Characterization of a new DyP-peroxidase from the alkaliphilic cellulomonad, *Cellulomonas bogoriensis*

Mohamed H. Habib,[1,2] Henriëtte J. Rozeboom,[1] and Marco W. Fraaije[1*]

[1]Molecular Enzymology Group, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, 9747AG Groningen, The Netherlands

[2]Department of Microbiology and Immunology, Faculty of Pharmacy Cairo University, Kasr El Aini 11562, Cairo, Egypt

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Table S1. Primers for making the mutants of *Cbo*DyP, *Tfu*Dyp and *Svi*DyP

Mutation	Sequence
*Cbo*DyP E201D Fwd	GTTGATGGCACCGCAAATCTGGATCC
*Cbo*DyP E201D Rvs	CGGTGCCATCAACTTGACCCATCAG
*Tfu*DyP D242E Fwd	CAGATCGAAGGCACCGCCAACC
*Tfu*DyP D242E Rvs	GGTGCCTTCGATCTGCCCCATGAG
*Svi*DyP D199E Fwd	CAGTTGGAAGGTACGAGGAATCT
*Svi*DyP D199E Rvs	CGTACCTTCCAACTGGCCCATG

Table S2. Buffer Preparations

Buffer	Composition
A	50 mM potassium phosphate buffer [KPi], 0.5 M NaCl, 5% [v/v] glycerol, pH 8
B	50 mM KPi, 0.5 M NaCl, 5% [v/v] glycerol, 500 mM imidazole, pH 8
C	50 mM KPi, 0.5 M NaCl, 5% [v/v] glycerol, 5 mM imidazole, pH 8
D	50 mM KPi, 150 mM NaCl, 10% [v/v] glycerol, pH 7.5

Table S3. Data collection and refinement statistics for *Cbo*DyP. Numbers in parenthesis are for the highest resolution shell.

	*wt Cbo*Dyp
Data collection	
Unit cell a, c (Å)	174.0, 283.0
Resolution (Å)	58.9 - 2.40
No. of observations	1827895 (88453)
No. of unique reflections	184630 (9000)
R_{pim} (%)	11.9 (56.9)
Completeness (%)	97.9 (96.2)
Mean I/σ (I)	7.0 (1.5)
$CC_{1/2}$	0.985 (0.463)
Redundancy	9.9 (9.8)
Wilson *B* factor ($Å^2$)	23.2
Refinement	
R / Rfree (%)	24.2 / 26.6
Protein residues in A.U.	2880 (8 x 23-383)
Heme	1 per monomer
Waters	446
Geometry:	
RMSD Bond lengths (Å)	1.49
RMSD Bond angles (°)	0.007
Ramachandran favored (%)	95.30
Ramachandran outliers (%)	0.28
PDB accession code	6QZO

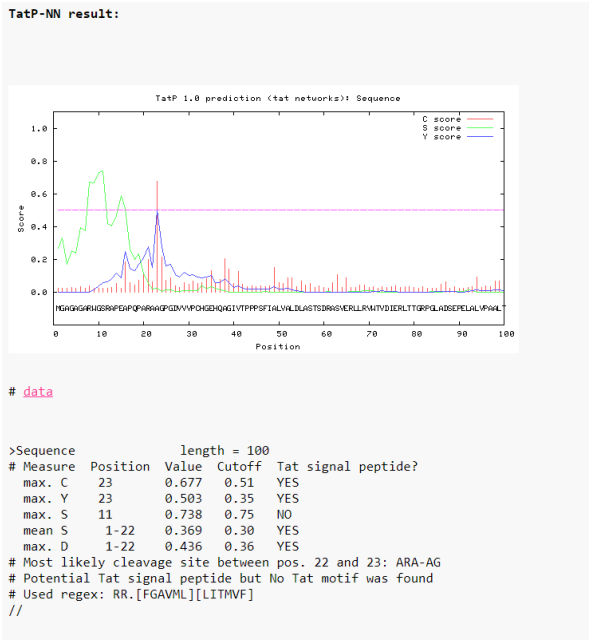


Figure S1. TatP 1.0 Server prediction of the Tat sequence for the *Cbo*DyP enzyme. The most likely cleavage site was identified by the server to be between position 22 and 23 as seen at ARA-AG.

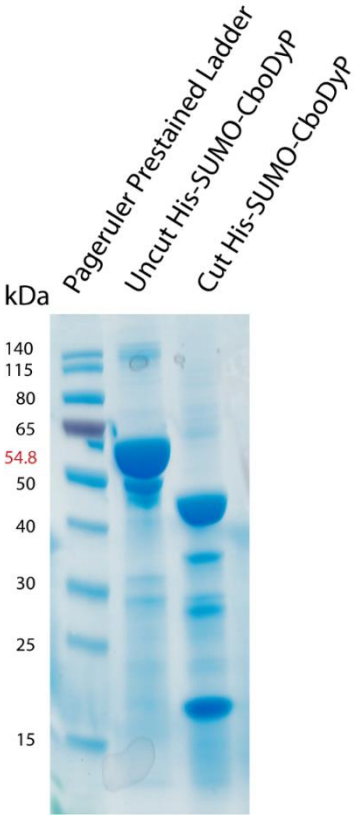
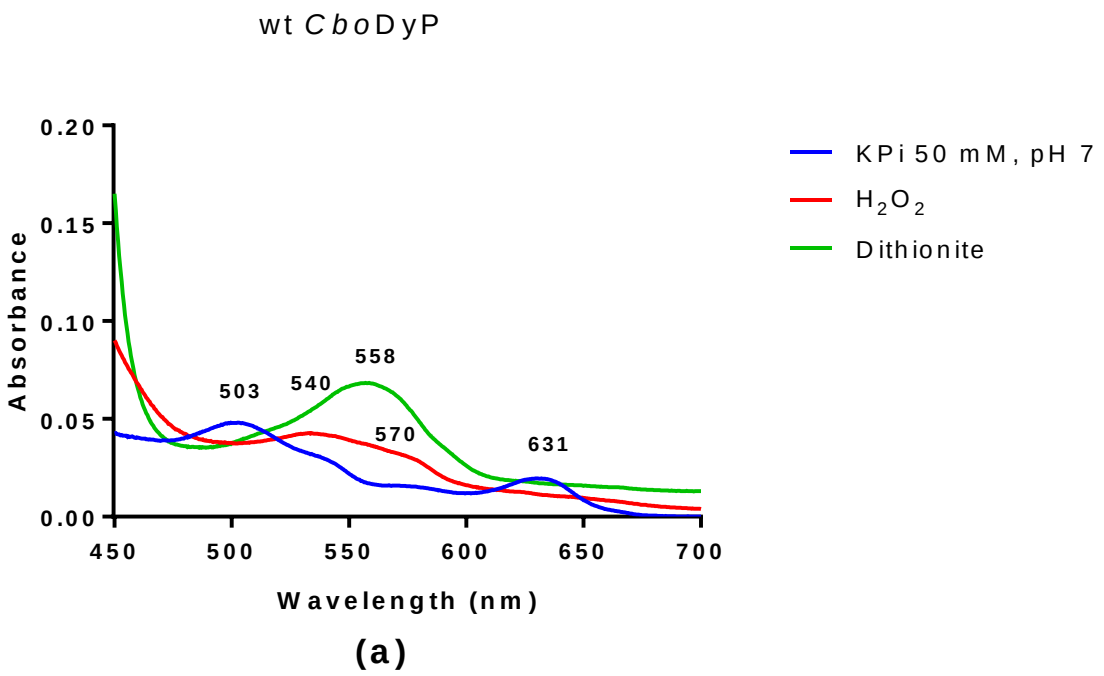


Figure S2. SDS-PAGE gel showing the uncut His-SUMO-*Cbo*DyP protein with a band at a size of ≈ 54 kDa and the *Cbo*DyP protein after cleavage of the His-SUMO using the SUMO protease with a band at a size of ≈ 41 kDa.



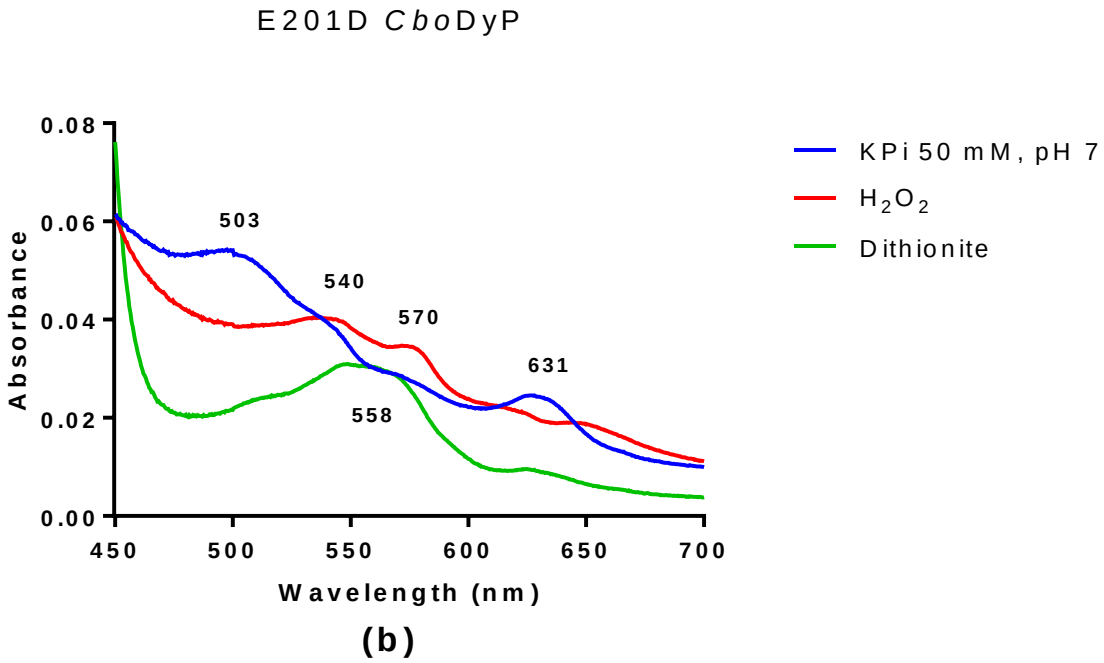


Figure S3. The UV-Vis spectra (450-700 nm) of a) wt *Cbo*DyP and b) E201D *Cbo*DyP showing the changes that occur upon addition of 1 mM hydrogen peroxide (red) or dithionite (green).

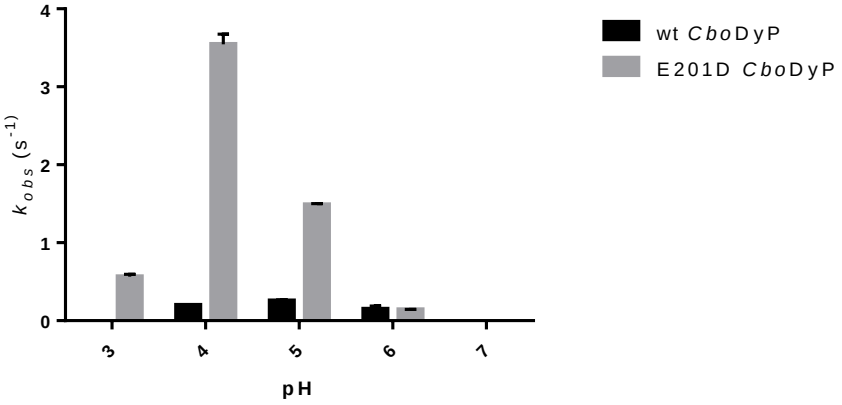


Figure S4. The pH profile of wt *Cbo*DyP versus the E201D mutant.

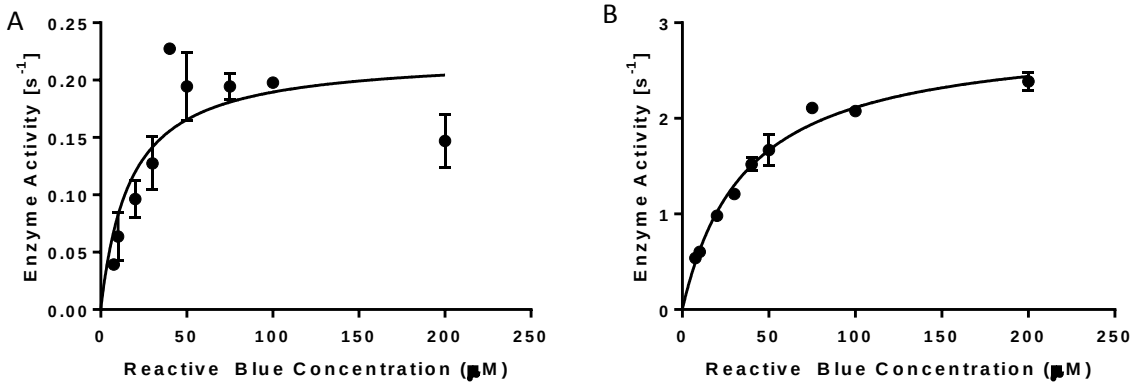


Figure S5. Steady-state kinetics for the peroxidase activity of the wild type *Cbo*DyP (A) and E201D *Cbo*DyP (B) against Reactive Blue-19 as a substrate.

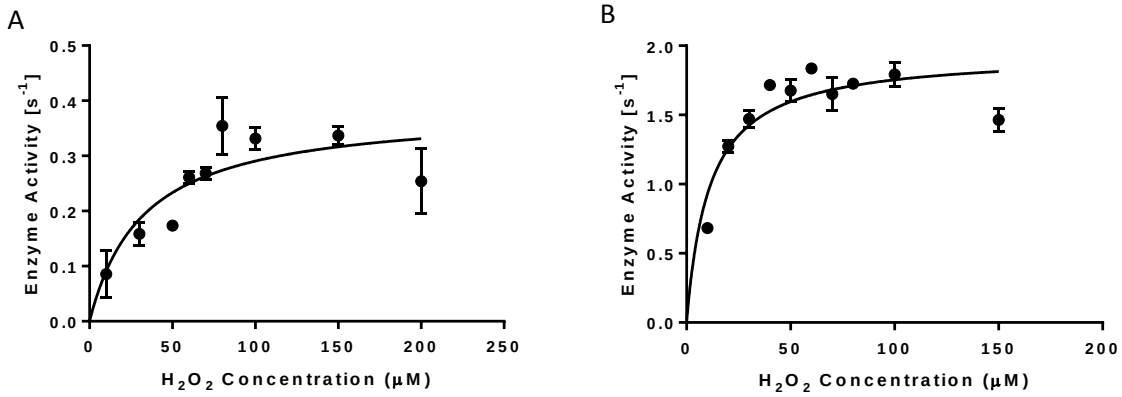


Figure S6. Steady-state kinetics for the peroxidase activity of the wild type *Cbo*DyP (A) and E201D *Cbo*DyP (B) against hydrogen peroxide as the substrate.

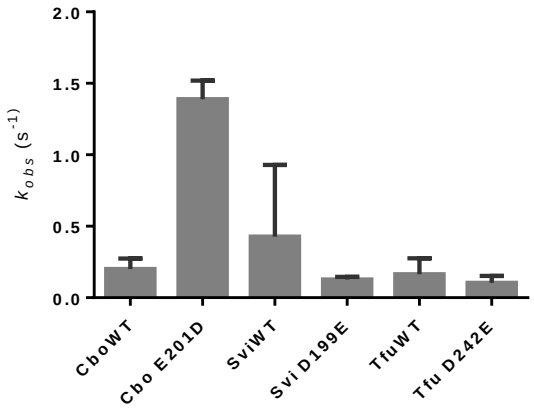


Figure S7. The peroxidase activity of the different DyPs using 50 μM RB19 as a substrate and 50 nM of enzyme in the presence of 100 μM H_2O_2.

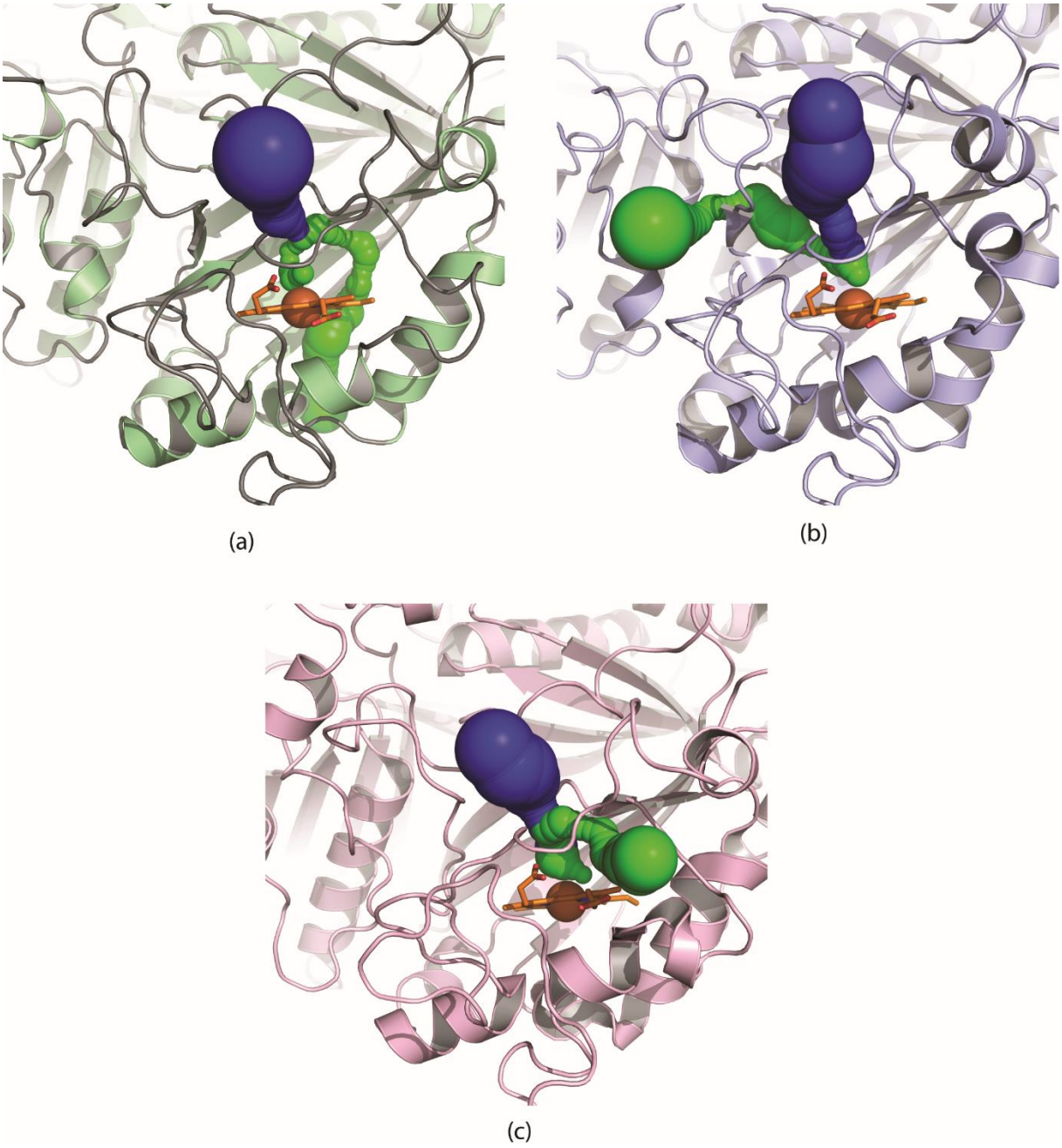
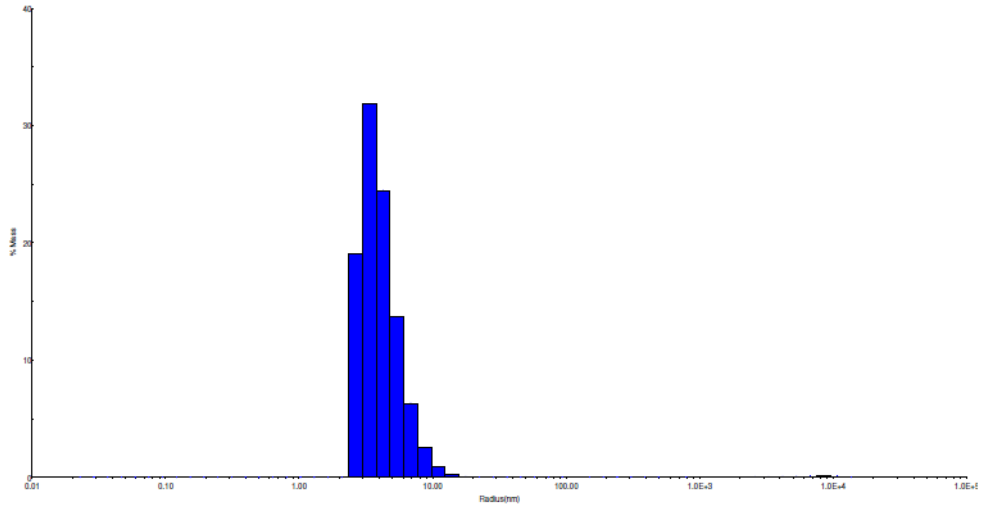


Figure S8. Accessibility of the heme binding cavity in *Cbo*Dyp (A), in *Thermomonospora curvata* heme-containing DyP-type peroxidase (B) and DtpA from *Streptomyces lividans* (C). The proteins are depicted in ribbon representation, the two most important access channels to the heme binding sites were determined with CAVER [1] and are depicted as blue and green spheres. The hemes are depicted as sticks. The green and blue channels overlap near the heme cofactor.



	Radius (nm)	%Pd	Mw-R (kDa)	%Intensity	%Mass
Peak 1	4.180	39.1	96	81.7	99.1
Peak 2	132.399	26.5	310430	8.7	0.1
Peak 3	305.448	19.1	2195000	3.3	0.0
Peak 4	645.334	18.1	12633200	2.0	0.1
Peak 5	5714.850	55.8	2078830000	4.3	0.7

Figure S9. DLS analysis of CboDyp showing the percentage mass distribution (from regularization analysis).

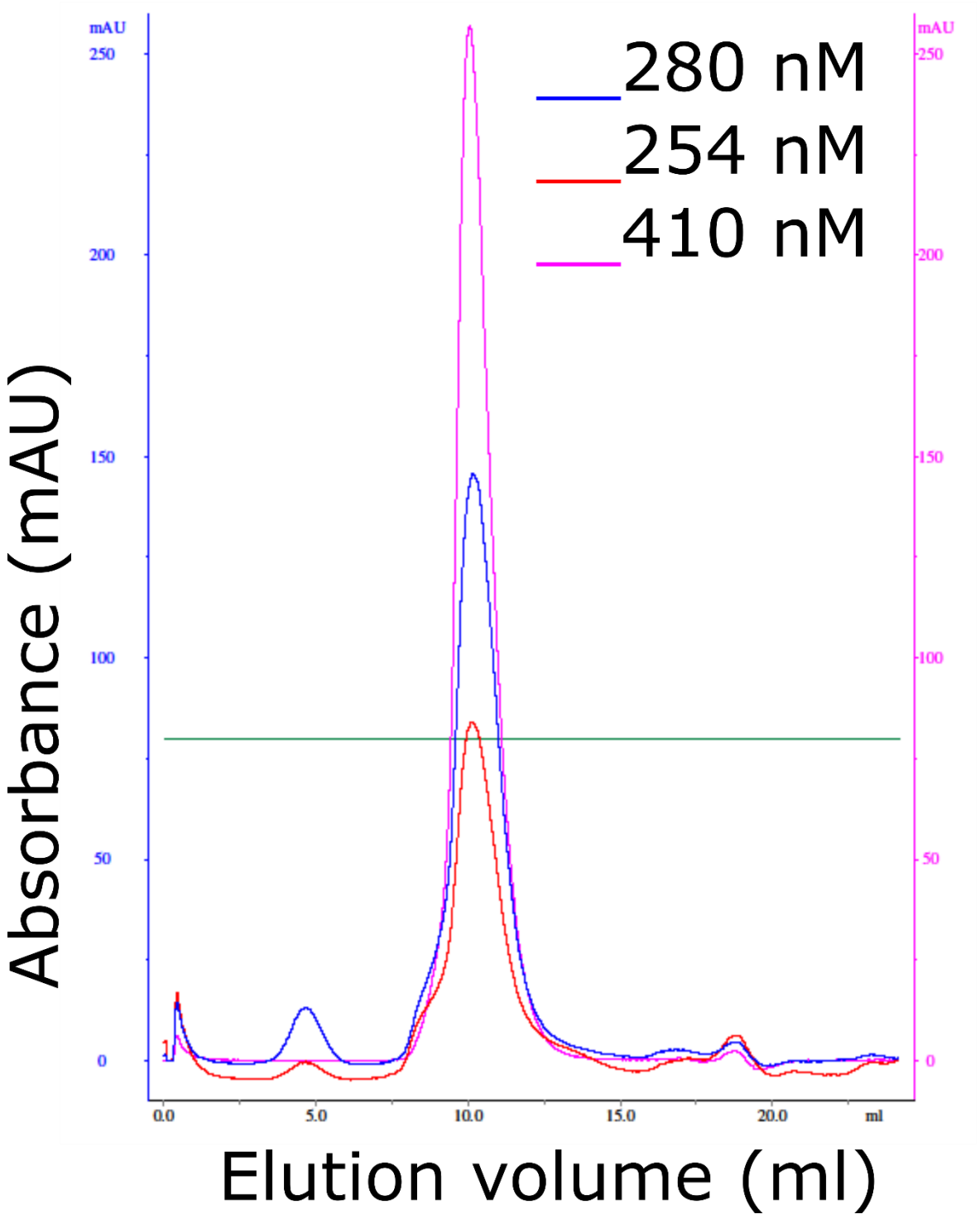


Figure S10. SEC elution profile of *Cbo*Dyp.

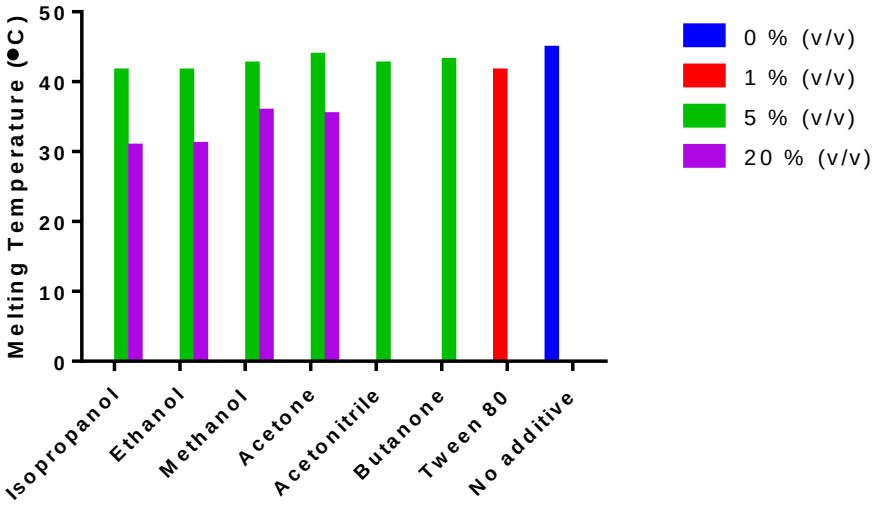


Figure S11. The melting temperature of *Cbo*DyP in the presence of different solvents at various concentrations.

References

1. Chovancova, E.; Pavelka, A.; Benes, P.; Strnad, O.; Brezovsky, J.; Kozlikova, B.; Gora, A.; Sustr, V.; Klvana, M.; Medek, P.; Biedermannova, L.; Sochor, J.; Damborsky, J. CAVER 3.0: A Tool for the Analysis of Transport Pathways in Dynamic Protein Structures. *PLOS Comput Biol* **2012**, *8* (10): e1002708.