Supplementary information for

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

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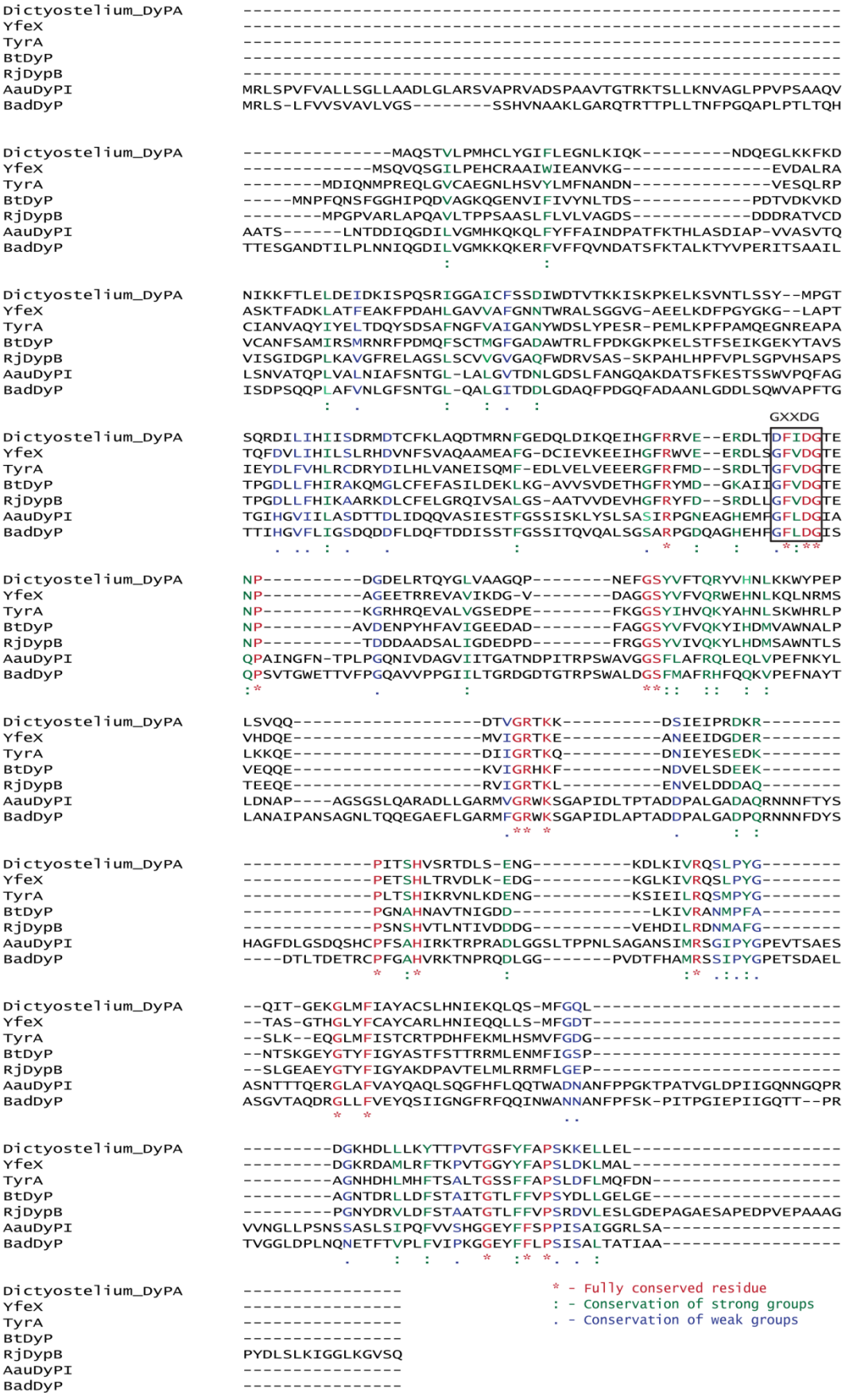

Figure S1: Sequence alignment of *Dictyostelium* DyPA with different bacterial and fungal DyPs. The conserved GXXDG motif is shown in a black box.

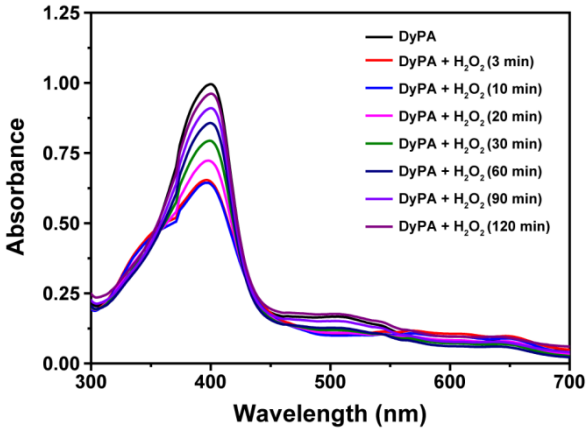

Figure S2: UV-visible absorption spectra of *Dictyostelium* DyPA in the presence of H_2O_2. Spectra of 10 μM *Dictyostelium* DyPA in the absence (black) or in the presence (red to magenta) of 10 μM H_2O_2 in 50 mM Tris pH 8.0 and 150 mM NaCl buffer at different time point.

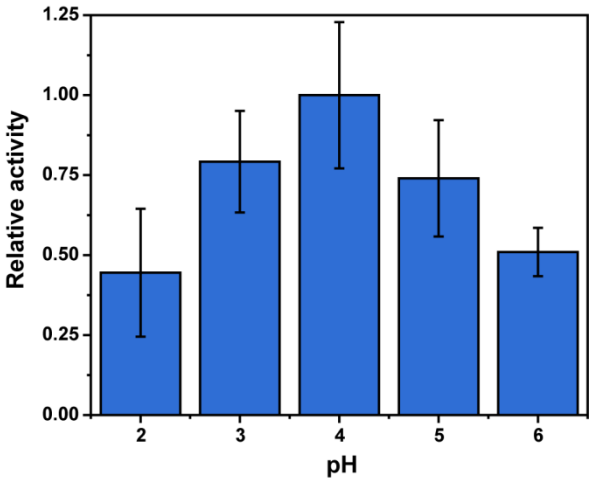

Figure S3: Optimum pH of *Dictyostelium* DyPA towards oxidation of veratryl alcohol. The standard assay was executed in 100 μl of 50 mM sodium acetate (pH 2.0 – 6.0) and 150 mM NaCl at 25 °C, containing 10 mM VA and with 4 μM of protein. The reaction was initiated upon the addition of 1 mM H_2O_2 and was monitored at 310 nm (VA ε_{310}= 9.3 mM^{-1} cm^{-1}). Data are average values of 3–6 independent measurements and bars represent the standard deviation.

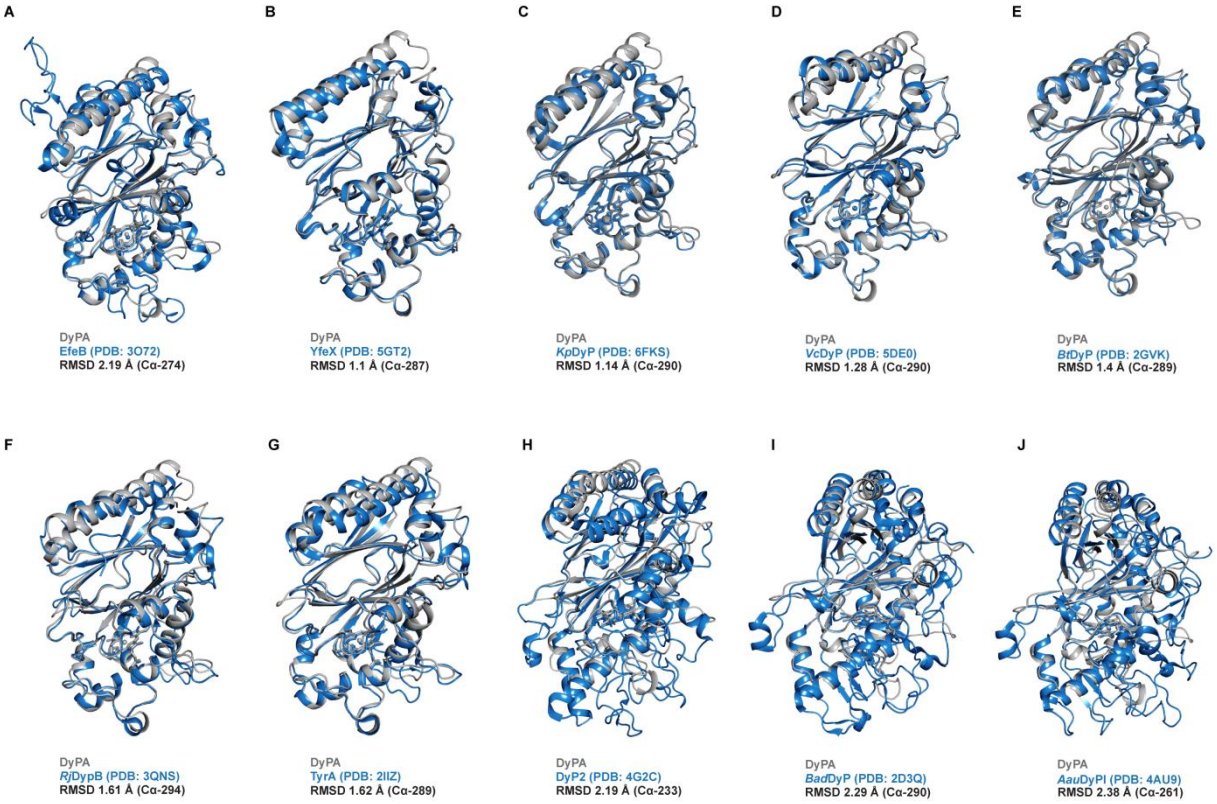

Figure S4. Structural comparison of *Dictyostelium* DyPA with related bacterial and fungal dye decolorizing peroxidases. **(A)** Structural alignment of *Escherichia coli* O157 EfeB (blue; class A) and *Dictyostelium* DyPA (gray). **(B)** Structural alignment *Escherichia coli O157* YfeX (blue; class B) and *Dictyostelium* DyPA (gray). **(C)** Structural alignment of *Klebsiella pneumoniae Kp*DyP (blue; class B) and *Dictyostelium* DyPA (gray). **(D)** Structural alignment of *Vibrio cholerae Vc*DyP (blue; class B) and *Dictyostelium* DyPA (gray). **(E)** Structural alignment of *Bacteroides thetaiotaomicron* VPI-5482 *Bt*DyP (blue; class B) and *Dictyostelium* DyPA (gray). **(F)** Structural alignment of *Rhodococcus jostii RHA1 Rj*DypB (blue; class B) and *Dictyostelium* DyPA (gray). **(G)** Structural alignment of *Shewanella oneidensis* TyrA (blue; class B) and *Dictyostelium* DyPA (gray). **(H)** Structural alignment of *Amycolatopsis sp.* 75iv2 DyP2 (blue; class C) and *Dictyostelium* DyPA (gray). **(I)** Structural alignment of *Bjerkandera adust*a *Bad*DyP (blue; class D) and *Dictyostelium* DyPA (gray). **(J)** Structural alignment of *Auricularia auricula-judae Aau*DyPI (blue; class D) and *Dictyostelium* DyPA (gray).

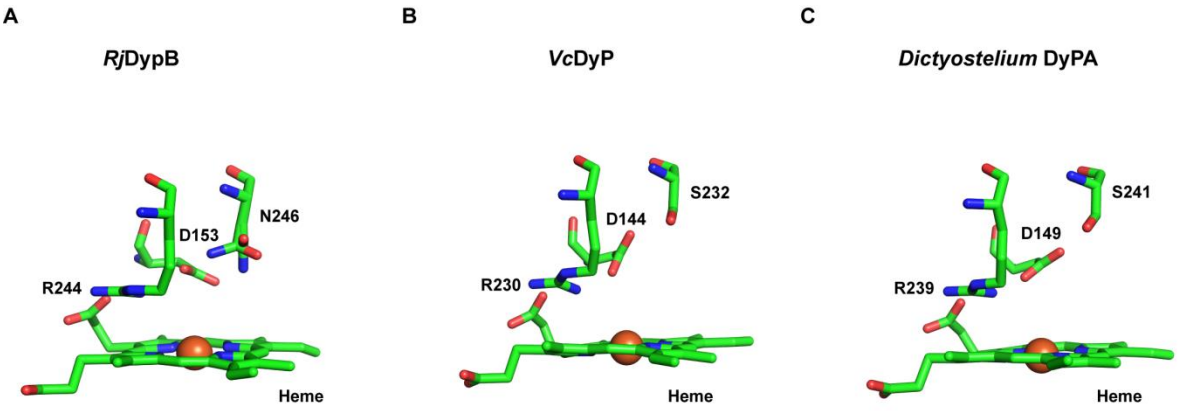

Figure S5. Heme microenvironment. **(A)** *Rj*DypB (PDB: 3QNS), **(B)** *Vc*DyP (PDB: 5DE0) and **(C)** *Dictyostelium* DyPA.

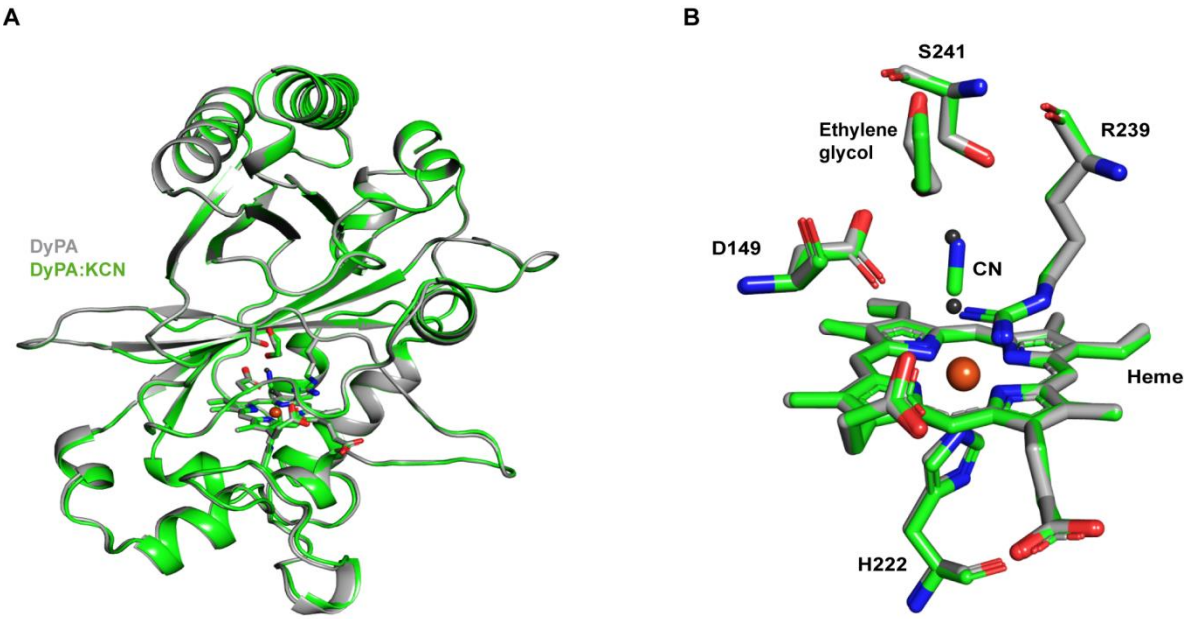

Figure S6: (A) Structural alignment of single monomers for the *Dictyostelium* DyPA native structure (gray) and *Dictyostelium* DyPA:KCN complex (green). **(B)** Heme microenvironment of the *Dictyostelium* DyPA in the presence and absence of CN. Gray spheres show the position of the water molecules in the *Dictyostelium* DyPA native structure.

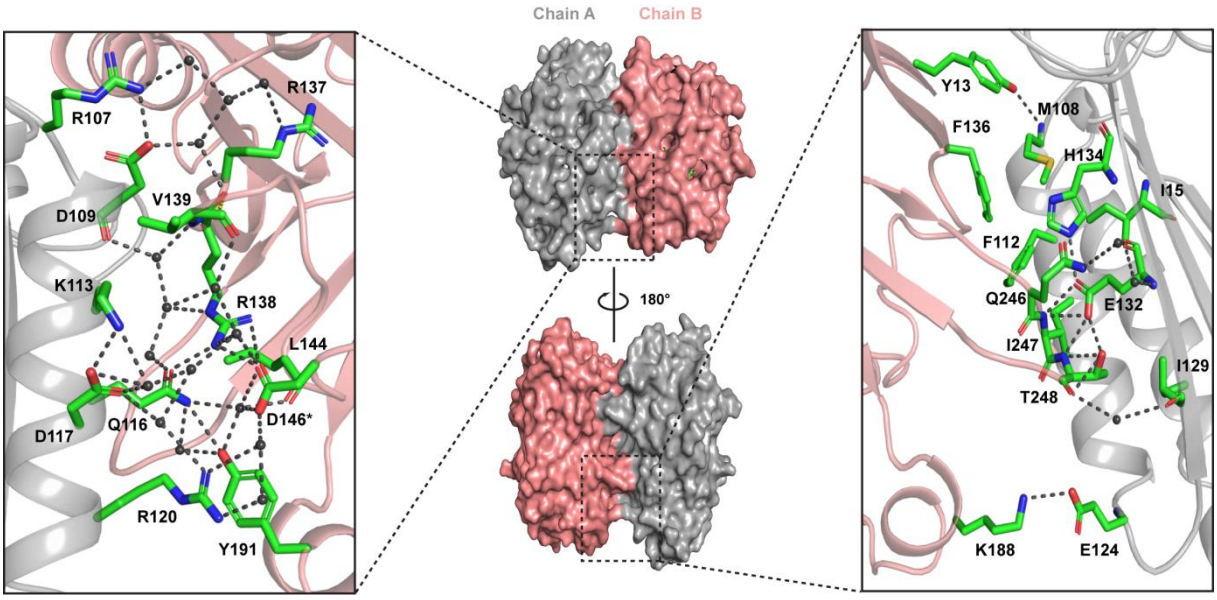

Figure S7: *Dictyostelium* DyPA dimer interface. *Dictyostelium* DyPA monomers are distinguished by the colors gray and salmon pink. Surface and cartoon representations of *Dictyostelium* DyPA dimer. Selected amino acids are colored in the atomic color scheme. The water molecules are shown as gray spheres. Key non-covalent bonds are represented as gray dashed lines.

Table S1. Data-collection and refinement statistics
(values in parentheses are for the outer shell).

	DyPA	DyPA:KCN	DyPA: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	$P4_12_12$	$P4_12_12$	$P4_12_12$
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 Macromolecules Ligands Water	5647 613 7 622	5429 612 11 393	5729 614 9 622
R.m.s deviations Bond length (Å) Bond angles (°)	 0.007 1.098	 0.007 1.103	 0.006 1.161
Ramachandran plot Favored (%) Additionally allowed (%) Outliers (%)	 99.18 0.82 0	 99.51 0.49 0	 99.18 0.82 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 % of reflections.