

Structural and Functional Characterization of a New Thermophilic-like OYE from *Aspergillus flavus*

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Nox		-----MTTAPTPTSIPEPARLIG-PLTLNRNIRVKAATFE--	31
YqiG		-----MNPKYKLPFFFTFKSGVTIDNRNAVAPMTTHYA	33
Chr-OYE1		-----MSTSELTFTPKYK-NLELKNRIVMAPMTRQA	30
AfoYel		MTQDIIDNIAAEGISYYTPAQVPVPPAGTQVEGSTKLFSPLITIR-GVTFPNRLFLA FLCQYS	59
YqjM		-----MARKLFPTPIIK-DMTLKNRIVMS PfMCMS	29
PETNR		-----MSAEKLTPLKVGV-AVTAPNRVFMAPLTRLR	30
OYE1		-----MSFVKDFKPQALGDNLFLFKPIKIG-NNELHRAVIPPLTRMR	41
		: * * : *	
Nox		GVMPRGAASDDL-INFHAEEVARGGAAMTTVAYCAVSP---GGRVHRDRLVMDERALPGLRR	88
YqiG		SNEDGTISEAE--LDYIIPR-SKEMGMVITACANVTDP-GKAFPGQPAlHDDSNIPGLKK	89
Chr-OYE1		SD---NGVPTQQI-ADYAYARRAAAEVLILSEGTVINRPASKNNQNIPDFYTEALNGWKN	87
AfoYel		AK---DG-YANDWHLTHIGGIVRQGPGLAIMEATAVQKV- GRITPDQLGLYDDGHIEPLKR	115
YqjM		SHEKDQ-KLTPPHMAHYISRAIQGVGLIIVEASAVNPQ- GRITPDQLGLWSDEHIEGFAG	87
PETNR		SIEPGDIPPTLM-GEYYRQRA---SAGLIISEATQISAQ-AKGYGAGPLGHSPEQIAAWKK	86
OYE1		ALHPGINPNRDWAVEYYYTQRARQRPQMITEGAFISQP-AGGYDNAPGVWSEEQMVETK	100
		. . . : . . . :	
Nox		LTDVAHGAELAAAIQHAGHLVANTLSNK-----TKTLAPSTRL-SPP	130
YqiG		LAQAIQAQGAQAVVQIHGGGIECPSEL-VPQQ-----DVVGPSDVF---DN	131
Chr-OYE1		VIDADHHNGMGMPQIWHVGDTRSTDY-YPL-----E	118
AfoYel		ITEFAHSSQSQTIGIQLAHAGRKASAVAPWLSGNAMAVEKVG---GWPDIDIVAPSAPQEEG	173
YqjM		LTEQVKKEGSKTGIGIQLAHAGRKAELEGDIFA-----PSAIFDEQ	123
PETNR		ITAGVHAEDGRIAVQLWHTGRISHSSI---QPGGQAPVSASALNANTRTSLRDENGNAIRV	144
OYE1		IFNAIHEKKSFWVVQLWLGVAAFPDN---LARDGLRYDSA---SDNVFMDAEQEAKAKK	154
		: : : * : *	
Nox		AMGLVKGATLAELDGVVDSDFERTARVAVDAGFDAIEV HLGN YLSSFSMSPNLNKRHDY	190
YqiG		GKQIARALKEEEVENIVKAFGEATTRAIRAIEAGFDGVEI HANG VLIQQFYSPKTNQRTDRW	191
Chr-OYE1		DMEKASTMTLEDIQDTIAQFAASAKSAKDLGFDVLEI HANG VLIDQFFWEGTNTRTDEY	178
AfoYel		INAPVKVLTEGIDIGLVKDWAEAARKAVRANFDAIE HAAG VLHQLFSPVNRRRTDKY	233
YqjM		-SATPPEAESAEVKETVQEFKQAAAARAKEAGFDVIE HAAG VLHIEFLSPSNNHRTDEY	186
PETNR		DTTTPRALMEDLPIGVINDVANAREAGFDDELVE HAAG VLHQLFSPSNNDTQDY	204
OYE1		ANNPQHSLSLKDEIKQYIKEYYVQAANKNSIAGADGVEI SAG VLNQFLDPHSNTRTDEY	214
		.: : .: . : * : * : * : * : *	
Nox		GGG-VAKRAEYPRRVIEAVRVAAGSSV----AV-----TAKFNMSDGVPKGLWLDQSLP	239
YqiG		GGG-DEKRLAFPALIVDEVKKAASEHAKGAFLVGYRLSPPEEPETPGLTMTET----	242
Chr-OYE1		GGKTIKERSFAVDVVKAIARAAGED-----PTIIIRLSQWKQDYSVKLAHTP--EE---	229
AfoYel		GGG-FENRVIRILLEICEEVRAVIPTA-----GPLLVRSITADFWEFDDNLTKEF--PESWT	286
YqjM		GGG-PENRVYFLREILIDEVKQVWD-----GPLFVRVASDSDYTDKG-----LD	227
PETNR		GGG-VENRARLVLEVVDVAVCNWSAD-----RIGIRVSPIGTFQNVNDGPNNE-----EA	252
OYE1		GGG-IENRARFTLEVVDVALVEAIGHGE-----KVGRLRSPYGVFNMSMGAET-----GI	262
		** . : * : . : : :	
Nox		IAQILEADGHLDAMQLTGGSSLLNGMYFFRGEVPLAEFVASQPKLVGYGLFYGPKIFPT	299
YqiG		-----YTLVDALGDK-----ELD-----YLHISLMDV-----NSK	267
Chr-OYE1		-----MEEWLLPLDKA-----GVD-----IFCHSQRRF-----WEP	255
AfoYel		VAQSIGRLALLADR-----GVD-----LVDDVSSGGI-----HAK	315
YqjM		IADHIGFAKWMEQ-----GVD-----LIDCSSGAL-----VH-	255
PETNR		DALY-LIEELAKR-----GIA-----YLHMSSETDL-----A-	277
OYE1		VAQYAYVAGELEKRAK-----AGKRIA-----FVHLVEPRV-----TN-	295
		: : : . . . :	
Nox		YPF----EEGFFLPFARQFQALRMVPLI-----LLGGINRV-----DTI-EHA	337
YqiG		AR---RGADP-TTRTMDLLNERNMKNVPL-----IavgSihsA---DDALAVI	308
Chr-OYE1		-----EFEGSDLNFAAGWAKKIYTGQPTITVGSVGLEGDFMAAFGGQGTEKADILTTRLR	309
AfoYel		SAIAIRSFGPGYQVHFQEIKAkVGER-LL-----IASVGGIKTG---ALAEVVV	360
YqjM		--ADINVPFGYQVSFAEKIREQA--D-MA-----TGAVGMTIDG-----SMAEELL	296
PETNR		-----CGKPYSEAFRQKVRERFHG--VI-----IGA-GAYTAe---KAE-DLI	313
OYE1		--PFLTETEgEgEyGGSndfvvysIWKG--PV-----IRA-GNFALH---PEVVREE	337
		. . . *	
Nox		LDEGEFFVAMARALLRDPQLVNKFQAESVDQGLICHNCNMCTIYTGTRCVVRDALVVRE	397
YqiG		EN-GIPLVAMGREILVDPDWTWVKVKEGREKQIETV-----IKGTD-----KEYYHL	353
Chr-OYE1		ERGDFDLVAVGRALLQDPEWAKVKVEQNTEALLDF-----SAESLG-----VL---	352
AfoYel		QSGIDA-VQAGRWFQONPGLVRAFANELGVKVRMATQ--IDWSFEGRG-----KKAKKS	411
YqjM		QNGRADLIPIGRELLRDPFFARTAAKQLNTEIPAPVQ--YERGw-----	338
PETNR		KGKLIDAVAFGRDYIANPDLVARLQKAElnPQRp-----ESFYGGG-----ABGYTD	361
OYE1		VKDKRTLIGYGRFFISNPDLVDRLEKGLPLNKYDR-----DTFYQMS-----AHGYID	385
		: . * : * .	
Nox		APRLGQ-----	403
YqiG		PEPLWQAIYNTPGWVPYKD-----	372
Chr-OYE1		Y-----	353
AfoYel		--SL-----	413
YqjM		-----	338
PETNR		YPSL-----	365
OYE1		YPTYEE--ALKLGWDKK--	400

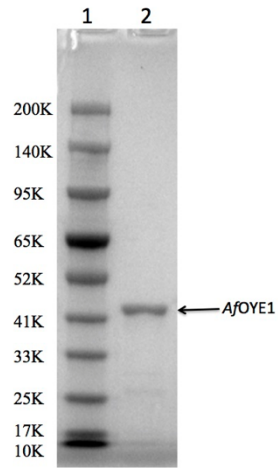


Figure S2. SDS-PAGE analysis of the purified AfOYE1. Lane 1: Protein ladder; Lane 2: The purified AfOYE1. The molecular weight of the purified AfOYE1 was at 43.4 KDa ranging between 41 KDa to 52 KDa. 10 uL of purified AfOYE1 was loaded on a 4-12% polyacrylamide gradient gel, and stained with Coomassie Blue.

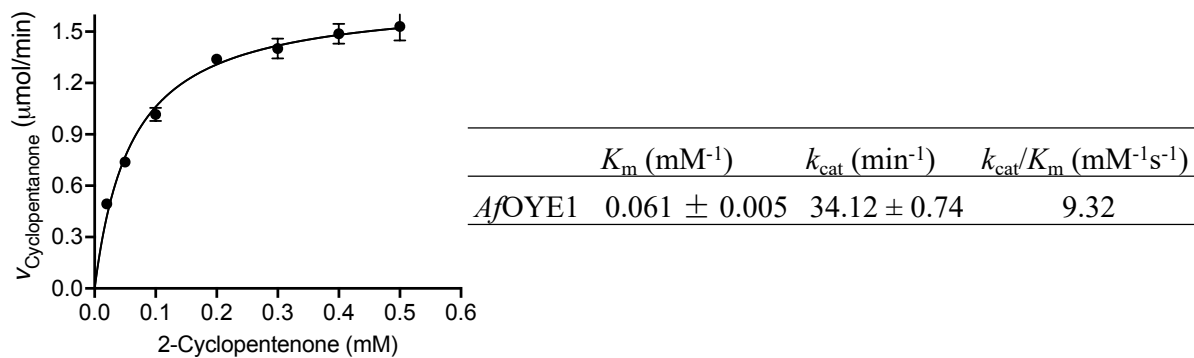


Figure S3. Steady-state kinetics of *AfOYE1*-catalyzed reduction of 2-cyclopentenone. Steady-state kinetics were measured in triplicates with varying concentrations of 2-cyclopentenone and fitting the data to the Michaelis-Menten equation. Errors limits are standard deviations.

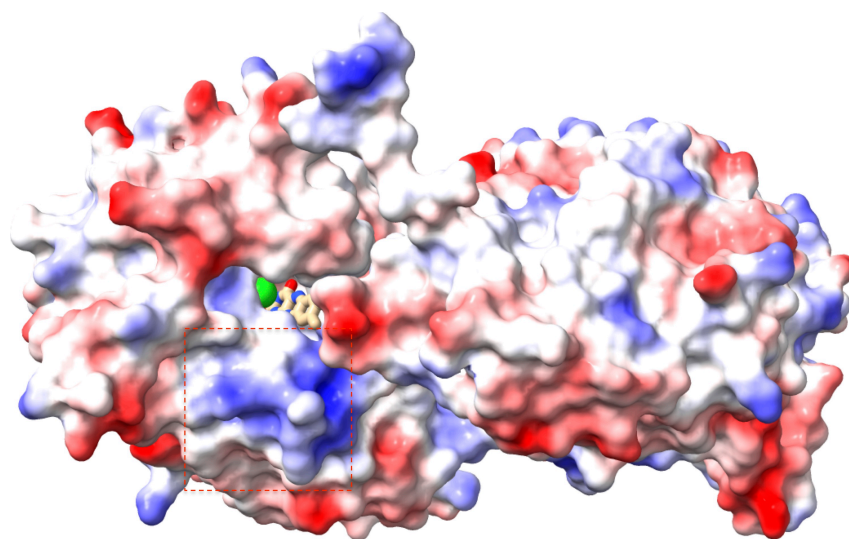


Figure S4. Surface charge distribution of *A/OYE1*, calculated by ChimeraX APBS software. Density with red potentially means negative, blue means positive, and white means neutral. Loop from S316 to G325 of *A/OYE1* labeled as the red dashed rectangle is brimming with the positive surface.

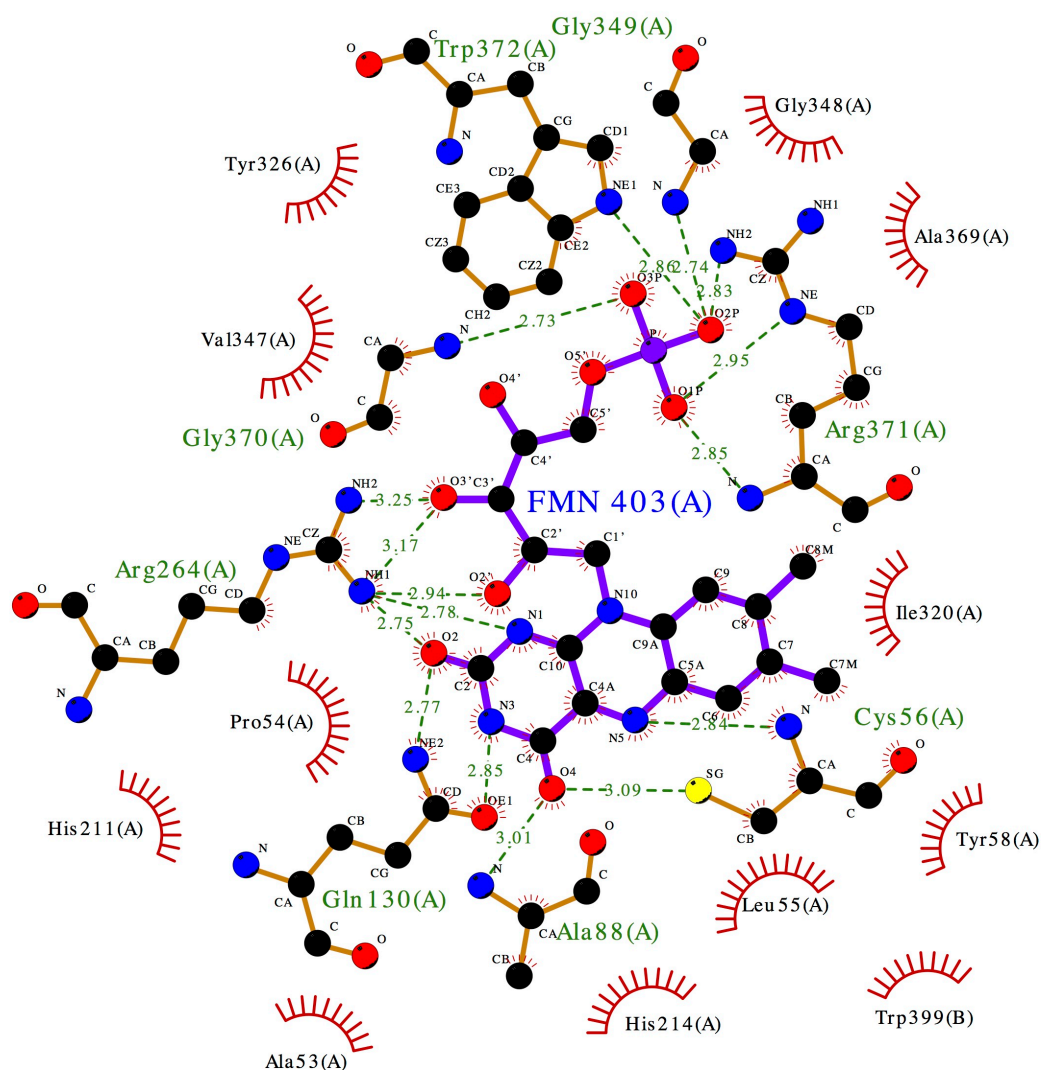


Figure S5. The plot of FMN cofactor interactions in the active site of *AfOYE1*. The interactions have been calculated and visualized by LigPlus software. The thin green dotted lines illustrate hydrogen bonds.

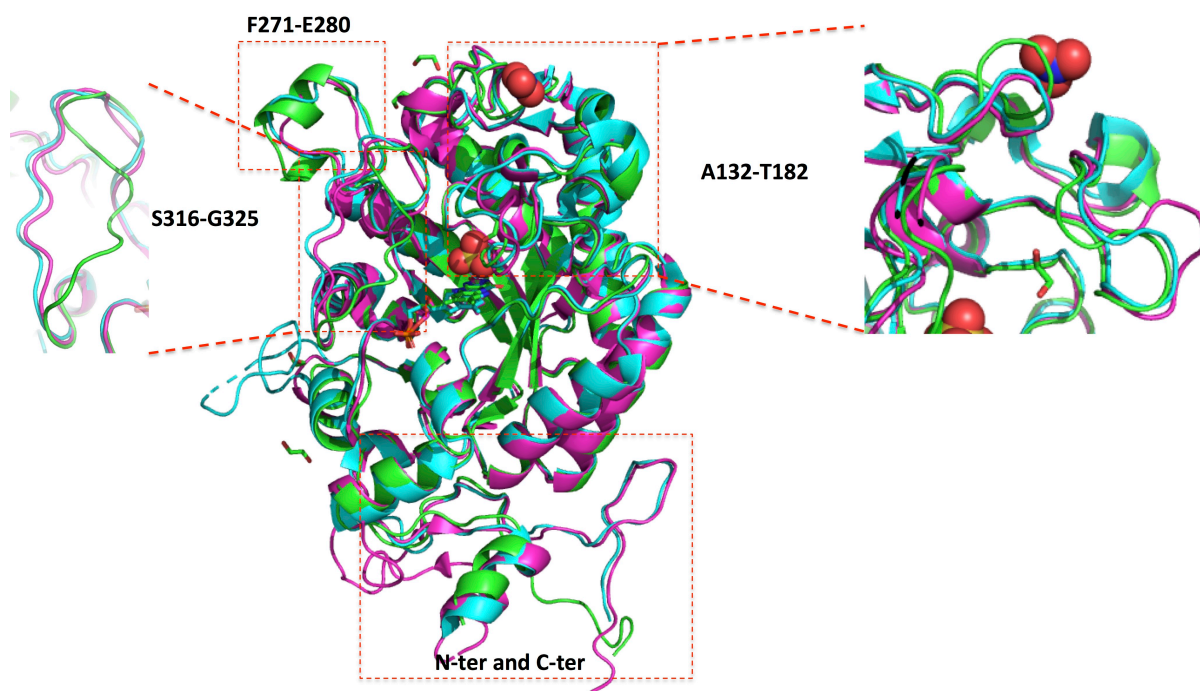


Figure S6. Superposition of monomers from the crystal structures of *AfOYE1* (green), *BfOYE4* (cyan), and *AnOYE8* (magenta). Red dashed rectangle labeled as F271-E280 means α 6-helix for the residues from Phe271 to Glu280 of *AfOYE1*, S316-G325 means the loop region from Ser316 to Gly325 of *AfOYE1*, A132-T182 means the cap subdomain region from Ala132 to Thr182, and N-ter and C-ter mean N terminal and C-terminal domains of *AfOYE1*.

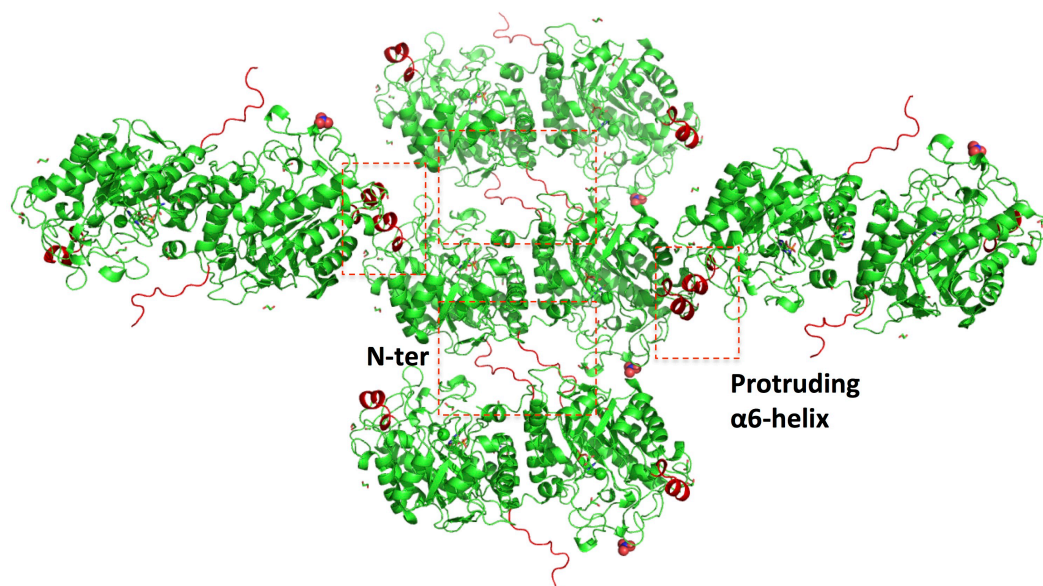


Figure S7. A view showing N-terminal loops (red) and protruding $\alpha 6$ -helix (red color, residues F271 to E280) of *AfOYE1* promote dimerization in five copies of crystallographically independent units.

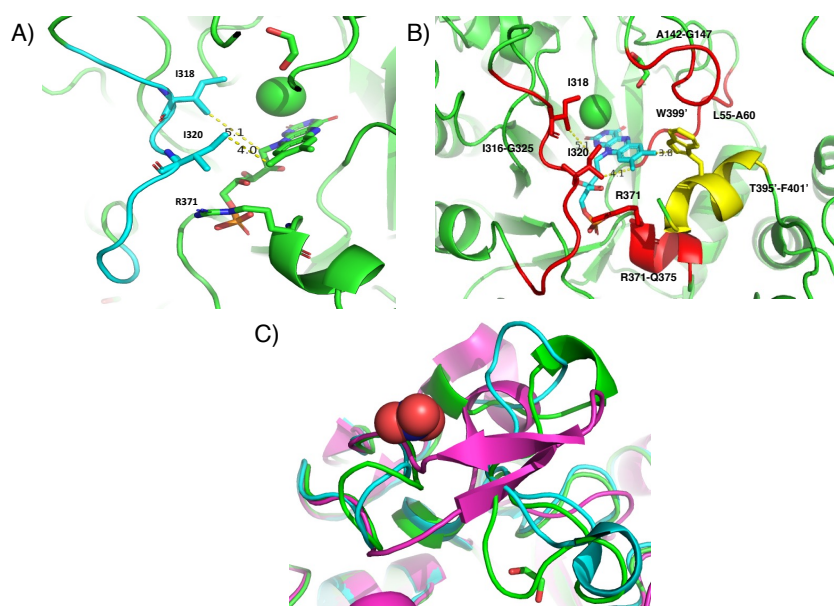


Figure S8. Details of the loop region from Ser 316 to Gly 325, the entrance of the catalytic pocket, and the superposition of cap regions from the crystal structures of *AfOYE1*. (A) The loop region from Ser 316 to Gly 325 (cyan) reaches the active site entrance of *AfOYE1* (green) and is close to FMN (green). (B) C-terminal α helix (red), residues T395 to F401 (labeled as T395'-F401'), from adjacent protomer pointing to FMN together with three loops and one α -helices (red) (labeled as L55-A60, A142-G147, I316-G325, and R371-Q375) contribute to the narrow entrance of catalytic pocket. (C) Superposition of cap regions from the crystal structures of *AfOYE1* (green), *CaOYE* (cyan), and *GSOYE* (magenta).

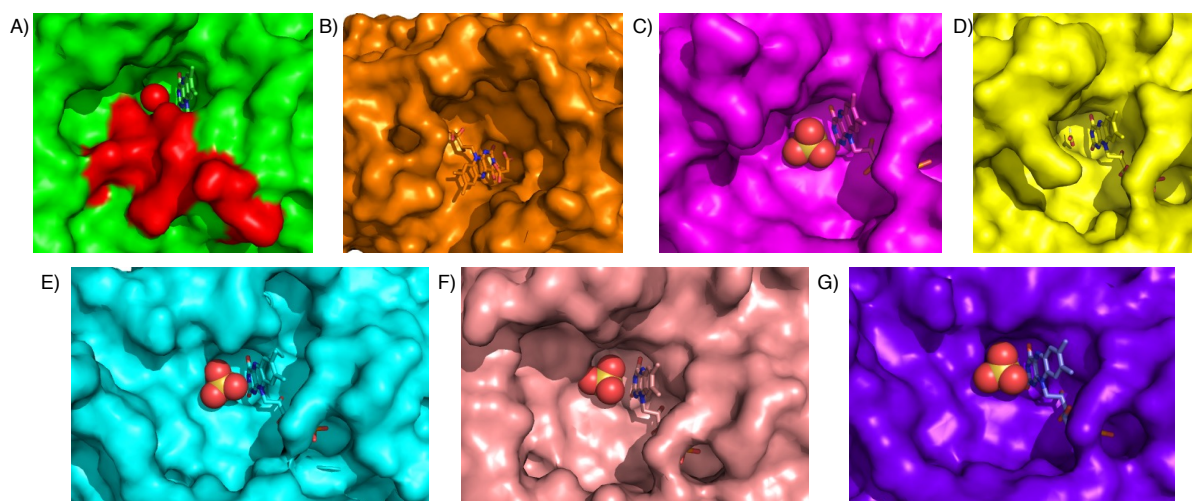


Figure S9. Surface representation of catalytic pocket among members of thermophilic-like Class III OYEs: (A) *AfOYE1*, (B) *CaOYE*, (C) *AnOYE8*, (D) *BfOYE4*, YqjM (cyans), (F) *TsOYE*, and (G) *GkOYE*. Residues 316 to 325 in *AfOYE1* close to the entrance of the catalytic pocket show red.

Table S1. Data collection and refinement statistics for *Af*OYE1.

Data collection	
Wavelength (Å)	0.9793
Resolution range (Å)	42.86 - 1.55 (1.605 - 1.55)
Space group	P 1 21 1
Unit cell	
a, b, c (Å)	47.47, 171.42, 50.48
α , β , γ (°)	90, 117.60, 90
Total reflections	667572 (53857)
Unique reflections	102393 (9786)
Multiplicity	6.5 (5.5)
Completeness (%)	99.28 (95.09)
$I/\sigma(I)$	25.71 (7.66)
R-merge (%)	0.05 (0.17)
R-meas (%)	0.05 (0.19)
R-pim (%)	0.02 (0.080)
CC1/2	0.99 (0.98)
Refinement	
Reflections used in refinement	102356 (9786)
Reflections/#R-free	4940 (467)
R-work/R-free (%)	0.1443 (0.1622) / 0.1676 (0.1954)
# of non-hydrogen	6997
Protein	5965
Ligands	112
Solvent	920
Protein residues	774
r. m. s. deviation	
Bond lengths (Å)	0.007
Bond angle (°)	0.95
Ramachandran plot	
Favored	96.88
Allowed	3.12
Outliers	0.00
Average B factor	11.12
Protein	9.78
Ligands	12.37
Solvent	19.66

Table S2. List of OYEs used for phylogenetic analysis and their accession numbers.

Enzyme	Class	Accession ID	Source
AcaryoER1	I	ABW29811	<i>Acaryochloris marina</i> MBIC11017
AcaryoER3	I	ABW32756	<i>Acaryochloris marina</i> MBIC11017
Achr-OYE4	I	AFK73188.1	<i>Achromobacter</i> sp. JA81
Chr-OYE2	I	ALE60337	<i>Chryseobacterium</i> sp. CA49
CtOYE	I	RUT11138.1	<i>Chroococcidiopsis thermalis</i>
CyanothER1	I	ACK64210	<i>Rippkaea orientalis</i> PCC 8801
CyanothER2	I	ACK65723	<i>Rippkaea orientalis</i> PCC 8801
GluER	I	AAW60280	<i>Gluconobacter oxydans</i> 621H
GsOYE	I	XP_005703492	<i>Galdieria sulphuraria</i>
MR	I	AAC43569.1	<i>Pseudomonas putida</i>
NCR	I	AAV90509	<i>Zymomonas mobilis</i>
NemA	I	BAA13186	<i>Escherichia coli</i>
NerA	I	CAA74280	<i>Agrobacterium tumefaciens</i>
NospuncER1	I	ACC84535	<i>Nostoc punctiforme</i> PCC 73102
NostocER1	I	BAB73564	<i>Nostoc</i> sp. PCC 7120
LyngbyaER1	I	EAW37813	<i>Lyngbya</i> sp. PCC 8106
PETNR	I	AAB38683.1	<i>Enterobacter cloacae</i>
SYE1	I	AAN55488	<i>Shewanella oneidensis</i> MR-1
SYE3	I	AAN57126	<i>Shewanella oneidensis</i> MR-1
SynER	I	ABB56505	<i>Synechococcus elongatus</i> PCC 7942
XenB	I	AAF02539.1	<i>Pseudomonas fluorescens</i>
XenB2	I	AGS77941.1	<i>Pseudomonas putida</i>
YersER	I	WP_032896199	<i>Yersinia bercovieri</i>
ArOYE1	II	AHL17019	<i>Ascochyta rabiei</i>
ArOYE2	II	AHL17020	<i>Ascochyta rabiei</i>
AtOPR1	II	NP_177794.1	<i>Arabidopsis thaliana</i>
AtOPR3	II	NP_001077884.1	<i>Arabidopsis thaliana</i>
CIER	II	EEQ40235	<i>Clavispora lusitaniae</i> ATCC 42720
CrOYE2	II	XP_001699408.1	<i>Chlamydomonas reinhardtii</i>
CYE	II	BAD24850	<i>Kluyveromyces marxianus</i>
HYE1	II	KAG7904883.1	<i>Ogataea polymorpha</i>
HYE2	II	AAN09953	<i>Ogataea polymorpha</i>
KYE1	II	AAA98815.1	<i>Kluyveromyces lactis</i>

<i>Le</i> OPR1	II	NP_001234781.1	<i>Solanum lycopersicum</i>
<i>Le</i> OPR2	II	NP_001233868	<i>Solanum lycopersicum</i>
<i>Le</i> OPR3	II	NP_001233873.1	<i>Solanum lycopersicum</i>
EBP1	II	AAA18013	<i>Candida albicans</i>
<i>Mg</i> ER	II	EDK41665	<i>Meyerozyma guilliermondii</i> ATCC 6260
OYE1	II	Q02899	<i>Saccharomyces pastorianus</i>
OYE2	II	Q03558	<i>Saccharomyces cerevisiae</i> S288C
OYE2.6	II	ABN66026	<i>Scheffersomyces stipitis</i> CBS 6054
OYE3	II	P41816	<i>Saccharomyces cerevisiae</i> S288C
Achr-OYE3	III	AFK73187.1	<i>Achromobacter</i> sp. JA81
<i>Af</i> OYE1	III	XP_041144250.1	<i>Aspergillus flavus</i> NRRL3357
AnabaenaER3	III	ABA25236.1	<i>Trichormus variabilis</i> ATCC 29413
<i>An</i> OYE8	III	XP_001399273.1	<i>Aspergillus niger</i>
<i>Bf</i> OYE	III	XP_001554780.1	<i>Botrytis cinerea</i> B05.10
<i>Ca</i> OYE	III	WP_015941499.1	<i>Chloroflexus aggregans</i>
<i>Chr</i> -OYE3	III	AFK73187.1	<i>Achromobacter</i> sp. JA81
<i>Cr</i> OYE1	III	XP_042928859.1	<i>Chlamydomonas reinhardtii</i>
<i>Dr</i> ER	III	AAF11740	<i>Deinococcus radiodurans</i> R1
FOYE-1	III	KRH78075	<i>Ferroplasma</i> sp. JA12
<i>Geo</i> ER	III	BAO37313	<i>Geobacillus</i> sp. #30
<i>Gk</i> OYE	III	BAD76617	<i>Geobacillus kaustophilus</i> HTA426
GloeoER	III	BAC91769	<i>Gloeobacter violaceus</i> PCC 7421
OYERo2	III	ALL54975	<i>Rhodococcus opacus</i> 1CP
PfvC	III	AFF18622.1	<i>Arthrobacter</i> sp. JBH1
RhrER	III	AMD82542.1	<i>Rhodococcus rhodochrous</i> ATCC 17895
<i>Rm</i> ER	III	ABF11721	<i>Cupriavidus metallidurans</i> CH34
TOYE	III	RUT11138.1	<i>Thermoanaerobacter pseudethanolicus</i> ATCC
<i>Ts</i> OYE	III	CAP16804	<i>Thermus scotoductus</i> SA-01
XenA	III	AAF02538	<i>Pseudomonas putida</i>
YqjM	III	BAA12619	<i>Bacillus subtilis</i>
<i>Chr</i> -OYE1	IV	ALE60336.1	<i>Chryseobacterium</i> sp. CA49
Ppo-ER1	IV	AHC19521	<i>Paenibacillus polymyxa</i> CR1
Ppo-ER2	IV	AIW41616	<i>Paenibacillus polymyxa</i> CR1
Ppo-ER3	IV	QBR53093	<i>Paenibacillus polymyxa</i>
Rer-ER7	IV	QBR53095.1	<i>Rhodococcus erythropolis</i>
<i>Ang</i> OYE11	V	XP_001390672.2	<i>Aspergillus niger</i> CBS 513.88

<i>Ang</i> OYE12	V	XP_001395504.2	<i>Aspergillus niger</i> CBS 513.88
<i>Ar</i> OYE3	V	AHL17021	<i>Ascochyta rabiei</i>
<i>Ar</i> OYE6	V	AHL17024	<i>Ascochyta rabiei</i>
<i>Bf</i> OYE6	V	XP_001547575.1	<i>Botrytis cinerea</i> B05.10
FbOYE	V	TAL24079.1	<i>Frankiales bacterium</i>
MoOYE4	V	XP_003720070.1	<i>Pyricularia oryzae</i> 70-15
Nox	V	WP_029256809.1	<i>Rhodococcus erythropolis</i>
PcOYE8	V	XP_002560444.1	<i>Penicillium rubens</i> Wisconsin 54-1255
BcOYE	VI	WP_098362597.1	<i>Bacillus cereus</i>
LacER	VI	ADK19581	<i>Lactocaseibacillus paracasei</i>
Lla-ER	VI	WP_046781786.1	<i>Lactococcus cremoris</i>
<i>Mf</i> OYE	VI	WP_039343246.1	<i>Burkholderia</i>
POYE	VI	WP_119715197.1	<i>Propionibacterium ruminifibrarum</i>
YqiG	VI	QBR53092	<i>Bacillus subtilis</i>

Table S3. Polymerase chain reaction primers for mutagenesis.

Oligonucleotides	
H211L-F	5'-TTC GAT GCC ATC GAG ATC CTG GCG GCG CAT GGT TAT CTG-3'
H211L-R	5'-CAG ATA ACC ATG CGC CGC CAG GAT CTC GAT GGC ATC GAA-3'
H214L-F	5'-ATC GAG ATC CAT GCG GCG CTG GGT TAT CTG CTG CAC CAG-3'
H214L-R	5'-CTG GTG CAG CAG ATA ACC CAG CGC CGC ATG GAT CTC GAT-3'
Y216F-F	5'-ATC CAT GCG GCG CAT GGT TTT CTG CTG CAC CAG TTT CTG-3'
Y216F-R	5'-CAG AAA CTG GTG CAG CAG AAA ACC ATG CGC CGC ATG GAT-3'

Table S4. Comparison of *Af*OYE1 characteristics with those of other thermophilic-like Class III OYEs. In the crystal, oligomerization data were checked from crystallographic structures; Interface area, numbers of interacting residues, and interactions involving the dimerization surface were calculated by PISA software; Catalytic pocket volume, surface, and depth were calculated by Proteins. Plus/DoGSiteScorer software. Hb: Hydrogen bonds. Sb: Salt bridges.

Protein (PDB ID)	In crystal	Interface (Å ²)	Interacting residues	Interactions (Hb, Sb)	Pocket Volume (Å ³)	Pocket Surface (Å ²)	Pocket Depth (Å)
<i>An</i> OYE8 (7QFX)	tetramer	2796	71	(18, 4)	703.38	864.99	16.97
<i>Ca</i> OYE (7O0T)	tetramer	1451	34	(16, 8)	758.08	748.47	22.74
<i>Bf</i> OYE4 (7BLF)	dimer	2121	53	(12, 3)	829.18	838.56	26.51
YqjM (1Z41)	dimer	660	31	(4, 3)	818.95	849.48	23.74
<i>Ts</i> ER (3HF3)	tetramer	1262	33	(17, 5)	653.90	690.12	20.21
<i>Gk</i> OYE (3GR7)	dimer	1199	30	(16, 0)	877.28	818.83	19.34
<i>Af</i> OYE1 (8J59)	dimer	492	41	(7, 6)	1203.07	1126.53	24.23

14. The data of the products from the *AfOYE1*-catalyzed bioreduction

Cyclopentanone (**1b**)

Colorless oil; $^1\text{H NMR}$ (CDCl_3): $\delta = 2.13\text{--}2.17$ (m, 4H), $1.93\text{--}1.97$ (m, 4H) ppm.

(*S*)-2-methylcyclopentanone (**2b**)

Colorless oil; $[\alpha]_{\text{D}}^{25} = +16.5^\circ$ (c 0.4, CHCl_3), the optical rotation indicates its configuration to be (*S*) (lit. (Shimoda et al. 2004), $[\alpha]_{\text{D}}^{25} = +114.9^\circ$ (c 0.52, CHCl_3); retention time: $t_{\text{R}}(S)$ 12.31 min (Chiral GC with CHIRASIL-DEX CB column); $^1\text{H NMR}$ (CDCl_3): $\delta = 2.19\text{--}2.33$ (m, 2H), $2.05\text{--}2.15$ (m, 2H), $1.95\text{--}2.03$ (m, 1H), $1.74\text{--}1.82$ (m, 1H), $1.42\text{--}1.53$ (m, 1H), 1.08 (d, $J = 6.96$ Hz, 3H) ppm.

Cyclohexanone (**3b**)

Colorless oil; $^1\text{H NMR}$ (CDCl_3): $\delta = 2.31\text{--}2.34$ (m, 4H), $1.82\text{--}1.88$ (m, 4H), $1.69\text{--}1.74$ (m, 2H) ppm.

(*S*)-2-methylcyclohexanone (**4b**)

Colorless oil; retention time: $t_{\text{R}}(S)$ 7.26 min (Chiral GC with CHIRASIL-DEX CB column); $^1\text{H NMR}$ (CDCl_3): $\delta = 2.29\text{--}2.35$ (m, 2H), $2.18\text{--}2.26$ (m, 1H), $1.98\text{--}2.04$ (m, 2H), $1.76\text{--}1.80$ (m, 1H), $1.55\text{--}1.64$ (m, 2H), $1.29\text{--}1.35$ (m, 1H), 0.96 (d, $J = 6.60$ Hz, 3H) ppm.

Propionamide (**5b**)

Colorless oil; $^1\text{H NMR}$ (CDCl_3): $\delta = 2.26$ (q, $J = 7.86$ Hz, 2H), 0.96 (t, $J = 7.86$ Hz, 3H) ppm.

Phenylnitroethane (**6b**)

Colorless oil; $^1\text{H NMR}$ (CDCl_3): $\delta = 7.30\text{--}7.37$ (m, 3H), $7.22\text{--}7.24$ (m, 2H), 4.64 (t, $J = 7.34$ Hz, 2H), 3.35 (t, $J = 7.34$ Hz, 2H) ppm.

1-Phenyl-2-nitropropane (**7b**)

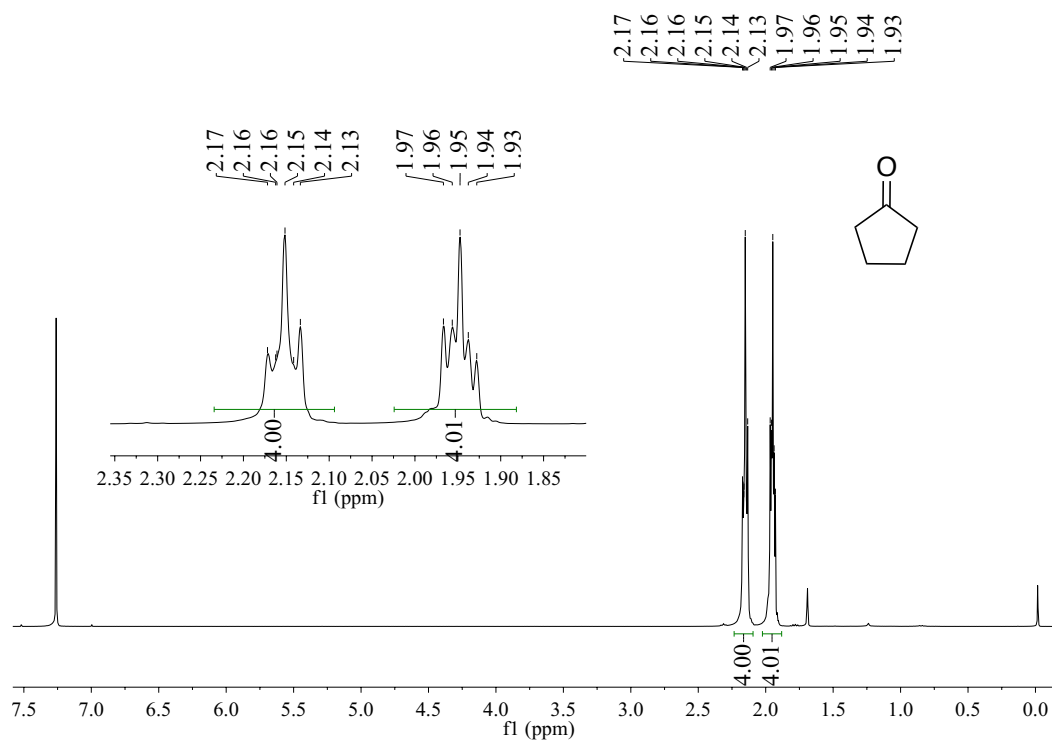
Colorless oil; $^1\text{H NMR}$ (CDCl_3): $\delta = 7.29\text{--}7.36$ (m, 3H), $7.18\text{--}7.20$ (m, 2H), $4.78\text{--}4.83$ (m, 1H), 3.36 (dd, $J = 7.52$ Hz, $J = 14.00$ Hz, 1H), 3.04 (dd, $J = 6.80$ Hz, $J = 14.00$ Hz, 1H), 1.57 (d, $J = 6.66$ Hz, 3H) ppm.

Citronellal (**8b**)

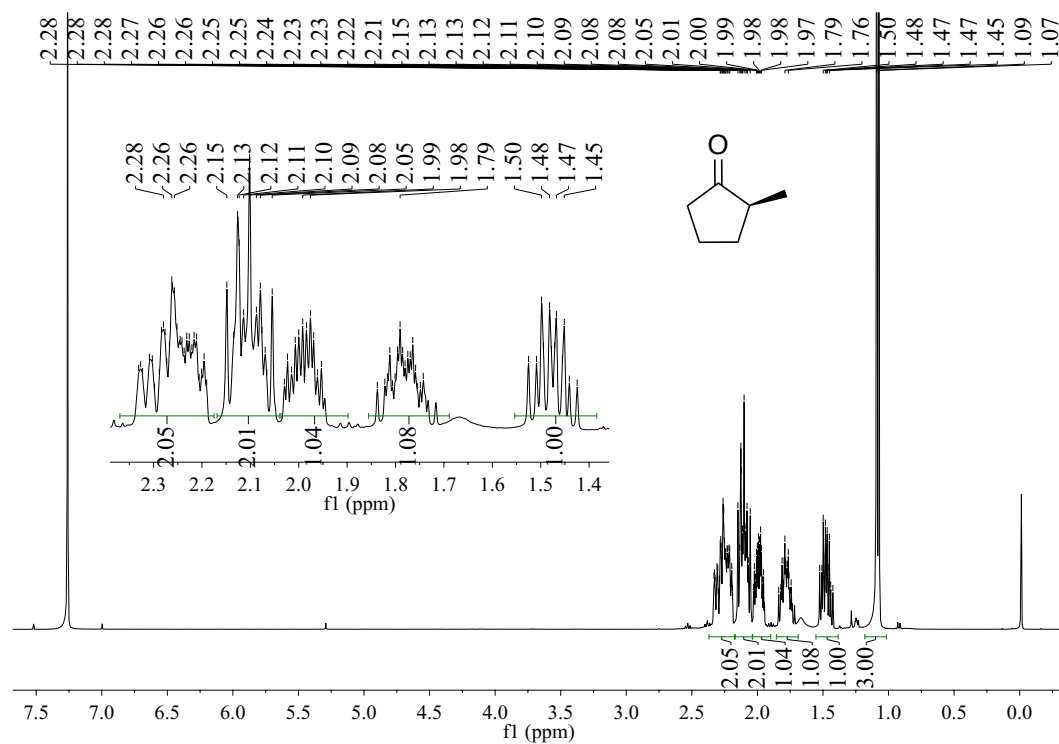
Colorless oil; ^1H NMR (CDCl_3): δ = 5.06-5.10 (m, 1H), 2.37-2.43 (m, 1H), 2.19-2.26 (m, 1H), 1.96-2.09 (m, 3H), 1.68 (s, 3H), 1.60 (s, 3H), 1.24-1.39 (m, 2H), 0.97 (d, J = 6.66 Hz, 3H) ppm.

15. Selected ^1H NMR spectra and GC chromatograms

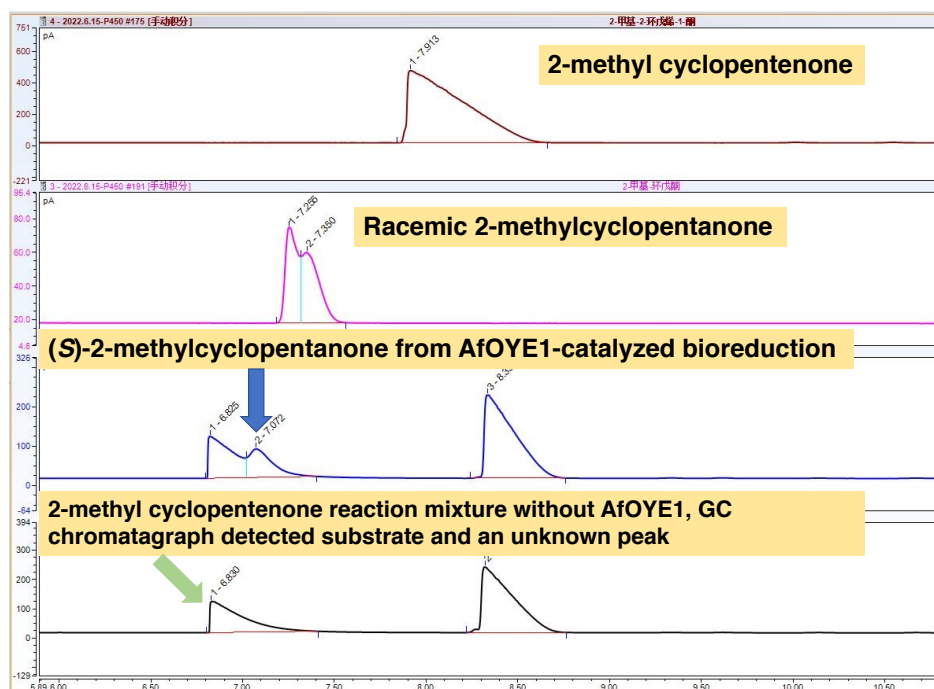
Cyclopentanone (**1b**)



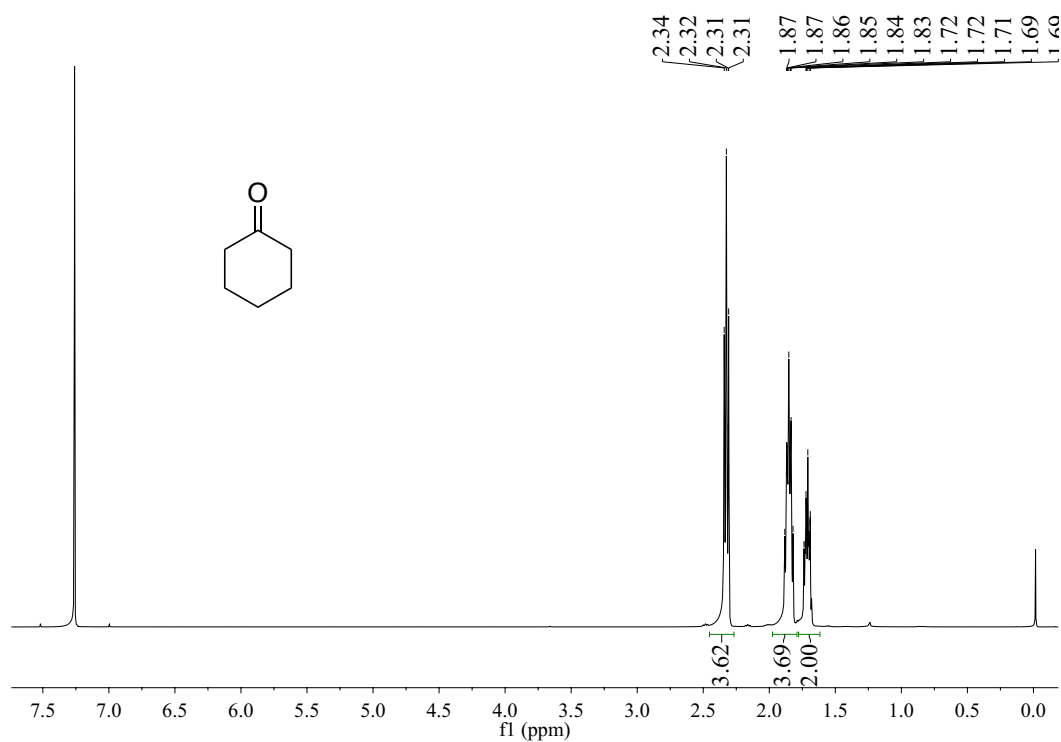
(*S*)-2-methylcyclopentanone (**2b**)



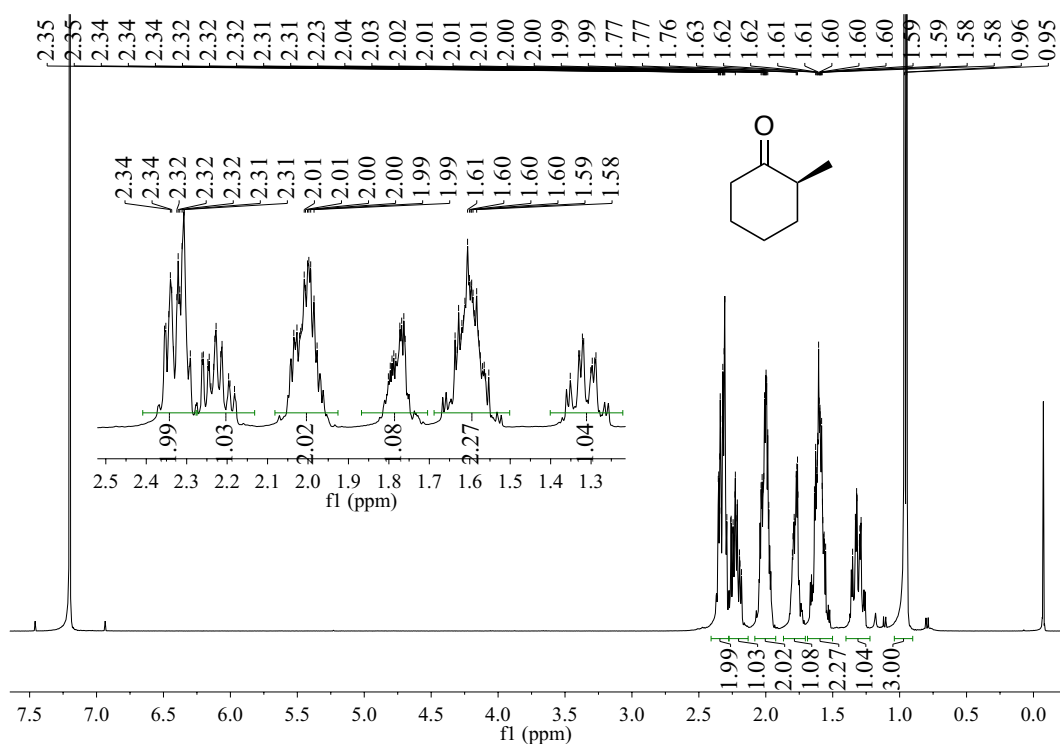
GC chromatogram of Racemic 2-methylcyclopentanone and (*S*)- 2-methylcyclopentanone (**2b**) from *Af*OYE1-catalyzed bioreduction



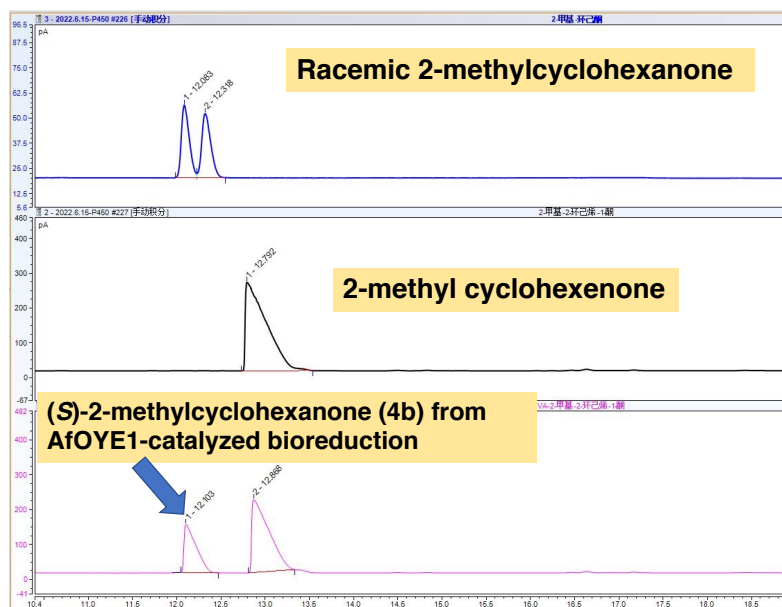
Cyclohexanone (**3b**)



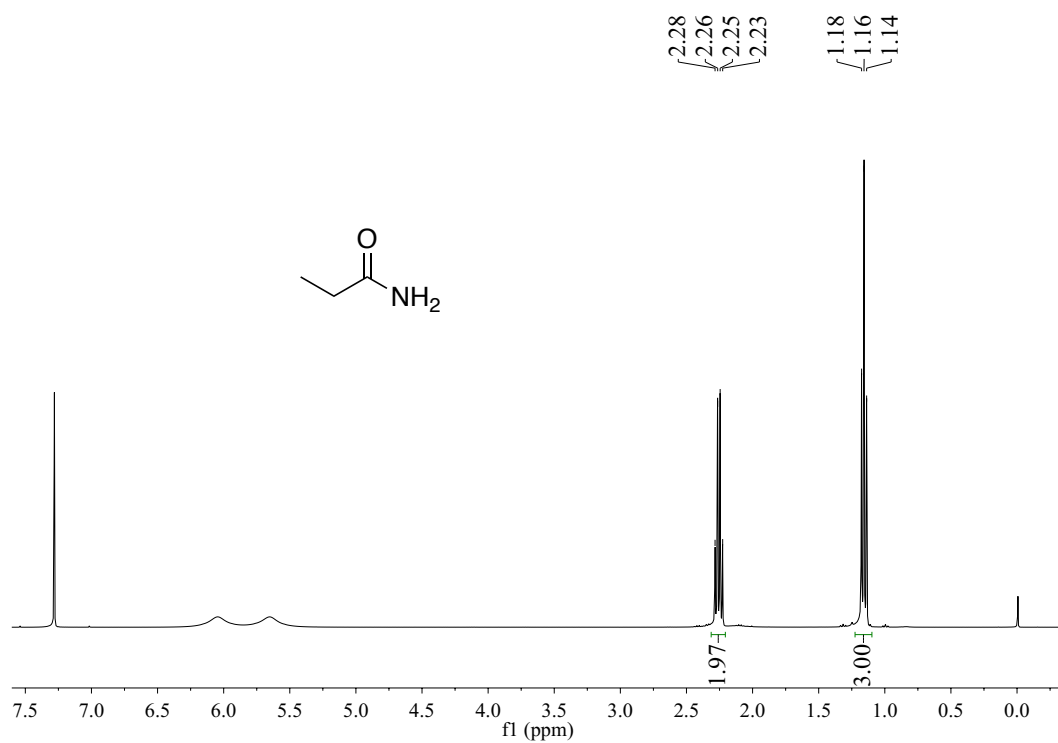
(*S*)-2-methylcyclohexanone (**4b**)



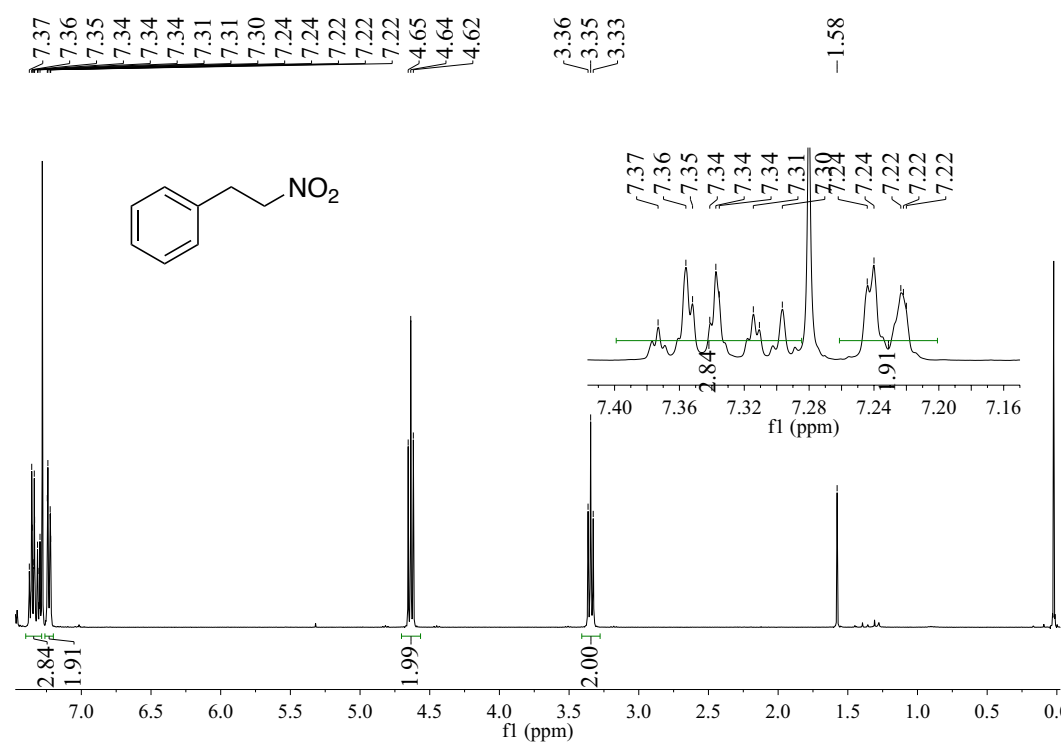
GC chromatogram of Racemic 2-methylcyclohexanone and (*S*)-2-methylcyclohexanone (**4b**) from *Af*OYE1-catalyzed bioreduction



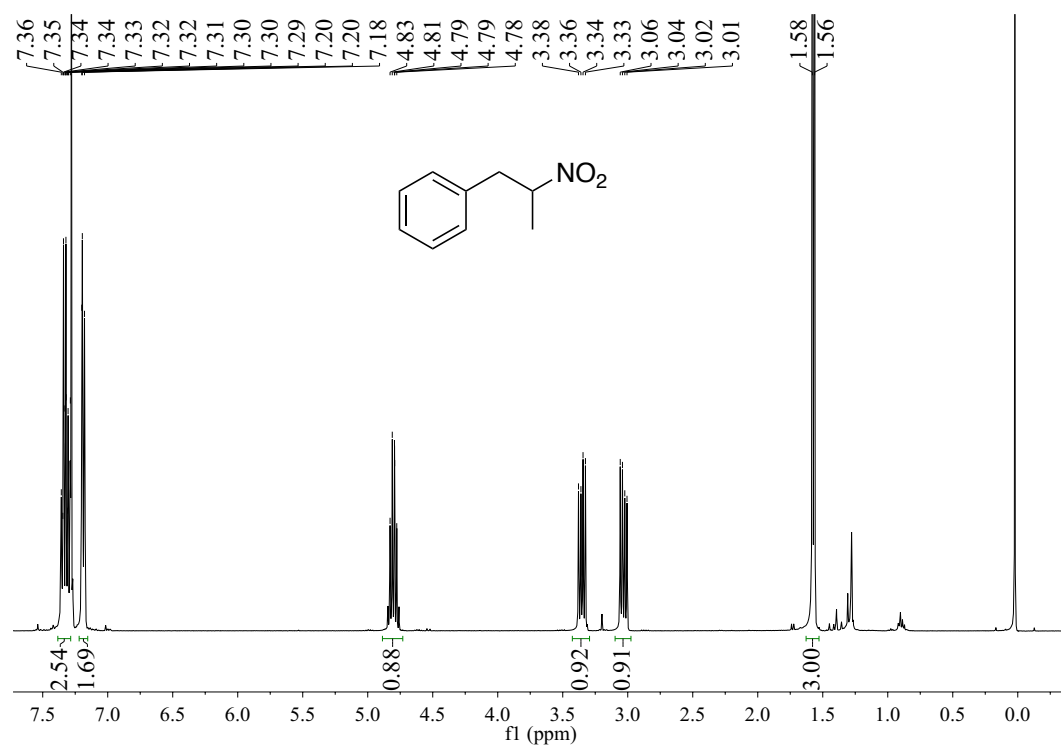
Propionamide (**5b**)



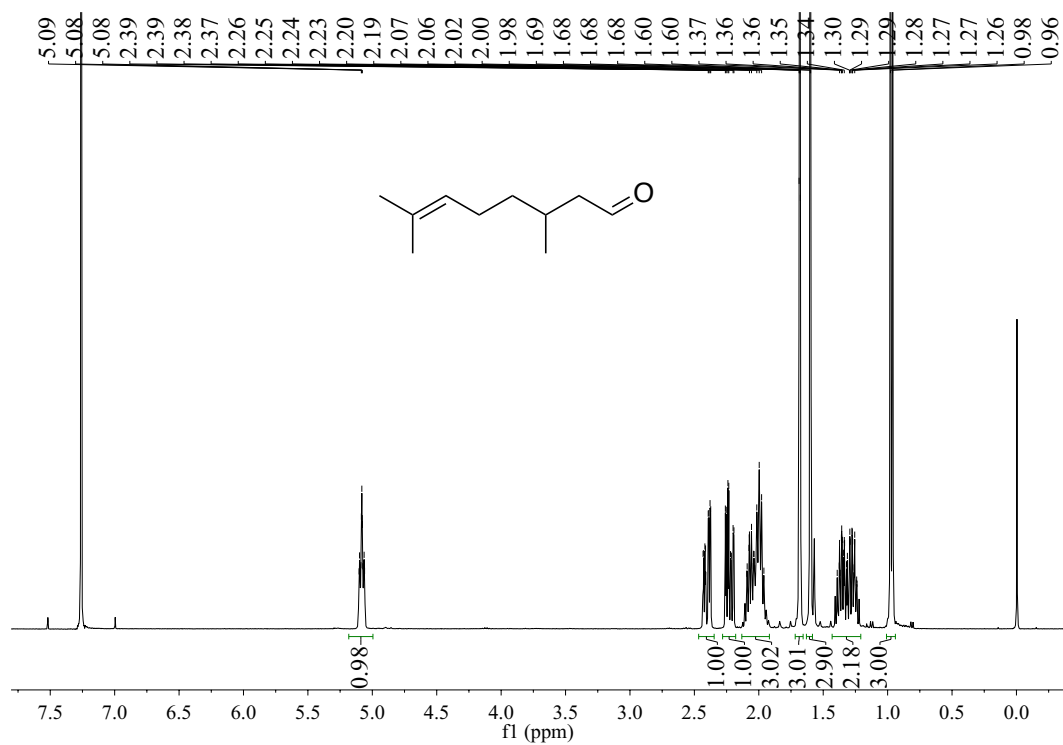
Phenylnitroethane (**6b**)



1-Phenyl-2-nitropropane (**7b**)



Citronellal (**8b**)



16. The sequence information of *Af*OYE1.

The amino acid sequence of *afvA* (UniProt: B8N8Q9, *Af*OYE1) with N-terminal His6-tag followed by a tobacco etch virus (TEV) protease cleavage site.

MGSSHHHHHHENLYFQGISYYTPAQVPPAGTQVEGSTKLFSPILTIRGVTFPNRLFLAP
LCQYSAKDGANDWHLTHIGGIVQRGPGLAIMEATAVQKVGRITPQDLGLYDDGHI
EPLKRTEFAHSQSQKIGIQLAHAGRKASAVAPWLSGNAMAVKEVGGWPDDIVAPS
AIPQEEGINAVPKVLTGEDIGVLKGDWAEAAKRAVRANFDAIEIHAAHGYLLHQFLS
PVSNNRRTDKYGGSFENRVRILLEICEEVRAVIPTAMPLLVRISATDWFEFDDNLTKEFP
ESWTVAQSIRLALLLADRGVDLVDVSSGGIHAKSIAAIRSGPGYQVHFAQEIKKAVG
EKLLISAVGGIKTGALAEVQSGIDAVQAGRWFQQNPGLVRAFANELGVKVRMAT
QIDWSFEGRGKKAKKSSL

The codon-optimized nucleotide sequence of *afvA*.

ATGGGCAGTAGTCATCACCACCATCACCACGAGAATCTGTACTTCCAAGGCATCA
GTTACTATACCCAGCGCAAGTTCCGCCGGCCGGTACGCAAGTTGAAGGTAGCA
CCAAGCTGTTTCAGCCCGCTGACGATTCGCGGCGTGACGTTTCCGAATCGTCTGTT
TCTGGCCCCACTGTGCCAGTACAGTGCAGAAAGATGGCTATGCGAACGACTGGCA
TCTGACCCACATTGGTGGCATTGTTTCAGCGTGGCCCCGGGTCTGGCCATCATGGAA
GCGACCGCCGTTCAAAAAGTTGGCCGTATCACGCCGCAAGATCTCGGTCTGTATG
ATGACGGCCACATCGAGCCACTGAAGCGCATCACCGAGTTCGCCCATAGTCAGA
GCCAGAAGATCGGTATCCAGCTGGCGCACGCGGGTCGTAAAGCCAGTGCCGTTG
CGCCGTGGCTGAGTGGTAATGCGATGGCCGTGAAAGAGGTTGGTGGCTGGCCGG
ATGACATCGTGGCGCCAAGCGCCATCCCGCAAGAAGAGGGCATCAATGCCGTGC
CAAAAGTGCTGACGGGTGAGGACATCGGCGTGCTGAAAAAAGACTGGGCGGAA
GCGGCGAAACGTGCGGTTTCGTGCCAACTTCGATGCCATCGAGATCCATGCGGCG
CATGGTTATCTGCTGCACCAGTTTCTGAGTCCGGTTAGCAACCGTCGCACGGACA
AATACGGCGGCAGCTTTGAAAACCGTGTGCGCATTCTGCTGGAGATCTGCGAAG
AAGTTCGCGCGGTGATTCCAACCGCCATGCCGCTCCTCGTTCGCATCAGCGCCAC
GGACTGGTTCGAGTTCGACGACAATCTGACGAAGGAATTCCCGGAGAGCTGGAC
CGTGGCGCAGAGTATCCGTCTGGCGCTGCTGCTGGCGGATCGTGGCGTTGATCTG
GTGGACGTGAGCAGCGGTGGCATTTCATGCGAAAAGTGCCATCGCCATCCGTAGT
GGCCCCGGGCTACCAAGTTCACTTCGCGCAAGAAATCAAAAAGGCGGTGGGTGAA
AAGCTGCTGATCAGTGCCGTTGGCGGCATTAAAACCGGTGCGCTGGCCGAAGAA
GTTGTTTCAGAGTGGCATTGACGCCGTTCAAGCCGGCCGTTGGTTTCAGCAGAACC
CGGGTCTGGTTCGCGCGTTCGCCAACGAGCTGGGTGTGAAGGTTTCGTATGGCCAC
GCAGATCGACTGGAGCTTCGAATAA

Reference:

Shimoda K, Kubota N, Hamada H, Kaji M, Hirata T (2004) Asymmetric reduction of enones with *Synechococcus* sp. PCC 7942. *Tetrahedron: Asymmetry* 15:1677-1679