

Supplementary information for

Structural and biochemical characterization of a dye decolorizing peroxidase from *Dictyostelium discoideum*

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thetaitaomicron VPI-5482 BtDyP (UniProt ID: Q8A8E8), *Rhodococcus jostii* RHA1 RjDyPB (UniProt ID: Q0SE24), *Auricularia auricula-judae* AauDyPI (UniProt ID: I2DBY1), *Bjerkandera adusta* BadDyP (UniProt ID: Q8WZK8)

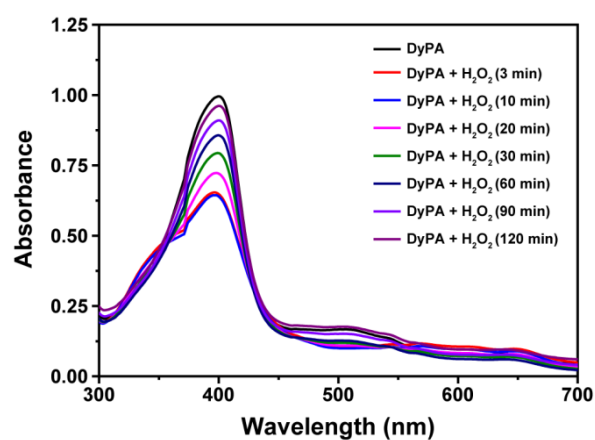


Figure S2: UV-visible absorption spectra of *Dictyostelium* DyPA in the presence of H₂O₂. Spectra of 10 μM *Dictyostelium* DyPA in the absence (black) or in the presence (red to magenta) of 10 μM H₂O₂ at different time points. Measurements performed in buffer containing 50 mM Tris pH 8.0, 150 mM NaCl at 23°C.

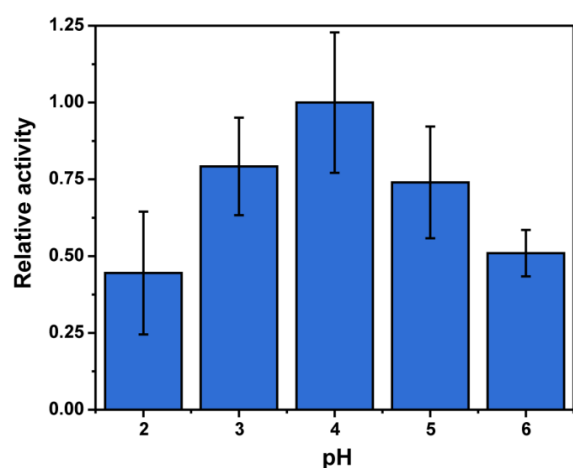


Figure S3: Optimum pH for the oxidation of veratryl alcohol by *Dictyostelium* DyPA. Assays were performed in 100 μl of 50 mM sodium acetate (pH 2.0 – 6.0) and 150 mM NaCl at 25 °C, containing 10 mM VA and 4 μM *Dictyostelium* DyPA. Reactions were initiated by the addition of 1 mM H₂O₂ and monitored at 310 nm (VA_ε₃₁₀ = 9.3 mM⁻¹ cm⁻¹). Data are average values of 3–6 independent measurements; bars represent the standard deviation.

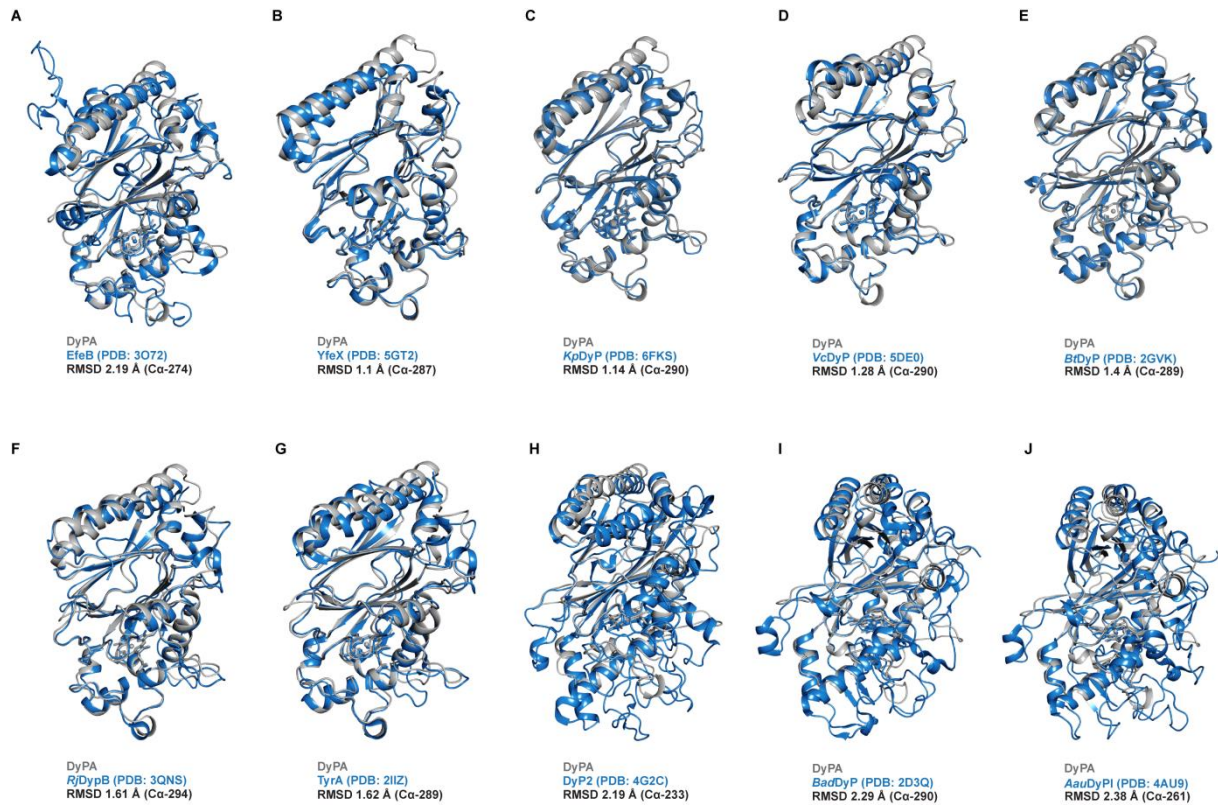


Figure S4. Structural comparison of *Dictyostelium* DyPA with related bacterial and fungal dye decolorizing peroxidases. Structural alignment of *Dictyostelium* DyPA (grey) with (A) *Escherichia coli* O157 EfeB (blue; class A). (B) *Escherichia coli* O157 YfeX (blue; class B). (C) *Klebsiella pneumoniae* KpDyP (blue; class B). (D) *Vibrio cholerae* VcDyP (blue; class B). (E) *Bacteroides thetaiotaomicron* VPI-5482 BtDyP (blue; class B). (F) *Rhodococcus jostii* RHA1 RjDypB (blue; class B). (G) *Shewanella oneidensis* TyrA (blue; class B). (H) *Amycolatopsis* sp. 75iv2 DyP2 (blue; class C). (I) *Bjerkandera adusta* BadDyP (blue; class D). (J) *Auricularia auricula-judae* AauDyPI (blue; class D).

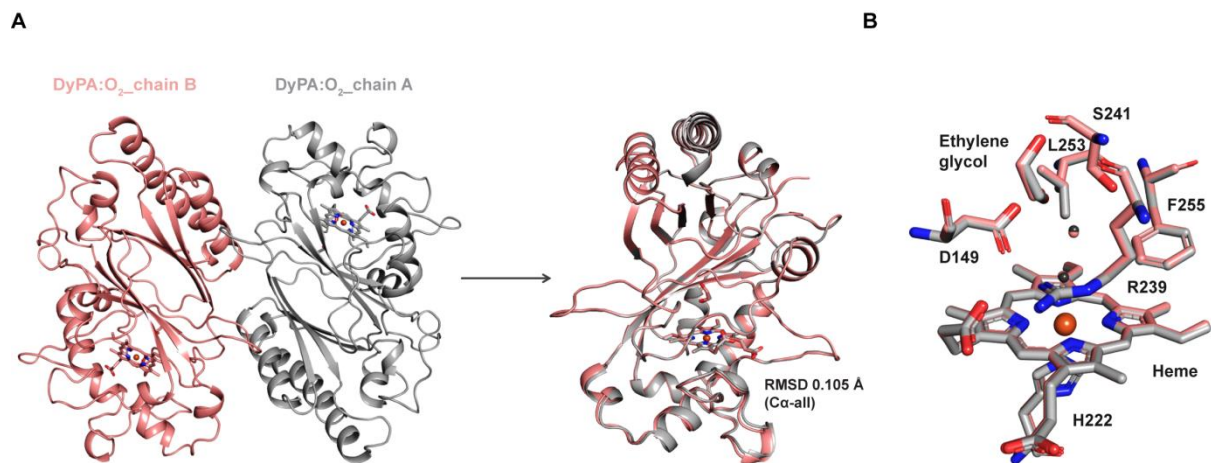


Figure S5: Structure of the *Dictyostelium* DyPA:O₂ complex. **(A)** The asymmetric unit contains two nearly identical copies of DyPA, as shown by the overlay of both structural models. **(B)** Heme microenvironment of the *Dictyostelium* DyPA:O₂ complex. Grey/salmon pink spheres show the position of the oxygen atoms in the *Dictyostelium* DyPA:O₂ complex structure.

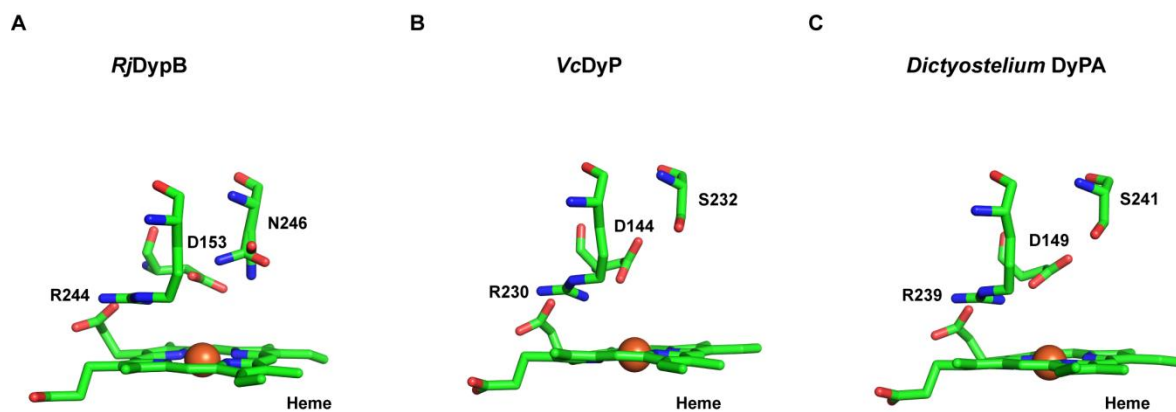


Figure S6. Heme microenvironment. **(A)** *RjDypB* (PDB: 3QNS), **(B)** *VcDyP* (PDB: 5DE0) and **(C)** *Dictyostelium* DyPA.

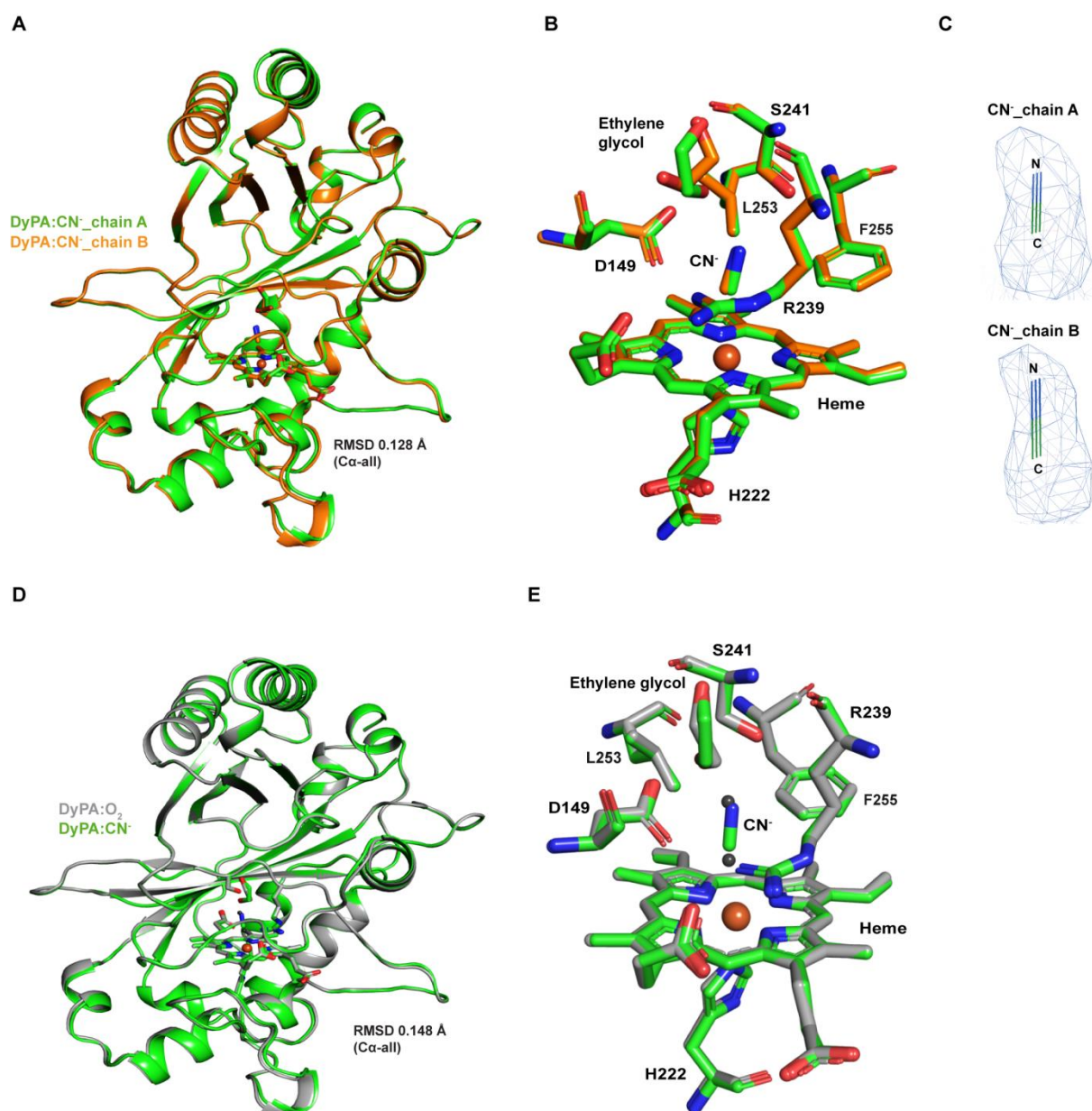


Figure S7: Structure of *Dictyostelium* DyPA:CN⁻ complex. **(A)** Structural alignment of both copies of the *Dictyostelium* DyPA:CN⁻ complex in the asymmetric unit reveals that both copies are nearly identical. **(B)** Heme microenvironment of the *Dictyostelium* DyPA:CN⁻. **(C)** OMIT electron density maps of CN⁻ moieties, contoured at 1.5 σ . **(D)** Structural alignment of single monomers for the *Dictyostelium* DyPA:O₂ complex structure (grey) and *Dictyostelium* DyPA:CN⁻ complex (green). **(E)** Overlay of the heme microenvironment of the *Dictyostelium* DyPA:CN⁻ (green) and activated O₂ (grey) complexes. The atoms of the activated oxygen molecule are shown as grey spheres and iron atom of the heme is shown as a red sphere, in the *Dictyostelium* DyPA:O₂ complex structure.

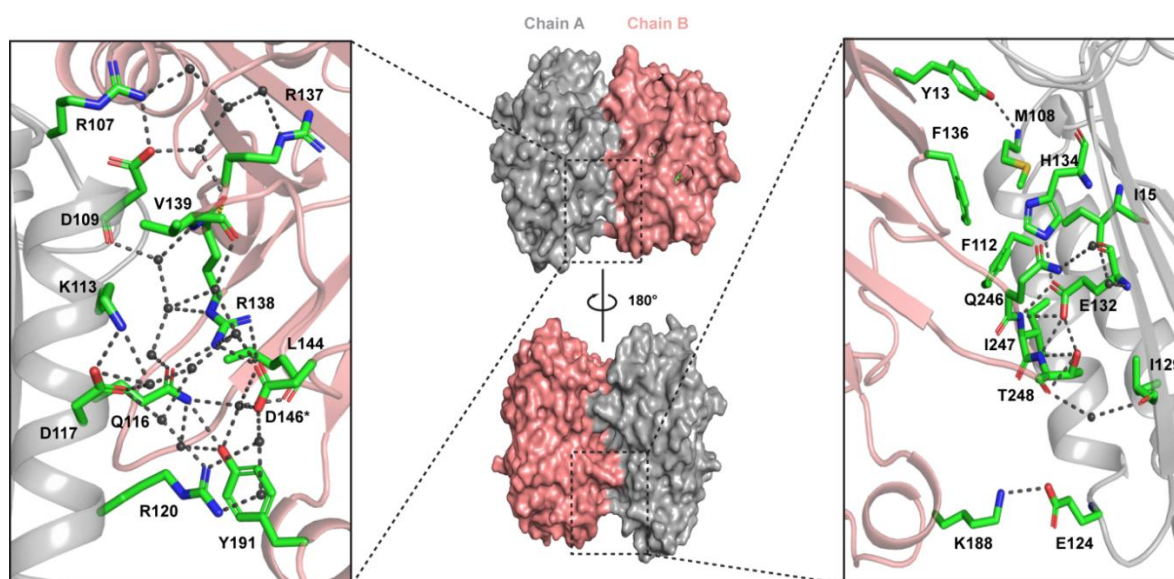


Figure S8: *Dictyostelium* DyPA:O₂ complex dimer interface. The individual *Dictyostelium* DyPA monomers are shown in grey and salmon pink. Surface and cartoon representations of *Dictyostelium* DyPA dimer. Selected amino acids are colored in the atomic color scheme. Water molecules are shown as grey spheres. Key non-covalent bonds are represented as grey dashed lines; the bond length cutoff is 3.5 Å.

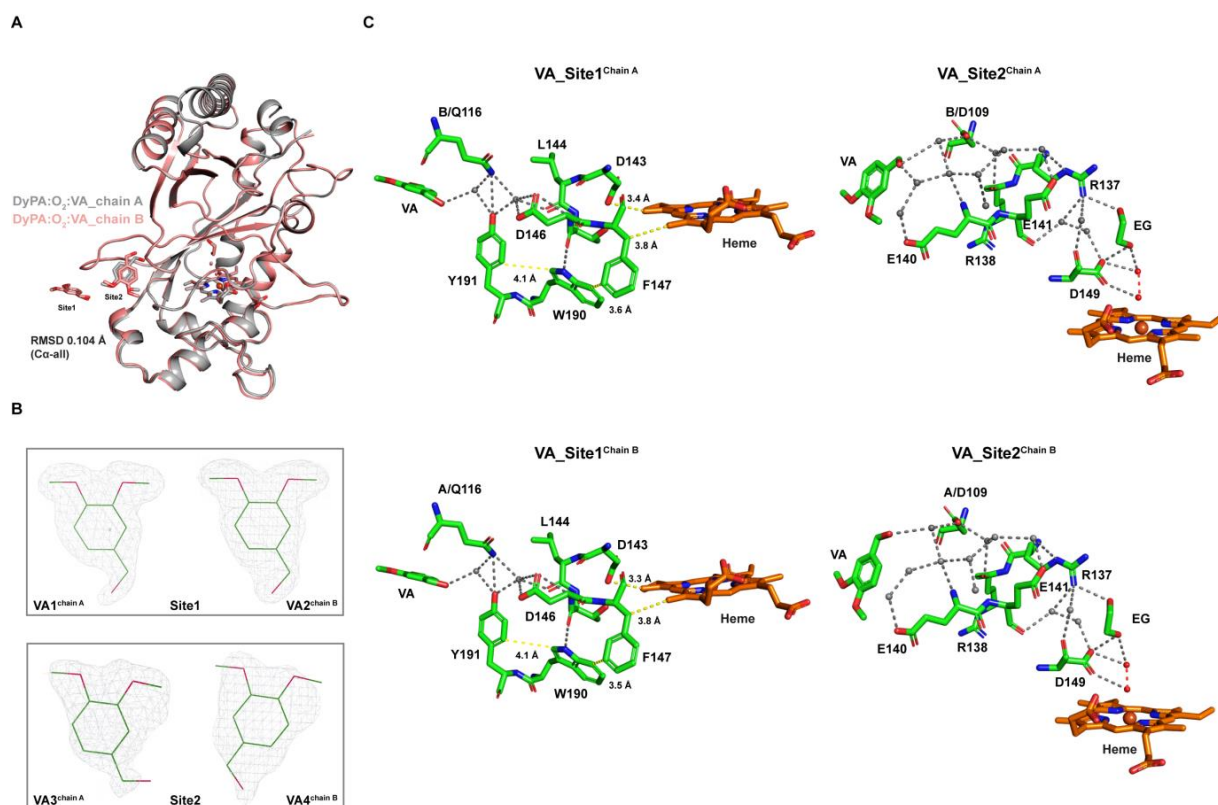


Figure S9: Structure of the *Dictyostelium* DyPA:O₂:VA complex. **(A)** Structure of *Dictyostelium* DyPA:O₂:VA complex. (A) Structural alignment of both copies of the *Dictyostelium* DyPA:O₂:VA complex in the asymmetric unit reveals that both copies are nearly identical (C α -RMSD of 0.105 Å). **(B)** OMIT electron density maps for bound VA molecules, contoured at 1 σ . **(C)** Proposed long-range electron transfer pathways from veratryl alcohol binding sites to the heme moiety of *Dictyostelium* DyPA. The atoms of the activated oxygen molecules are shown as red spheres and the water molecules are shown as grey spheres. Hydrogen bonds are represented as grey dashed lines.

Table S1. Data-collection and refinement statistics

(values in parentheses are for the outer shell).

	DyPA:O ₂	DyPA:CN ⁻	DyPA:O ₂ :VA
Data collection[#]			
X-Ray Source	ID29, ESRF Grenoble	ID29, ESRF Grenoble	ID23-1, ESRF Grenoble
Wavelength (Å)	0.91985	0.91985	0.910003
Crystal-to-detector distance (mm)	313.233	288.91	318
Exposure time per image (s)	0.0375	0.0375	0.0375
No. of images	1200	1200	3000
Oscillation range (°)	0.3	0.3	0.15
Resolution range (Å)	47.78-1.7(1.79-1.7)	47.74-1.85 (1.94-1.85)	47.64-1.60 (1.63-1.60)
Space group	<i>P</i> 4 ₁ 2 ₁ 2	<i>P</i> 4 ₁ 2 ₁ 2	<i>P</i> 4 ₁ 2 ₁ 2
Unit cell a,b,c (Å) α, β, γ (°)	141.03, 141.03, 95.56 90, 90, 90	141.05, 141.05, 95.48 90, 90, 90	140.44, 140.44, 95.28 90, 90, 90
Crystal mosaicity (°)	0.13	0.115	0.097
No. of molecules in AU	2	2	2
Wilson B-factor	23.4	25.8	21.1
Total reflections	2811648	2170478	4163950
Unique reflections	105810	82374	125165
Multiplicity	26.6 (25.4)	26.4 (26.2)	33.26 (33.48)
Completeness (%)	100 (100)	100 (100)	100 (100)
Mean I/sigma(I)	18.5 (2.9)	15.4 (2.9)	25.13 (3.7)
R _{sigma} (%)	3.1 (36.1)	3.9 (36.2)	2.3 (27.9)
R _{int} (%)	13.67 (77)	15.14 (81)	10.38 (74)
Refinement			
Resolution range (Å)	45.25-1.7	45.22-1.85	45.11-1.60
Reflections used in refinement	105721	82292	125077
Reflections used for R-free	5281	4123	6292
R _{work} (%)	14.71	15.59	15.2
R _{free} (%)	16.5	17.99	16.8
Stereochemical parameters			
Number of non-hydrogen atoms	5647	5429	5729
Macromolecules	613	612	614
Ligands	9	11	10
Water	618	393	618
R.m.s deviations			
Bond length (Å)	0.007	0.007	0.006
Bond angles (°)	1.098	1.103	1.161
Ramachandran plot			
Favored (%)	99.18	99.51	99.18
Additionally allowed (%)	0.82	0.49	0.82
Outliers (%)	0	0	0
B-factors (Å ²)	30.00	33.00	29.00
PDB ID	7O9J	7O9L	7ODZ

[#] R_{free} is calculated for a randomly chosen 5 % subset of reflections.