

Microorganisms

Supplementary Material to the Article

A new thermophilic ene-reductase from the filamentous anoxygenic phototrophic bacterium *Chloroflexus aggregans*

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YqjM	-----	MARKLFTPITIKDMT	15
XenA	-----	MSALFEPYTLKDVT	14
RmER	-----	MPHLFDPYRIGNLE	14
DrER	-----	MTVSSAAAPQPASPAAPLLFTPLKLRSLE	29
RhrER2718	-----	VSVLFEPPLTLRGVT	14
OYERo2	-----	MSVLFEPIITFRGVVT	14
PfvC	-----	MPALFRPPLTLRSLE	14
CrOYE1	MGSATKASGSACHVPGAASTAFACPVRKHQPMRQPLHAVGTPGPSGRPLLFEPITLRLQT	60	
AnabaenaER3	-----	-MDLFTPPLIIRDIT	13
GloeER	-----	-MHLFAPPLTLRDIT	13
CaOYE	-----	-MQPHLFPTPLTIGSVT	15
FOYE1	-----	MSLLFSPYQLGSLS	14
ChrOYE3	-----	-MLYTPVNFRNVE	12
GkOYE	-----	MNTMLFSPYTIIRGLT	15
GeoER	-----	METMLFSPYTIIRGVVT	15
TsOYE	-----	MALLFTPPLELGGLR	14
TOYE	-----	MSILHMPKIKDIT	14
		* . * : :	
YqjM	LKNRIVMSPMCQYSSHEKD---	GKLTPFHMAYHISRAIGQVGLIIVEASAVNPQGRITDQ	72
XenA	LRNRRIAPPMCQYMA-ED---	GLINDWHQVHYASMARGGAGLLVVEATAVAPEGRITPG	69
RmER	LANRRIAIAPMCQYSA-QE---	GNATDWHMIHLGQMALSGAGLLIEATAVSPEGRITPT	69
DrER	LPNRRVVSPMCQYSA-TD---	GVAINEFHVLHGLQYALGGAGLILEATAVSPEGRITPE	84
RhrER2718	IPNRRVWAMPMCQYSA-ADVIGDQVGVPNEWWRTHLVSRRAIGGTGLLITEATAVSPEGRISAA	74	
OYERo2	VPNRRVWAMPMCQYSA-ADVTGRDVGVPGDWRHTLVTRAIGGAGLILTEATAVSPEGRISPA	74	
PfvC	LTHRGWVSPMCQYSCGPDG-APGVPNDWHLMLHLSFAAGGAALILTEAAVNAAGRISPR	73	
CrOYE1	IPNRIIWLSPMCQYSA-VD---	GAPNTWHLVHLGARAAAGGCCVLMTEACAVVPEGRISPS	115
AnabaenaER3	LPSRVAMSPMCQYSA-EN--	-GFANDWHFWVHLGSRAVGGTGLIMVEATAVTPQGRITPG	68
GloeER	LRNRRIAVSPMCQYSS-ID--	-GLANDWHFWVHLGSRAVGGALVIFEAAVEARGRISPO	68
CaOYE	LRNRIGMSPMCQYSA-VD--	-GFPTDWHLMLHGLARAAGGVLILTEATAVSPEGRISPF	70
FOYE1	LANRLVIAPMCQYSA-VD---	GIAQDWHMLHGLRILAISGAGLVIVEATGVNPEGRITPF	69
ChrOYE3	LKNRWRVMSPMCQYSS-ED--	-GLPNDFHVHYGSRAQGGAGLILLEATGVPEPRGRITNH	67
GkOYE	LKNRIVMSPMCQYSCDTKD--	-GAVRTWHKIHYPARAVQGVGLIIVEATGVTPQGRISER	72
GeoER	LKNRIVMSPMCQYSCDTE--	-GKVRTWHKIHYTRAVQGVGLIIVEATVTAQGRISAR	72
TsOYE	LKNRLAMSPMCQYSATLE--	-GEVTDWHLHYPTRALGGVGLLVEATAVEPLGRISPY	70
TOYE	IKNRIMMSPMCQYSASTD--	-GMPNDWHVHYATRAIGGVGLIMQEATAVESRGRITDH	70
	:	*	***:
YqjM	DLGIWSDEHIEGFAKLTEQVKEQGS---KIGIQLAHAGRKAEL-----G-	114	
XenA	CAGIWSDAHAQAFVPVVAQIAKAGS---VPGIQIAHAGRKASANRPWE-GDDHIG-ADDA	124	
RmER	DLGLYNDANEAALGRVLGAVRNHSP---IAVTQLAHAGRKASSEAPWD-GGGQIRPD-QP	125	
DrER	DLGLWDDROQIVPLGHITDFVHQHGG---HIGVQLAHAGRKASTYAPWGR-GKGAVPA--EL	138	
RhrER2718	DLGIWNNDTQQAFAEINAQLAYFGA---VPGIQLAHAGRCASTQVWPW-GKSLP-ADDR	129	
OYERo2	DLGIWNNDTQTEFAEINAQLEYFGA---VPGIQLGHAGRKGSAHVPWR-GGSSLG-DDDR	129	
PfvC	DAGLYNDEQAEAWQRITSFVHRHGAAEAKIGQLAHAGRKASTYWFPSGORGSVPESD--	131	
CrOYE1	DAGMWNDAAQPLRQTSVHGRKASTFIRSHGA---VPSVQLAHAGRKASTFPFWVQGGRPLYPGKDA	172	
AnabaenaER3	DLGLWDDKKQIEPLTRIVFLRQQGS---VTGQLAHAGRKASCNPWVW-GCTPLTPEQ--	122	
GloeER	DLGIWSDAHIEPLRRINDFIHRQES---VAGIQIAHAGRKASTARPWE-GGGPLTEGE--	122	
CaOYE	DLGIWSDDHIAALSRIVKLIESLGA---VAGIQLAHAGRKASVGRPWE-GCKPIAPAN--	124	
FOYE1	CLGLYNDEQAEAWQRITSVHGRKASTFIRSHGA---VAGIQLAHAGRKASTRFPWD-PGSPSPE--E	124	
ChrOYE3	CMGIWNSDDHIAQLRQEVEVHKNSE---SKIGIQLSHSGRKGSTWNTK----QIS--VE	117	
GkOYE	DLGIWSDDHIAQLRELVGLVKEHGA---AIGIQLAHAGRKSQVP-----G-	114	
GeoER	DLGIWSDEHVQGLRELVSLVKEHGA---KIGIQLAHAGRKAEV-----G-	114	
TsOYE	DLGIWSSEDHPLGLKELARRIREAGA---VPGIQLAHAGRKAGTARPWE-GGKPL-----	120	
TOYE	DLGIWNDEQVKELKKIVDICKANGA---VMGQLAHAGRKCNCIS-----	111	
	* : : :	: * : * .	***: * * . * ***
YqjM	----DIFAPSIAFDEQSA-TPVEMSAEKVKETVQEFKQAAARAKEAGFDVIEIHAAGY	169	
XenA	RGW-E-TIAPSIAFGAHLPNPVRAMTLDDIARVKQDFVDAARRARDAGFEWIELFAGY	183	
RmER	RGWQ-TFAPSAPVHAAGEV-PPAALDKAGMKKLRDDFVAAAARKAALGIEGIEVREGAGY	183	
DrER	GGWQ-VIGPDENSFHDLFPT-TPAMMGADELRRGVDAFSAARRAQVAGFDAVEVHAAGY	196	
RhrER2718	LSWQ-TVAPSAPVFG-HLA-DPVELTTEGIEKVVADFAAAATRALKAEFKVEVHAAGY	186	
OYERo2	LSWQ-TVAPSAPVFG-DHT-PPAATTADIRKVVADFAAAERASRAGFVKVEVHAAGY	186	
PfvC	GGWA-TVGPSPSAFD-GYA-EPAAMTEEQIQQGVISDFAAAARAVDAGFDTLELREGAGY	188	
CrOYE1	AAWQ-VVAPSAPVFA-DFQ-TPREMTEADIREVIDAFAAAERSIKAGFQVVEIHAAGY	229	
AnabaenaER3	GGWQ-PVAPSPIPFQENAP-VPISLDERGIQETIFAFVAAAQRALQVGFQMIIEIHAAGY	180	
GloeER	GGWADSVAPSALPFDAGYP-VPEALDEAGIAATVQAFAAAARRSLAAGFVLEIHAAGY	181	
CaOYE	GGWP-VVGPTAEPFPAGYP-TPIPLDAAGIARVVADPATATKRARAAGFRWEIHAAGY	182	
FOYE1	GGWQ-TWAPSIAKFYEESSL-TPHPMSIEDLETVKQDFVNSAIRAERAGFKAIELREGAGY	182	
ChrOYE3	EGWE-TIAPSIPYHPTER-IPHVLTQEEEIKEQVRNFREARRAVEAGFDVIEIHAAGY	175	
GkOYE	----EIIAPSAPVFDSSP-TPKEMTKADIEETVQAFQNGARRAKEAGFDVIEIHAAGY	169	
GeoER	----EIIAPSAPVFDSSP-TPKEMTKDIEETIQAQFQNGARRAKEAGFDVIEIHAAGY	169	
TsOYE	--GWR-VVGPSPIPFDEGYP-VPEPLDEAGMERILQAFVEGARRALRAGFQVIELBMAAGY	177	
TOYE	--YEDVVGPSPIKAGDRYK-LPRELSVVEEIKSIVKAFGEAKRANLAGYDVVEIHAAGY	168	
	* : :	* . : * .	: * : * ***

YqjM	LIHEFLSPLSNHRTDEGGSPENRYRFLREIIDEVK Q QVWD--GPLFVRVSASDYTD----	223
XenA	LGOSFFSEHSNKRTDAYGGSFDNRSRFLLLETAAV R EWPENLPLTARFGVLEYDGR---	240
RmER	LLHQFLSPIANHRTDEGGSL E NRMRFPLEVFDAV R EAFPAERPVWMRVSATDWVP---	239
DrER	LLHQFLSPLANTRTDYGGSFENR T RLLLEILTAV R EWPAAELPLFVRVSATDWLTERG	252
RhrER2718	LIHQFLSPESNKRTDRYGGSFENR I RLLLEILTAV R EWPAAELPLFVRVSATDWLTERG	246
OYERo2	LLHQFLSPVSNHRTDEYGGSFAGR I RLLLEEVDAVRGVWPAELPLFVRVSATDWLSEEPG	246
PfvC	LLHQFLSPNTRTDWSGGNEAGRNRMLIA D AVREVMPESMPLLRIASATDWAE---	244
CrOYE1	LLHQFLSPQANKRKD O YGGSLANRMRPLAVAAEAVRAV I PPPELPLAVRISATDWGP---	286
AnabaenaER3	LLHSFLSPLSNRRTDRYGGSL E NRMRLLEVVRRV D VLPNGMPLFVRISATDWVE---	236
GloeOER	LLHSFLSPLSNRRTDRWGG F ENR I RLLLAVVAEAVRGWPERLPLFVRISATDWTE---	237
CaOYE	LLHNFLSPLGNDRNDEGGDLRGRVRLLSEVTAAVRAEWPSDLPLAVRLCSDWTP ---	238
FOYE1	LIHQFLSPLSNRQD O YGGSL E NRMRF I RFLVEIVDAVN Q ELNDNVALFVRISGTEYAE---	231
ChrOYE3	LINEFLSPLSNRQD E YGGSPENRYRFLGEVIDAV R EVWWD--GPLFVRISASDYHP---	223
GkOYE	LINEFLSPLANKRQD E YGGSSENRYRFLGEVIDAV R QVWD--GPLFVRISASDYHP---	223
GeoER	LLSSFLSPLSNQRTDAYGGSL E NRMRFPLQVAQAVREVVPRELPLFVRVSATDWGE	233
TsOYE	LIHEFLSPLSNKRD E YGN S ENR A RFLIEV I DEVR K NKPEN K IFVRVSADYME---	224
TOYE	* . * * : * : * . * . : * . . . :	
YqjM	---KGLDIADHIGFAKWMKEQGVLDIDCSSGALV-HADINVPGYQVFSFAKIR-EQADM	278
XenA	---DEQTLESIELARRFKAGLDLLSVSGFTIPETNIWPWGA M GP <i>IA</i> ERVR-REAKL	296
RmER	---NGWDIEGTIALSH E LKARGSAAVHVGSTGGVSPQQAIKIGPGYQVPGYQVFSFAKIR-EAEVL	295
DrER	---GGWDL E QTQVQLSKLLKYEGVDVLDI S SSGG I AAQQIEVPGYQVPGYQVFSFAKIR-EAEV	309
RhrER2718	LEVD E SWTADQTVANILNSDYGVL D VDS T GGNSPAAOQIPVEPGYQVPGYQVFSFAKIR-NESLL	305
OYERo2	LDADS E WTQTVLSVQ A LAQ D LG V LDV D VSSGGVA-SARI P IGPGYQVPGYQVFSFAKIR-NEETV	304
PfvC	---CG E D I EAS V LAQA H E G V D LDVSSGG A HH Q Q I Q P GP G Y Q T C FS A R I R-RET R	300
CrOYE1	LDGPAW D VQS V LC G AL R LG C LD I D V SSGG Q LP R AL I P V PG G Y Q V F SE R IR-REAR R	345
AnabaenaER3	---GGWDL Q QS I LS R EL K TL G V D LD V ST T GG V PH A RI P VE K GY Q V F AA K IR-EEAGI	292
GloeOER	---GGWDL Q QS V LA Q VL A RS G V D LD V SSGG V PH A RI P AG P GY Q T R FA E IR-AEADM	293
CaOYE	--- E GLTI A DT V EV A ML R LE Q GV D LD C SSGG I AP G IT I P V GE G Y Q V F AA Q VR-REANI	294
FOYE1	---CG E LT I E S IT F S Q CE K R G AG F IV H V T GG V AH Q Q I P V PG G Y Q EH A Q I K-Q N VI	294
ChrOYE3	---NGWEIT D SV E LS V KL N HS V DL D V D VSSGG N I H GV K IP L FD G Y Q V PL AS Q VR-NEADV	287
GkOYE	---DGLTA K D V PY A CRM K EQ G V D LD V SSGG A IV-PARMNVY P GY Q V F AA E IR-READI	278
GeoER	---EGLTA K D V Y P Y A CRM K EQ G V D LD V SSGG A IV-PATINAY P GY Q V F AA E IR-REAEI	278
TsOYE	---CGWSLED T LA F ARR L KE V LD D CCSSGG V LR V IP L AP G F Q V F AD A VR-KR V GL	289
TOYE	---GGINIDMMVEYINMIKD-KV D LD V SSGG L L-NVDINLY P GY Q V K YA E TIK-KRCNI	278
	: : * * : : : : : : : :	
YqjM	ATGAVGM I TDGSMAEE I LQNQGRAD I LF I GRE L RD P FF F ART A Q L N T E I --PAPV Q Y E	336
XenA	PVTS A WG G PT P Q L AE A AL Q AN Q LD L VS V GR A HL D PH W AY F AA K EL G VE K A-SW T LP P Y	355
RmER	PTMAVG L TE E AE Q AE A I AN NE AD I I S I AR A ML D P R W P W H AA A KL G AS V --NAP K Q Y E	353
DrER	SVM A VG L I E T Q A E AI L Q Q AD A DL I AL G RP F LR D P H WA Q RA A RE L GP R P V -S- I Q Y E	367
RhrER2718	PAA A VG L I E TE P Q E AE K I V ED G AV A V V I GRE L RD P Y W RA R ARE E LN A EV G PH I PS Q Y E	365
OYERo2	PAA A VG L I E TE P Q E AE I V E SE A VA V V I GRE L RD P Y W RA R ARE E LN A EV G PH I PS Q Y E	364
PfvC	PTGT V G L IT T SP G Q E AE H AV A T G Q D GF V I RA A AL R DP H WW L RA A FE L GH D L--AWAP Q Y E	358
CrOYE1	ATGT V G L IT T KG Q AE K I L Q E KG A D V V I GRE M LR D P N PL R AA E EL G Y E GA-RYPP Q Y E	404
AnabaenaER3	MTG A VG L IN E AE Y AD Q I T RG C AD V L I GRE L RR N P Y W S I Y ARC S LD E EP--NWPV P Y G	350
GloeOER	LTG A VG G Q T SA E Q D H I V R T Q AD L V I L GRE L RR D P Y W P L K AA V EL R AP G --PW P Q Y E	351
CaOYE	ATA AV G L I TT P E H AD A IV R NG D AD L V I L GRE L RD P HW P LA R AL G HD L --APP Q Y E	352
FOYE1	PTMAVG L I H TH S QA E TI L K S EQ A DM I I AR A AL K NP H WP W TA A EL G DK P --FAPP Q Y E	352
ChrOYE3	KTG A VG L IK V K S HA E II L KG D AD L I FI I ARE M LR N P Y LA V Q G F E M N E E C--FFPH Q Y E	345
GkOYE	PTGA V G L IT T SG W Q E EE I LL Q NG R AD L V F L ARE E LL R NP Y W P Y A ARE L G A K I --SAP Q Y E	336
GeoER	ATGA V G L IT T SG W Q E EE I LL Q NG R AD L V F L ARE E LL R NP Y W P Y A ARE L G A K I --PAP V Q Y E	336
TsOYE	RTGA V G L IT T PE Q A E T L L Q AG S AD L V F L L G R V LL R DP Y PL R AA K AL G V A --EVPP Q Y E	347
TOYE	KTS A VG L IT T Q E LA E E I LS N ER A DL V A L GRE L RR D P Y W V L H TY T S----KEDWPK Q Y E	333
	* : : * : : : : : * : * : * : :	
YqjM	GW-----	338
XenA	AH T LER R -----	363
RmER	SQPRGLE K LFDAHF Q R	371
DrER	AGW-----	370
RhrER2718	AF-----	367
OYERo2	AY-----	366
PfvC	AVPRHFS-----	365
CrOYE1	GKFPIK-----	410
AnabaenaER3	AVKRQR-----	357
GloeOER	AKP-----	354
CaOYE	AW -----	354
FOYE1	AR-----	354
ChrOYE3	AKISS-----	350
GkOYE	GWRF-----	340
GeoER	GWRF-----	340
TsOYE	GF-----	349
TOYE	AFKK-----	337

Figure S1. Sequence alignment of the putative ER from *Chloroflexus aggregans* performed by Clustal Omega. The catalytic residues are highlighted in pink and the finger print motifs of thermophilic-like OYE reported by Oberdorfer et al. (2011) are highlighted in green and cyan. The residues shaded in grey are conserved among OYE family, and the one in bold indicate similar residues. Residues highlighted in yellow show the sequence conservation of FMN binding. Residues highlighted in petrol green are involved in dimer formation. The accession numbers are reported in Table S3.

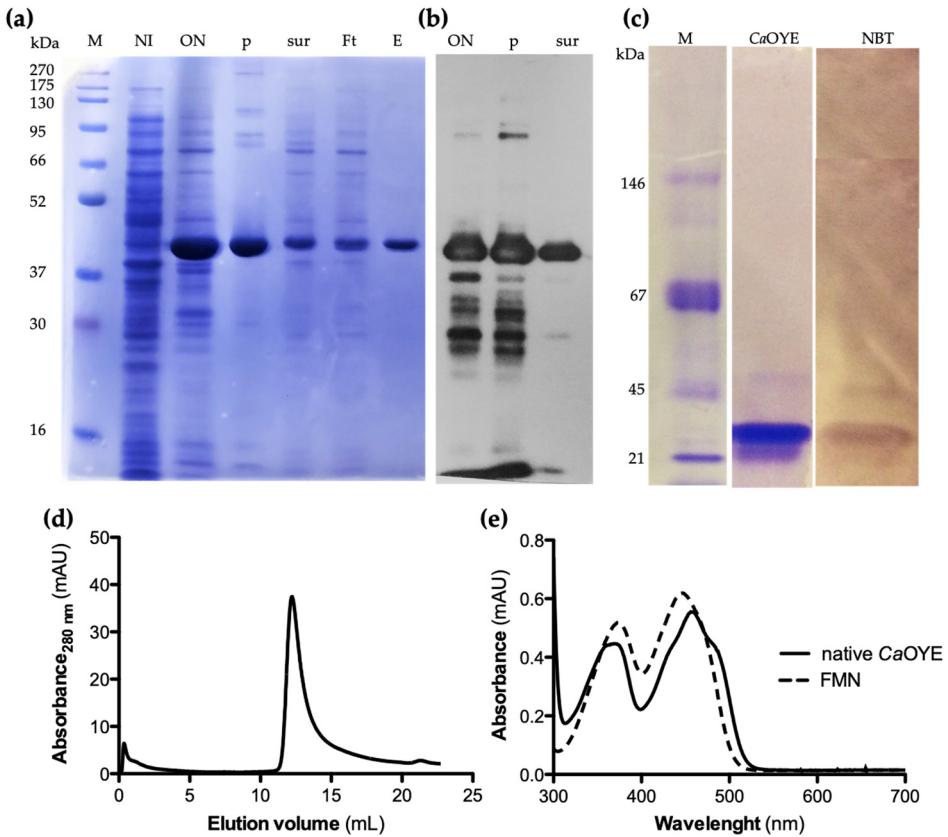


Figure S2. (a) SDS-PAGE 12% acrylamide: BL21(DE3) grown at 25 °C expressing *CaOYE*: Prestained SharpMass™ VII protein ladder (M), total cell extracts from not-induced cells (NI) and overnight induced cells with IPTG (ON), pellet fraction (p), soluble protein fraction (sur), flow-through (Ft) and pooled eluted fractions from IMAC (E); (b) Western blot assay with anti-His tag antibodies with samples of protein induced at 25 °C; (c) Native-PAGE using precast NuPAGE™ Tris-Acetate 3-8% gel from ThermoFisher: SERVA native marker (M), *CaOYE* samples stained by Commassie brilliant blue and by NTB stain; (d) Analytical gel filtration of purified *CaOYE* sample eluted from a Superose 12 300/GL column.; (e) Determination of *CaOYE* concentration based on flavin absorption spectra of purified enzymes and released flavin after thermal denaturation (dotted line).

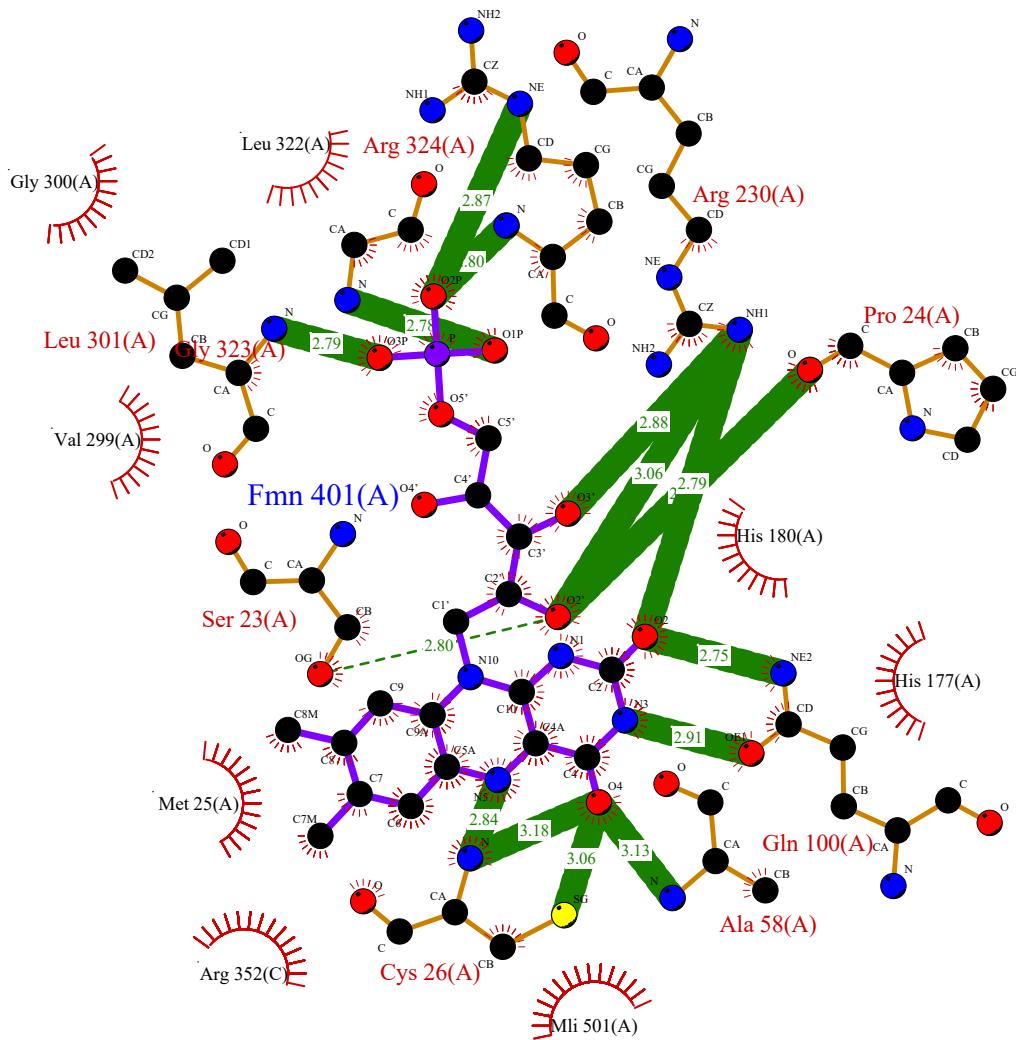


Figure S3. Schematic illustration of the interaction of FMN with active site residues of *CaOYE*. The thin green dotted lines illustrate hydrogen bonds.

Supplementary Tables

Table S1. Oligonucleotides used for PCR amplification of *CaOYE* sequence and cloning.

Primer	Sequence (5' → 3') ¹
CaOYE 1_for	ATGATGCAATTAAAGCAGCACGTCCG
CaOYE 2_rev	GCGCGCATGACGAATTATCGTACC
CaOYE 3_for	TTCAACTACTGAGAGAGCC <u>CATATG</u> CAACCACATTATTAC
CaOYE 4_rev	TATA <u>GGATC</u> CTCACCACGCCGCAA <u>ACTGC</u>

¹ Restriction sites are shown in bold. Modifications introduced to mutate the original sequences are underlined.

Table S2. X-ray crystallographic data collection and refinement statistics of CaOYE (pdb 7O0T).

Data collection statistics	
Wavelength	0.97
Space group	C 2 2 21
Cell constants	
a, b, c (Å)	108.194 162.274 216.2
α, β, γ (°)	90 90 90
Resolution range for refinement (Å)	38.01 - 2.40 (2.641 - 2.40)
Total reflections	343346 (22759)
Number of Unique Reflections	73908 (4571)
R _{merge}	0.185 (0.522)
<I / sigma(I)>	4.9 (1.8)
Completeness (%)	99.3 (100.00)
Multiplicity	4.6 (5.0)
Refinement statistics	
R _{work} / R _{free}	0.22/0.26
RMSD bond lengths (Å)	0.0068
RMSD bond angles (°)	1.47
Average B factor	34.24
Main chain B factor	34.38
Ligands B factor	26.97
Water molecules B factor	29.45
No. of non-hydrogen atoms	
Number of protein atoms	10754
Number of ligands atoms	159
Number of water molecules atoms	188
Ramachandran plot (%)	
Most favoured	95.03
Generously allowed	4.54
Outliers	0.43

Table S3. List of OYE sequences used for the phylogenetic analysis, and their accession numbers from the National Center for Biotechnology Information (NCBI) or Phytozome (*).

Protein name	Organism	Accession number
AcaryoER1	<i>Acaryochloris marina</i> MBIC11017	ABW29811
AcaryoER3	<i>Acaryochloris marina</i> MBIC11017	ABW32756
AnabaenaER3	<i>Trichormus variabilis</i> ATCC 29413	ABA25236
AtOPR1	<i>Arabidopsis thaliana</i>	NP_177794
AtOPR3	<i>Arabidopsis thaliana</i>	NP_001077884
CaOYE	<i>Chloroflexus aggregans</i>	WP_015941499.1
Chr-OYE3	<i>Cryseobacterium</i> sp. CA49	AHV90721
CrOYE1	<i>Chlamydomonas reinhardtii</i>	Cre01.g050150*
CrOYE2	<i>Chlamydomonas reinhardtii</i>	Cre03.g210513*
CrOYE3	<i>Chlamydomonas reinhardtii</i>	Cre17.g727300*
CtOYE	<i>Chroococcidiopsis thermalis</i>	WP_015152687.1
CyanothER1	<i>Rippkaea orientalis</i> PCC 8801	ACK64210
CyanothER2	<i>Rippkaea orientalis</i> PCC 8801	ACK65723
DrER	<i>Deinococcus radiodurans</i> R1	AAF11740
FOYE-1	<i>Ferrovum</i> sp. JA12	KRH78075
GeoER	<i>Geobacillus</i> sp. #30	BAO37313
GkOYE	<i>Geobacillus kaustophilus</i> HTA426	BAD76617
GloeoER	<i>Gloeobacter violaceus</i> PCC 7421	BAC91769
GsOYE	<i>Galdieria sulphuraria</i>	XP_005703492.1
LeOPR1	<i>Solanum lycopersicum</i>	NP_001234781
LeOPR2	<i>Solanum lycopersicum</i>	NP_001233868
LeOPR3	<i>Solanum lycopersicum</i>	NP_001233873
LyngbyaER1	<i>Lyngbya</i> sp. PCC 8106	EAW37813
NospuncER1	<i>Nostoc punctiforme</i> PCC 73102	ACC84535
NostocER1	<i>Nostoc</i> sp. PCC 7120	BAB73564
OYERo2	<i>Rhodococcus opacus</i> 1CP	ALL54975
Pfvc	<i>Arthrobacter</i> sp. JBH1	AFF18622
RhrER2718	<i>Rhodococcus rhodochrous</i> ATCC 17895	AMD82542
RmER	<i>Cupriavidus metallidurans</i> CH34	ABF11721
SynER	<i>Synechococcus elongatus</i> PCC 7942	ABB56505
TOYE	<i>Thermoanaerobacter pseudethanolicus</i> ATCC	ABY93685
TsOYE	<i>Thermus scotoductus</i> SA-01	CAP16804
XenA	<i>Pseudomonas putida</i>	AAF02538
YqjM	<i>Bacillus subtilis</i>	BAA12619

Table S4. Thermostability fingerprints. Parameters referring to primary sequence have been calculated with ProtParam Expasy; oligomerization states in solution have been obtained from literature data and derived from size exclusion, native gel or light scattering measurements; in crystal oligomerization data were deduced from crystallographic structures, as well as interface area and number of interactions (Hydrogen bonds, Hb, and Salt bridges, Sb) involving the dimerization surface, calculated by PISA software; T_{opt} corresponds to the optimal temperature for enzymatic activity and it has been deduced from literature data, while T_m corresponds to melting temperature of the corresponding recombinant enzymes, measured either by Fluorescence (ThermoFAD or Thermofluor) or Circular dichroism.

Enzyme	Oligomerization								
	Sequence length (aa)	Arg/Lys ratio	Total proline content (%)	In solution	In crystal	T_{opt} (°C)	T_m (°C)	Interface (\AA^2 , %tot)	Interactions (Hb, Sb)
<i>CaOYE</i>	354	7.0	8.2	monomer	dimer	-	79	1451 (10.4%)	(16; 8)
<i>FOYE1</i>	354	1.6	6.2	-	-	50 ($t_{1/2}=5\text{h}$)	-	-	-
<i>GkOYE</i>	340	2.2	6.2	tetramer	dimer	70	76-82	1211 (8.9%)	(16; 0)
<i>GeoER</i>	340	1.7	5.0	trimer or tetramer	-	70	-	-	-
<i>TsOYE</i>	349	4.6	8.0	dimer	octamer of dimers	65	-	1273 (9.3%)	(16; 2)
<i>TOYE</i>	337	0.7	3.6	tetramer, octamer, dodecamer	dimer of dimers	-	> 70	1073 (8.0%)	(15; 0)
<i>YqjM</i>	338	0.9	3.8	tetramer	dimer of dimers	25-30 ($t_{1/2}=9\text{ days}$)	-	1152 (8.5%)	(13; 0)
<i>XenA</i>	363	2.9	6.1	dimer	dimer	-	50.4	1346 (9.7%)	(2; 2)
<i>RmER</i>	371	2.0	7.0	monomer	dimer	35	-	2132 (14%)	(28; 7)
<i>DrER</i>	370	4.4	6.2	dimer	-	30	-	-	-
<i>ChrOYE3</i>	350	1.3	4.0	dimers, tetramers	-	35 ($t_{1/2}=233.5\text{h}$)	-	-	-
<i>RhrER2718</i>	367	3.4	6.3	dimer	-	40	-	-	-
<i>OYERo2</i>	366	6.3	6.8	dimer/ tetramer conc. dependent	-	up to 20	-	-	-

PfvC	365	12.5	6.0	-	-	-	-	-	-
<i>CrOYE1</i>	410	2.5	9.0	-	-	40	-	-	-
Anabaena ER3	357	4.8	7.0	-	-	-	-	-	-
GloeoER	354	10.7	6.2	-	-	-	-	-	-