FOR THE RECORD

Observed octameric assembly of a *Plasmodium yoelii* peroxiredoxin can be explained by the replacement of native “ball-and-socket” interacting residues by an affinity tag

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Abstract: Peroxiredoxins (Prxs) are ubiquitous and efficient antioxidant enzymes crucial for redox homeostasis in most organisms, and are of special importance for disease-causing parasites that must protect themselves against the oxidative weapons of the human immune system. Here, we describe reanalyses of crystal structures of two Prxs from malaria parasites. In addition to producing improved structures, we provide normalizing explanations for features that had been noted as unusual in the original report of these structures (Qiu *et al.*, BMC Struct Biol 2012;12:2). Most importantly, we provide evidence that the unusual octameric assembly seen for *Plasmodium yoelii* Prx1a is not physiologically relevant, but arises because the structure is not of authentic *P. yoelii* Prx1a, but a variant we designate *PyPrx1a^N* that has seven native N-terminal residues replaced by an affinity tag. This N-terminal modification disrupts a previously unrecognized, hydrophobic “ball-and-socket” interaction conserved at the B-type dimer interface of Prx1 subfamily enzymes, and is accommodated by a fascinating two-residue “ β -slip” type register shift in the β -strand association at a dimer interface. The resulting change in the geometry of the dimer provides a simple explanation for octamer formation. This study illustrates how substantive impacts can occur in protein variants in which native residues have been altered.

Keywords: malaria; structural genomics; protein structure; artifact; peroxidase; dimer interface; radiation damage; hyperoxidation

Abbreviations: PDB, Protein Data Bank; Prx, peroxiredoxin; SGC, Structural Genomics Consortium.

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Introduction

Peroxiredoxins (Prxs) are ubiquitous antioxidant enzymes crucial for redox homeostasis and in some organisms involved in redox regulation and signaling.^{1,2} They reduce reactive nitrogen and oxygen species (ROOH, e.g. peroxynitrite, organic peroxides, or hydrogen peroxide) via a three-part catalytic cycle

Table I. Data Quality and Refinement Statistics for Original and Rerefined Plasmodium Prx Structures^a

	<i>PvPrx1a</i> (S–S)		<i>PyPrx1a</i> ^{N*} (S–S)	
Conformation	LU		LU	
Oligomeric state	(α ₂) ₅		(α ₂) ₄	
Space group	<i>P2</i> ₁		<i>P4</i> ₂₂	
Unit cell (Å)	<i>a</i> = 70.55 <i>b</i> = 149.59 <i>c</i> = 131.91		<i>a</i> = 105.08 <i>b</i> = 105.08 <i>c</i> = 41.83	
B angle (°)	104.88		90	
Resolution (Å)	41.6–2.48 (2.53–2.48) ^b		50.0–2.3 (2.38–2.3)	
Completeness	99.2 (99.8)		94.6 (99.3)	
< <i>I</i> / <i>σ</i> >	10.8 (1.3)		26.6 (4.3)	
Unique reflections	86,438		10,337	
Data redundancy	3.4 (3.3)		13.9 (12.8)	
<i>R</i> _{merge} ^c	0.115 (0.488)		0.153 (0.471)	
	Deposited	Current	Deposited	Current
PDB code	2H66	4L0U	2H01	4L0W
Redox state	S ₅₀ –S ₁₇₀ '	S ₅₀ –S ₁₇₀ '	SH ₄₄ /SH ₁₆₄ '	S ₅₀ –S ₁₇₀ '
<i>R</i> _{work} (%)	19.4	17.6	25.8	19.6
<i>R</i> _{free} (%)	23.2	21.2	28.8	24.6
Molecules in AU	10	10	1	1
Protein residues	1684	1707	174	173
Total atoms	13448	13720	1422	1442
Water	154	114	54	67
Acetate	0	3	0	1
Sulfate	0	0	0	1
< <i>B</i> factors> (Å ²) ^d				
Main chain	45	49.5	28.7	29.9
Side chains, H ₂ O	46.1	55.7	29.3	35.3
rmsd bonds (Å)	0.016	0.01	0.018	0.009
rmsd angles (°)	1.42	1.2	1.54	1.19
φ,ψ angles				
Favored (%) ^e	95.4	96.7	91.9	95.3
Outliers (%)	0.6	0.1	2.3	0

^a All crystal parameters and data quality statistics are those given in the PDB entries for the previously deposited models.^b Values in parentheses correspond to those of the highest-resolution shell.^c $R_{\text{merge}} = \sum |I_{hkl}| - \langle I \rangle / \sum I_{hkl}$.^d Greater average *B*-factors in our current models may be ascribed to refinement in Buster (Global Phasing Ltd.) rather than REFMAC5 (CCP4; used for the deposited structures).^e φ,ψ data from <http://kinemage.biochem.duke.edu/databases/top500.php>.

involving two key thiols which, in 2-Cys Prxs, consist of the peroxidatic cysteine (C_P) and the resolving cysteine (C_R). The reduced C_P (C_P–SH) reacts with ROOH to yield ROH and C_P–SOH, which in turn reacts with C_R–SH to form the disulfide C_P–S–S–C_R and H₂O. Finally, C_P–SH and C_R–SH are regenerated, typically via a thioredoxin-like reductant.

According to a recent survey we completed,³ all parasites that cause a significant burden of disease feature Prxs among their countermeasures against the host immune system, and these enzymes have been considered as potential targets for drug development.⁴ As part of our survey, we found that the naming of many Prxs in various parasites was highly inconsistent, making comparisons difficult. To address this problem, we proposed a new nomenclature based on evolutionary relationships that assigns Prxs to one of five common subfamilies (Prx1, Prx6, Prx5, Tpx, PrxQ),³ and we will use this nomenclature here.

As malaria parasites, genus *Plasmodia*, cause a massive and widespread disease burden,⁵ we and collaborators have pursued studies of their Prxs.^{6–8} Thus with particular interest, in our review we noted four Protein Data Bank (PDB) entries for *Plasmodial* Prx structures that were deposited by the Structural Genomics Consortium (SGC) in 2007, but not otherwise published: Prx1a from *P. vivax* (*PvPrx1a*) in thiol and disulfide forms (PDB codes 2I81 and 2H66); Prx1a from *P. yoelii* (*PyPrx1a*) in disulfide form (PDB code 2H01); and Prx6 from *P. yoelii* (*PyPrx6*) in disulfide form (PDB code 1XCC)[†]. In the spirit of the SGC mission to increase structural knowledge of proteins from neglected protozoan parasites, we undertook analysis of the four structures in order to describe them in the literature. As we were completing our analyses, SGC scientists published descriptions of all four structures⁹

[†]In Qiu et al. the Prx1a enzymes are called Trx-Px1.

(with a further refined *PyPrx6* structure—PDB entry 3TB2—replacing entry 1XCC). Regarding the plasmodial *Prx1a* structures, while we can substantiate the basic structural descriptions made by Qiu *et al.*,⁹ we report here that the evidence does not support their conclusions that the disulfide bonds in these enzymes are unusual, nor that these *Prxs* are sensitive to hyperoxidation, nor that the octameric assembly reported for *PyPrx1a* is physiologically relevant. In particular, we report that the *PyPrx1a* structure that was solved is of a variant that has seven N-terminal residues replaced by an affinity tag. To emphasize that this structure is not native *PyPrx1a*, we designate it *PyPrx1a*^{N*}.

Results and Discussion

Polishing refinements of *PvPrx1a* and *PyPrx1a*^{N*} in their disulfide states combined manual model building in Coot¹⁰ and maximum-likelihood minimization using BUSTER-TNT¹¹ to yield models with R/R_{free} values lower by 2–6% and somewhat improved overall geometry (Table I; deposited as PDB ID 4LOU and 4LOW respectively). Noteworthy changes in the structure of *PyPrx1a*^{N*} include refining the C_P–C_R disulfide distance to ~2.6 from ~3.1 (between symmetry mates since there is only one chain in the asymmetric unit) and adjusting the residue numbering to correspond to the native sequence. For *PvPrx1a*, 17 additional residues were built at the C-terminus of chain J, including a previously unmodeled C_P–C_R disulfide linkage so that, in all, five of the ten chains now have modeled disulfides. Otherwise, changes included rebuilding and adding to some weakly ordered parts of the structure and adjusting the solvent models, in some cases modeling acetate and sulfate molecules.

Features of *Prx1* family disulfide bonds are not unusual

Given mass spectrometric evidence that both *PvPrx1a* and *PyPrx1a*^{N*} are purified in the disulfide state, Qiu *et al.*⁹ highlighted that “the apparent absence of detectable disulfide bonds” in most *PvPrx1a* chains and that the “long Cys–S_P to Cys–S_R distances [observed in *PyPrx1a*^{N*} are] not easily accounted for, yet prevalent in the *Prx1* subfamily.” Key to this latter conclusion is that the *PyPrx1a*^{N*} structure was solved using a rotating anode X-ray source and the perception that this would be insufficient to open disulfide bonds. We agree that five of the ten expected *PvPrx1a* disulfides are sufficiently disordered that they cannot be modeled, and that the modeled disulfides in *PvPrx1a* and *PyPrx1a* have long apparent S–S bond distances of up to ~2.8 Å. However, this phenomenon is readily explained by the partial reduction of the disulfide bonds by X-ray radiation, as has been commonly observed in the literature for synchrotron

radiation,^{12–15} and has been documented to occur even with weaker laboratory-based, rotating anode (Cu–K_α) radiation.¹³ This is consistent with past descriptions of *Prx1* structures, in which authors attribute weak or missing disulfide density and long apparent S–S bond distances to disorder and radiation damage^{16,17} and do not view these results as indicative of special chemical properties.

PvPrx1a and *PyPrx1a* need not be “sensitive” to overoxidation

“Sensitive” as opposed to “robust” in the *Prx* literature denotes *Prx1* subfamily enzymes that can be rapidly converted to a hyperoxidized, inactive C_P–SO₂ (C_P-sulfinate) form by concentrations of H₂O₂ as low as 100 μM.^{18–20} In some organisms, the C_P–SO₂ form can be reactivated by the enzyme sulfiredoxin, enabling a modulation of *Prx* activity that can influence redox regulation and H₂O₂-based signaling.¹ It is unknown whether *Prxs* play such a signaling role in any protozoan parasite.³ Qiu *et al.* assigned the *PvPrx1a* and *PyPrx1a* enzymes as “sensitive” based on the presence of sequence motifs and analyses showing that 500 μM to 5 mM H₂O₂ for unspecified lengths of time led to apparent formation (at an unquantified yield) of the C_P-sulfinate or -sulfonate (–SO₃) forms. However, such demonstrated “susceptibility” to hyperoxidation need not imply “sensitivity” to hyperoxidation. Also, despite the original observation that certain “GGLG,” “YF” sequence motifs are associated with “sensitivity,”¹⁹ it has since been learned that “sensitivity” cannot be assigned based solely on these sequence motifs. Notably, for two closely related cyanobacterial *Prxs* both featuring “GG(V/DG,” “YF” motifs, one is “sensitive” and the other is not.²¹ Thus, to assess the “sensitivity” of these plasmodial enzymes, studies are needed that define the rate of hyperoxidation as a function of H₂O₂ concentration. As noted in our review,³ as of yet no *Plasmodial* *Prx* is known to be sensitive, consistent with none of these organisms having the sulfiredoxin enzyme that has the physiological role of reactivating hyperoxidized *Prxs*.

The *PyPrx1a*^{N*} octamer appears to be an artifact caused by a modified N-terminus

Subfamily *Prx1* enzymes are obligate dimers, associating at what is termed the B-type interface,⁶ with each active site incorporating C_P from one chain and C_R from the C-terminal region of the other. Furthermore, all characterized *Prx1* subfamily enzymes form oligomeric rings, principally (α₂)₅ decamers with five B-type dimers associating via A-type (or oligomer building) interfaces² and with decamers and dimers existing in solution in a dynamic equilibrium influenced especially by redox state.^{22,23} Two (α₂)₆ dodecamers have been reported.^{17,24}

Figure 1. Multimeric assemblies of plasmodial *PvPrx1a* and *PyPrx1a*^{N*}. A: The *PvPrx1a* (α_2)₅ decameric ring highlighting alternating subunits (purple/magenta) and indicating the A-type and B-type subunit interfaces. The upper view into the face of the ring shows a secondary structure ribbon within a transparently rendered solvent-accessible surface and the lower image is an edge-on view of the ring as above, but with the ribbon showing only the central β -sheet that extends across the two subunits of the B-type dimers. B: *PvPrx1a* (purple hues) and *PyPrx1a*^{N*} (green hues) showing only the β -strands and superimposed based on a single monomer (as indicated). The directions of the $\sim 17^\circ$ rotation (upper panel) and the ~ 6 Å translation (lower panel) of the B-type dimeric partner of *PyPrx1a*^{N*} (emerald green) relative to that of *PvPrx1a* (magenta) are indicated. C: Depictions of the *PyPrx1a*^{N*} (α_2)₄ octamer (green hues). Views are as in panel A.

As reported by Qiu *et al.*,⁹ *PvPrx1a* forms the typical (α_2)₅ decamers but *PyPrx1a*^{N*} forms a novel (α_2)₄ octamer (Fig. 1). The authors noted that the octamer had normal A-type interfaces, and its formation was related to altered B-type interfaces that they described by the relative positions of two conserved Leu residues (see Fig. 5 in Qiu *et al.*).⁹ While noting that this alternate association also involved a large conformational difference in the $\beta 7$ - $\alpha 5$ loop (Asn143-Ser149) moving Arg148 from buried (in the *PvPrx1a* decamer) to exposed (in the *PyPrx1a*^{N*} octamer), a change in the packing of the C-terminal tail, and an altered conformation of the N-terminal residues, the authors were not able to define how these changes drove octamer formation.

Given the $\sim 85\%$ sequence identity of *PyPrx1a* to *PvPrx1a* and the conservation of decamer formation over much more divergent Prxs, we were skeptical that native *PyPrx1a* would behave so differently and we sought to discover the specific structural features favoring this alternate B-type interface. Comparing the conformations of the *PvPrx1a* and *PyPrx1a*^{N*} monomers, we confirmed the changes listed above. However, in seeking to understand the change in the B-type interface, we discovered that for dimers overlaid based on one chain, the second chain is

shifted ~ 7.2 Å of which ~ 6 Å is due to a translation along the interface that corresponds to a two-residue register shift in the alignment of strand $\beta 7'$ with strand $\beta 7$ [Figs. 1(B) and 2(A)]. Termed a “ β -slip,” such a structural change has been seen to occur as a consequence of point mutations in other proteins having a β -strand subunit interface.^{25,26}

The ~ 6 Å translation along strand $\beta 7$ is accompanied by a 17.2° rotation around an axis parallel to the β -strands [Figs. 1 and 2(A)]. This “butterfly” type bending of the dimer toward the center of the toroid explains the octamer formation, because the narrowing of the internal angle of the multimeric ring by $\sim 17^\circ$ is a near perfect match to the difference between 108° angles that form a pentagon and 90° angles that form a square (Fig. 1). The next question we focused on was: what caused the β -slip and the $\sim 17^\circ$ rotation? It could not be the interfacing β -strands themselves as they are identical in sequence between *PvPrx1a* and *PyPrx1a* [Fig. 2(B)].

Our attention was drawn to the conformational differences of the N-terminal residues, because these residues occupy the B-type dimer interface above strands $\beta 7$ and $\beta 7'$ [Fig. 2(C,D)]. Interestingly, although the natural *PvPrx1a* and *PyPrx1a* N-terminal sequences are quite similar, the *PyPrx1a*^{N*}

Figure 2. The origins of the PyPrx1a^N octamer. **A:** View from the center of the toroid down the twofold symmetry axis of C_α-traces of the B-type dimers of PvPrx1a (purple hues) and PyPrx1a^N (green hues) overlaid based on the left-hand chain. The ~ 6 Å downward shift due to the two-residue β-slip is indicated (arrow). The lower panel shows an orthogonal view and highlights the $\sim 17^\circ$ rotation around an axis perpendicular to the page. Secondary structure elements near the interface are labeled. An interactive view is available in the electronic version of the article. **B:** Segments of native PvPrx1a and PyPrx1a sequences aligned with sequences of the constructs crystallized. Residues in gray had no interpretable electron density and are not included in our rerefined models; red boxes surround residues that differ from the native sequences, and conserved residues in strand β7 lining the B-interface trench are colored along the visible spectrum (blue through red) for easy identification in panels C and D. Identical residues are marked by *, hydrophobic ball-and-socket residues (see text) are marked with ψ , and the positions of secondary structure elements are indicated. **C:** The B-interface trench of the decameric PvPrx1a, rendered as solvent-accessible surface, with the N-terminal residues 2–7 shown as sticks (purple hues for carbon atoms). Val5 is labeled with a ψ . H-bonds are dashed lines and the surface coloring is gray for most residues and otherwise as in panel B for strand β7. Residues belonging to the right-hand subunit are numbered with a prime. The upper panel view matches that of the upper panel of A, and the lower panel is an orthogonal view from the twofold axis looking into the upper half of the cleft, roughly corresponding to the view in the lower panel of A. **D:** The same as panel C, but for the PyPrx1a^N octamer, and showing residues 4–7 as sticks (green hues for carbon atoms). In panels C and D, pairs of arrows (colored by subunit, as above) indicate the relative positions of the subunits to emphasize the 6 Å downward shift in the right hand subunit of PyPrx1a^N. In the upper panels of A, C, and D, the twofold axis between the subunits is indicated (●).

construct that crystallized has substantial sequence differences, with native residues 1–7 replaced by a His₆ tag with a TEV protease cleav-

age site [Fig. 2(B)]. Furthermore, Qiu *et al.* state that for PyPrx1a “only a structure with an N-terminal truncation of 6 residues [plus the

initiator Met] crystallized sufficiently well for data collection.” This strongly implies that the substitution of *PyPrx1a* residues 1–7 [Fig. 2(B)] was indeed responsible for altering the protein conformation in a way that enabled the growth of the octameric crystal form.

A side-by-side comparison of the B-type dimer interfaces of *PvPrx1a* (decamer) and *PyPrx1a*^{N*} (octamer) allows the structural change to be understood. A view down the two-fold symmetry axis reveals that in the *PvPrx1a* decamer, N-terminal residues 2–7 from the two chains fill a deep hydrophobic cleft that is above the $\beta 7$ and $\beta 7'$ strands and lined with residues conserved between the two Prxs [Fig. 2(B–D)]. In particular, Gly6, Val5, Tyr4, and Thr3 are nestled in the cleft and are nearly as well ordered as the surrounding residues (*B*-factors $\sim 50 \text{ \AA}^2$ compared with $\sim 40 \text{ \AA}^2$ for surrounding residues). Gly6 packs in a position with no room for a side chain and the hydroxyl of Thr3 receives a key H-bond from Val6-NH stabilizing the backbone path [Fig. 2(C)].

In native *PyPrx1a*, Gly6 and Val5 are conserved and Tyr4 and Thr3 are replaced with the Ile4 and Ser3, conserving the respective hydrophobic and H-bonding qualities at those positions. However, in *PyPrx1a*^{N*} the replacement of Gly6 with a Gln is incompatible with this chain path and the cleft cannot be effectively filled. This destabilizes the normal B-type interface that supports decamer formation, and apparently stabilizes the new β -slipped arrangement with an altered cleft [Fig. 2(D)]. In this new arrangement, Gln6, Phe5, and Tyr4 of *PyPrx1a*^{N*} occupy the cleft, but are less deeply nestled in it and are rather poorly ordered (*B*-factors $\sim 75 \text{ \AA}^2$ compared with $\sim 30 \text{ \AA}^2$ for surrounding residues, with sufficiently weak electron density such that the conformations should not be considered definitive). That these three residues do not fill the cleft as completely as do the five ordered residues in the *PvPrx1a* decamer provides an explanation for the $\sim 17^\circ$ rotation in the subunit, as the rotation serves to bring the walls of the cleft closer together [Fig. 2(A), lower panel]. Given the above explanations, it is clear that the conformational changes in the $\beta 7$ - $\alpha 5$ loop and the C-terminal tail result from and help stabilize the beta-slipped structure, but are not the underlying causes of the change in *Prx1a* oligomerization.

Residues Val5 and its surroundings (Leu124/Val136/Leu139/Val141 on strands $\beta 6$ and $\beta 7$) interact in a manner analogous to what has been described in other systems as a ball-and-socket interaction, which can be important for dimer stabilization.^{27,28} Interestingly, hydrophobic residues at these positions [denoted with ψ in Fig. 2(B)] are conserved among *Prx1* enzymes and it is clear that the loss of Val5 in *PyPrx1a*^{N*} coincides with the β -slip that leads to the disappearance of the socket [Fig. 2(D)] mostly due to a backbone conformation change

of Asn142 moving its side chain into the cleft. This backbone change of Asn142 explains the change in the path of the Asn143-Ser149 ($\beta 7$ - $\alpha 5$) loop in *PyPrx1a*^{N*} and the accompanying repositioning of Arg148 emphasized by Qiu *et al.* Finally, although beyond the scope of this report, the dodecameric assembly of the *Prx1 Mycobacterium tuberculosis* AhpC appears to result exclusively from an altered B-type dimer, in which the cleft is expanded rather than contracted. Interestingly, here the hydrophobic ball residue Val5 is substituted with the bulkier Ile, with socket residue Val141 replaced with Ala and, strikingly, Leu124 with the negatively-charged Asp.

From this analysis, we conclude that the *PyPrx1a*^{N*} octamer is not physiologically relevant. It does, however, provide a valuable case study for how small sequence changes can lead to striking differences in oligomeric organization, such as might occur during the natural evolution of a protein family. Since the ball-and-socket residues at the Val5 position and the contacting cleft residues Leu124/Val136/Leu139/Val141 are chemically conserved as hydrophobic residues among *Prx1* enzymes, the study makes clear that the N-terminal residues of *Prx1* family enzymes are significant contributors to the dimer interface and that it will be important for researchers to be cognizant of and explicit about modifications made to their native N-terminal sequences.

References

- Hall A, Karplus PA, Poole LB (2009) Typical 2-Cys peroxidoreductases—structures, mechanisms and functions. *FEBS J* 276:2469–2477.
- Hall A, Nelson K, Poole LB, Karplus PA (2011) Structure-based insights into the catalytic power and conformational dexterity of peroxidoreductases. *Antioxid Redox Signal* 15:795–815.
- Grete MC, Poole LB, Karplus PA (2012) Peroxidoreductases in parasites. *Antioxid Redox Signal* 17:608–633.
- Deponte M, Rahlfs S, Becker K (2007) Peroxidoreductase systems of protozoal parasites. *Subcell Biochem* 44:219–229.
- Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY, Ali MK, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Bahalim AN, Barker-Collo S, Barrero LH, Bartels DH, Basáñez MG, Baxter A, Bell ML, Benjamin EJ, Bennett D, Bernabé E, Bhalla K, Bhandari B, Bikbov B, Bin Abdulhak A, Birbeck G, Black JA, Blencowe H, Blore JD, Blyth F, Bolliger I, Bonaventure A, Boufous S, Bourne R, Boussinesq M, Braithwaite T, Brayne C, Bridgett L, Brooker S, Brooks P, Brugh TS, Bryan-Hancock C, Bucello C, Buchbinder R, Buckle G, Budke CM, Burch M, Burney P, Burstein R, Calabria B, Campbell B, Canter CE, Carabin H, Carapetis J, Carmona L, Cella C, Charlson F, Chen H, Cheng AT, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahiya M, Dahodwala N, Damsere-Derry J, Danaei G, Davis A, De Leo D, Degenhardt L, Dellavalle

- R, Delossantos A, Denenberg J, Derrett S, Des Jarlais DC, Dharmaratne SD, Dherani M, Diaz-Torne C, Dolk H, Dorsey ER, Driscoll T, Duber H, Ebel B, Edmond K, Elbaz A, Ali SE, Erskine H, Erwin PJ, Espindola P, Ewoigbokhan SE, Farzadfar F, Feigin V, Felson DT, Ferrari A, Ferri CP, Fèvre EM, Finucane MM, Flaxman S, Flood L, Foreman K, Forouzanfar MH, Fowkes FG, Fransen M, Freeman MK, Gabbe BJ, Gabriel SE, Gakidou E, Ganatra HA, Garcia B, Gaspari F, Gillum RF, Gmel G, Gonzalez-Medina D, Gosselin R, Grainger R, Grant B, Groeger J, Guillemin F, Gunnell D, Gupta R, Haagsma J, Hagan H, Halasa YA, Hall W, Haring D, Haro JM, Harrison JE, Havmoeller R, Hay RJ, Higashi H, Hill C, Hoen B, Hoffman H, Hotez PJ, Hoy D, Huang JJ, Ibeanusi SE, Jacobsen KH, James SL, Jarvis D, Jasrasaria R, Jayaraman S, Johns N, Jonas JB, Karthikeyan G, Kassebaum N, Kawakami N, Keren A, Khoo JP, King CH, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Laden F, Lalloo R, Laslett LL, Lathlean T, Leasher JL, Lee YY, Leigh J, Levinson D, Lim SS, Limb E, Lin JK, Lipnick M, Lipshultz SE, Liu W, Loane M, Ohno SL, Lyons R, Mabweijano J, MacIntyre MF, Malekzadeh R, Mallinger L, Manivannan S, Marcenes W, March L, Margolis DJ, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGill N, McGrath J, Medina-Mora ME, Meltzer M, Mensah GA, Merriman TR, Meyer AC, Miglioli V, Miller M, Miller TR, Mitchell PB, Mock C, Mocumbi AO, Moffitt TE, Mokdad AA, Monasta L, Montico M, Moradi-Lakeh M, Moran A, Morawska L, Mori R, Murdoch ME, Mwaniki MK, Naidoo K, Nair MN, Naldi L, Narayan KM, Nelson PK, Nelson RG, Nevitt MC, Newton CR, Nolte S, Norman P, Norman R, O'Donnell M, O'Hanlon S, Olives C, Omer SB, Ortblad K, Osborne R, Ozgediz D, Page A, Pahari B, Pandian JD, Rivero AP, Patten SB, Pearce N, Padilla RP, Perez-Ruiz F, Perico N, Pesudovs K, Phillips D, Phillips MR, Pierce K, Pion S, Polanczyk GV, Polinder S, Pope CA 3rd, Popova S, Porrini E, Pourmalek F, Prince M, Pullan RL, Ramaiah KD, Ranganathan D, Razavi H, Regan M, Rehm JT, Rein DB, Remuzzi G, Richardson K, Rivara FP, Roberts T, Robinson C, De León FR, Ronfani L, Room R, Rosenfeld LC, Rushton L, Sacco RL, Saha S, Sampson U, Sanchez-Riera L, Sanman E, Schwebel DC, Scott JG, Segui-Gomez M, Shahraz S, Shepard DS, Shin H, Shivakoti R, Singh D, Singh GM, Singh JA, Singleton J, Sleet DA, Sliwa K, Smith E, Smith JL, Stapelberg NJ, Steer A, Steiner T, Stolk WA, Stovner LJ, Sudfeld C, Syed S, Tamburlini G, Tavakkoli M, Taylor HR, Taylor JA, Taylor WJ, Thomas B, Thomson WM, Thurston GD, Tleyjeh IM, Tonelli M, Towbin JA, Truelsen T, Tsilimbarris MK, Ubeda C, Undurraga EA, van der Werf MJ, van Os J, Vavilala MS, Venketasubramanian N, Wang M, Wang W, Watt K, Weatherall DJ, Weinstock MA, Weintraub R, Weisskopf MG, Weissman MM, White RA, Whiteford H, Wiebe N, Wiersma ST, Wilkinson JD, Williams HC, Williams SR, Witt E, Wolfe F, Woolf AD, Wulf S, Yeh PH, Zaidi AK, Zheng ZJ, Zonies D, Lopez AD, AlMazroa MA, Memish ZA. (2012) Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380:2197–2223.
6. Sarma GN, Nickel C, Rahlfs S, Fischer M, Becker K, Karplus PA (2005) Crystal structure of a novel *Plasmodium falciparum* 1-Cys peroxiredoxin. *J Mol Biol* 346:1021–1034.
7. Nickel C, Trujillo M, Rahlfs S, Deponte M, Radi R, Becker K (2005) *Plasmodium falciparum* 2-Cys peroxiredoxin reacts with plasmoredoxin and peroxi-redoxin. *Biol Chem* 386:1129–1136.
8. Kehr S, Sturm N, Rahlfs S, Przyborski JM, Becker K (2010) Compartmentation of redox metabolism in malaria parasites. *PLoS Pathog* 6:e1001242.
9. Qiu W, Dong A, Pizarro JC, Botchkarev A, Min J, Wernimont AK, Hills T, Hui R, Artz JD (2012) Crystal structures from the *Plasmodium* peroxiredoxins: new insights into oligomerization and product binding. *BMC Struct Biol* 12:2.
10. Emsley P, Lohkamp B, Scott WG, Cowtan K (2010) Features and development of Coot. *Acta Crystallogr D* 66:486–501.
11. Bricogne GBE, Brandl M, Flensburg C, Keller P, Paciorek W, Roversi P, Sharff A, Smart OS, Vonnrhein C, Womack TO (2011) BUSTER. 2.11.2. Cambridge, United Kingdom: Global Phasing Ltd.
12. Hall A, Sankaran B, Poole LB, Karplus PA (2009) Structural changes common to catalysis in the Tpx peroxiredoxin subfamily. *J Mol Biol* 393:867–881.
13. Berkholtz DS, Faber HR, Savvides SN, Karplus PA (2008) Catalytic cycle of human glutathione reductase near 1 Å resolution. *J Mol Biol* 382:371–384.
14. Roberts BR, Wood ZA, Jonsson TJ, Poole LB, Karplus PA (2005) Oxidized and synchrotron cleaved structures of the disulfide redox center in the N-terminal domain of *Salmonella typhimurium* AhpF. *Protein Sci* 14:2414–2420.
15. Sarma GN, Manning VA, Ciuffetti LM, Karplus PA (2005) Structure of Ptr ToxA: an RGD-containing host-selective toxin from *Pyrenophora tritici-repentis*. *Plant Cell* 17:3190–3202.
16. Boucher IW, McMillan PJ, Gabrielsen M, Akerman SE, Brannigan JA, Schnick C, Brzozowski AM, Wilkinson AJ, Muller S (2006) Structural and biochemical characterization of a mitochondrial peroxiredoxin from *Plasmodium falciparum*. *Mol Microbiol* 61:948–959.
17. Guimaraes B, Souchon H, Honore N, Saint-Joanis B, Brosch R, Shepard W, Cole S, Alzari P (2005) Structure and mechanism of the alkyl hydroperoxidase AhpC, a key element of the *Mycobacterium tuberculosis* defense system against oxidative stress. *J Biol Chem* 280:25735–25742.
18. Yang KS, Kang SW, Woo HA, Hwang SC, Chae HZ, Kim K, Rhee SG (2002) Inactivation of human peroxiredoxin I during catalysis as the result of the oxidation of the catalytic site cysteine to cysteine-sulfinic acid. *J Biol Chem* 277:38029–38036.
19. Wood ZA, Poole LB, Karplus PA (2003) Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. *Science* 300:650–653.
20. Rhee SG, Woo HA (2011) Multiple functions of peroxiredoxins: peroxidases, sensors and regulators of the intracellular messenger H₂O₂, and protein chaperones. *Antioxid Redox Signal* 15:781–794.
21. Pascual MB, Mata-Cabana A, Florencio FJ, Lindahl M, Cejudo FJ (2010) Overoxidation of 2-Cys peroxiredoxin in prokaryotes: cyanobacterial 2-Cys peroxiredoxins sensitive to oxidative stress. *J Biol Chem* 285:34485–34492.
22. Barranco-Medina S, Lázaro J-J, Dietz K-J (2009) The oligomeric conformation of peroxiredoxins links redox state to function. *FEBS Lett* 583:1809–1816.
23. Wood ZA, Poole LB, Hantgan RR, Karplus PA (2002) Dimers to doughnuts: redox-sensitive oligomerization of 2-cysteine peroxiredoxins. *Biochemistry* 41:5493–5504.
24. Cao Z, Roszak AW, Gourlay LJ, Lindsay JG, Isaacs NW (2005) Bovine mitochondrial peroxiredoxin III forms a two-ring catenane. *Structure* 13:1661–1664.

25. Evans MR, Card PB, Gardner KH (2009) ARNT PAS-B has a fragile native state structure with an alternative beta-sheet register nearby in sequence space. *Proc Natl Acad Sci USA* 106:2617–2622.
26. Eneqvist T, Andersson K, Olofsson A, Lundgren E, Sauer-Eriksson AE (2000) The beta-slip: a novel concept in transthyretin amyloidosis. *Mol Cell* 6:1207–1218.
27. Thompson LC, Walters J, Burke J, Parsons JF, Armstrong RN, Dirr HW (2006) Double mutation at the subunit interface of glutathione transferase rGSTM1-1 results in a stable, folded monomer. *Biochemistry* 45:2267–2273.
28. LeFevre KR, Cordes MH (2003) Retroevolution of lambda Cro toward a stable monomer. *Proc Natl Acad Sci USA* 100:2345–2350.