

A self-rotation puzzle

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Introduction

The peroxiredoxins (Prxs) are a ubiquitous family of antioxidant enzymes that regulate intracellular levels of H₂O₂ where they are implicated in both tissue protection against oxidative stress and H₂O₂-mediated signalling pathways (Wood *et al.* 2003). In recent years, their key role in antioxidant defence has been emphasised by their high abundance in both bacterial and mammalian cells. Peroxiredoxin III (Watabe *et al.* 1994) is a typical member of the 2-Cys PrxIII subclass with catalytic cysteines at its N(Cys47) and C(Cys168) termini and with a dimer as the functional unit. Electron Microscopy (EM) studies (Gourlay *et al.* 2003; Wood *et al.* 2003) have shown that PrxIII exists as an oligomeric ring. We have determined the crystal structure of bovine mitochondrial PrxIII C168S mutant at 3.3Å resolution (Cao *et al.* 2005).

What is the puzzle?

Crystals production, data collection and processing were described previously (Cao *et al.* 2005). The crystals belong to the monoclinic space group C2 with the Matthews coefficient (Matthews 1968) suggesting 10 ($V_m=2.8 \text{ Å}^3/\text{Da}$) or 12 ($V_m=2.3 \text{ Å}^3/\text{Da}$) monomeric subunits in the crystal asymmetric unit. The usual statistical indicators (CCP4 1994) gave no indication of crystal twinning. Since most (6 out of 8) known typical 2-Cys Prxs structures are decamers, a self-rotation function was calculated using the program MOLREP to locate the expected NCS two-fold and five-fold axes. The

results are shown in Figure 1 as stereographic projections of polar angles. The absence of substantial peaks on the $\chi=72^\circ$ section indicate there is no 5-fold symmetry in the crystal structure (Fig 1a). However, 3 peaks on the $\chi=60^\circ$ section indicate three 6-fold symmetry axes, each of which is perpendicular to 6 two-fold axes shown as lines of 6 peaks in the $\chi=180^\circ$ section (Fig 1b). In each line the peaks occur at an angle of about 30 degrees to each other and are perpendicular to a 6-fold peak, which also coincident with a 2-fold peak. Taken together, these facts suggest a dodecameric rather than a decameric structure. However, as the Matthews coefficient suggested only one dodecameric ring in the asymmetric unit, the indication of three different 6-fold axes of rotation really puzzled us.

Initial attempts to solve the structure by molecular replacement by MOLREP (CCP4 1994), AMoRe (Navaza 1994) and PHASER (Storoni *et al.* 2004) with the default parameters using thioredoxin peroxidase B (TPxB) (PDB Code 1qmv) monomer, dimer and decamer as the starting model were all tried without any successful solutions. A solution was finally obtained by the molecular replacement program PHASER (Storoni *et al.* 2004) using the dimer of TPxB as a search model (Cao *et al.* 2005).

What is the answer to the puzzle?

The structure shows that stable PrxIII dimers are formed across a non-crystallographic 2-fold axis that extends the central β -sheet. The 6-fold NCS-related dimers are assembled into a dodecameric ring structure with outer and inner diameters of 150 and 70 Å respectively (*cf* 130 and 60 Å for the decameric Prxs) (Fig 2a and 2b).

The surprising feature of the crystal structure of PrxIII C168S is its presence as a 2-ring catenane comprising two interlocking dodecameric toroids (Fig. 2c), which are arranged such that half of one ring is related to half of the other ring by the crystallographic 2-fold axis. The planes of the rings are not at right angles, but are inclined at an angle of 55° , which allows a larger contact surface between the rings. This structure provides the solution to the self-rotation puzzle. The two 6-fold peaks ($\chi=60^\circ$) at $\phi=66^\circ$, $\phi=104^\circ$ and $\phi=66^\circ$, $\phi=-104^\circ$, with coincident 3-fold ($\chi=120^\circ$) and 2-fold ($\chi=180^\circ$) axes, are the two 6-

fold axes perpendicular to the planes of the rings. The two lines of six 2-fold peaks ($\phi = 90, \varphi = +165; \phi = 64, \varphi = +150; \phi = 38, \varphi = +130; \phi = 25, \varphi = +75; \phi = 38, \varphi = +23; \phi = 64, \varphi = 0$) represent the 2-fold axes in the plane of the ring. Because the ring is composed of six homodimers, there are 12 2-fold axes in the plane, giving a 30 degree angle between adjacent axes. What is the large 6-fold, peak at $\phi = 64, \varphi = 0$? This is actually the tail of a peak arising from an improper rotation of 55 degrees (Fig 3a), which is the angle between the planes of the two rings (Fig 3b). Finally, the 2-fold peak at $\phi = 26, \varphi = 180$ is perpendicular to this and relates the two rings as shown in Fig 3b where the axis is in the plane of the page and runs vertically through the centre of the model. The two peaks at $\phi = 90, \varphi = 90$ and $\phi = 90, \varphi = -90$ are the crystallographic 2-fold symmetry axes of the spacegroup C2.

Other catenanes

There are three previous examples of protein catenanes cited in the literature and two of them are rather specialised cases. One is a totally artificially -produced peptide catenane based on a small segment of a dimeric mutant of the p53^{tet} protein generated *in vitro* using chemical techniques (Yan and Dawson 2001). Another one is a viral capsid assembly of 420 subunits where the subunits are topologically linked by covalent (isopeptide) bonds creating a form of protein 'chain mail' which is highly resistant to degradation (Wikoff *et al.* 2000). The third example is the crystal structure of RecR from *Deinococcus radiodurans*, which is involved in homologous recombinational DNA repair in prokaryotes (Lee *et al.* 2004) (PDB ID: 1VDD). Four RecR monomers form a ring-shaped tetramer of 222 symmetry with a central hole of 30-35 Å diameter. In the crystal, two tetramers are concatenated (Fig 4).

How is the catenane formed?

We have no data indicating how the 2-ring catenane structure is formed but a model described previously (Cao *et al.* 2005) is shown in Fig 5. Briefly, dimeric units can interact in two different modes that are not mutually exclusive. One mode produces the dimer-dimer contacts, primarily hydrophobic, associated with ring generation in this and other Prx structures. The other mode gives polar contacts that could potentially initiate

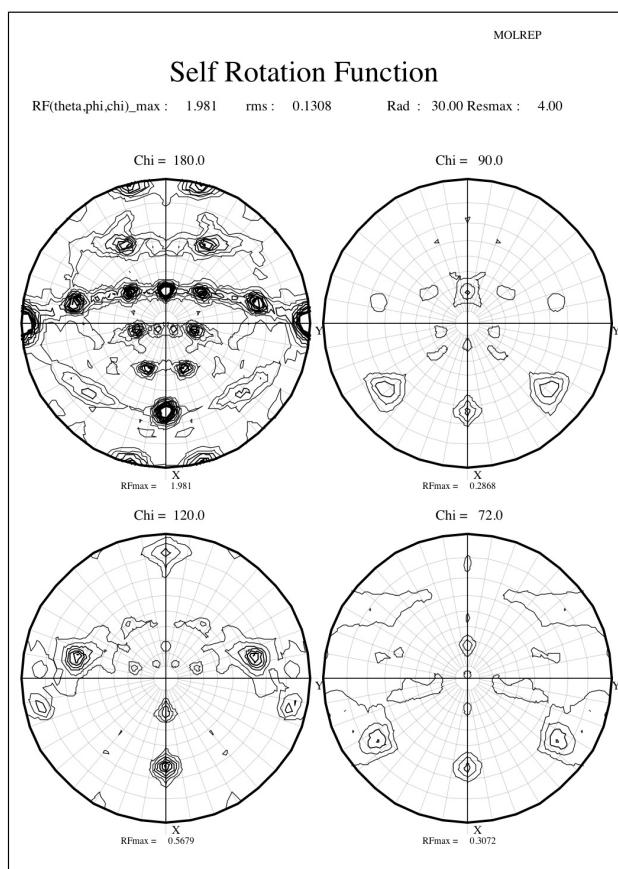
catenane formation at any stage during single toroid assembly by allowing two rings to form simultaneously around each other.

At present it is unclear whether the catenane structure has any physiological relevance, but it provides interesting new insights into protein topology and mechanisms of subunit assembly.

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a)



b)

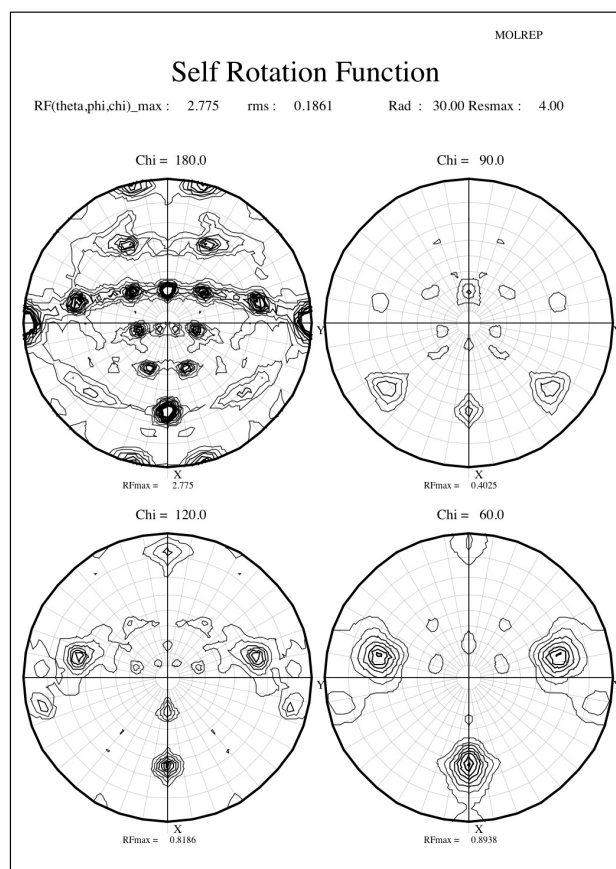


Figure 1 Self-rotation functions calculated by MOLREP with the chi angles shown.

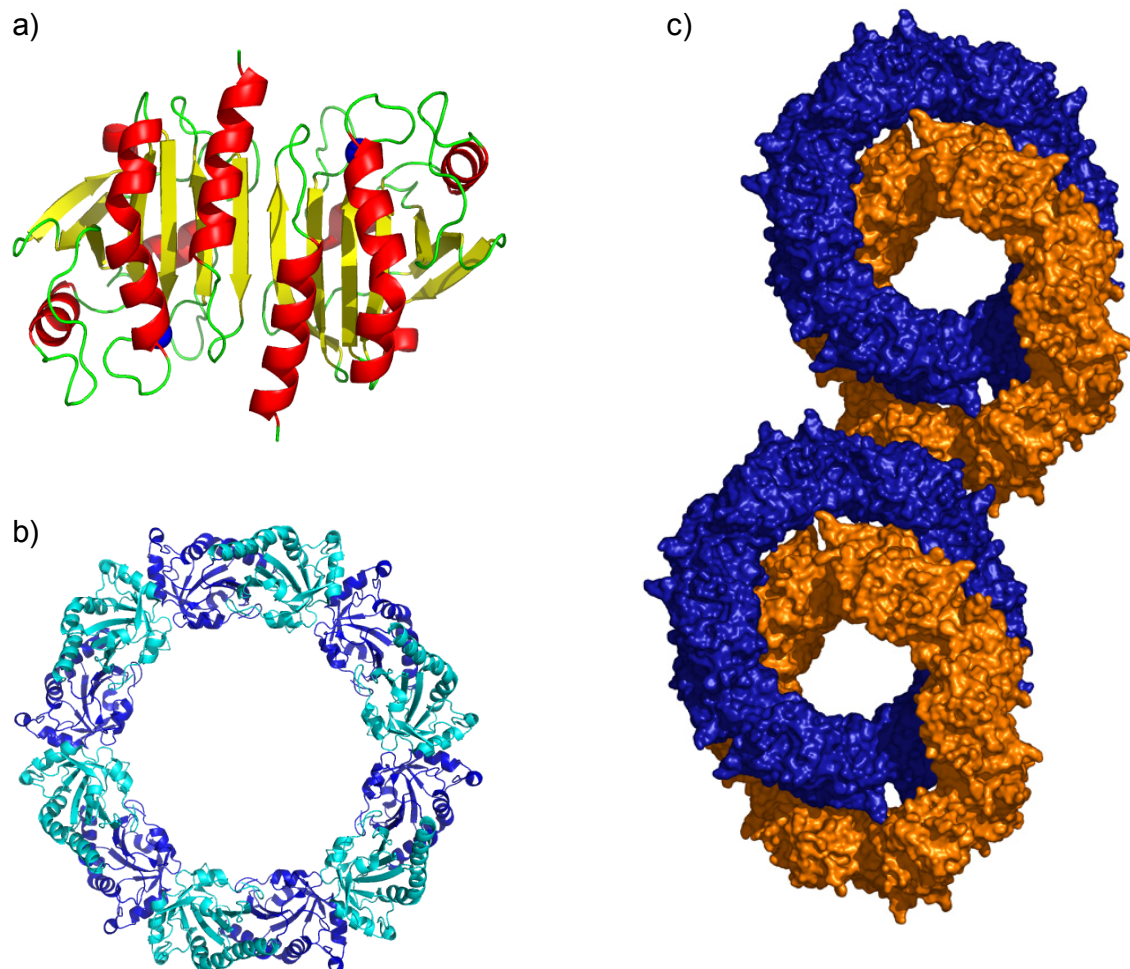


Figure 2 Crystal structure of PrxIII C168S

- a) PrxIII dimer. The active site Cys47 is highlighted by blue ball presentation.
- b) PrxIII dodecamer
- c) Two dodecamer rings (gold and blue) of PrxIII form the interlocked 2 -ring protein catenane structure in the unit cell.

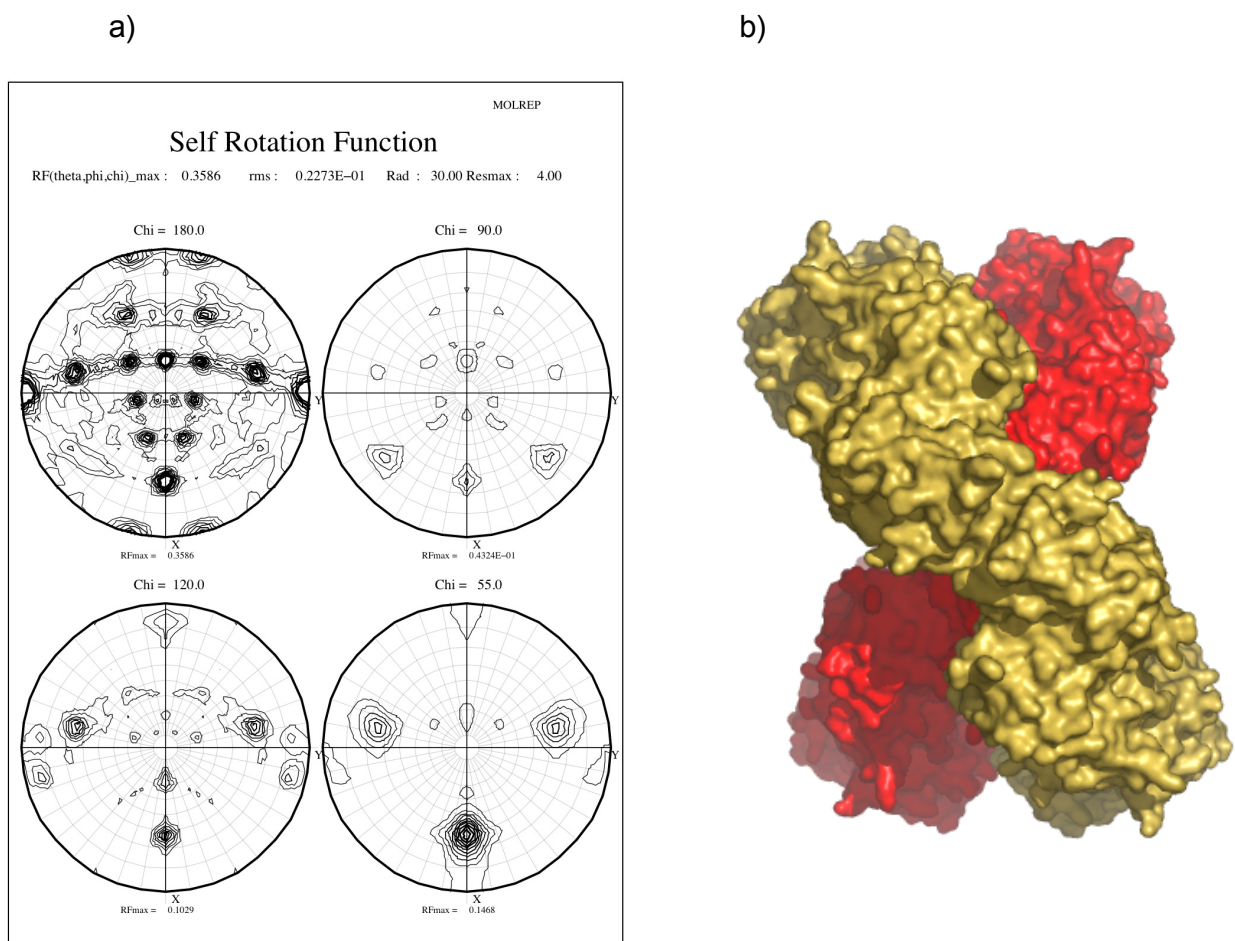


Figure 3

- a) The self-rotation function calculated by MOLREP with chi angle at 55 degrees.
- b) The side view of the 2-ring catenane structure. The figure was take from (Cao *et al.*

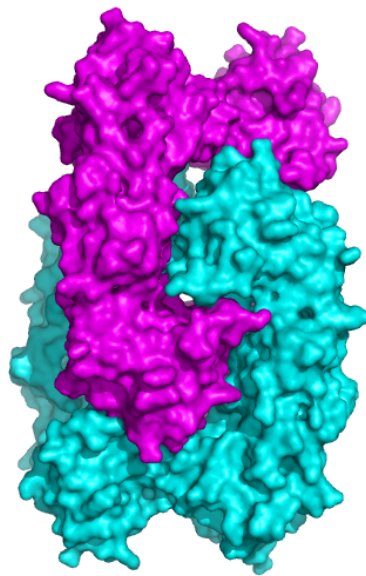


Figure 4 The crystal structure of *Deinococcus radiodurans* RecR octamer. Two tetramers (pink and cyan) are concatenated to form an octameric structure in the crystal.

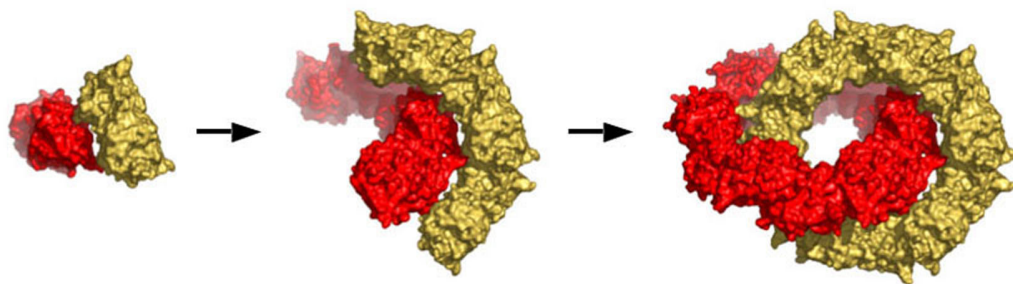


Figure 5 Proposed mechanism of assembly of the 2 -ring catenane structure.

Polar contacts between dimers (shown in red and gold), potentially occurring at any stage during single toroid formation, provide the basis for initiating the generation of a second topologically -linked ring leading to the overall 2-ring catenane structure. The figure was take from (Cao *et al.* 2005).