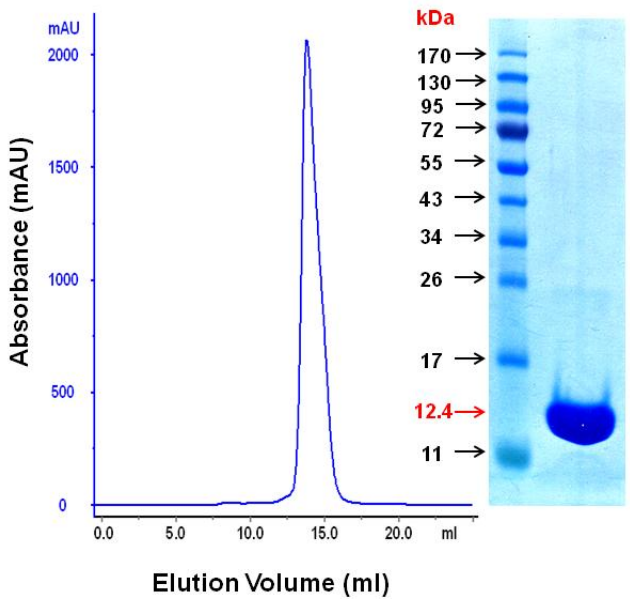
Bound MOPS and MPD molecules

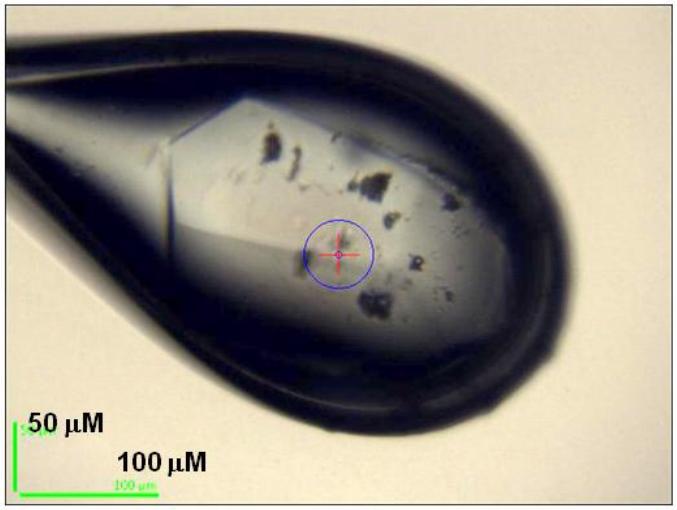
Solvent-exposed GSH binding channel in PfGrx1 is filled with several water molecules, and $^{\gamma}$Glu portion of GSH binding pocket is occupied by MOPS (Figs. 1c, 5a, Supplementary Fig. S4a). The binding mode of sulfonic group of MOPS is akin to bound 2-(n-morpholino)-ethane sulfonic acid (MES) in oxidized ScGrx1 (PDB code 3C1R, Yu et al., 2008), SO_4 in SyGrxA (PDB code 3QMX, Kim et al., 2011) from *Synechocystis sp*. PCC 6803 as well as the Grx-domain of human thioredoxin reductase 3 (PDB code 3H8Q). In PfGrx1, S atom from sulfonic group occupies the carboxylic carbon atom position of $^{\gamma}$Glu of GSH, while O1 and O2 atoms interact with main-chain N atoms of Asp99 and Cys88 respectively. Sulfonic group oxygen atom from MOPS hydrogen bonds with four water molecules. Propane chain carbon atoms (C1 and C3) stack with the side chain of Tyr32 and the distance between them is ~3.8 Å. The morpholine ring is in a chair conformation, and the protonated N^{+} atom forms a salt bridge with both carboxylate oxygen atoms of Asp99. The solvent-exposed portion of MOPS is masked by more than ten water molecules which form four and five-member ring structures (Fig. 5a, Supplementary Fig. S4a). A molecule of 2-methyl-2,4-pentanediol (MPD) used for crystallization occupies the hydrophobic void created by residues Asn13, Ile16, and Glu17 from α1-helix, residues Tyr65 and Leu69 from the α3-helix, and residues Phe79, Lys82, and Val84 from β3/β4-strands (Supplementary Fig. S4b). The 4-hydroxyl group of MPD H-bonds with main-chain carbonyl O atom of Lys82 and two water molecules, while the 2-hydroxyl group H-bonds with side-chain $O^{\delta 1}$ atom of Asn13.

The secondary structural elements connecting loop/turn conformations in Grxs and Grx-like domain

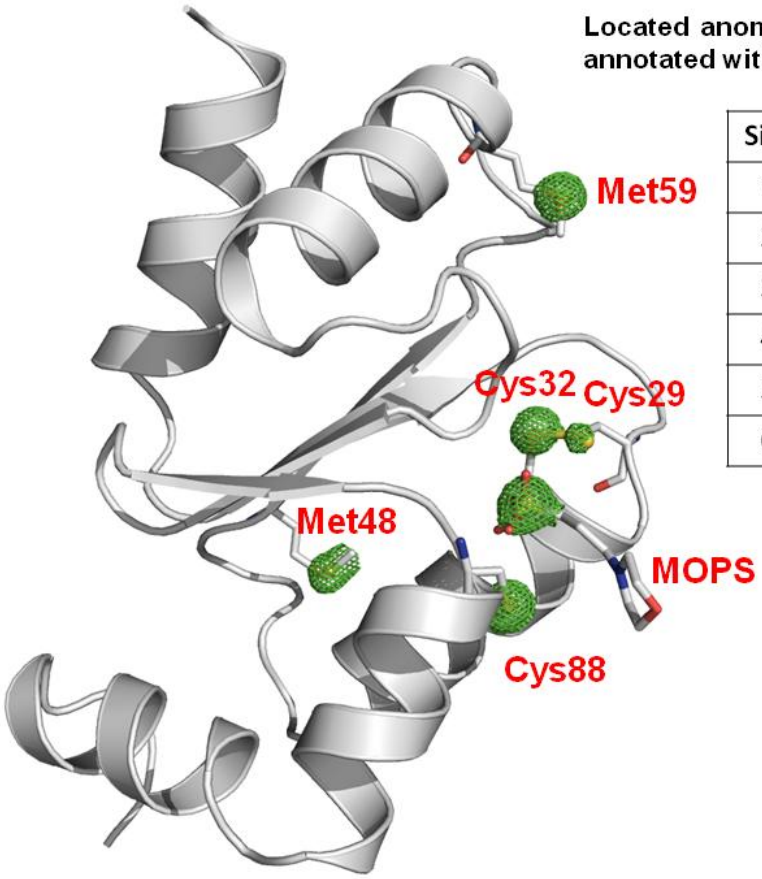
Three different types of hydrogen bonding networks are observed in the C-terminal regions of β1- and β2-strands and N-cap of α2-helix (Supplementary Fig. S6). The loop between β1-strand and α2-helix in both CXXC and CSYS Grxs adopts two different conformations except for EpGrx which has a four residue insertion. This loop in PfGrx1, ScGrx1, and SsGrx1 adopts similar conformations, and the β-turn motif is absent due to flipping the carbonyl oxygen at position 27 when compared to remaining structures. This loop has a five residues insertion in CGFS Grxs (AtGrxCp, EcGrx4, HsGrx5, HsGrx3D1, HsTXLN2 and ScGrx5) which adopts a β-hairpin motif and is structurally conserved. Three different types of hydrogen bonding networks are observed in the C-terminal regions of β1- and β2-strands and N-cap of α2-helix (Supplementary Fig. S7). In some Grxs (PfScGrx1, ScGrx2, ScGrx5, SsGrx1, and EvGrx), a β-turn motif occurs due to two or three-residue insertions in loop between α2-helix and β2-strand. This β-turn motif induces an additional hydrogen bond between the β1 and β2-strands. In ScGrx1, ScGrx2, and ScGrx6, the C-terminal portion of α2-helix has an extra residue insertion, and H-bonds in helix-capping region differ from other Grxs. In CXXC and CSYS containing Grxs, the α3-helix ends in a Gly-based capping motif and this feature is absent in CGFS containing Grxs (Supplementary Fig. S8a). In most of mono- and dithiol Grxs, α4-helix ends with Gly-based Schellman motif (Supplementary Fig. S8b).



Supplementary Figure S1. GPC profile and SDS-PAGE gel for purified PfGrx1. (c). Active site CPYC motif in PfGrx1-SAD structure.



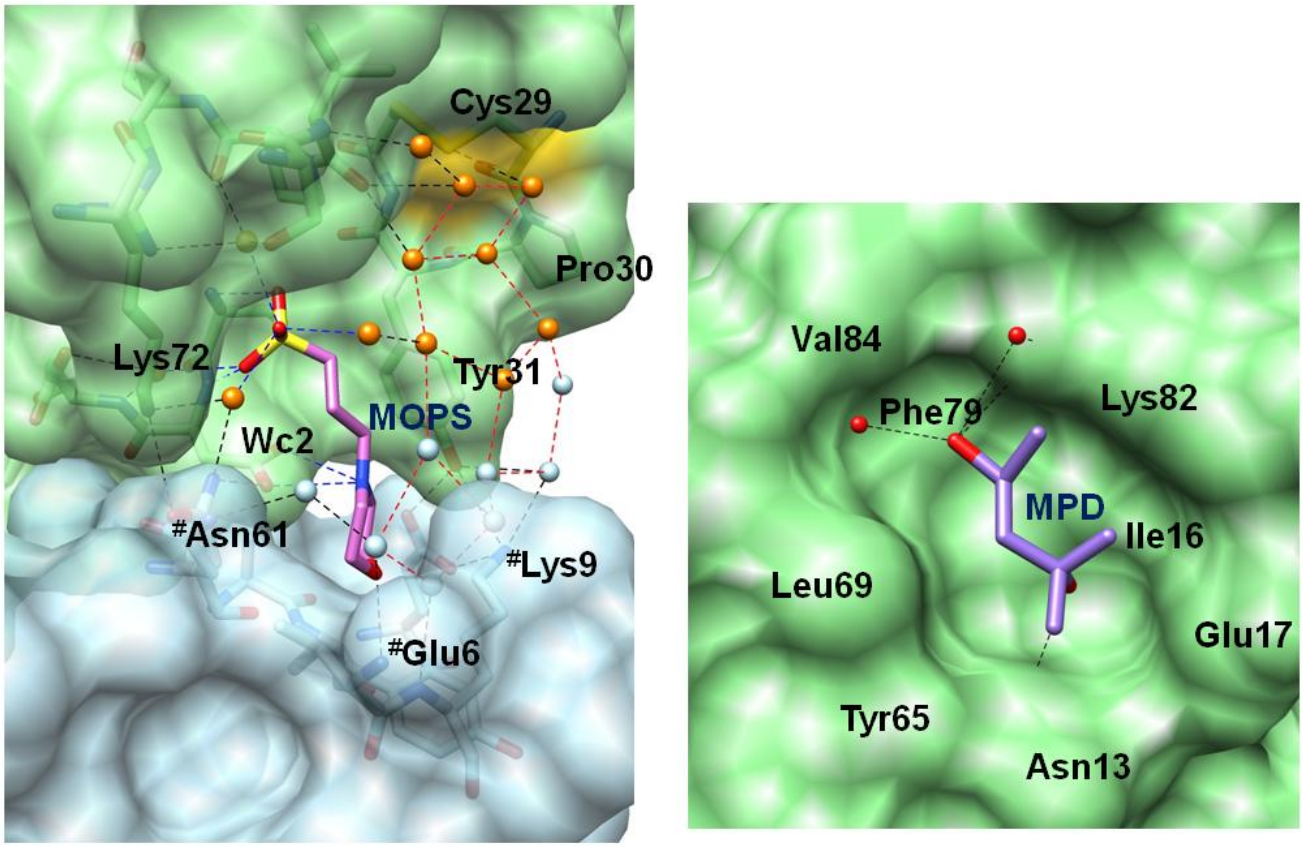
Supplementary Figure S2. The crystal used for PfGrx1-AR2 data collection, which was grown in a hanging-drop setup.



Located anomalous scatterers and each site annotated with the corresponding atom

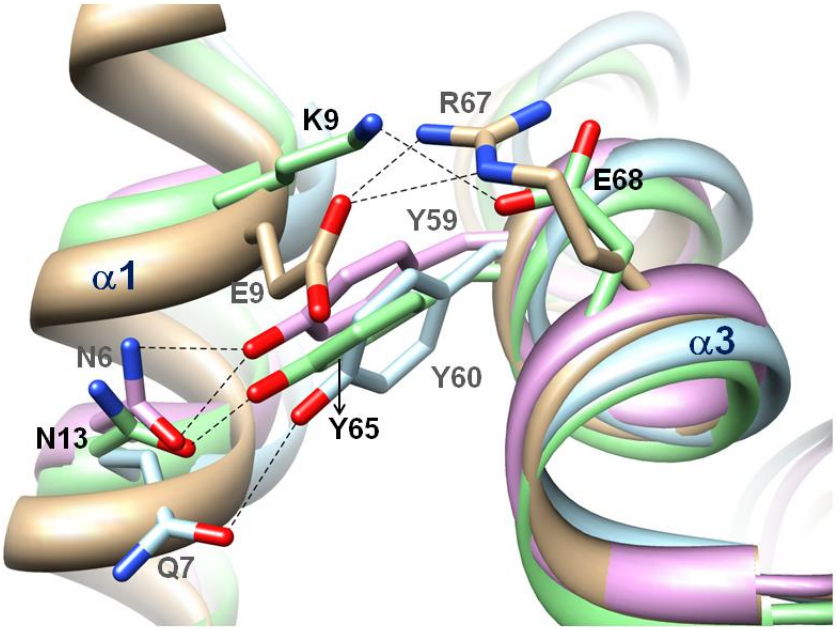
Site	Occupancy	Atom
1	1.00	S - MOPS
2	0.97	S^{γ} – Cys88
3	0.95	S^{γ} – Cys32
4	0.84	S^{δ} – Met59
5	0.75	S^{γ} – Cys29
6	0.56	S^{δ} – Met48

Supplementary Figure S3. Anomalous difference Fourier maps around S atoms at 4 σ (green).

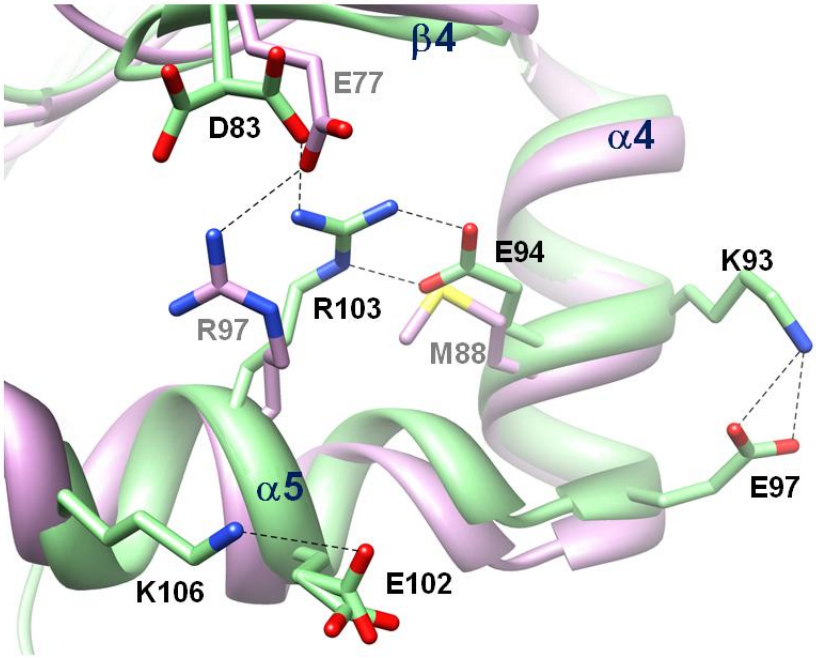


(a) **(b)**

Supplementary Figure S4. (a) Hydrogen-bonding network around MOPS binding site. Four and five-membered water molecule rings are shown as red dashed lines. Reference and symmetry-related molecules are shown in green and blue, respectively. Bound MOPS is shown as a stick model. (b) Surface representation of MPD binding site and bound MPD is shown as stick model.

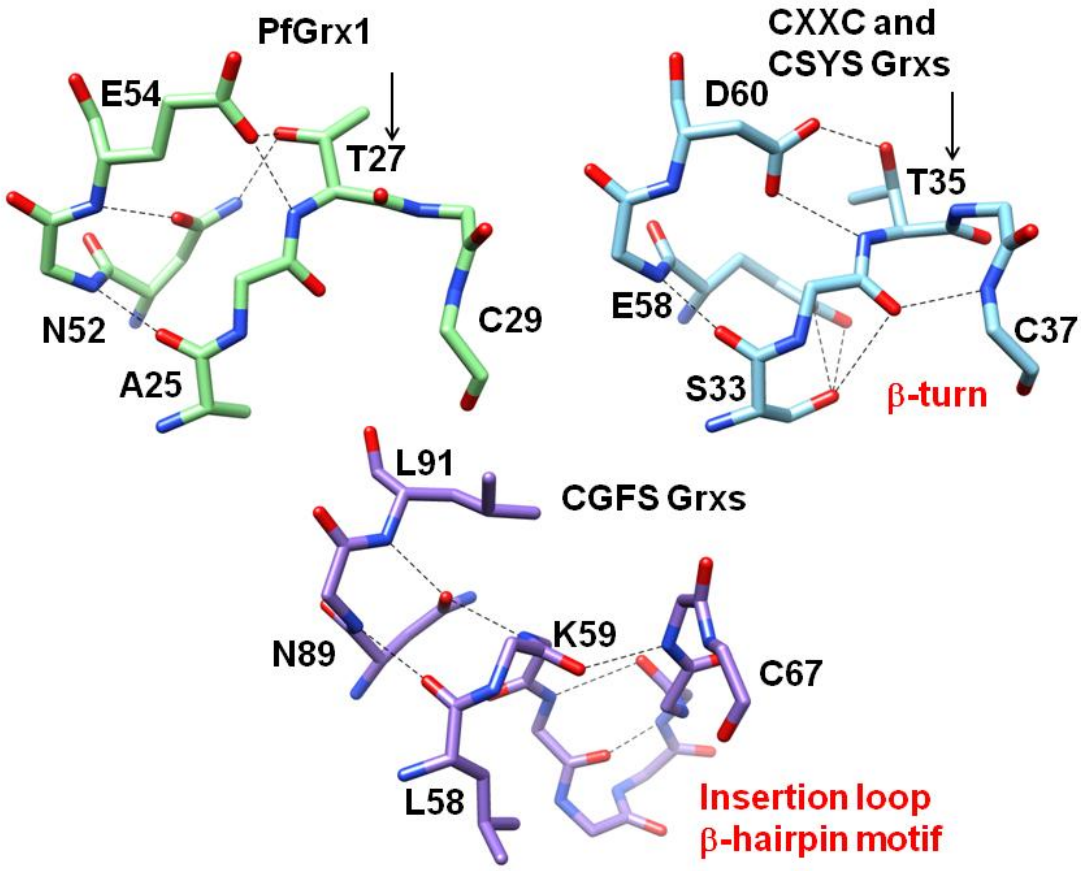


(a)

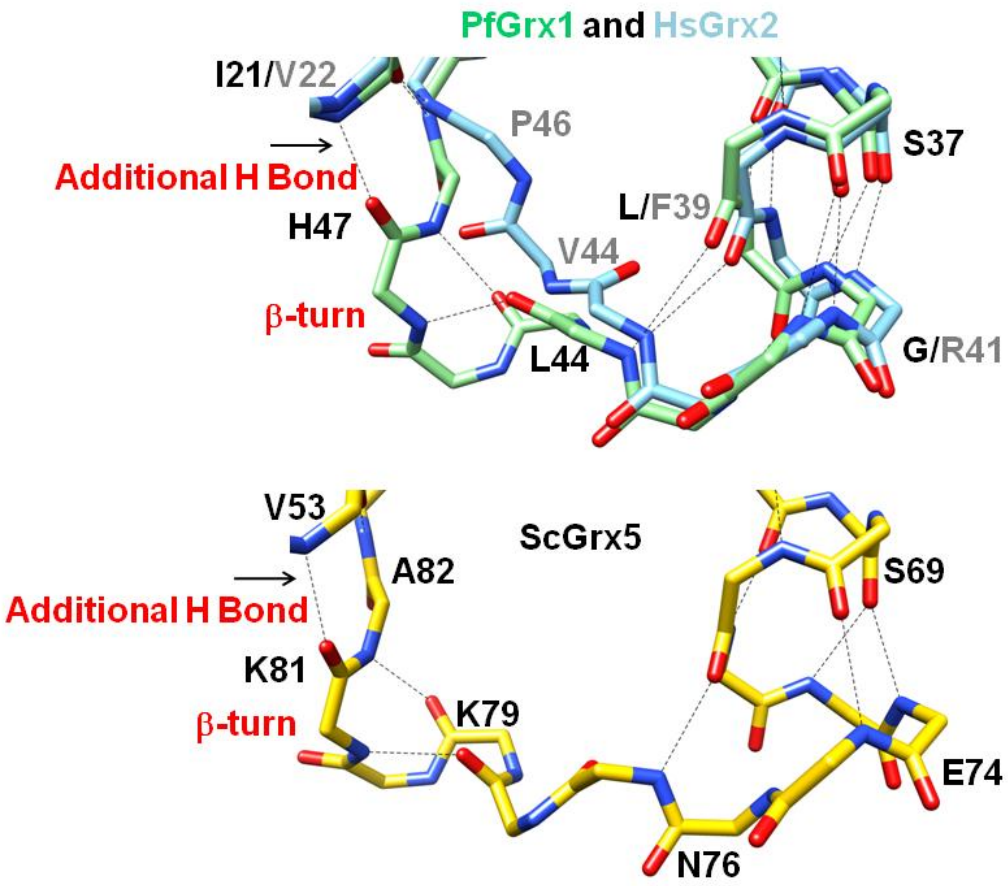


(b)

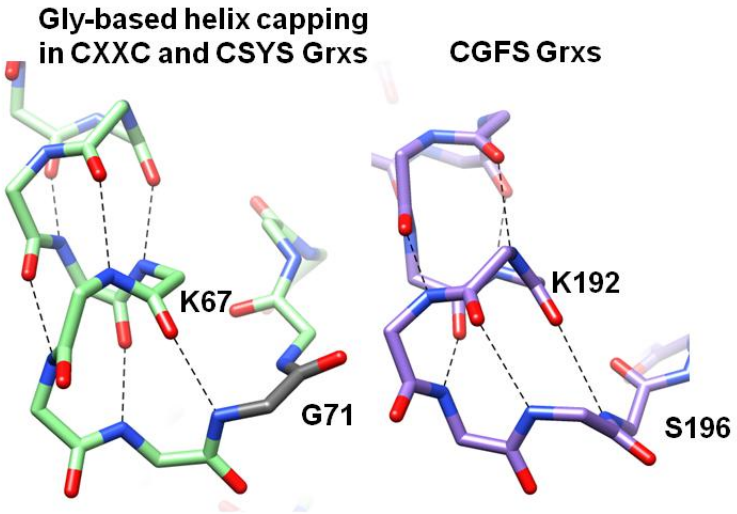
Supplementary Figure S5. (a). Unique interactions between the α1- and α3-helices in PfGrx1, SsGrx1 (pink), EvGrx (blue), and PtGrxS12 (brown). (c). Unique salt ridges between the β4-strand, α4, and α5-helices in PfGrx1 (green) and SsGrx1 (pink).



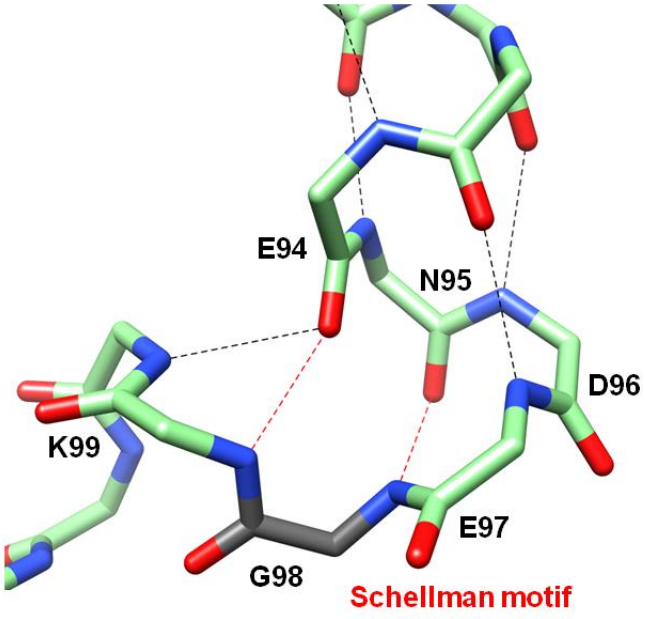
Supplementary Figure S6. Comparison of N-Cap region of α2-helix. The flipping of the carbonyl group is indicated by an arrow. The five-residue insertion in CGFS Grxs adopts β-hairpin motif.



Supplementary Figure S7. Two different types of C-cap in α2-helix and two different conformations of loop between α2-helix and β2-strand.



(a)



(b)

Supplementary Figure S8. (a). Comparison of α3-helix capping in CXXC and CXXS Grx structures. (b). Gly-based Schellman motif in α4-helix capping in both mono- and dithol-Grx structures.

Table S1. Anomalous signal-to-noise ratio ($\langle \Delta F/\sigma(\Delta F) \rangle$)

Resolution	∝-8	8-6	6-5	5-4	4-3.5	3.5-3.0	3.0-2.5	2.5-2.2	2.2-2.0	2.0-1.8	1.8-1.55
No. Reflections	143	185	236	491	507	859	1690	1859	1899	2828	5877
$\langle I/\sigma(I) \rangle$	104.1	103.1	103.9	112.5	109.7	102.8	86.7	74.3	61.1	42.4	19.2
% Complete	91.7	98.9	99.2	99.4	99.6	99.8	99.8	99.8	99.5	99.3	97.7
$\langle \Delta F/\sigma(\Delta F) \rangle$	1.61	2.12	2.14	1.43	1.41	1.42	1.39	1.29	1.2	1.12	0.99

Table S2. List of double conformations in all PfGrx1 models. The occupancy of the major conformation is indicated and the other conformation is complementary. The sign '-' indicates that no additional conformation was modeled.

Residue	PfGrx1-SAD	PfGrx1-AR1	PfGrx1-AR2
Lys14	0.50	0.74	0.55
Glu17	0.54	0.51	-
Glu18	0.54	0.51	-
Ile21	0.52	0.57	0.58
Lys26	0.50	0.74	0.58
Cys29	0.65	-	-
Pro30	0.65	-	-
Ile33	0.63	-	-
Ser37	0.64	0.50	0.50
Asn43	0.57	0.58	0.55
Ser46	0.60	0.55	0.61
Met48	0.55	0.65	-
His49	0.55	-	-
Lys55	0.53	0.87	0.73
Ans61	0.53	0.67	-
Lys72	0.51	0.61	0.67
Arg77	0.52	0.51	0.55
Asn81	0.51	0.51	-
Lys82	0.51	0.51	0.55
Asp83	0.51	0.51	0.55
Asp90	0.51	-	-
Asn99	0.53	-	0.71
Glu102	0.52	-	0.62
Lys101	-	0.74	-
Gln105	-	0.74	-