

# Biochemical and Structural Insights into the Cytochrome P450 reductase from *Candida tropicalis*

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## Supplementary Information

Figure S1: Multiple sequence alignment of CPRs of different species.

Figure S2: Saturation curves of C<sub>t</sub>CPR for (A) ferricyanide and (B) cytochrome *c*.

Figure S3: GC-MS chromatograms and mass spectra of biotransformations of dodecanoic acid by CYP52A21.

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Figure S5: Control reactions of the quantification of H<sub>2</sub>O<sub>2</sub> stability.

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Table S1. Data collection and refinement statistics. Values in parentheses are for highest resolution shell.

Figure S7: Interactions of the FMN group of C<sub>t</sub>CPR with the residues and water molecules.

Figure S8: Interactions of the FAD group of C<sub>t</sub>CPR with the residues and water molecules.

Figure S9: Alignment between the FMN and FAD groups of the different CPR structures.

Figure S10: Interaction of the hinge region with the FMN and FAD domains for CPRs from different species.

Table S2: Uncoupling percentage (%) relative to NADPH consumed at different ionic strength.

Table S3: Uncoupling percentage (%) relative to NADPH consumed at different pH.

Table S4: Uncoupling percentage (%) relative to NADPH consumed at different temperatures.

Figure S11: Full-length SDS-PAGE gel used in the preparation of the cropped insert for Figure 1 in the main text.

10 20 30 40 50 60 70 80 90 100

CtCPR  
 Ca1CPR  
 CapCPR  
 yCPR  
 ATR2 MKNMMNYKLLKCSVSKNSKGVLSPTPHLTKPPTIHTErdLLPSSSFFLLSSSSYNIYNAMSSSSSSSSTSMIDLMAAIKIGEFPVIVSDPANASAYES  
 CanCPR -----MESSESLV-----R  
 ApaCPR1 -----MDSRIELL-----R  
 ApaCPR2 -----MDSNYEKLSPFDLMFAIIEAVKLDRMH-----G  
 GhiCPR1 -----MSSSSDLV-----G  
 GhiCPR2 -----MDSSSSSSSGSPFLDLSALVKAK-----MDFSNASS-----D  
 CsuCPR -----MNSKMSESAQVVQ-----D  
 MdoCPR -----M-----M  
 hCPR -----MINMGDSHVD-----T  
 rCPR -----MGDSHED-----T

110 120 130 140 150 160 170 180 190 200

CtCPR -----MALDKLDLYVIIITLVVAVA-AYFAKNQFLDQP--ODTG-----FLNTDSGSSNRDLVST-LKKNKNTLLLLFGSQGTAEADYAN  
 Ca1CPR -----MALDKLDLYVIIITLVVAVA-AYFAKNQFLSKP--ODTG-----FLSNDGAGSSRNILET-LKKNKNTLLLLFGSQGTAEADYAN  
 CapCPR -----MVDNLLASVAVALVVVFAV-YKYFNGGLEVQS--SNAGSSTPFGNAKADDDGDSRNFVAL-MEKNKNTLVVYFSQGTAEADLAS  
 yCPR -----MPFG-IDNTDFTVLACLVLAVLLVYVKNRSIKELLSMDD-----GDI TAVSSGNRDIQV-VTEKNKNTLVVYASQGTAEADYAK  
 ATR2 VAELSSMLIE--NRQFAMIVTTSIAVLIGCIV-MLVWRRSSGNS--KRVE-----PLKPLVVKPRE--EE-IDGRKVTIFFGTQGTAEAGFAK  
 CanCPR SIESAIGVSLG--SDAVLMLLTSFAVIVGLVV--FFLKRSSDRS--KEVK-----PVVFPKSNVPEPEEIE--VEPGKVTIFFGTQGTAEAGFAK  
 ApaCPR1 SVEQALGVKLG--DET VVLVLTTSVAVILGLLV--FLWKRSGDNRS--KDR-----QVVVVKPVSLKDDDEDEY-VGPKDKTLTIFFGTQGTAEAGFAK  
 ApaCPR2 FPQAVVARLME--NKDLLMLITTSIAVLVGCVV-VLLYRSATGSAK--KVAE-----PPKIVVRKEPEETEY-VDDGKVKVTIFFGTQGTAEAGFAK  
 GhiCPR1 FVESVLGVSLGEGSVTDSMIVIAITTSIAVLIGLLV--FFWKRSSGERS--RDVK-----PLVAPKPVSLKDEEDDDAVIAAGKTKVTIFYGTQGTAEAGFAK  
 GhiCPR2 SAAQVTVLFE--NRE FVMILTTSIAVLIGCVV-LLIWRSSAQKP--KQIQ-----LPLKPSIIEKEPE--LE-VDDGKVKVTIFFGTQGTAEAGFAK  
 CsuCPR AAAAVAG-----TSLSTFDIVMLVLLGAAV-WWLYSSRKNKK--DEIVLSKYSIQTAGS IQVAENSFIKK-LKSSGRSLVVVYFSQGTGTEGFAK  
 MdoCPR SAHEVVEVSE--EFLGLTDIALLVLLVVGAT-WYFMRSRKKEEAPIRSYS--IQPTTVSTVSTENSFIKK-LKASGRSLVVVYFSQGTAEAGFAK  
 hCPR SSTVSEAVAE--VSLSMTDMILFSLIVGLLTYWFLFRKKKEEVP--EFTK-----IQTLTSSVRESSFVEK-MKKTGRNIIIVFYGSQGTAEAFAN  
 rCPR SATMPEAVAE--VSLSTTDMVLFSLIVGLVITYWFLFRKKKEEIP--EFSK-----IQTTAPPVKESSFVEK-MKKTGRNIIIVFYGSQGTAEAFAN

Membrane anchor FMN binding

210 220 230 240 250 260 270 280 290 300

CtCPR KLSRELHSRFGIKTM-VADFADYDFDN---FGDITEDILVFFIVATYGEGETDNADEFHTWLTE-----EADTLSTLRVTVFGLGNSTYEFFNAIGRK  
 Ca1CPR KLSRELHSRFGIKTM-VADFADYDFDN---FGDITEDILVFFIVATYGEGETDNADEFHTWLTE-----EADTLSTLRVTVFGLGNSTYEFFNAIGRK  
 CapCPR KLAKELESSEYGLRTM-TADPENFDYDK---FDFPESHAVFITASYGDGEPDNDNAQDLYSFLGNSPFSQDGETLENLNFVAVFGLGNVLYEYFNKAGRD  
 yCPR KFSKELVAKFNINVM-CADVENYDFES---LNDVP--VIVSIFISTYGEDEFPDGAVNFEDFICNA-----EAGALSNLRYNMFGGLGNSTYEFFNGAAKK  
 ATR2 ALGEEAKARYEKTRKIVDLDYAADDEYEEKLLKEDVAFFFLATYGDGEPDNDNAARFYKWFTEG--NDRGEWLNKLVYGVFGLGNRQYEHFNKIAKV  
 CanCPR ALSEEIKARYEKAVKVVDDMDYAADDDLYEEKLLKETIAFFMVATYGDGEPDNDNAARFYKWFTEG--QERGVWLQHLTYGVFGLGNRQYEHFNKIGKV  
 ApaCPR1 ALAEEIKARYDKAVKVVDDMDYAADDDLYEEKLLKETIAFFMVATYGDGEPDNDNAARFYKWFTEG--ERE PWLQQLSYGIFGLGNRQYEHFNKIGKV  
 ApaCPR2 ALAEEAKARYQQAHFTVLDLDFADDEEYEEKMKKETAIAFFFLATYGDGEPDNDNAARFYKWFTEG--KERGNWLDLYGVFGLGNRQYEHFNKIAKV  
 GhiCPR1 ALAEEIKARYEKAAVKVVDDLDYAMDDEYEEKLLKETIAFFMVATYGDGEPDNDNAARFYKWFTEG--NERLPWLQQLTYGVFGLGNRQYEHFNKIAKV  
 GhiCPR2 ALVEEAKARYEKATNIVDLDYAADDEEYEEKMKKDNLAFFFLATYGDGEPDNDNAARFYKWFTEG--KERGEWLNQMKYGIFFGLGNRQYEHFNKIAKV  
 CsuCPR RLAKEGV-RYKMKGM-VADPEECMEELTKLQDIPNSLAV-FCMATYGEDEPTDNADEFYEWLKS-----GEVDLTGLNYAVFGLGNKTYEHYNAVAIY  
 MdoCPR RLAKEGV-RYKMKGM-VADPEECMEELTKLQDIPNSLAV-FCMATYGEDEPTDNADEFYEWLKS-----GEVDLTGLNYAVFGLGNKTYEHYNAVAIY  
 hCPR RLSKDAH-RYGMGRM-SADPEEYDLADLSSLEPEIDNALV-FCMATYGEDEPTDNAQDFYDNLQE-----TDVDSLGVKFAVFGGLGNKTYEHFNAMGKY  
 rCPR RLSKDAH-RYGMGRM-SADPEEYDLADLSSLEPEIDKSLV-FCMATYGEDEPTDNAQDFYDNLQE-----TDVDSLGVKFAVFGGLGNKTYEHFNAMGKY

FMN binding

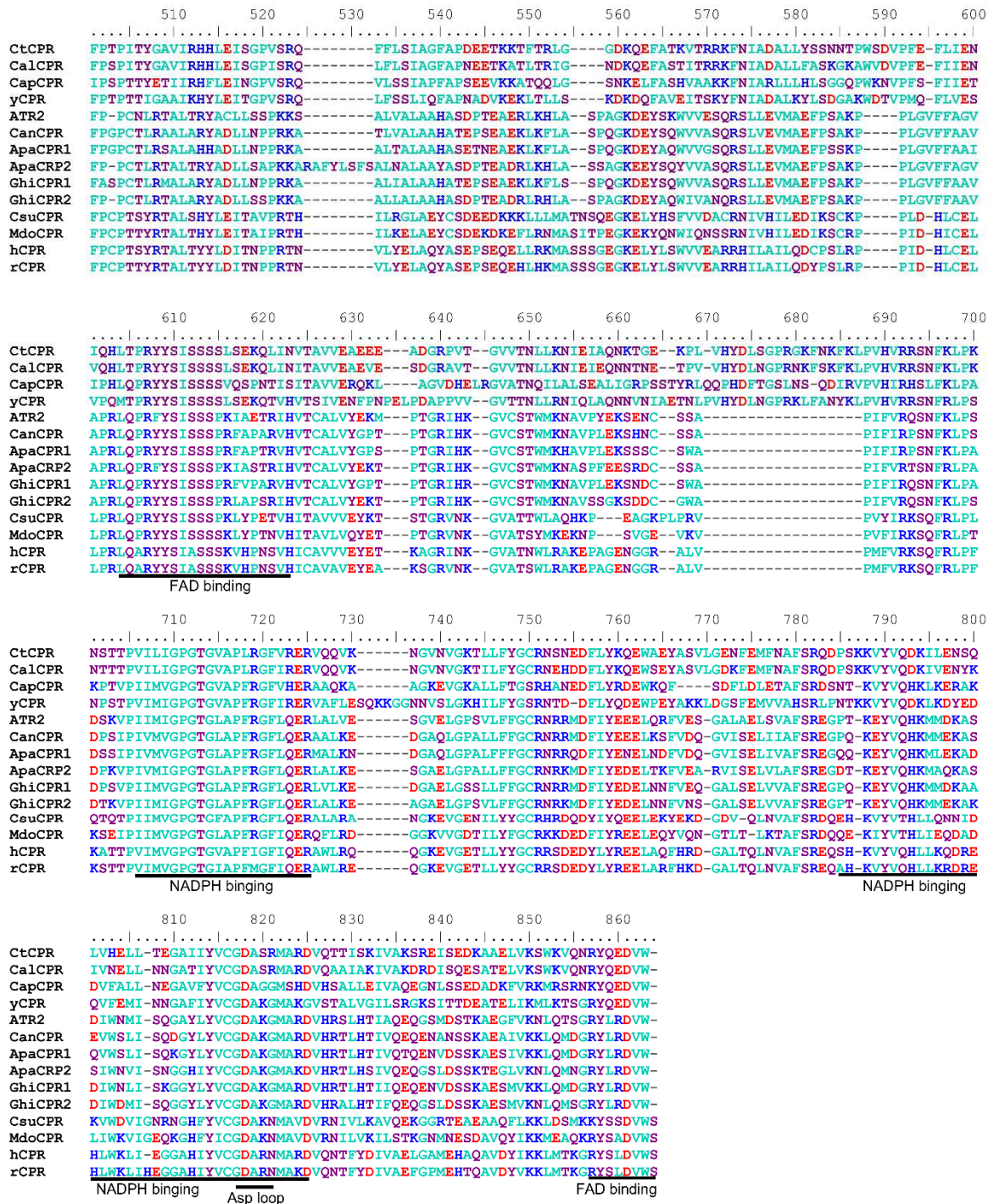
310 320 330 340 350 360 370 380 390 400

CtCPR FDRLLSEKGGDRFAEYAGDDGTGLDDED FMAWKDNVFDALKNLNFEEK-ELKYEY-----NVKLTERRDLSAADSQVSLGEPNKKYI-NSE---GI  
 Ca1CPR FDQLLEEKGGERFAEYAGDDGTGLDDED FLSWKDGVFDSLKNLNYEEK-ELKYEY-----NVKLTERRDLDVDDSNVSLGEPNKKYI-NSQ---GV  
 CapCPR MHEFTLDLGGHSIGPYGEGDDSKGML EEDYMAWKDEFLAALVTKWGLKER-EAVVEP-----ATSVKDIIE-DAQSHD VYLGEPNLKHLQASK---AR  
 yCPR AEKHLAAGAIRLKLGLGADDGAGTDEDYMAWKDSILEVLKDELHLDEQ-EAKFTS-----QFYTVLNEIT---DMSLSEGPSAHLPSHQLNLRNAD  
 ATR2 VDDLVVEQGAQLRVQVGLGDDD-QCIEDDFTAWREALWPELDITLREEDG-TAVAFYTAAVLEYRVSIHDSADAKDNINMANGNY-----  
 CanCPR IDEQLSEQGAQLRVQVGLGDDD-QCIEDDFTAWREQLWPELDQILRDEDDASSASTPYTAALILEYRLVIHDT-TMSLEDKHAMANGN-----  
 ApaCPR1 IDEKLESEQGAQLRVQVGLGDDD-QCIEDDFTAWREQLWPELDQILRDEESLDSVSTPYTAALILEYRVVIHDAIIPSYDDNHAFAVANGD-----  
 ApaCPR2 VDNLVVEQGGQRLVQVGLGDDD-QCIEDDFTAWRETVWPELDQILRNEED-SAAATAYTAAVLEYRVVVFHD--ELDESINSHANGAN-----  
 GhiCPR1 LDEQLSEQGAQLRIVQVGLGDDD-QCIEDDFTAWRELLWPELDQLRDEEDENATSTPYTAALILEYRVVVDPAVMHVEENYSNKANGN-----  
 GhiCPR2 VDELLTEQGAQLRVQVGLGDDD-QCIEDDFTAWRELVWPELDQLRDEED-ATVSTPYTAAVLEYRVVVFHDPAAPLEDKNWSNANGH-----  
 CsuCPR LDKRLEELGATRVHELGLGDDD-ANI EDDFITWKRDFWPAVCEKFNIESTGEEELTR-----QFRVLSHQPEEIPSENVFTGEIARL---H---TY  
 MdoCPR VDKRLEELGATRVHELGLGDDD-ANI EDDFITWKRDFWPAVCEKFFIEGSGEEVLMR-----QFRLEEQP--DVQPDRIYTGTEIARL---H---SM  
 hCPR VDKRLEQLGAQRIFELGLGDDD-GNLEEDFITWREQFWPAVCEFFVGEATGEESSIR-----QYELVVHT--DIDAAKVYMGEMGRL---K---SY  
 rCPR VDQRLBQLGAQRIFELGLGDDD-GNLEEDFITWREQFWPAVCEFFVGEATGEESSIR-----QYELVVHT--DMDVAKVYTGEMGRL---K---SY

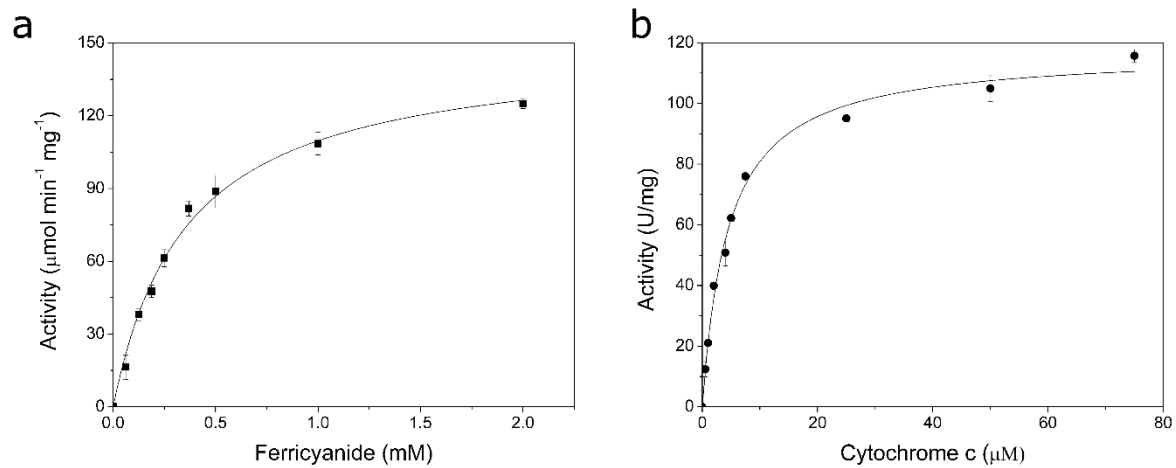
Hinge

410 420 430 440 450 460 470 480 490 500

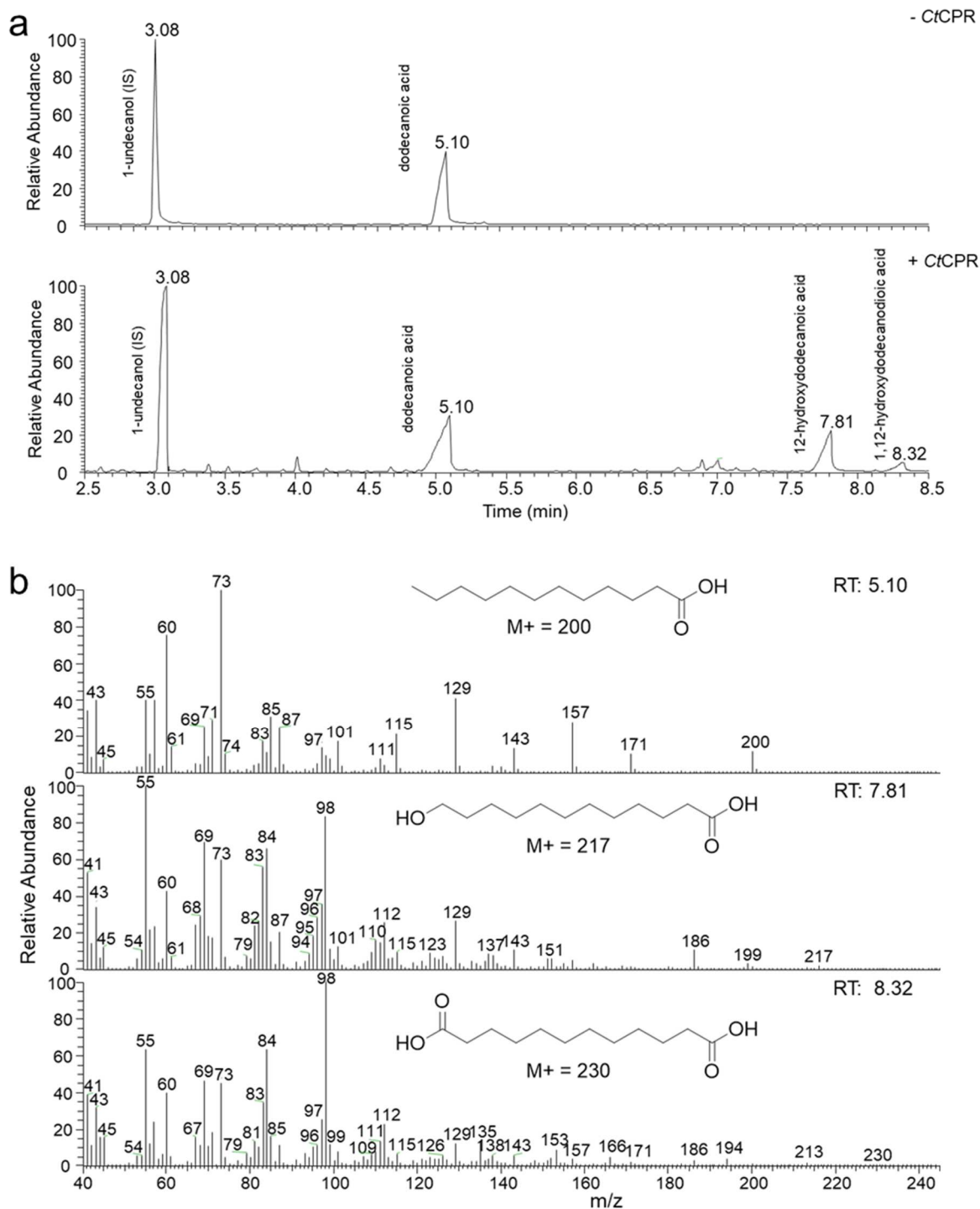
CtCPR DLTGKPPFDHHPYLARI TETRELFSSKE RHCIVHEFDISESNLKYTTGDHLAIWPSNSDENIKQFAKCFGLEDKLDTVIELKALDSTYTT-----IP  
 Ca1CPR DLSKPPFDHHPYLAKITKTKELEN-SKDRNCVHVHEFDISESNLKYTTGDHLAVWPSNSDENIKQFLKCFGLEDKENTVIELKALDSTYS-----IP  
 CapCPR EVKQPPYNASNPLAKVFAAQLEFNTDRHCIHMEFDITGA--RYTGDHLAFWQCNNEEVRQFAKALGITN-PQOPIAISVLDKSTYR-----  
 yCPR GIQLGPFDISQPYIAPVKSRELFSSNDRNCIHSFELDLSGNSIKYSTGDHLAVWPSNPLEKVEQFLSIFNLD--PETIFDLKPLDPTVK-----VP  
 ATR2 ----TVFDAQHPYKANVAVKRELHTPESDRSCIHLEFDISGTLIYETGDHVGVLCDNLSETVDEALRLDMS--PDYFSLHAEKEDGTPI-SSSLPPP  
 CanCPR ----TTYDIIHPCKNVAVQRELHTPESDRSCIHLEFDISGTLIYETGDHVGVAENSEETVEEAARLLGQS--LDLTFSLHADKEDGTAL-CGSLPPP  
 ApaCPR1 ----ASYDLHPCPNVAVQRELHTPESDRSCIHLEFDISGTLIYETGDHVGVAENDEETVEEAARLLGQP--LDLTFSLHADKEDGTPI-CGSLPPP  
 ApaCPR2 ----TYVDAQHPYKANVAVKRELHTPASDRSCIHLEFDISGTLIYETGDHVGVAENLETVEEAARLLNLP--PQTYFSLHADKEDGTAL-CGSLPPP  
 GhiCPR1 ----ATYDLHPCPNVAVQRELHAPESDRSCIHLEFDISGTLIYETGDHVGVAENDEETVEEAARLLGQP--LDLTFSLHADKEDGTAL-CGSLPPP  
 GhiCPR2 ----ATYDLHPCPNVAVQRELHAPESDRSCIHLEFDISGTLIYETGDHVGVAENDEETVEEAARLLGQS--LDLTFSLHADKEDGTAL-CGSLPPP  
 CsuCPR QVQRPPYDAKNPFLAQITVNRLEHK-GGDRSCIHLEFDISGTLIYETGDHVGVAENDEETVEEAARLLGQP--LDLTFSLHADKEDGTAL-CGSLPPP  
 MdoCPR QNQRPPYDAKNPFLAVVIVNRELEHK-GGDRSCMHLELDIDGSKMRYDAGDHVAMPINDKILVEKLGKLDAN--LDTVSLINTDTSKSK-----HP  
 hCPR ENQKPPYDAKNPFLAAVTTNRKLNQ-GTERHMLHLELDISDSKIRYESGDHVAVYPANDSALVNLQKILGAD--LDVMSLNLNDEESNKK-----HP  
 rCPR ENQKPPYDAKNPFLAAVTNRKLNQ-GTERHMLHLELDISDSKIRYESGDHVAVYPANDSALVNLQIGELGAD--LDVMSLNLNDEESNKK-----HP



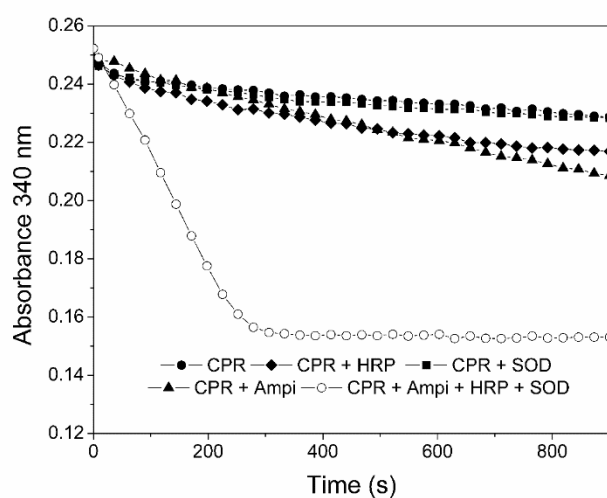
**Figure S1.** Multiple sequence alignment of CPRs of different species generated by ClustalW. CtCPR, *Candida tropicalis* (AAV84084.1); CaICPR, *Candida albicans* (KGQ86567); CapCPR, *Candida apicola* (JQ015264); Ycpr, *Saccharomyces cerevisiae* (P16603); ATR2, *Arabidopsis thaliana* CPR2 (AEE85738.1); CanCPR, *Capsicum annuum* (AFV95075); ApaCPR1, *Andrographis paniculate* CPR1 (AQT38168); ApaCPR2, *A. paniculate* CPR2 (AQT38169); GhiCPR1, *Gossypium hirsutum* CPR1 (ACN54323); GhiCPR2, *G. hirsutum* CPR2 (ACN54324); CsuCPR, *Chilo suppressalis* (AGM20565); MdoCPR, *Musca domestica* (Q07994); hCPR, human (NP\_000932); rCPR, rat (NP\_113764).



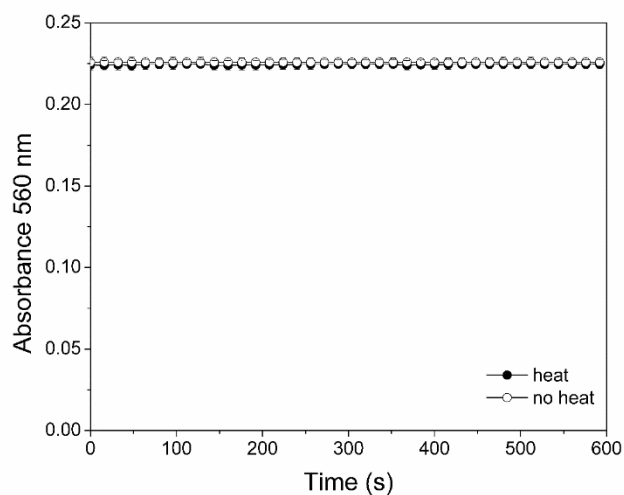
**Figure S2.** Saturation curves of CiCPR for (a) ferricyanide and (b) cytochrome c. Values are the average of three independent measurements. Data was fit into the Michaelis-Menten equation.



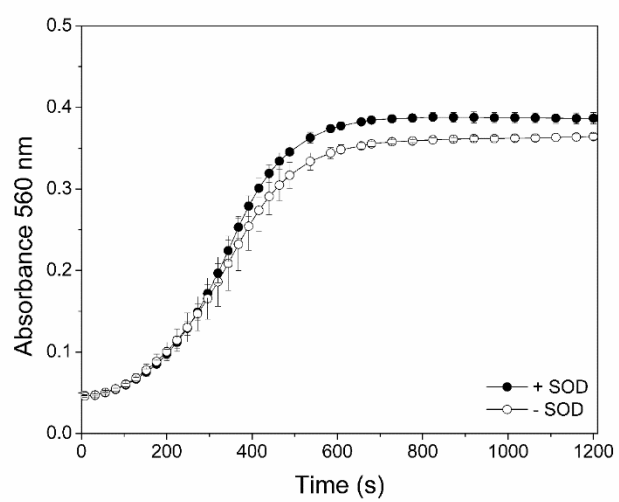
**Figure S3.** GC-MS chromatograms and mass spectra of biotransformations of dodecanoic acid by CYP52A21. **(a)** Chromatograms of the biotransformations without (- CtCPR) and with (+ CtCPR) addition of CtCPR. **(b)** Mass spectra of substrate dodecanoic acid (RT: 5.10), and products 12-hydroxydodecanoic acid (RT: 7.81), and 1,12-dodecanedioic acid (RT: 8.32). Reaction conditions: buffer = 200 mM potassium phosphate pH 8.0, [CYP52A21] = 2  $\mu$ M, [CtCPR] = 50  $\mu$ M, [dodecanoic acid] = 4 mM, [glucose] = 200 mM, [glycerol] = 100 mM, [1,2-diodecanoyl-*sn*-glycero-3-phosphocoline] = 45  $\mu$ M, [*BmGDH*] = 0.2 U, [NADP<sup>+</sup>] = 0.5 mM. Reactions were incubated at 30°C for 24 hours with shaking (200 rpm).



**Figure S4.** Control of the consumption of NADPH with the different components of the reaction. The assays were carried in a final volume of 200  $\mu$ L, containing 20 mM Tris-HCl pH 8.0, 0.2  $\mu$ M CtCPR and started with the addition of 12.5 mM NADPH (black circle). The different reactions contained: 2 U/mL superoxide dismutase (SOD, square), 0.2 U/mL horseradish peroxidase (HRP, diamond), 50  $\mu$ M Ampiflu™ Red (Ampi, triangle) and HRP, SOD and Ampiflu™ Red (white circle).



**Figure S5.** Control reactions of the quantification of  $H_2O_2$  stability. Ampiflu™ Red/HRP reaction was incubated with 8 mM  $H_2O_2$ , one sample was heated at 80 °C (black) for 1 min and, after cooling down, the absorbance was measured and compared to reaction without the heating step (white).

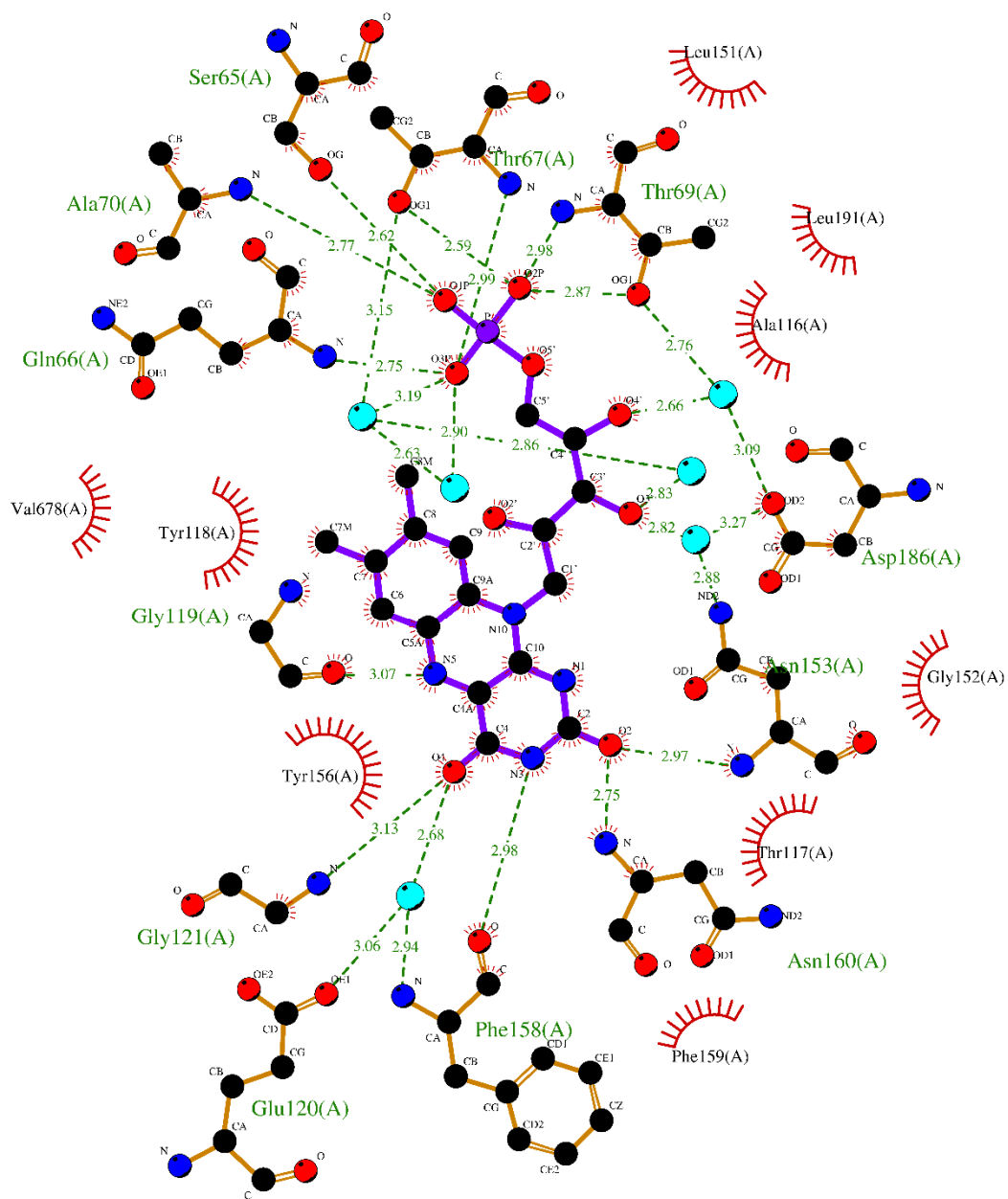


**Figure S6.** Control reactions of the total  $\text{H}_2\text{O}_2$  detected during the reactions with (black) and without (white) addition of SOD. Plot shows increase on absorbance at 560 nm over time.

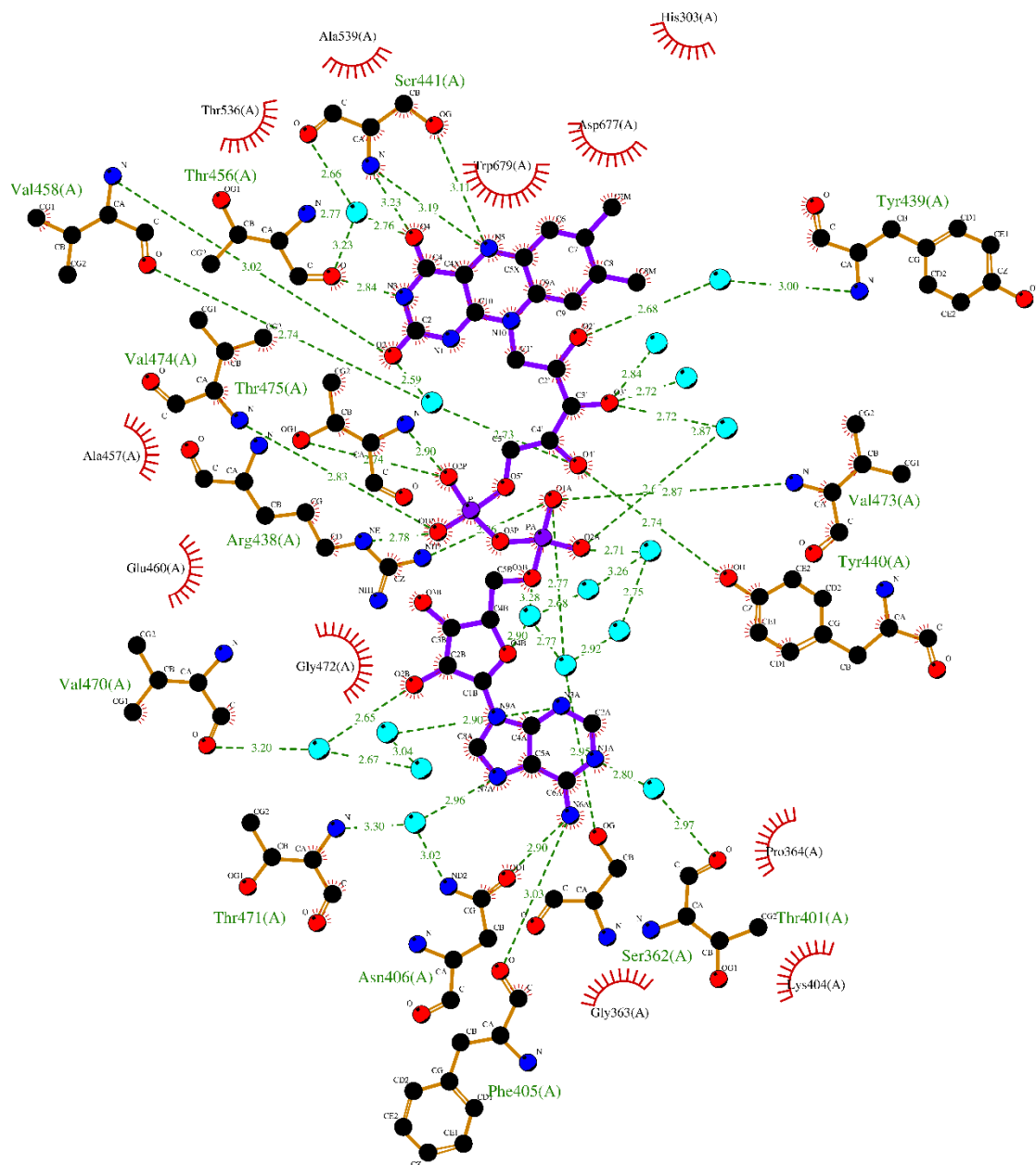
	CtCPR	CtCPR with NADPH
<b>Data collection</b>		
Space group	P 1 21 1	P 1 21 1
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	74.29, 67.18, 143.47	74.66, 67.50, 143.99
$\alpha$ , $\beta$ , $\gamma$ (°)	90.00, 91.50, 90.00	90.00, 91.55, 90.0
Resolution (Å)	52.27 - 1.50 (1.55 - 1.50)*	74.63 - 2.08 (2.16 - 2.08)
<i>R</i> <sub>merge</sub>	0.081 (0.687)	0.191 (2.247)
$\langle I/\sigma \rangle$	7.7 (1.5)	4.0 (0.5)
Completeness (%)	97.5 (96.8)	95.4 (86.9)
Redundancy	3.4 (3.5)	3.0 (2.9)
<i>CC</i> <sub>1/2</sub>	0.99 (0.72)	0.99 (0.36)
<b>Refinement</b>		
Resolution (Å)	52.27 - 1.50	74.63 - 2.08
No. reflections used	220 256	82 237
<i>R</i> <sub>work</sub> / <i>R</i> <sub>free</sub>	0.176/0.202	0.219/0.264
Molecules in ASU	2	2
No. atoms		
Protein	10 253	10 184
Ligand/ion	62 (FMN), 106 (FAD)	62 (FMN), 106 (FAD), 62 (NADPH)
Water	964	371
<i>B</i> -factors		
Protein	23.0	41.7
Ligand/ion	16.2 (FMN), 14.7 (FAD)	41.5 (FMN), 28.7 (FAD), 36.4 (NADPH)
Water	30.1	38.9
R.m.s. deviations		
Bond lengths (Å)	0.014	0.007
Bond angles (°)	1.683	1.474
Ramachandran distribution (%) Favoured/Allowed/Outliers	98.0/1.9/0.2	95.7/3.9/0.4

**Table S1.** Data collection and refinement statistics. Values in parentheses are for highest resolution shell.

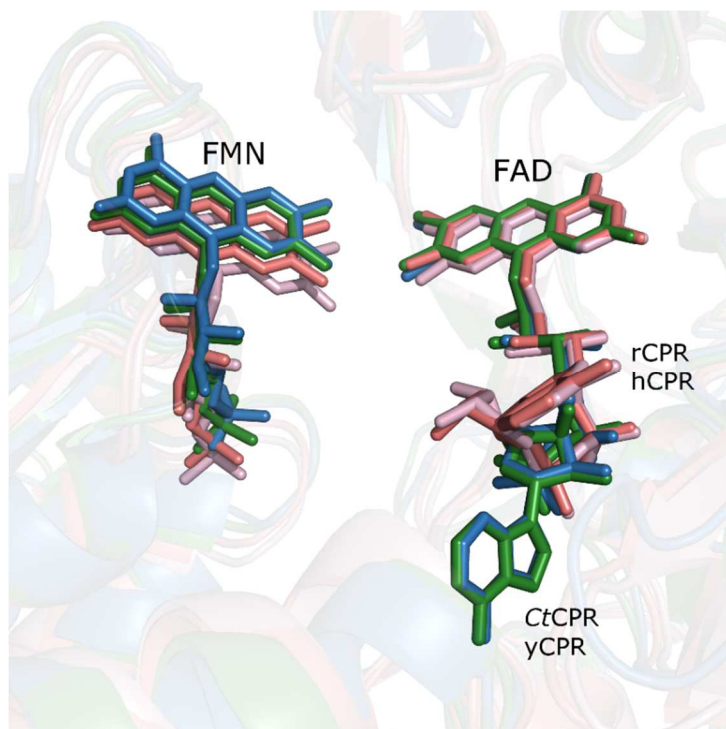




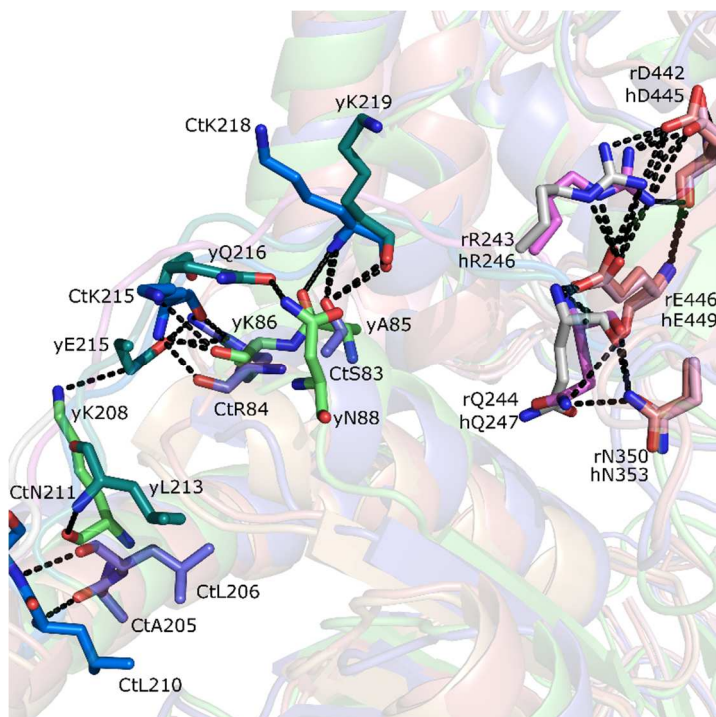
**Figure S7.** Interactions of the FMN group of CcPR with the residues and water molecules.



**Figure S8.** Interactions of the FAD group of CtCPR with the residues and water molecules.



**Figure S9.** Alignment between the FMN and FAD groups of the different CPR structures. CtCPR (green) CPR from *C. tropicalis*. yCPR (blue) *S. cerevisiae* CPR. hCPR (pink) human CPR. rCPR (salmon) rat CPR.



**Figure S10.** Interaction of the hinge region with the FMN and FAD domains for CPRs from different species. *C. tropicalis* (Ct), yeast *S. cerevisiae* (y), rat (r) and human (h) CPR structures are aligned. Residues from the hinge are displayed Ct (blue), y (green), r (grey), h (magenta) and their interactions with amino acids from the FMN domain (light blue for Ct and light green for y) and the FAD domain (salmon for r and pink for h).

NaCl (M)						
	0	0.1	0.15	0.2	0.5	1
NADPH	100	100	100	100	100	100
H <sub>2</sub> O <sub>2</sub>	27	33	33	31	36	51
Cytochrome c	73	67	67	69	64	49

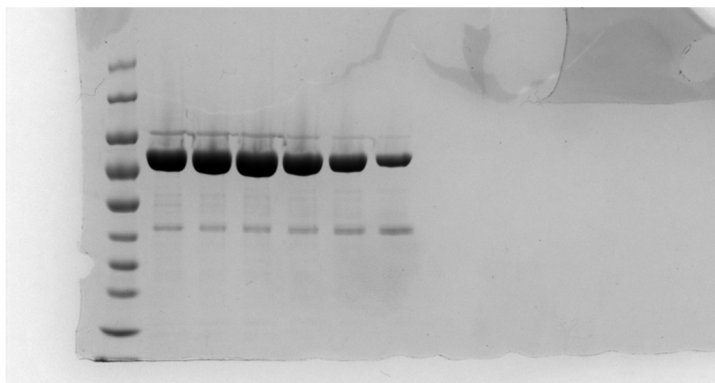
**Table S2.** Uncoupling percentage (%) relative to NADPH consumed at different ionic strengths.

pH						
	5.5	6.0	6.5	7.0	7.5	8.0
NADPH	100	100	100	100	100	100
H <sub>2</sub> O <sub>2</sub>	34	30	27	28	29	28
Cytochrome c	66	70	73	72	71	72

**Table S3.** Uncoupling percentage (%) relative to NADPH consumed at different pH.

T (°C)				
	20	25	37	42
NADPH	100	100	100	100
H <sub>2</sub> O <sub>2</sub>	28	31	24	29
Cytochrome c	72	69	76	71

**Table S4.** Uncoupling percentage (%) relative to NADPH consumed at different temperatures.



**Figure S11:** Full-length SDS-PAGE gel used in the preparation of the cropped insert for Figure 1 in the main text. Lane 1 represent the molecular weight marker, with subsequent lanes the eluted fractions from gel filtration chromatography.