

**Anika Scholtissek, Dirk Tischler, Adrie H. Westphal, Willem J. H. van Berkel and
Caroline E. Paul**

Class I	Class II	Class III
PETNR	-----SAEKLFTPLKVG--AVTAPNRVFMALPLTRLRSIEPGD	35
MR	-----MPDTSFSNPLGFTPLQLG--SLSLPNRVIMAPLTRSR--TPDS	39
SYE3	-----MSVFTAYESG--ALTLONRIVMAPVTRARTTPQGN	33
XenB	-----MATIFDPIKLG--DLELSNRIIMAPLTRCRA--DEGR	33
AcaryoER1	-----MSISSVFEPRLG--AVDLNRNVMAPLTRGRS--GPDR	35
OYE1	-----MSFVKDFKPKQALGDTNLFKPIKIG--NNELHRAVIPPLTRMRALHPGN	46
ArOYE1	-----MSDSRLFKPLKVG--SIELKNRVAMAPLTRFRASDSA	36
CYE	-----MSYMNFDPKPLGDTNIFKPIKIG--NNELKHRVMPALTRMRATAPGN	46
EBP1	MTIESTNSFVVPSTKLIDVTPGLSTKFOPIKVG--NNVLQPIAYVPTTRFRASKDHI	58
MgER	-----MSVNIPLGETQVFQPTKLG--KNTLSHRVFPPTTRRSLEDHT	43
XenA	-----MSALFEPYTLK--DVTLRNRIAPPMCMQYMAE--DG	32
FOYE-1	-----MSLLFSPYQLG--SLSLANRLVIAPMCMQYSAY--DG	32
TsOYE	-----MLFTPLELG--GLRLKNRLMASPMCMQYSAT--LEG	31
TOYE	-----MSILHMPCLKYK--DITIKNRIMSPCMQYSAS--TDG	33
YqjM	-----MARKLFTPTITIK--DMTLKNRIVMSPCMQYSSHEKDG	35
LacER	-----MSGYHFLKPFTFKHQTITLKNRIVIPMTITRLSFEDGT	38
YqiG	-----MNPKYKLPFEPFTFKS--GVTINNRIAVPMTHYASNEDGT	39
Chr-OYE1	-----MSTESLFTPFYK--NLELKNRIVMAPMTRAQS--DNG	34
SYE4	-----MTIENTVNSVENLFDYTKLND--TITLKNRILMAPLTRCMA--DANL	43
Nox	-----MTAPTPSIFEPARLG--PLTLNRNIVKAATFEGV--MPRG	37
SecStruc:	βαβ βββ βββ	
PETNR	IP-TPLMGEYYRQRAS--AGLIISEATQIS-AQAKGYAGAPGLHSPEQIAAWKKITAGVH	91
MR	VP-GRLQQIYYQGRAS--AGLIISEATNIS-PTARGVYVTPGIWTDQAEAGWKGVVEAVH	95
SYE3	IP-NDLMVQYYAQRSS--AGLIITEATQIS-NDSQGYSFTPGVYTEAQIDGWKKVTAAPH	89
XenB	VP-NALMAEYYRQRAS--AGLILSEATSVT-PMGVGYPDTPGIWSDQVQRGWNITKAVH	89
AcaryoER1	VP-NALMAEYYRQRAS--AGLIITEATSVT-EQAAGWSETPGIYSEADQIAWROQVTDVAH	91
OYE1	IPNRDWAEEYTRQAQRPGTMIITEGAFIS-POAGGYDNAPGVWSEEQMVETWKIFNATH	105
ArOYE1	IL--PFAAEYYSQRASVPGTLILSEATLIT-QGHGYSNVPAIENQEQIDAWKKVTEAVH	93
CYE	IPNTEWAEYYYRQRSQYPGTLIITEGTFPS-AQSGGYPNVPGIWSKEQLAEWKKIFNATH	105
EBP1	--PSDLQLNYYNARSQYPGTLIITEATFAS-ERGGIDLVHPGIYNDAAQSKWKINEAIH	115
MgER	--PSNLAYKYYDERSKFPGTLIISEGTFPS-AQAGLYEGVPGIWTERTQTKKHIDKIH	100
XenA	LI-NDWHQVHYASMARGGAGLVVEATAVA-PEGRIITPGCAGIWSAHAQAFVPPVQAIK	90
FOYE-1	IA-QDWHLMHLGRLATSGAGLVIVEATGYN-PEGRIITPFCLGLYNDEQEAALGRIVAFAR	90
TsOYE	EV-TDWHLLHYPTRALGGVGLILVEATAVE-PLGRITSPYDLGIWSEDLHPLGLKELARRIR	89
TOYE	MP-NDWHYHYATRAIGGVGLIMQEGATAVE-SRGRITDHDGLIWNDEQVKELKHIYDICK	91
YqjM	KL-TPFHMAHYISRATGOVGLIIVEASAVN-POGRITDODLGIWSEDEHIEGFAKLTEQVK	93
LacER	VT--RDEIRYYQQRAG-GYGMFITGTANYN-ALGKGFEGLSVADDRFIPGLSKLAAMK	94
YqiG	IS--EAELDYYIPRSK-EMGMVITACANYN-PDGKAFPGQPAITHDLSNIPGLKKLAQAIQ	95
Chr-OYE1	VP-TQKIIDYYARRAAE--GLILSEGTVINRPAKMNQNPIDFYGTEALNGWKNVTDVAH	93
SYE4	VP-TDDMVAYYARRAE--AGLIISEATIIR-PDAQGYPNTPGLIFTQAQIAGWRKVTDAVH	99
Nox	AV-SDDLINFHAEVARGGAAMTTVAYCAVS-PGGRVHRDRTL-VMDERLALPGLRRLTDVAH	94
SecStruc:	α1α1α1α1α1 β2β2β2β β2β2β2β	
PETNR	AED-GRIAVQLWHTGRISHSSSIQPGGQAP-----VSASALNANTRTSL-RDEN-	137
MR	AKG-GRIALQLWHVGRVSHELVQPDGQQP-----VAPSALKAEGAEFCFEFED-	142
SYE3	EAG-GKIFNQIWHVGRVSHPIF-QQGNAP-----IAPSAIAPVQKTVWVDEAH	136
XenB	AAG-GKIVLQLWHVGRISHPLY-LNGEAP-----VAPSAIQPKGHVS-----	129
AcaryoER1	QQD-GKIFLOIWHHTGRASHPDFQLNGARP-----TSASAIKPAGEVH-----	132
OYE1	EKK-SFVWVQLVNLGWAAPFDNLARDGLR---YDSASDN-----VFMDAEQ	147
ArOYE1	KKG-SFIYLQLWALGRVANKEFSEAHGIT-----VKSSSAT-----QLS-	141
CYE	ENK-SFVWVQLVNLGRQAWPEVLKKEGLR-----YDSATDD-----LYMGEE	137
EBP1	GNG-SFSSVQLWYLGRVANAKDLKDSGLP-----LIAPSA-----VYWDENS	156
MgER	ENK-SFASITLWNLGRTGDPALLKKAQKP-----FLAPSA-----IYFDEES	141
XenA	AAG-SVPGIQAIAHAGRKASANRPWEGDDHIGADDARGWETIAPSAI-----	136
FOYE-1	EFGQAKMAIQLAHAGRKASTRRPWPDPGSPYP-EEGGWQTWAPSAIK-----	136
TsOYE	EAG-AVPGIQLAHAGRKAGTARPWEGGKPL-----GWRVVGSPSPI-	129
TOYE	ANG-AVMGIQLAHAGRKCNISY-----EDVVGSPSPIK-----	122
YqjM	EQG-SKIGIQLAHAGRKAELE-----GDIFAPSAI-----	123
LacER	TGG-TKAILQIFSAGRMNSKI-LRGEQP-----VSASAVA-----	128
YqiG	AQG-AKAVVQIHGGITECPSEL-VPQQDV-----VGPSPV-----	128
Chr-OYE1	HNG-GKMGPOIWHVGDTRSTPD-----GKIFVQLWHTGRVAHPHF-FGGGDV-----LAPSAQKIEGSPVR-----	114
SYE4	ANG-GKIFVQLWHTGRVAHPHF-FGGGDV-----LAPSAQKIEGSPVR-----	140
Nox	AEG-ALAAAOIHAGLVANTLSN-----KTKTLAPSTRL-----	127
SecStruc:	α B3B383	

Class I	PETNR	GNAIRVDT-TTPRALELDE	IPGIVN-DFRQAVANAREAGFDLVELHSAHGYLLHQFL	SPS	195				
	MR	GTAGLHPT-STPRALETDE	IPGIVE-DYRQAAQRAKRAAGFDMVEVHAANAQLPNO	FLATG	200				
	SYE3	PEGQMVC-PEPREMTQAD	IDRVVA-DFAKAGANATAAGFDGTEIHHGGNGYLLIDQ	FLRTN	194				
	XenB	LVRPLADY-PTPRALETAETAE	IVE-AYRTGAENAKAAGFDGVEIHHGANGYLLDQ	FLQSS	187				
	AcaryoER1	TPQGGKPF-VTPRAVSLDE	IPSVIQ-DFAQATGNARKAGFDGVEIHHGANGYLLIDQ	FLRDG	190				
	Class II	OYE1	EAKAKKAN-NPQHS�TKDE	IKQYIK-EYVQAAKNSIAAGADGVEIHSANGYLLNQ	FLDPH	205			
		ArOYE1	-----EDL-AVPKEMTVEE	IKETVS-AYAQAANKNAIKAGFDGVEIHAANGYLLIDQ	FLQDT	184			
		CYE	KERALKAN-NPQHGITKEE	IKQYIK-EYVDAAKKAIDAGADGVQIHHGANGYLLNQ	FLDPI	205			
		EBP1	EKLAKEAG-NELRALTEEE	IDHIVEVEYPNAAKHAEAGFDYVEIHHGAHGYLLDQ	FLNLA	215			
		MgER	KKAAEKAG-NPLRAMTEEE	IKDMIYEQYTTAAKNALAEAGFDYTELHSAHGYLLHFE	LEES	200			
Class III		XenA	---FGAHLPNVPRAMTLD	DIARVKQ-DFVDAARRARDAGFEWIELHFAHGYLLGQS	FFSEH	192			
		FOYE-1	---FYEES-LTPHMSIED	LETVKQ-DFVNSAIRAERAGFKATIELHGAHGYLLIHO	FLSPL	191			
		TsOYE	---FDEGY-PVPEPLDEAG	MERILQ-AFVEGARRALRAGFQVIELHMAHGYLLSS	FLSPL	184			
		TOYE	---AGDRY-KLPRELSVEE	IKSTVK-AFGEAAKRALAGYDVVEIHAAGYLLIHE	FLSPL	177			
		YqjM	---FDEQS-ATPVEMSAEK	VKETVQ-EFKQAAARAKEAGFDVIEIHAAGYLLIHE	FLSPL	178			
	LacER	--APRAGY-ETPRALTSAE	IEATIH-DFQAVRRAILAGFDGIELHGANTYLIQ	QFYSPN	184				
	YqiG	---FDNGK-QIARALTEEE	VENIVK-AFGEATRRATEAGFDGVEIHHGANGYLLIQ	QFYSPK	183				
	Chr-OYE1	--YPLEDM-EKASTMTLED	IQDTIA-QFAASAKSAKDLGFDVLEIHHGAHGYLLIDQ	FFWEG	170				
	SYE4	--MRELT-YTPKAVTVED	IQGLVR-DYAKAAENAIEAGFDGVEIHHGANGYLLIDQ	FLHHD	196				
	Nox	---SPPAM-GLVKGATLAE	LDGVVS-DEERTARVAVDAGFDAIEVHLGHNYLLSS	EMSPN	182				
SecStruc:		$\alpha 3 \alpha 3 \alpha 3 \alpha 3 \alpha 3 \alpha 3 \alpha 3 \alpha$ $\beta 4 \beta 4 \beta$ $\alpha D \alpha D \alpha$							
Class I	PETNR	SNQRTDQYG-GSVENR	ARLVLEVVD	AVCNEWS----	ADRI-GIRVSP	IGTFQNV	DNG---	246	
	MR	TNRRTDQYG-GSIENR	ARFPLEVVD	AAEVFG----	PERV-GIRLTP	FLEL	FGLTDD---	251	
	SYE3	SNHRTDAYG-GSPEKR	IRFLLEVVE	AAVSAQIG----	ADKV-GVRLAP	YVTFK	DMACP---	245	
	XenB	TNORTDNYG-GSLENR	ARLLLEVTD	AAIDVWG----	AGRV-GVHLAP	RADSH	DMGDD---	238	
	AcaryoER1	TNERODAYG-GTIENR	TRFLEVVE	AAVAVWS----	ADHV-GVRLSPT	NAFND	MRDS---	241	
	OYE1	SNTRTDQYG-GSIENR	ARFTLEVVD	ALVEAIG----	HEKV-GLRLSP	YGVFN	SMS-GGAE	258	
	ArOYE1	VNKRTDNYG-GSIENR	SRFAMEVTRA	IVDAIG----	ADKT-GIRLSP	FSTFQ	GMKMA---	235	
	CYE	SNNRTDQYG-GSIENR	ARFTLEVVD	AVDAVG----	AERT-SIRFSP	YGTFT	GTMS-GGEN	258	
	EBP1	SNKRTDKYGG-GSIENR	ARLLLRVVD	KLIEVVG----	ANRL-ALRLSP	WASFQ	GMEIEGEE	270	
	MgER	SNKRTDKYGG-GSIENR	ARFVLE	LDVHMISIVG----	AERL-GIRLSP	WATFQ	GMKSVHGE	254	
Class II	XenA	SNKRTDAYG-GSFDNR	SRFLLLET	LAARVWP----	ENLPLTAR	FGVLE	YD-----GR	240	
	FOYE-1	SNQRQDQYG-GSLENR	VRYPLEILSAV	KHALS----	AEMVVGMR	ISAVD	WA-----P-	238	
	TsOYE	SNQRTDAYG-GSLENR	VRFPQLQAQAV	REVVP----	RELPLFVR	VSATD	WDWG-----E-	231	
	TOYE	SNKRKDEYG-NSIENR	ARFLIEVIDE	VRKNWP----	ENKPIFVR	VASAD	DYD-----E-	224	
	YqjM	SNHRTDQYG-GSPENR	YRFLREIIDE	VKQVWD----	GPLFVR	VASAD	YD-----D-	223	
	LacER	SNRRTDEWG-GDRDKR	VRFPPLAVVHAE	KVIATIDRPFLL	GYRISPEELEQ-----			235	
	YqiG	TNQRTDRWG-GSDEKR	LAFPLAIVDE	VKAASEHAKGAF	LVGYRLSPEEPET-----			234	
	Chr-OYE1	TNTRTDQYGGGKTIKERS	RFADVVKAI	TRAAGV----	EDFTIIIRL	SQWKQ	QDYSVKLAHT	226	
	SYE4	SNRRTDQYG-GTPVNM	SRFALVVDA	IIARIG----	HDRT-GLRIS	PGAYF	NMAS-D---	246	
	Nox	LNKRHDRYG-GSAKRAE	YPRRVIEAVR	VAAGV----	SSVAVTAK	FNMSD	GV-----P-	229	
SecStruc:		$\alpha 4 \alpha 4 \alpha 4 \alpha 4 \alpha 4 \alpha 4 \alpha 4$ $\beta 5 \beta 5 \beta 5 \beta 5$							
Class I	PETNR	PNEEA-DALYLIEE	LAKR-----	GI-AYLHMSE-----			TD-	274	
	MR	-EPEA-MAFYLAGEL	DRR-----	GL-AYLHFNE-----			PD-	278	
	SYE3	-EIVE-TILLAAKQL	SFA-----	GV-AYLHSE-----			AD-	272	
	XenB	-NLAE-TFTYVARE	LGKR-----	GI-AFICSRE-----			KE-	265	
	AcaryoER1	-NPIS-TFTHAAQA	LNTY-----	NL-AYLHVLE-----			AL-	268	
	OYE1	TGIVA-QYAYVAGE	LEKRAKAGKRL-AFV	HLVE-----			PR-	291	
	ArOYE1	-EPIP-QFSDI	IKKL-----	NSLNL-AYLHMVD-----			SR-	262	
	CYE	PGIVA-QYAYVIGE	LEKRARAGKRL-AFID	LVE-----			PR-	291	
	EBP1	IH-----	SYILQQLQQRAD	NGQQL-AYISLVE-----			PR-	298	
	MgER	VHPLT-TYSYLVNE	LEKRAQAGNRL-AYIS	LVE-----			PR-	287	
Class II	XenA	DEQTL	EESIELARRFKAG-----	GL-DLLSVSVGF-----				269	
	FOYE-1	GGLTIEESIT	FSQECEKR-----	GA-GFIHVSTGG-----				267	
	TsOYE	GGWSLED	TAFARRLKEL-----	GV-DLLDCSSGG-----				260	
	TOYE	GGINIDM	MVEYINMI-KD-----	KV-DLIDVSSGG-----				252	
	YqjM	KGLDIADHIG	FAKWMKEQ-----	GV-DLIDCSSGA-----				252	
	LacER	PGITLDD	TALIDALKQT-----	KI-DYLHVSQSDV-----			WR-----	TS-	269
	YqiG	PGLTMTET	YTLVDALGDK-----	EL-DYLHISLMDV-----			NS-----	KA-	268
	Chr-OYE1	PEEME-EWLL	PLKDA-----	GV-DIFHCSQRRF-----			WE-----	PE-	256
	SYE4	SRDRV-VFDYLL	PELEKR-----	DL-AFVHIGI-----			FD-----		274
	Nox	KGLWLDQSL	PIAQIL	LEAD-----	GHLDAMQLTG	SSLLNGMYFFRGEV	PLAEFVASQPKL		284
SecStruc:		$\alpha 5 \alpha 5 \alpha 5 \alpha 5 \alpha 5 \alpha 5 \alpha$ $\beta 6 \beta 6 \beta$							

Class I	PETNR	-----LAGG-KPYS-----EAFRQKVRERFH--GVIIGAGA-----	302
	MR	-----WIGGDITYP-----EGFREQMRQRFK--GGLIYCGN-----	307
	SYE3	-----WDDAPQ-IP-----ESFRIELRNVEFK--GSIIIVAGR-----	300
	XenB	-----GA-----DSLGPQLKEAFG--GAYIANER-----	287
	AcaryoER1	-----QGHMLAVEG-----ERVTPYIRQVFQ--GPLMINGG-----	297
Class II	OYE1	-----VTNPFLTEGEGEYEYEGSNDVYSIWK--GPVIRAGN-----	325
	ArOYE1	-----VSGDASIEGYDSLE----PLIPLYTS--SPLL IAGG-----	292
	CYE	-----VTDPFLEFEKWFKEGTNEFIYSIWK--GPVLRVGN-----	325
	EBP1	-----VTGIYDVSL-KDQQGRSNEFAYKIWK--GNFIRAGN-----	331
	MgER	-----VDGINSVEK-KDQTG--NNDFVKDLWK--GTILKAGN-----	319
Class III	XenA	-----TIPETNIPWGPAFMGPIAERVVREAK--LPVTSAWG-----	303
	FOYE-1	-----LVAHQQIPVGPQGYQVEHAQAIKQNVN--IPTMAVGL-----	301
	TsOYE	-----VVLRVRIPLAPGFQVPPFADAVRKRVG--LRTGAVGL-----	294
	TOYE	-----L-LNVLDINLYPGYQVKYAEIKKRCN--IKTSAVGL-----	285
	YqjM	-----L-VHADINVFPQGYVSFAEKIREQAD--MATGAVGM-----	285
	LacER	-----LRNPEDTAI-----MNEQIRDHVAGAFPVIVVGG-----	298
	YqiG	-----RRGADPTRT-----RMDLLNERVGNKVPLIAVGS-----	297
	Chr-OYE1	-----FEGSDL-----NFAGWAKKITG--QPTITVGSVGLGDFMAAFGGQG	296
	SYE4	-----DSIEFDYLG-----GTASSYVRAHYG--KTLVGVGS-----	303
	Nox	VGYGLKFYGPKIFPTYPFEEGFLPFARQFRQALR--MPLILLGG-----	327
SecStruc:		α6α6α6α6α6 β7β7	
Class I	PETNR	-YTAEKAE--DL--IGKGLIDAVAFGRDYIANPDLVARLQKKAEL--N----PQRPESEFY	351
	MR	-YDAGRAQ--AR--LDDNTADAVAFGRPFIANPDLPERFRLGAAL--N----EPDPSTFY	356
	SYE3	-YDVERAN--DV--IEKGYADLVAFGRAFIANPDLPYRLANQLPL--S----PFDKGPLF	349
	XenB	-FTKDSAN--AW--LAEGKADAVAFGVPIANPDLPARLKADAPL--N----EPRPELFY	336
	AcaryoER1	-YDAVSGA--AA--IANQEAADVAYGVPIANPDLPERFAKQAPL--N----EPDPSTFY	346
Class II	OYE1	-FALHPE---VVREEVKDKRTLIGYGRFFISNPDLVDRLEKGLPL--N----KYDRDTFY	375
	ArOYE1	-FKADSAR-RLDDEEHKGRDVVVVGRYFISTPDLVYRLNKGIEF--T----PYDRDTFY	344
	CYE	-YALDPD---QATLDSKKPNTLIGYGRSFIANPDLVYRLEKGLPL--N----KYDRNTFY	375
	EBP1	-YTYDAPEFKTLINDLKNDRSIIGFSRFFTSNPDLVEKLKLGKPL--N----YYNREEFY	384
	MgER	-YTYDAPKFGQLDDVSDGRTLVGFSRYFISNPDLISRLEKGHQL--A----PYERETFY	372
Class III	XenA	-FGTPQL---AEALQANQLDLVSVGRAHLADPHWAYFAAKELGVEKA----SWTLAPY	355
	FOYE-1	-ITHSAQ---AETILKSEQADMIATARAALKNPHWPWTAAL ELGDKPF-----APPOY	350
	TsOYE	-ITTPEQ---AETLLQAGSADLVLLGRVLLRDPYFPLRAAKALGVAP-----VPPQY	343
	TOYE	-ITTQEL---AEELISNERADLVALGRELRLNPPYWLHTYT--SKED-----WPKQY	331
	YqjM	-ITDGS---AEELQNGRADLIFIGRELRLDPFFARTAAKQLNTEIP-----APVOY	334
	LacER	-IKT--PA--DAEK-AAESFDLVAIGHMIREPHHWQKVLHDHDEKAIRYQIAPADLEELG	352
	YqiG	-IHS--AD--DALAVIENGIPLVAMGREILVDPNWTVKVKEGREKQIETVIKGTDKKEYH	352
	Chr-OYE1	TEKADLTE--LTKRLERGDFDLVAVGRALLQDPEWAKKVKEQNTALL----DFSAESLG	350
	SYE4	-YSAETAS--KA--IAEDKFDLIAIGRPFIANPDYVAKVRNSEEL--V----AYSDEMLA	352
	Nox	-INRVDT---IEHALDE-GFEFVAMARALLRDPQLVNKFQAESVDQGLCTHCNCKMPTTY	382
SecStruc:		α7α7α7α7α7α7 β8β8 α8α8α8α8α8α8 αFα	
Class I	PETNR	GG-----GAEGYTDYPSL-----	364
	MR	GG-----AEVGYTDYPFLDNHGDRLG-----	377
	SYE3	GG-----SAAGYTDYPSYQAALRAVIRSSDDEVA----	378
	XenB	GK-----GPVGYIDYPTL-----	349
	AcaryoER1	TR-----GAEGYTDYPFLDPLITAA-----	366
Class II	OYE1	QM-----SAHGYIDYPTYEEALKLGWDKK-----	399
	ArOYE1	TPK-----SEKGYTDYPFSEEFKASANL-----	367
	CYE	TF-----TKEGYTDYPSYEESSVAKGYKKEEKY----	403
	EBP1	KY-----YNYGYNSYDESEKQVIGKPLA-----	407
	MgER	GR-----SDFGYNDYPKYGEKREDAEVAKKRVPEELVV	405
Class III	XenA	AH-----WLERYR-----	363
	FOYE-1	QR-----AR-----	354
	TsOYE	QR-----GF-----	347
	TOYE	ER-----AFKK-----	337
	YqjM	ER-----GW-----	338
	LacER	IAPTFLDFIESISGGAKGVPLTTAQSVT---SSNVTQD-----	387
	YqiG	LPEPLWQAIVNTQG---WVPYKD-----	372
	Chr-OYE1	VL-----Y-----	353
	SYE4	SL-----I-----	355
	Nox	TG-----TRCVVRDALVVREAPRLGQ-----	403

Figure S1. Multiple sequence alignment of OYEs from class I, II and III and non-assignable OYEs. Shading indicates conservation of amino acids among the sequences according to each class. Black shading represents conserved residues among all OYE family members. Yellow shading represents unique conserved residues for class I. Grey shading represents unique conserved residues for class II. Green shading highlights unique conservation for class III. When the conservation was found for another class as well, it was highlighted in the same colour. LacER, YqiG, Chr-OYE1, SYE-4 and Nox represent non-assignable OYEs according to the phylogenetic analysis (Figure 2 in the original manuscript). Conserved residues for non-assignable OYEs were highlighted according to the three OYE classes as described. The multiple sequence alignment was performed via Web Service applying Clustal Omega [1]. Secondary protein structure prediction was performed via Jpred4 Incorporating Jnet server [2].

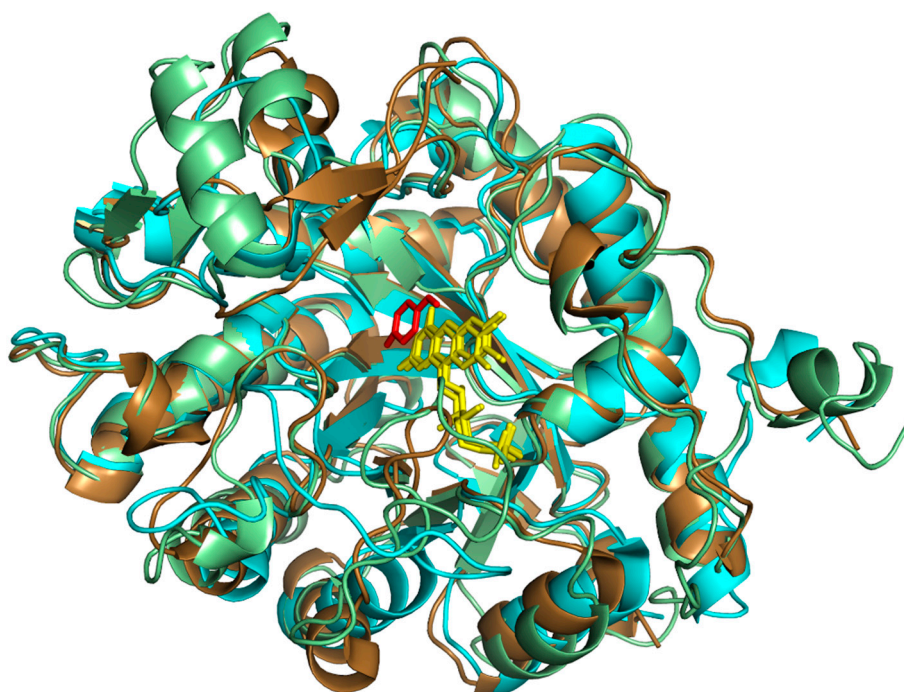


Figure S2. Overlay of three-dimensional model of the crystal structure of SYE1 (in gold, pdb entry: 2QG9; class I) from *Shewanella oneidensis*, OYE1 (in light green, pdb entry: 1OYA; class II) from *Saccharomyces pastorianus* and YqjM (in turquoise, pdb entry: 1Z41; class III) from *Bacillus subtilis*. The FMN cofactor is shown as stick model and coloured in yellow. The rms distances (rmsd) were obtained from overlaid structures between SYE1 and OYE1 (0.667 Å), SYE1 and YqjM (1.055 Å), OYE1 and YqjM (1.262 Å).

References

1. McWilliam, H.; Li, W.; Uludag, M.; Squizzato, S.; Park, Y.M.; Buso, N.; Cowley, A.P.; Lopez, R. Analysis tool web services from the EMBL-EBI. *Nucleic Acids Res.* **2013**, *41*, W597–W600.
2. Drozdetskiy, A.; Cole, C.; Procter, J.; Barton, G.J. JPred4: A protein secondary structure prediction server. *Nucleic Acids Res.* **2015**, *43*, W389–W394.