Structure, mechanism and ensemble formation of the Alkylhydroperoxide Reductase subunits AhpC and AhpF from *Escherichia coli*

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Supplemental Figure Legends

Figure S1: (*A*) The homo-dimeric form of *Ec*AhpF, revealing a maximum length of 170 Å. The second symmetry related *Ec*AhpF molecule is depicted with lighter shaded color. The 90° view shows the distance (125 Å) between the NTD redox centers (C129/132 and C129'/132') of the molecule. The dimer interface covers mainly the FAD-FAD'-domain with the solvent accessible area of 2,520 $Å^2$, which is around 10% of the total monomeric solvent accessible area of 25,103 $Å^2$. The interface is stabilized by hydrophobic- and hydrophilic interactions, like hydrogen bonds and salt bridges. (*B*) The N-terminal domain redox-active dithiol (C129/132) is reduced in the 2 Å resolution structure, while oxidized in the 2.65 Å one. Stereo view of the omit map (σ-weighted Fo-Fc (mFo-DFc) electron density map) contoured at 3 σ for the region around the N-terminal domain redox center (C129/132) shows that NTD is oxidized in the 2.65 Å structure (*i*) and reduced in the 2 Å resolution structure (*ii*). (*C*) The *E. coli* AhpF crystal structure at 2 Å and 2.65 Å resolution. The four segments of both *Ec*AhpF structures, the N-terminal domain (orange), linker segment (green), the FAD-domain (yellow) and the NADH-domain (brown) were aligned (the structure at 2.65 Å is depicted in lighter shades). The NTD adopts different conformation and the redox-active dithiol (C129/132) (purple and red) is reduced in the 2 Å resolution structure, while oxidized in the 2.65 Å structure. (*D*) Cadmium ion is located in the interface between neighboring *Ec*AhpF molecules. Two highly conserved and catalytically important histidine side chains (H130 and H85) are protruded on the same side to make a positively charged groove, wherein one cadmium ion from the crystallizing solution is bound. The cadmium ion forms six coordinated bonds, two to E110'and each to H85, His114', H130 and one water molecule, respectively. The symbol (') highlight the symmetry related residue.

Figure S2: In order to identify the NADH binding pocket, the *Ec*AhpF structures were compared with the *E. coli* TrxR (PDB ID: 1TDF), which has a bound $NADP^+$. The $NADP^+$-binding motif

($_{152}$GGGNTA$_{157}$) is conserved with the sequence of Gly-X-Gly-X-X-Ala in all NADP-binding domains, in which the alanine is replaced with glycine in *Ec*AhpF structure ($_{362}$GGGNSG$_{367}$). Another important substitution is the replacement of the polar R176 to non-polar F386 in the *Ec*AhpF structure. R176 in *Ec*TrxR interacts with the phosphate group of $NADP^+$ and this interaction is hindered by the substitution to F386 in the *Ec*AhpF structure, which might explains its higher preference for NADH over NADPH. A significant structural difference was observed in the nicotinamide interacting loop region in the FAD-domain. In *Ec*AhpF, the loop is displaced away from the nicotinamide ring by about 3.5 Å relative to *Ec*TrxR, wherein the nicotinamide interacting residues, H290 and R293, are replaced by V492 and K495 in *Ec*AhpF, respectively. In the 2.65 Å *Ec*AhpF structure, two sulphate molecules are found. The first sulphate is hydrogen bonded to NADP binding motif residues N365 and S366 and the second one is close to the FAD molecule and is hydrogen bonded with W326, Q448 and G450. In the 2.0 Å *Ec*AhpF structure, the NADH binding pocket is filled with one sulfate, two glycerol and one PEG molecules. The sulfate occupies similar position as that in the 2.65 Å *Ec*AhpF structure and has interactions with N365 and S366. The first glycerol has interaction with K391 and the other one has interaction with F386 and E385. The PEG molecule interacts with V447, I449, G450, L451, S366 and R327. These interacting residues may likely represent the NADH binding pocket residues of AhpF. It is also been seen from both the structure that the sulfate molecule bound in the NADH binding pocket may have addition role by enhancing the NADH binding and hence in the catalytic activity of AhpF.

Figure S3: Solution X-ray scattering studies of *EcAhpF*. (*A*) Small angle X-ray scattering pattern (○) and its corresponding fitting curves (—; green: experimental, red: calculated) at 8 mg/ml concentration. The curves of *E. coli* AhpF are displayed in logarithmic unit for clarity. (*B*) Distance distribution functions of *Ec*AhpF at 8 mg/ml concentration. (*C*) *Ec*AhpF solution shape as calculated by GASBOR program. (*D*) Theoretical scattering curves for the monomer (magenta) and dimer (orange) of open conformation and monomer (blue) and dimer (cyan) of closed conformation for *Ec*AhpF calculated

using the CRYSOL program fits with experimental scattering pattern (○) with a χ-value of 4.4 and 2.1 and 5.1 and 2.2, respectively.

Figure S4: (*A*) Electron density for the redox active C_p47'/C_R166 disulfide bond between molecule A (green) and molecule B (cyan). The omit map (σ-weighted Fo-Fc (mFo-DFc) electron density map) is contoured at 2 σ (green lines) around the disulphide bond. The symbol ‘ is used for residues of the symmetry subunit in the dimer formation. (*B*) The redox active cysteines (C47/C166’ and C47’/C166) of *St*AhpC are depicted in magenta and are not involved in an intermolecular disulfide bond. The C-terminal amino acids of *St*AhpC are ordered and form intermolecular hydrogen bonds.

Figure S5: The FAD- and NADH-domain are responsible for the head-to-tail dimer formation in the *Ec*AhpF. The second symmetry related *Ec*AhpF molecule is depicted with lighter shaded color. The dimer interface is stabilized by the hydrophobic, hydrogen and salt bridge interactions. There is no significant difference observed in the dimer interface between the *Ec*AhpF- and *St*AhpF structures, except for an additional salt bridge between K234 and D373 and a bifurcated hydrogen bond between the side chain nitrogen atom of R233 with main chain oxygen atoms of H347 and C348, that could have occurred in *Ec*AhpF. Such bridge is missing in *St*AhpF, due to the rotational motion of the NADH-domain. The symbol * indicates the residues, belonging to symmetry molecule.

Figure S6: The high purity of *E. coli* AhpF-CTD (*A*) and NTD_{1-196} (*B*) are shown in the SDS-PAGE. Small angle X-ray scattering pattern (○) of *Ec*AhpF-CTD (*F*) and *Ec*NTD_{1-196} (*C*), and the corresponding experimental fitting curves (green; the red curve represents the calculated one). (*D, G*) Distance distribution function of *Ec*AhpF-CTD (*G*) and NTD_{1-196} (*D*). The solution shape of *Ec*AhpF-CTD and *Ec*NTD_{1-196} are revealed in column (*H*) and (*E*), which have been calculated by GASBOR program.

(A)

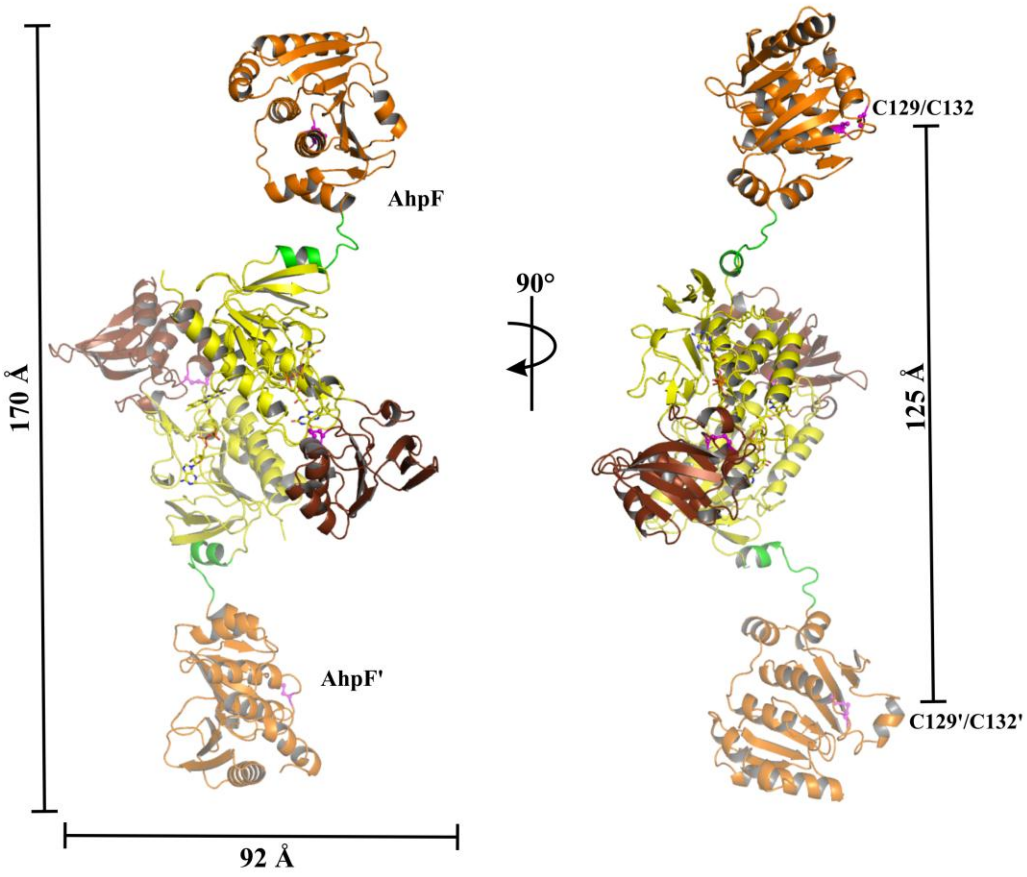


Figure S1A

(B(i)) **(B(ii))**

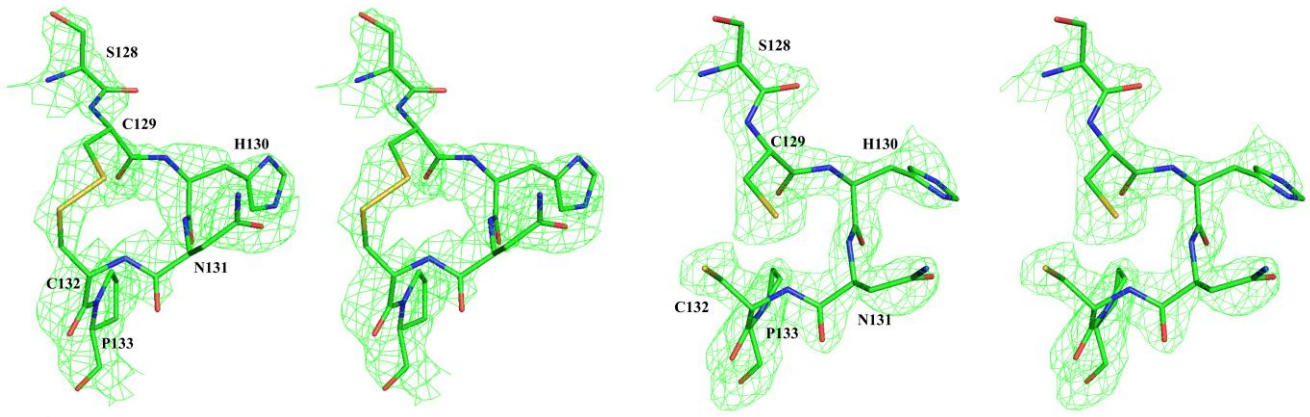
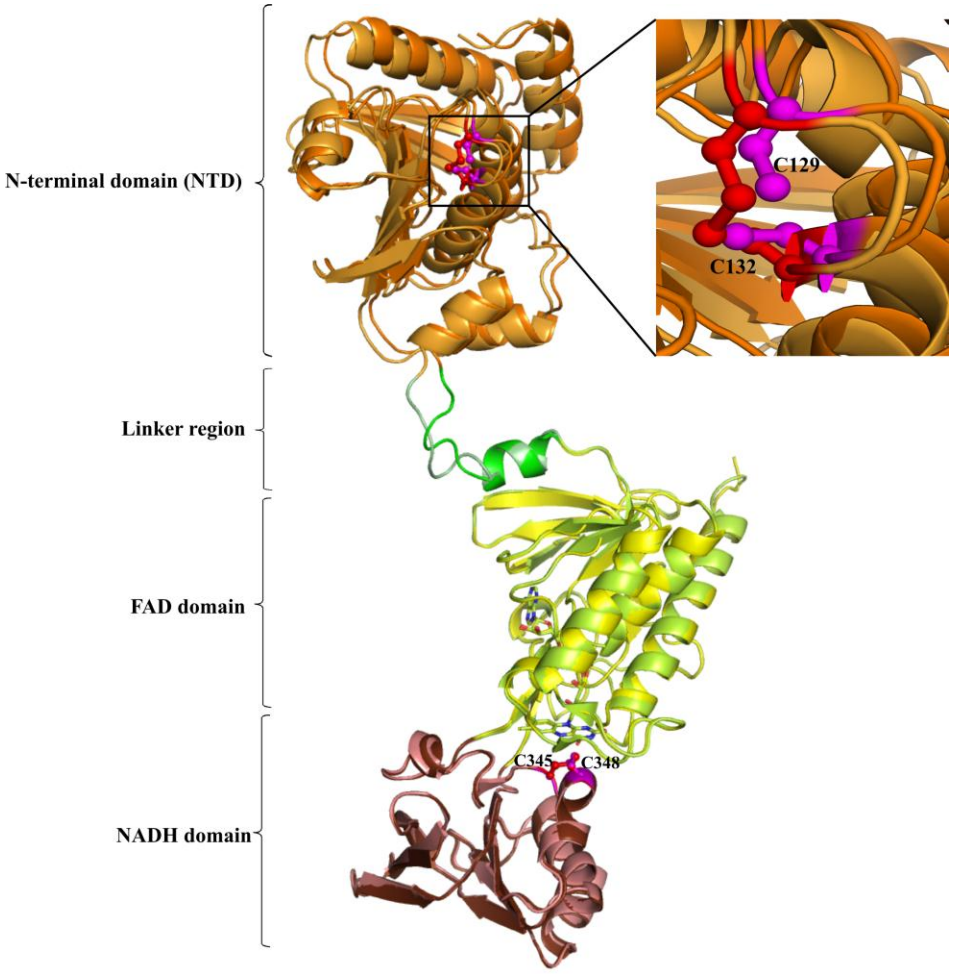


Figure S1B

(C)



(D)

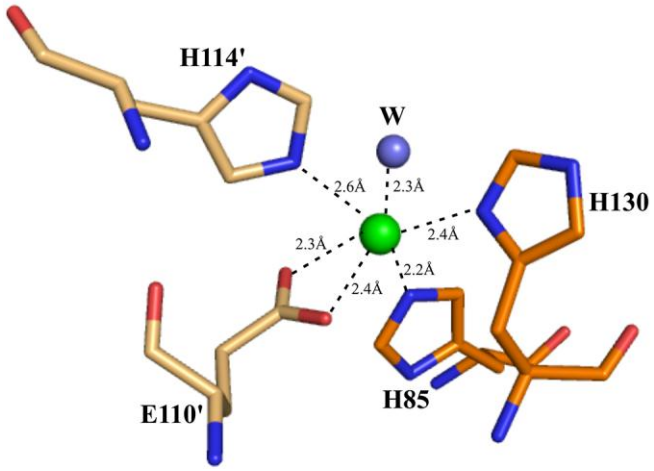


Figure S1C-D

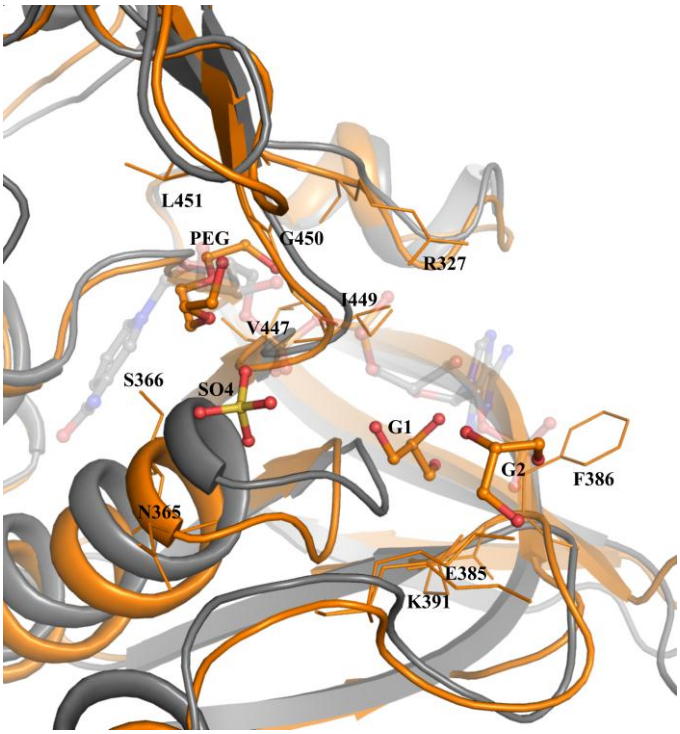
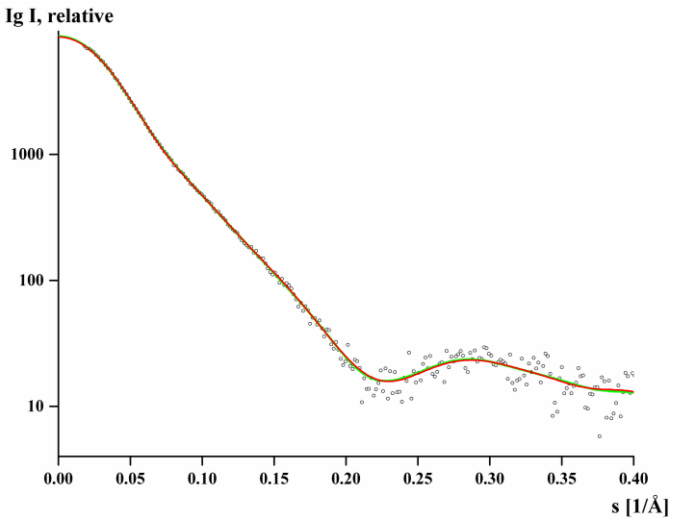
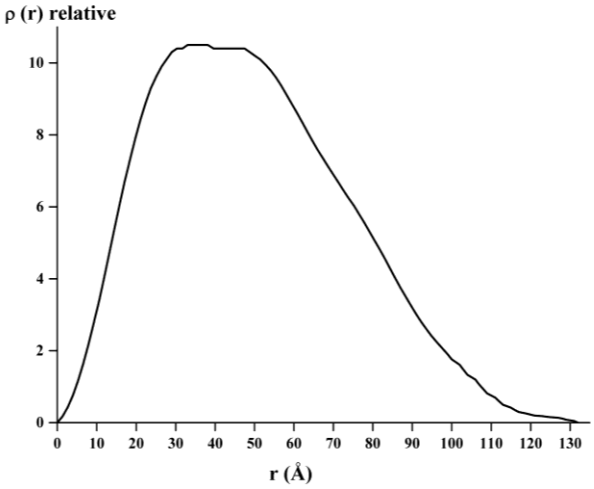

L451
PEG
G450
R327
I449
V447
S366
SO4
G1
G2
F386
N365
E385
K391

Figure S2

(A)



(B)



(C)

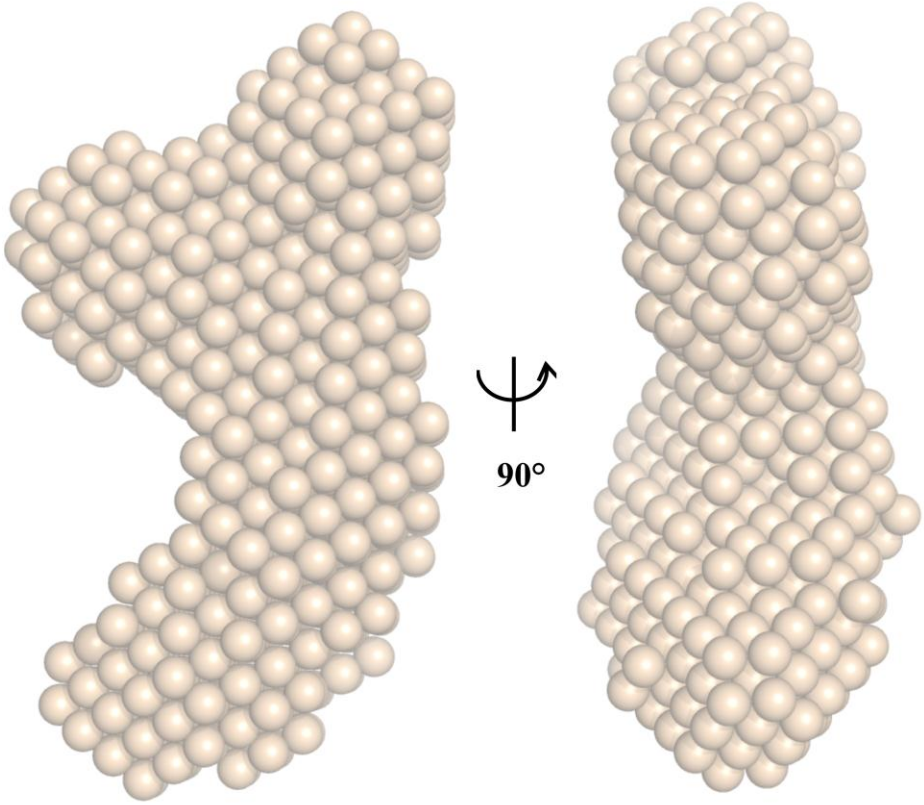


Figure S3A-C

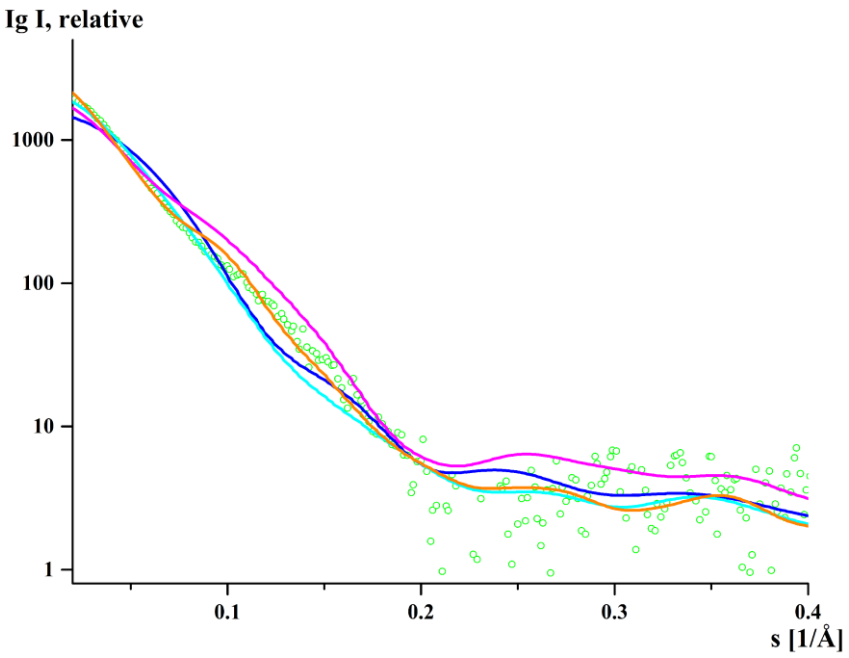
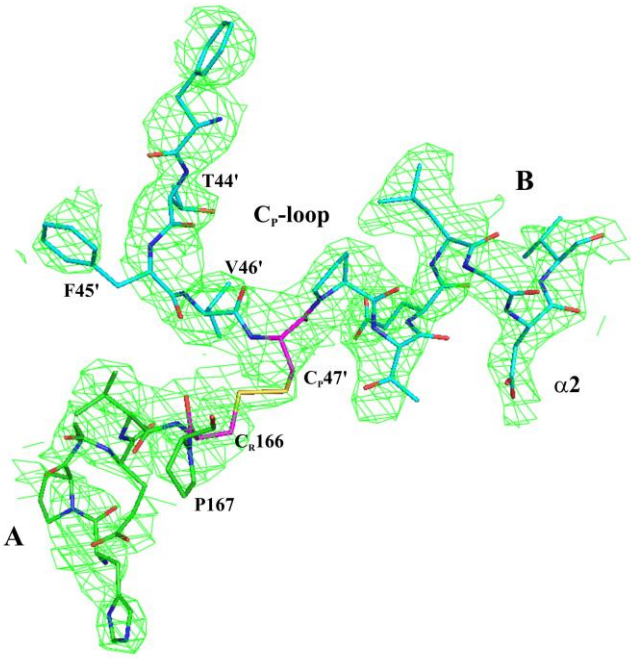


Figure S3D

(A)



(B)

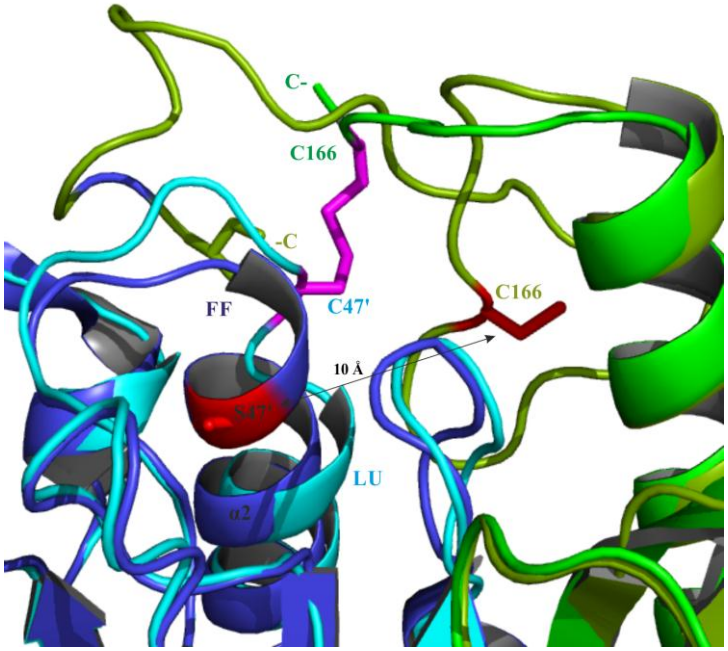


Figure S4A-B

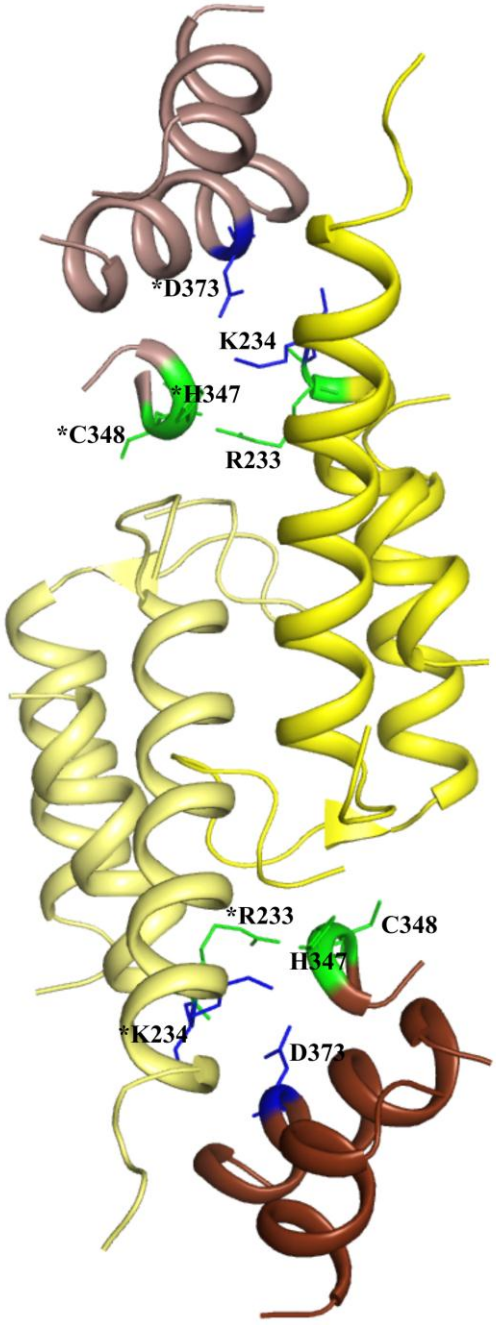


Figure S5

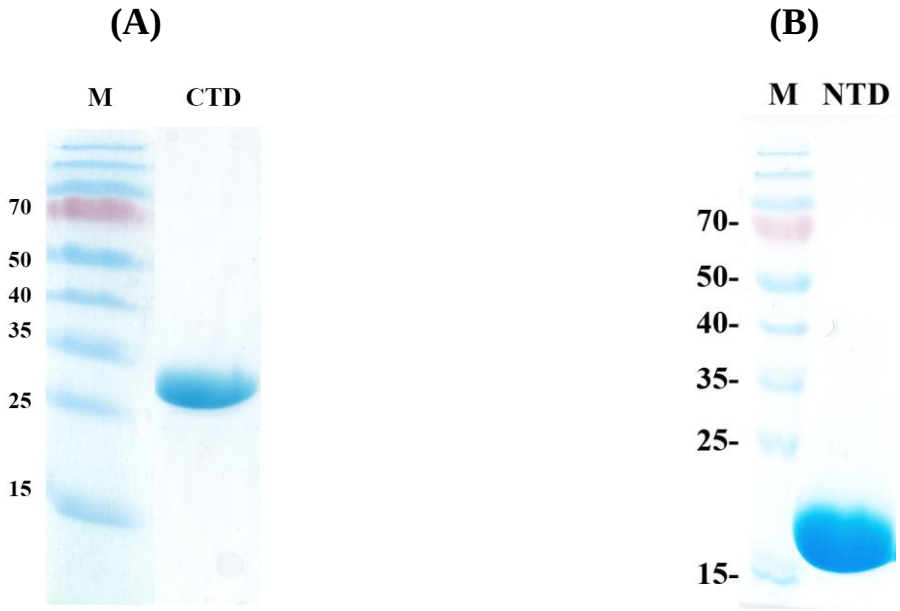


Figure S6A-B

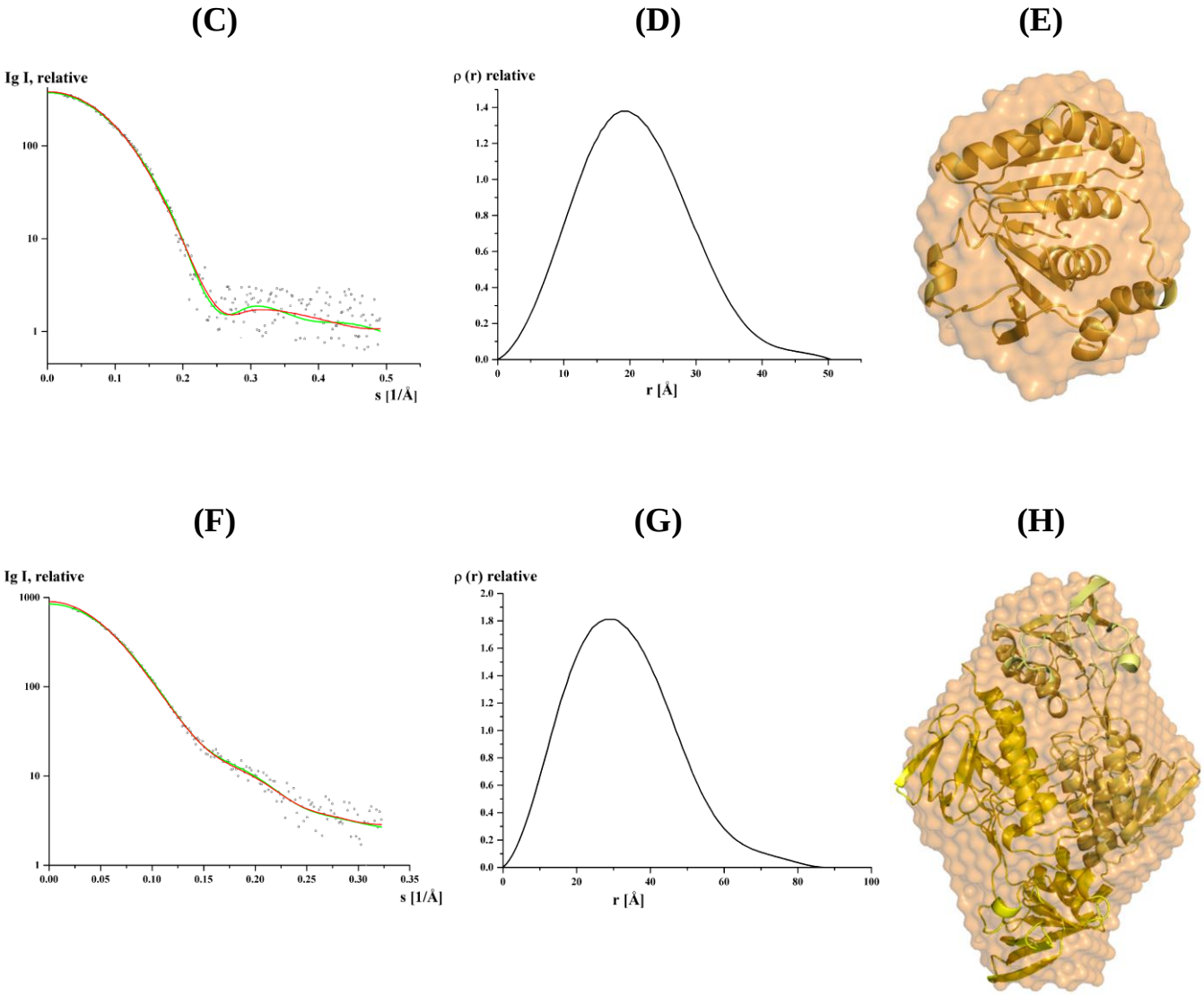

(C)
(D)
(E)
Ig I, relative
100
10
1
0.0
0.1
0.2
0.3
0.4
0.5
s [1/Å]
ρ (r) relative
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
r [Å]
(F)
(G)
(H)
Ig I, relative
1000
100
10
1
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
s [1/Å]
ρ (r) relative
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
r [Å]

Figure S6C-H