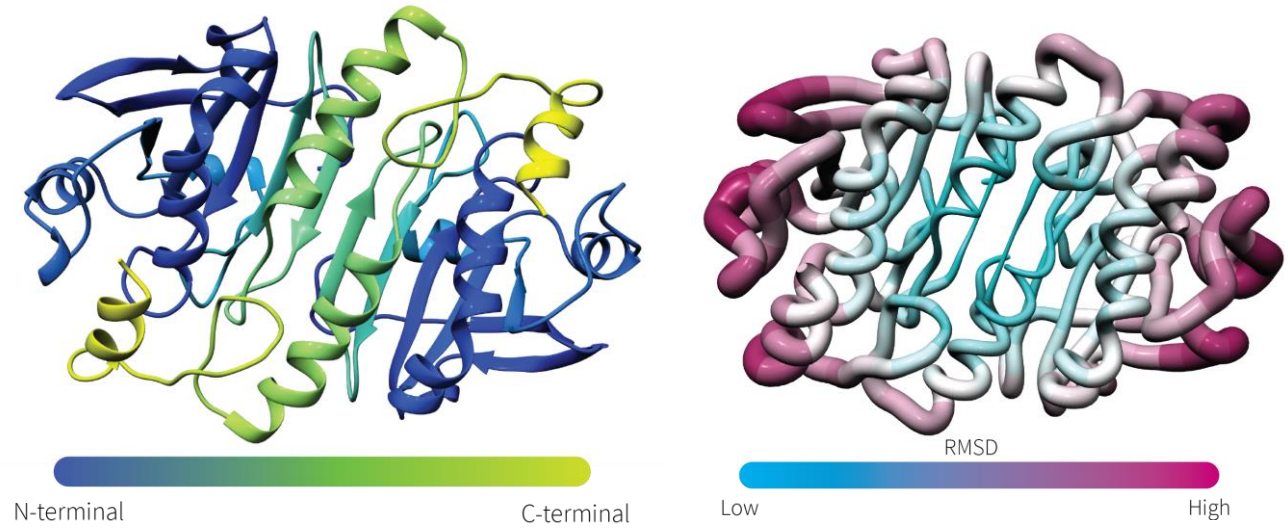

Figure 1

Figure 1. Solution of conformations of Prx3. Left, atomic map of a Prx3 dimer was build using NAMD simulations guided by the 4.4 ÅVPP cryo-EM reconstruction [6]. Decomposition of ROIs is mediated by catalytic cysteines that act in pairs with each monomer within a dimer providing a cysteine residue to an active site. Hydrogen peroxide first binds to the peroxidatic cysteine located in helix α2 (blue). The other monomer provides a free cysteine located in the C-terminal region (yellow) for resolving and forming a disulphide link with the peroxidatic cysteine. Right, the MD simulation shows a rigid β-sheet through the centre of the dimer (cyan). The active sites and inter-dimeric regions (pink) appear to be most flexible suggesting concerted movements that can translate to adjacent dimers.

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