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Supporting information for article:

Structural basis of regioselective tryptophan dibromination by the single-component flavin-dependent halogenase AetF

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Table S1 Pairwise *DALI* alignment of AetF against the *AlphaFold2* model of Bmp5 (top row) and *DALI* hits for AetF against the PDB90.

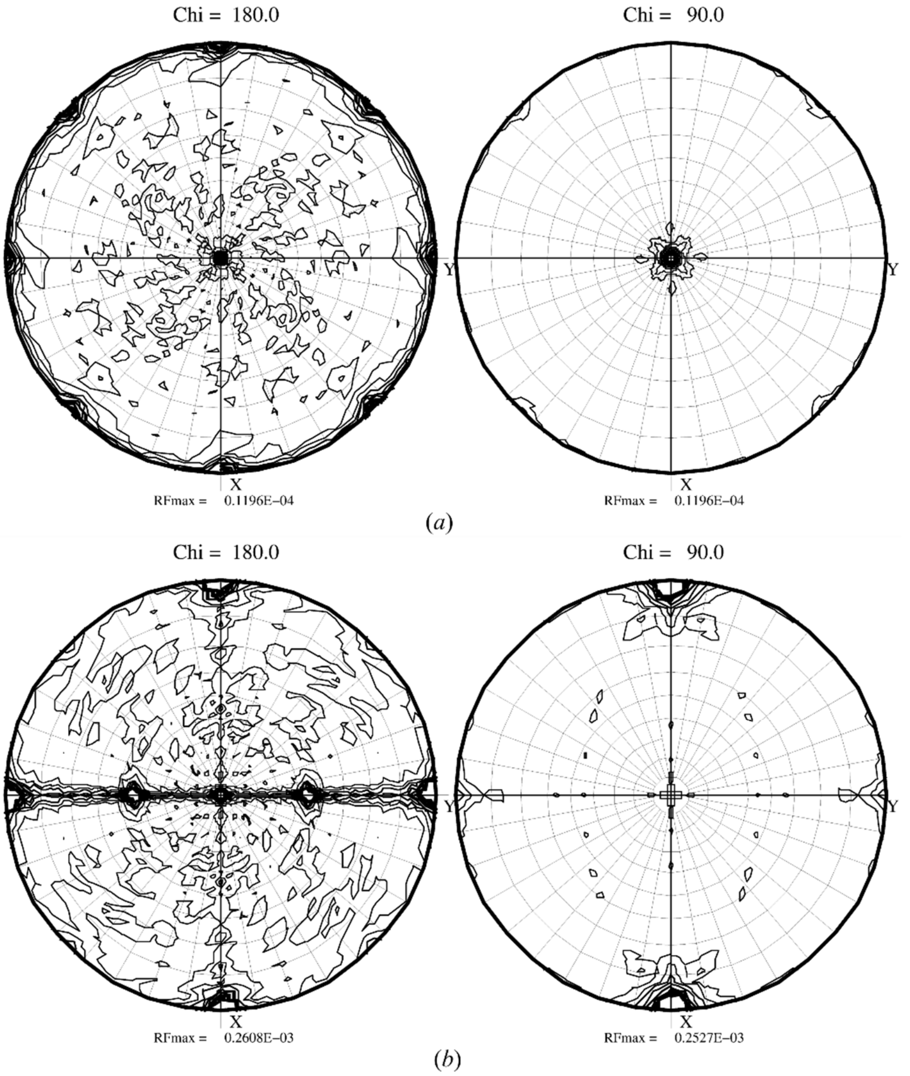
The *DALI* run against the PDB was performed on 01.03.2023. The structure of Bmp5 used in the pairwise alignment is AF-U6BHD3-F1-model_v4.

No.	Protein	PDB	Z	rmsd	lali	nres	%id	reference
n.a.	flavin-dependent phenol brominase (Bmp5)	n.a.	27.6	3.5	438	515	16	(Agarwal *et al.*, 2014)
1	ancestral flavin-containing monooxygenase 2 (ancFMO2)	6sem	25.9	3.6	432	530	14	(Nicoll *et al.*, 2020)
2	ancestral flavin-containing monooxygenase 5 (ancFMO5)	6sek	25.6	3.1	426	504	15	(Nicoll *et al.*, 2020)
3	ancestral flavin-containing monooxygenase 3-6 (ancFMO3-6)	6se3	25.2	3.1	436	528	13	(Nicoll *et al.*, 2020)
4	ancestral flavin-containing monooxygenase 1 (ancFMO1)	7al4	20.8	3.2	437	524	14	(Bailleul *et al.*, 2021)
5	phenylacetone monooxygenase, a Baeyer-Villiger monooxygenase	1w4x	18.8	3.5	295	533	12	(Malito *et al.*, 2004)
6	protein with similarity to flavin-containing monooxygenases	1vqw	18.6	3.2	321	442	13	(Eswaramoorthy *et al.*, 2006)
7	pyrrolizidine alkaloid *N*-oxygenase (*Zv*PNO)	5nmx	18.3	3.3	321	409	13	(Kubitza *et al.*, 2018)
8	cyclohexanone monooxygenase	3gwd	18.2	3.6	301	531	13	(Mirza *et al.*, 2009)
9	steroid monooxygenase	4aos	18.2	3.5	297	532	14	(Franceschini *et al.*, 2012)
10	*S*-allyl-L-cysteine S-oxygenase	6wpu	17.9	3.2	332	439	14	(Valentino *et al.*, 2020)

Table S2 Boundaries of the FAD-binding and NADP-binding domains shown in Fig. 2

Protein	FAD-binding domain	NADP-binding domain	reference
AetF	1-132, 327-441	133-176, 294-326	this work
Bmp5	1-139, 324-432	140-199, 288-323	this work
AncFMO2	1-154, 331-442	155-213, 296-330	(Nicoll *et al.*, 2020)
*Zv*PNO[1)]	1-155 260-371	156-259	this work
	1-155, 260-425	156-259	(Kubitza *et al.*, 2018)

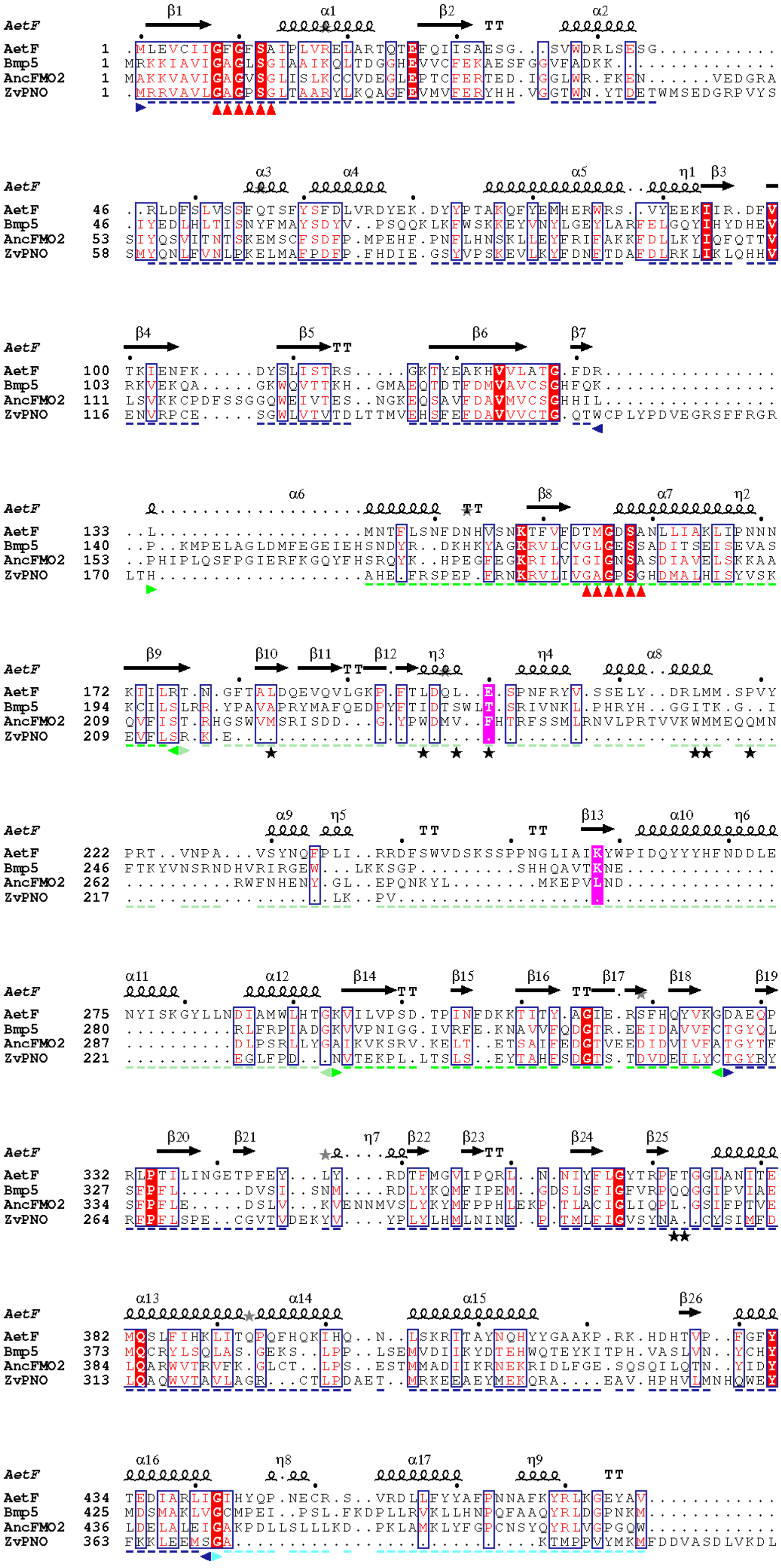
[1)] For *Zv*PNO the authors of the structure assigned the whole sequence either to the NADP- or to the FAD-binding domain. For Fig. 2, we defined the FAD-binding domain of *Zv*PNO based on structural elements conserved in the other three proteins. This leaves a short C-terminal extension (residues 372-425) as additional structural elements shown in light blue in Fig. 2.



(*a*)

(*b*)

Figure S1 Self-rotation functions for χ=180 ° and χ=90 ° showing the location of 2-fold and 4-fold axes. (*a*) Untwinned $P4_3$ crystals (empty AetF). The short axis is the crystallographic 4-fold *c*-axis (*b*) Almost perfectly non-merohedrally twinned $P2_12_12_1$ crystals. The short axis is the *a*-axis. A non-crystallographic 4_3-axis lies parallel to *a* and the crystals are twinned by 90 ° rotation around *a*.



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AetF
AetF        1  .MLEVCIIGFGFSAIPLVRELARTQTEFQIISAESG...SVWDRLSESG..........
Bmp5        1  MRKKIAVIGAGLSGIAAIKQLTDGGHEVVCFEKAESFGGVFADKK...............
AncFMO2     1  MAKKVAVIGAGVSGLISLKCCVDEGLEPTCFERTED.IGGLWR.FKEN......VEDGRA
ZvPNO       1  .MRRVAVLGAGPSGLTAARYLKQAGFEVMVFERYHH.VGGTWN.YTDETWMSEDGRPVYS

AetF
AetF       46  ..RLDFSLVSSFQTSFYSFDLVRDYEK.DYYPTAKQFYEMHERWRS..VYEEKIIR.DFV
Bmp5       46  .IYEDLHLTISNYFMAYSDYV..PSQQKLKFWSKKEYVNYLGEYLARFELGQYIHYDHEV
AncFMO2    53  SIYQSVITNTSKEMSCFSDFP.MPEHF.PNFLHNSKLLEYFRIFAKKFDLLKYIQFQTTV
ZvPNO      58  SMYQNLFVNLPKELMAFPDFP.FHDIE.GSYVPSKEVLKYFDNFTDAFDLRKLIKLQHHV

AetF
AetF      100  TKIENFK.....DYSLISTRS.....GKTYEAKHVVLATG.FDR................
Bmp5      103  RKVEKQA.....GKWQVTTKH..GMAEQTDTFDMVAVCSGHFQK................
AncFMO2   111  LSVKKCPDFSSGGQWEIVTES..NGKEQSAVFDAVMVCSGHHIL................
ZvPNO     116  ENVRPCE.....SGWLVTVTDLTTMVEHSFEFDAVVVCTG.QTWCPLYPDVEGRSFFRGR

AetF
AetF      133  ..L...................MNTFLSNFDNHVSNKTFVFDTMGDSANLLIAKLIPNNN
Bmp5      140  ..P..KMPELAGLDMFEGEIEHSNDYR..DKHKYAGKRVLCVGLGESSADITSEISEVAS
AncFMO2   153  ..PHIPLQSFPGIERFKGQYFHSRQYK..HPEGFEGKRILVIGIGNSASDIAVELSKKAA
ZvPNO     170  LTH...................AHE.FRSPEP.FRNKRVLIVGAGPSGHDMALHISYVSK

AetF
AetF      172  KIILRT.N.GFTALDQEVQVLGKP.FTLDQL..E.SPNFRYV.SSELY..DRLMM.SPVY
Bmp5      194  KCILSLRR.YPAVAPRYMAFQEDPYFTIDTSWLT.SRIVNKL.PHRYH..GGITK.G..I
AncFMO2   209  QVFIST.RHGSWVMSRISDD...G.YPWDMV..FHTRFSSMLRNVLPRTVVKWMMEQQMN
ZvPNO     209  EVFLSR.K.E..................................................

AetF
AetF      222  PRT..VNPA...VSYNQFPLI..RRDFSWVDSKSSPPNGLIAIKYWPIDQYYYHFNDDLE
Bmp5      246  FTKYVNSRNDHVRIRGEW...LKKSGP.........SHHQAVTKNE..............
AncFMO2   262  ..........RWFNHENY.GL..EPQNKYL........MKEPVLND..............
ZvPNO     217  ...................LK..PV...................................

AetF
AetF      275  NYISKGYLLNDIAMWLHTGKVILVPSD.TPINFDKKTITY.AGIE.RSFHQYVKGDAEQP
Bmp5      280  ..........RLFRPIADGKVVPNIGG.IVRFE.KNAVVFQDGTR.EEIDAVVFCTGYQL
AncFMO2   287  ..........DLPSRLLYGAIKVKSRV.KELT..ETSAIFEDGTVEEDIDVIVFATGYTF
ZvPNO     221  ..........EGLFPD...NVTEKPL.LTSLS..EYTAHFSDGTS.TDVDEILYCTGYRY

AetF
AetF      332  RLPTILINGETPFEY...LY....RDTFMGVIPQRL..N.NIYFLGYTRPFTGGLANITE
Bmp5      327  SFPFL......DVSI..SNM....RDLYKQMFIPEM..GDSLSFIGFVRPQQGGIPVIAE
AncFMO2   334  SFPFLE.....DSLV...KVENNMVSLYKYMFPPHLEKP.TLACIGLIQPL.GSIFPTVE
ZvPNO     264  RFPFLSPE..CGVTVDEKYV....YPLYLHMLNINK..P.TMLFIGVSYNA..CYSIMFD

AetF
AetF      382  MQSLFIHKLITQPQFHQKIHQ..N..LSKRITAYNQHYYGAAKP.RK.HDHTVP..FGFY
Bmp5      373  MQCRYLSQLAS.GEKS..LPP..LSEMVDIIKYDTEHWQTEYKITPH.VASLVN..YCHY
AncFMO2   384  LQARWVTRVFK.GLCT..LPS..ESTMMADIIKRNEKRIDLFGE.SQSQILQTN..YIDY
ZvPNO     313  LQAQWVTAVLAGR...CTLPDAET..MRKEEAEYMEKQRA....EAV.HPHVLMNHQWEY

AetF
AetF      434  TEDIARLIGIHYQP.NECR.S..VRDLLFYYAFPNNAFKYRLKGEYAV............
Bmp5      425  MDSMAKLVGCMPEI..PSL.FKDPLLRVKLLHNPQFAAQYRLDGPNKM............
AncFMO2   436  LDELALEIGAKPDLLSLLLKD..PKLAMKLYFGPCNSYQYRLVGPGQW............
ZvPNO     363  FKKLEEMSGA.......................KTMPPVYMKMFDDVASDLVKDL
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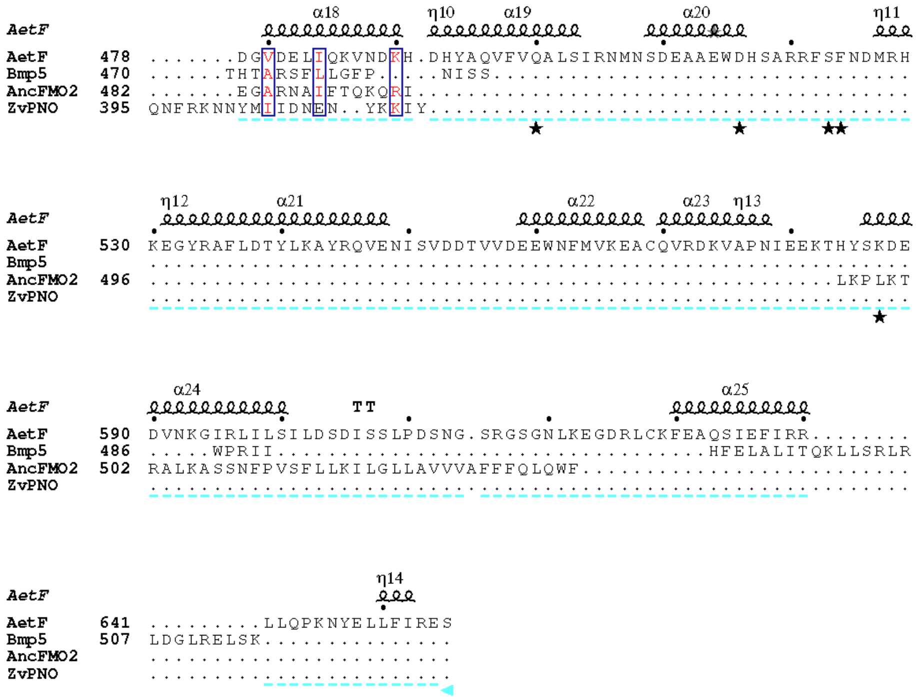


Figure S2 Pairwise *DALI* alignment of AetF against Bmp5 (AF-U6BHD3-F1-model_v4), AncFMO2 (6sf0 *B*) and *Zv*PNO (5nmx *A*). Missing structural elements were copied from the respective *AlphaFold2* models. Domains in AetF are marked by dashed lines and domain borders by arrows (same color code as in Fig. 2). Consensus sequences for dinucleotide binding (GXGXXG and GXGXXA) are marked by red triangles and residues involved in binding of Trp or 5-Br-Trp are indicated by black stars. The catalytic amino acids found in AetF (E200 and K258) are highlighted in magenta.

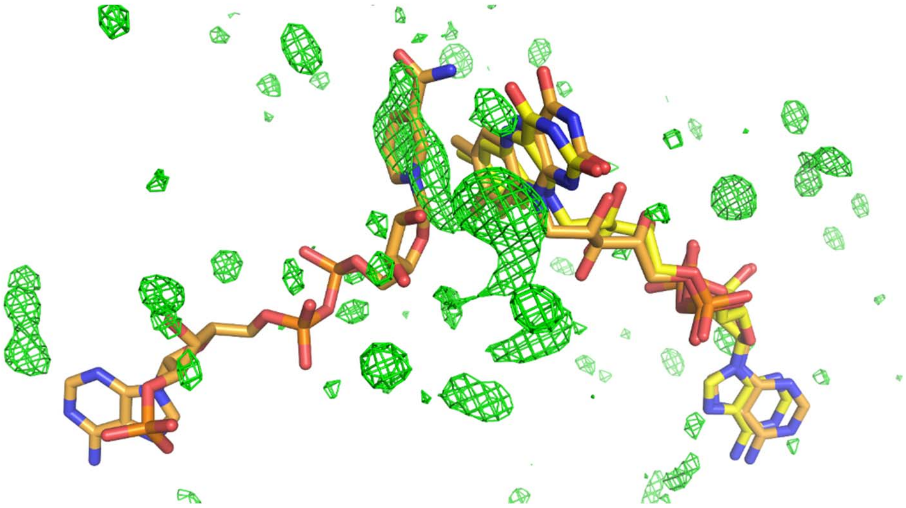


Figure S3 Difference density (mF_o-DF_c density, 3 σ, green mesh) at the expected location of the NADP-binding site. The larger contiguous difference density near the FAD in AetF (yellow carbon atoms) could be interpreted by a nicotinamide riboside. For comparison, NADP and FAD from 6sf0 (light orange carbon atoms) are shown (structures superimposed on the FADs). The NADP in AetF would need to adopt a different binding pose compared to 6sf0 to fit the shown difference density.

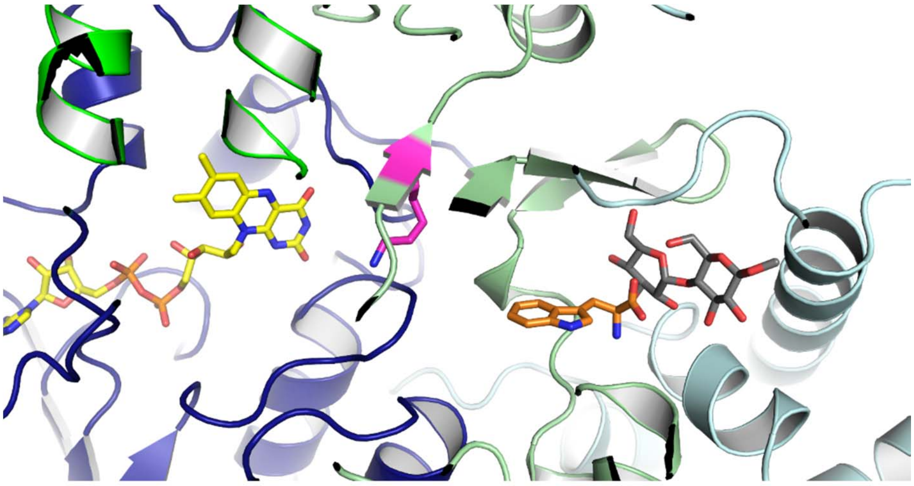


Figure S4 Comparison of Trp binding in AetF and CYMAL6 in AncFMO2. Our structure for AetF–Trp (cartoon, domains coloured as in Fig. 2) and AncFMO2 (6sf0 *D*) were superimposed at the FADs. Both Trp (orange carbon atoms) and CYMAL6 (grey carbon atoms) bind to a similar site in the respective enzyme.

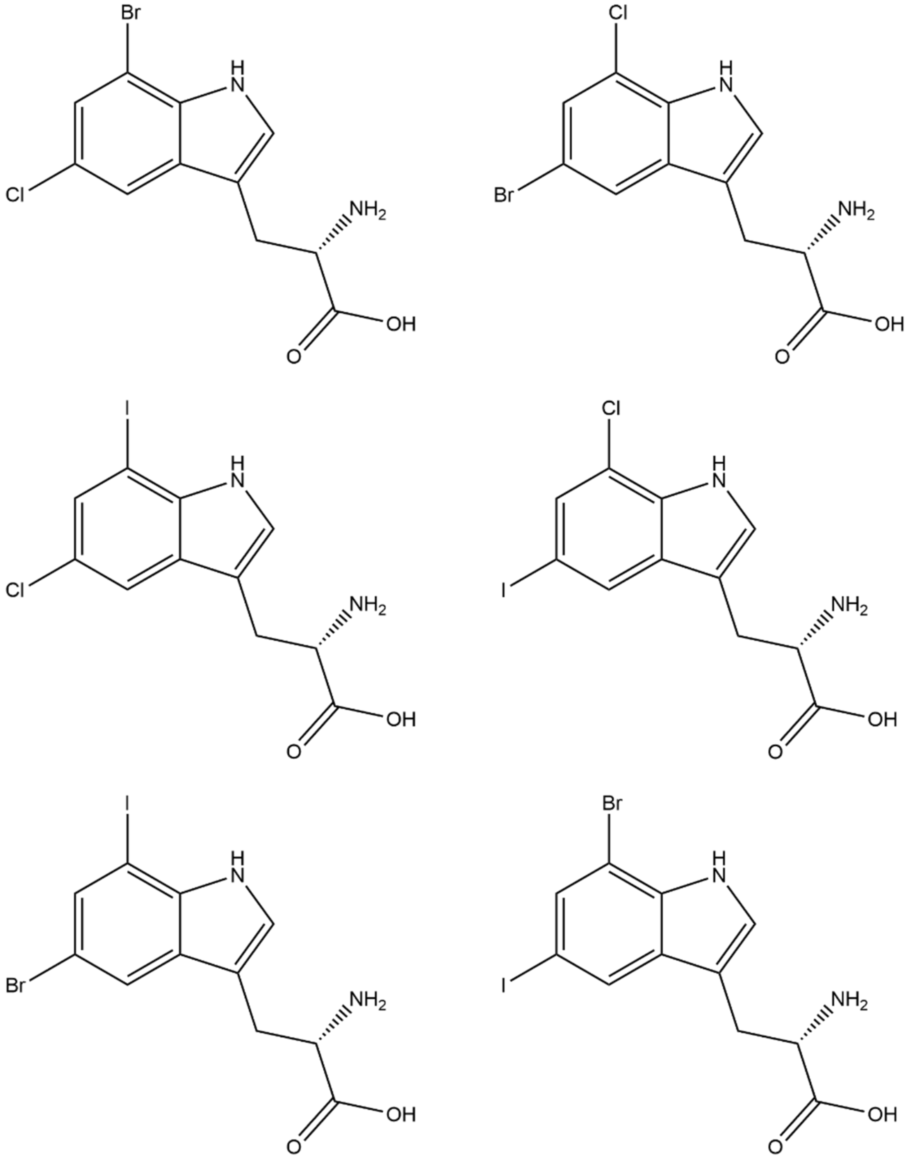
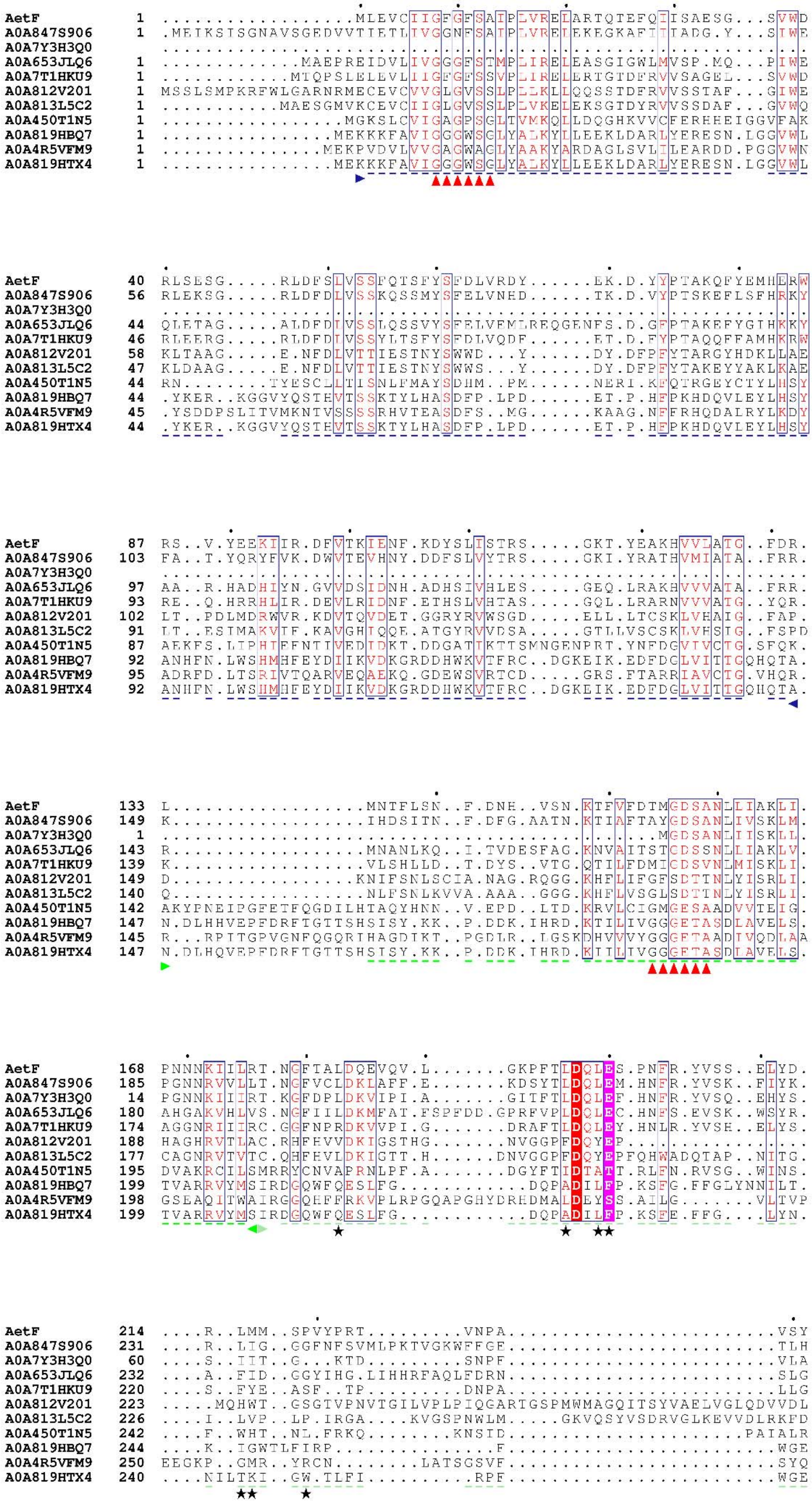


Figure S5 Possible products of tryptophan halogenation with two different halogens. With prior use of a suitable two-component halogenase, a regioselective second halogenation by AetF should be possible. This would allow site-specific differential double halogenation of Trp.



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AetF          1  ..................MLEVCIIGFGFSAIPLVRELARTQTEFQIISAESG...SVWD
A0A847S906    1  .MEIKSISGNAVSGEDVVTIETLIVGGNFSAIPLVRELEKEGKAFIITADG.Y...SIWE
A0A7Y3H3Q0       ............................................................
A0A653JLQ6    1  .............MAEPREIDVLIVGGGFSTMPLIRELEASGIGWLMVSP.MQ...PIWE
A0A7T1HKU9    1  ............MTQPSLELEVLIIGFGFSSVPLIRELERTGTDFRVVSAGEL...SVWD
A0A812V201    1  MSSLSMPKRFWLGARNRMECEVCVVGLGVSSLPLLKLLQQSSTDFRVVSSTAF...GIWE
A0A813L5C2    1  ...........MAESGMVKCEVCIIGLGVSSLPLVKELEKSGTDYRVVSSDAF...GVWQ
A0A450T1N5    1  .................MGKSLCVIGAGPSGLTVMKQLLDQGHKVVCFERHHEIGGVFAK
A0A819HBQ7    1  ................MEKKKFAVIGGGWSGLYALKYLLEEKLDARLYERESN.LGGVWL
A0A4R5VFM9    1  ...............MEKPVDVLVVGAGWAGLYAAKYARDAGLSVLILEARDD.PGGVWN
A0A819HTX4    1  ................MEKKKFAVIGGGWSGLYALKYLLEEKLDARLYERESN.LGGVWL

AetF         40  RLSESG.....RLDFSLVSSFQTSFYSFDLVRDY......EK.D.YYPTAKQFYEMHERW
A0A847S906   56  RLEKSG.....RLDFDLVSSKQSSMYSFELVNHD......TK.D.VYPTSKEFLSFHRKY
A0A7Y3H3Q0       .............................................................
A0A653JLQ6   44  QLETAG.....ALDFDLVSSLQSSVYSFELVEMLREQGENFS.D.GFPTAKEFYGTHKKY
A0A7T1HKU9   46  RLEERG.....RLDFDLVSSYLTSFYSFDLVQDF......ET.D.FYPTAQQFFAMHKRW
A0A812V201   58  KLTAAG.....E.NFDLVTTIESTNYSWWD...Y......DY.DFPFYTARGYHDKLLAE
A0A813L5C2   47  KLDAAG.....E.NFDLVTTIESTNYSWWS...Y......DY.DFPFYTAKEYYAKLKAE
A0A450T1N5   44  RN........TYESCLLTISNLFMAYSDHM..PM......NERI.KFQTRGEYCTYLHSY
A0A819HBQ7   44  .YKER..KGGVYQSTHVTSSKTYLHASDFP.LPD......ET.P.HFPKHDQVLEYLHSY
A0A4R5VFM9   45  .YSDDPSLITVMKNTVSSSSRHVTEASDFS..MG......KAAG.NFFRHQDALRYLKDY
A0A819HTX4   44  .YKER..KGGVYQSTHVTSSKTYLHASDFP.LPD......ET.P.HFPKHDQVLEYLHSY

AetF         87  RS..V.YEEKIIR.DFVTKIENF.KDYSLISTRS.....GKT.YEAKHVVLATG..FDR.
A0A847S906  103  FA..T.YQRYFVK.DWVTEVHNY.DDFSLVYTRS.....GKI.YRATHVMIATA..FRR.
A0A7Y3H3Q0       ............................................................
A0A653JLQ6   97  AA..R.HADHIYN.GVVDSIDNH.ADHSIVHLES.....GEQ.LRAKHVVVATA..FRR.
A0A7T1HKU9   93  RE..Q.HRRHLIR.DEVLRIDNF.ETHSLVHTAS.....GQL.LRARNVVVATG..YQR.
A0A812V201  102  LT..PDLMDRWVR.KDVTQVDET.GGRYRVWSGD.....ELL.LTCSKLVHAIG..FAP.
A0A813L5C2   91  LT..ESIMAKVTF.KAVGHTQQE.ATGYRVVDSA.....GTLLVSCSKLVHSIG..FSPD
A0A450T1N5   87  AEKFS.LIPHTFFNTIVEDTDKT.DDGATTKTTSMNGENPRT.YNFDGVIVCTG.SFQK.
A0A819HBQ7   92  ANHFN.LWSHMHFEYDIIKVDKGRDDHWKVTFRC..DGKEIK.EDFDGLVITTGQHQTA.
A0A4R5VFM9   95  ADRFD.LTSRIVTQARVEQAEKQ.GDEWSVRTCD.....GRS.FTARRIAVCTG.VHQR.
A0A819HTX4   92  ANHFN.LWSHMHFEYDIIKVDKGRDDHWKVTFRC..DGKEIK.EDFDGLVITTGQHQTA.

AetF        133  L..................MNTFLSN..F.DNH..VSN.KTFVFDTMGDSANLLIAKLI.
A0A847S906  149  K..................IHDSITN..F.DFG.AATN.KTIAFTAYGDSANLIVSKLM.
A0A7Y3H3Q0    1  ..............................................MGDSANLIISKLL.
A0A653JLQ6  143  R..................MNANLKQ..I.TVDESFAG.KNVAITSTGDSSNLLIAKLV.
A0A7T1HKU9  139  K..................VLSHLLD..T.DYS..VTG.QTILFDMIGDSVNLMISKLI.
A0A812V201  149  D.................KNIFSNLSCIA.NAG.RQGG.KHFLIFGFSDTTNLYISRLI.
A0A813L5C2  140  Q..................NLFSNLKVVA.AAA..GGG.KHFLVSGLSDTTNLYISRLI.
A0A450T1N5  142  AKYPNEIPGFETFQGDILHTAQYHNN..V.EPD..LTD.KRVLCIGMGESAADVVTEIG.
A0A819HBQ7  147  N.DLHHVEPFDRFTGTTSHSISY.KK..P.DDK.IHRD.KTILIVGGGETASDLAVELS.
A0A4R5VFM9  145  R...RPITGPVGNFQGQRTHAGDIKT..PGDLR..LGSKDHVVVYGGGFTAADIVQDLAA
A0A819HTX4  147  N.DLHQVEPFDRFTGTTSHSISY.KK..P.DDK.THRD.KTILIVGGGFTASDLAVELS.

AetF        168  PNNNKIILRT.NGFTALDQEVQV.L.......GKPFTLDQLES.PNFR.YVSS..ELYD.
A0A847S906  185  PGNNRVVLLT.NGFVCLDKLAFF.E.......KDSYTLDQLEM.HNFR.YVSK..FIYK.
A0A7Y3H3Q0   14  PGNNKIIIRT.KGFDPLDKVIPI.A.......GITFTLDQLEF.HNFR.YVSQ..EHYS.
A0A653JLQ6  180  AHGAKVHLVS.NGFIILDKMFAT.FSPFDD.GPRFVPLDQLEC.HNFS.EVSK..WSYR.
A0A7T1HKU9  174  AGGNRIIIRC.GGFNPRDKVVPI.G.......DRAFTLDQLEY.HNLR.YVSH..ELYS.
A0A812V201  188  HAGHRVTLAC.RHFHVVDKIGSTHG.......NVGGPFDQYEP.................
A0A813L5C2  177  CAGNRVTVTC.QHFHVLDKIGTT.H......DNVGGPFDQYEPFQHWADQTAP..NITG.
A0A450T1N5  195  DVAKRCILSMRRYCNVAPRNLPF.A.......DGYFTIDTATT.RLFN.RVSG..WINS.
A0A819HBQ7  199  TVARRVYMSIRDGQWFQESLFG............DQPADILFP.KSFG.FFGLYNNILT.
A0A4R5VFM9  198  GSEAQITWAIRGGQHFFRKVPLRPGQAPGHYDRHDMALDEYSS.AILG.......VLTVP
A0A819HTX4  199  TVARRVYMSIRDGQWFQESLFG............DQPADILFP.KSFE.FFG....LYN.

AetF        214  ....R..LMM..SPVYPRT.........VNPA.........................VSY
A0A847S906  231  ....R..LIG..GGFNFSVMLPKTVGKWFFGE.........................TLH
A0A7Y3H3Q0   60  ....S..IIT..G...KTD.........SNPF.........................VLA
A0A653JLQ6  232  ....A..FID..GGYIHG.LIHHRFAQLFDRN.........................SLG
A0A7T1HKU9  220  ....S..FYE..ASF..TP.........DNPA.........................LLG
A0A812V201  223  .....MQHWT..GSGTVPNVTGILVPLPIQGARTGSPMWMAGQITSYVAELVGLQDVVDL
A0A813L5C2  226  ....I..LVP..LP.IRGA....KVGSPNWLM.....GKVQSYVSDRVGLKEVVDLRKFD
A0A450T1N5  242  ....F..WHT..NL.FRKQ........KNSID......................PAIALR
A0A819HBQ7  244  ....K..IGWTLFIRP...............F.........................WGE
A0A4R5VFM9  250  EEGKP..GMR..YRCN........LATSGSVF.........................SYQ
A0A819HTX4  240  ....NILTKI..GW.TLFI..........RPF.........................WGE
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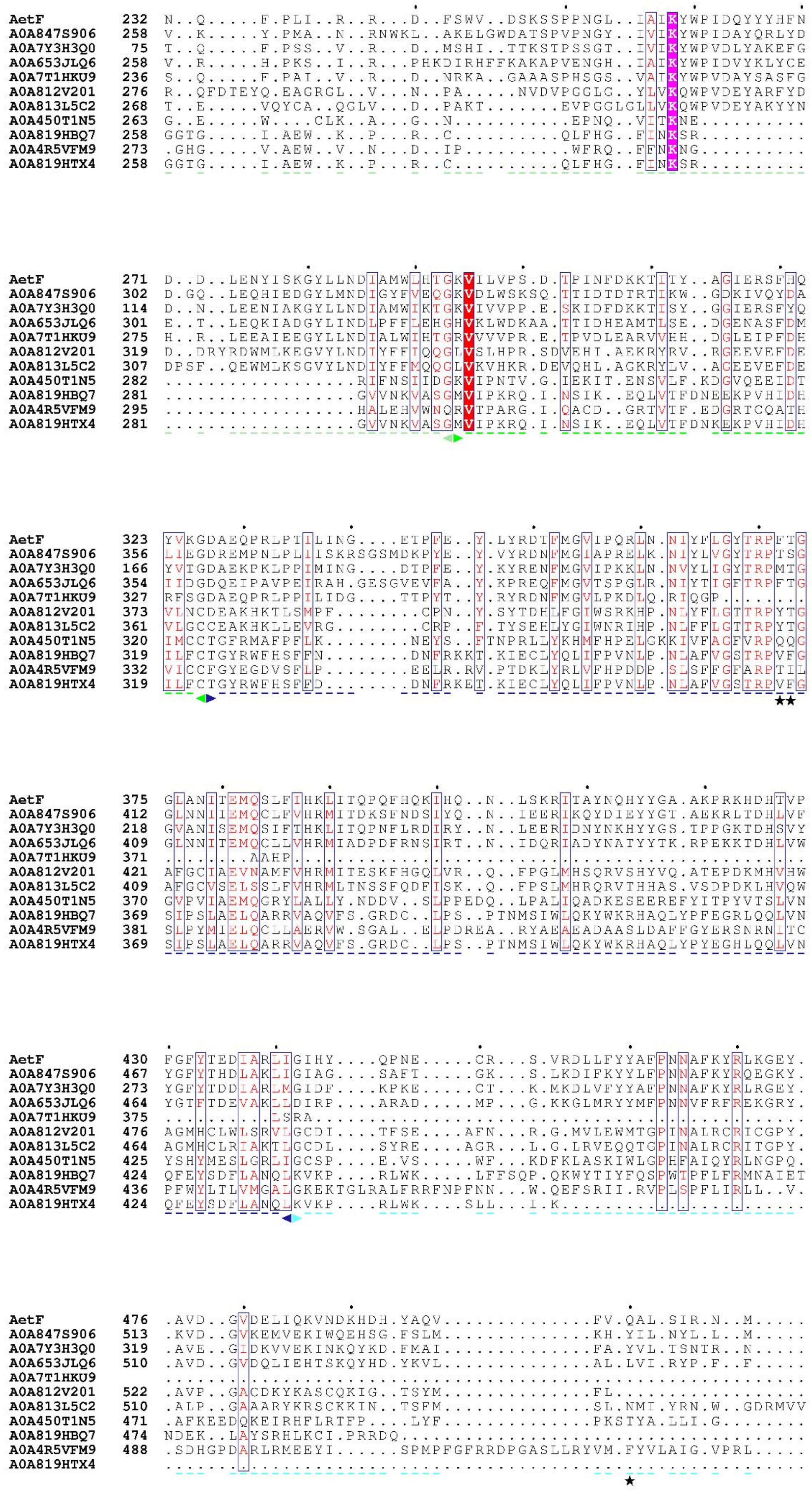

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AetF        232  N..Q.....F.PLI..R..R...D..FSWV..DSKSSPPNGL..IAIKYWPIDQYYYHFN
A0A847S906  258  V..K.....Y.PMA..N..RNWKL..AKELGWDATSPVPNGY..IVIKYWPIDAYQRLYD
A0A7Y3H3Q0   75  T..Q.....F.PSS..V..R...D..MSHI..TTKSTPSSGT..IVIKYWPVDLYAKEFG
A0A653JLQ6  258  V..R.....H.PKS..I..R...PHKDIRHFFKAKAPVENGH..IAIKYWPIDVYKLYCE
A0A7T1HKU9  236  S..Q.....F.PAT..V..R...D..NRKA..GAAASPHSGS..VATKYWPVDAYSASFG
A0A812V201  276  R..QFDTEYQ.EAGRGL..V...N..PA.....NVDVPGGLG..YLVKQWPVDEYARFYD
A0A813L5C2  268  T..E.....VQYCA..QGLV...D..PAKT.......EVPGGLGLLVKQWPVDEYAKYYN
A0A450T1N5  263  G..E.....W....CLK..A...G..N..........EPNQ..VITKNE..........
A0A819HBQ7  258  GGTG.....I.AEW..K..P...R..C..........QLFHG..FINKSR..........
A0A4R5VFM9  273  .GHG.....V.AEW..V..N...D..IP.........WFRQ..FFNKNG..........
A0A819HTX4  258  GGTG.....I.AEW..K..P...R..C..........QLFHG..FINKSR..........

AetF        271  D..D..LENYISKGYLLNDIAMWLHTGKVILVPS.D.TPINFDKKTITY..AGIERSFHQ
A0A847S906  302  D.GQ..LEQHIEDGYLMNDIGYFVEQGKVDLWSKSQ.TTIDTDTRTIKW..GDKIVQYDA
A0A7Y3H3Q0  114  D..N..LEENIAKGYLLNDIAMWIKTGKVIVVPP.E.SKIDFDKKTISY..GGIERSFYQ
A0A653JLQ6  301  E..T..LEQKIADGYLINDLPFFLEHGHVKLWDKAA.TTIDHEAMTLSE..DGENASFDM
A0A7T1HKU9  275  H..R..LEEAIEEGYLLNDIALWIHTGRVVVVPR.E.TPVDLEARVVEH..DGLEIPFDH
A0A812V201  319  D..DRYRDWMLKEGVYLNDTYFFTQQGLVSLHPR.SDVEHT.AEKRYRV..RGEEVEFDE
A0A813L5C2  307  DPSF..QEWMLKSGVYLNDIYFFMQQGLVKVHKR.DEVQHL.AGKRYLV..ACEEVEFDE
A0A450T1N5  282  ..................RIFNSIIDGKVIPNTV.G.IEKIT.ENSVLF.KDGVQEEIDT
A0A819HBQ7  281  ..................GVVNKVASGMVIPKRQ.I.NSIK..EQLVTFDNEEKPVHIDH
A0A4R5VFM9  295  ..................HALEHVWNQRVTPARG.I.QACD..GRTVTF.EDGRTCQATH
A0A819HTX4  281  ..................GVVNKVASGMVIPKRQ.I.NSIK..EQLVTFDNKEKPVHIDH

AetF        323  YVKGDAEQPRLPTILING....ETPFE..Y.LYRDTFMGVIPQRLN.NIYFLGYTRPFTG
A0A847S906  356  LIEGDREMPNLPLIISKRSGSMDKPYE..Y.VYRDNFMGIAPRELK.NIYLVGYTRPTSG
A0A7Y3H3Q0  166  YVTGDAEKPKLPPIMING....DTPFE..Y.KYRENFMGVIPKKLN.NVYLIGYTRPMTG
A0A653JLQ6  354  IIDGDQEIPAVPEIRAH.GESGVEVFA..Y.KPREQFMGVTSPGLR.NIYTIGFTRPFTG
A0A7T1HKU9  327  RFSGDAEQPRLPPILIDG....TTPYT..Y.RYRDNFMGVLPKDLQ.RIQGP........
A0A812V201  373  VLNCDEAKHKTLSMPF........CPN..Y.SYTDHLFGIWSRKHP.NLYFLGTTRPYTG
A0A813L5C2  361  VLGCCEAKHKLLEVRG........CRP..F.TYSEHLYGIWNRIHP.NLFFLGTTRPYTG
A0A450T1N5  320  IMCCTGFRMAFPFLK........NEYS..FTNPRLLYKHMFHPELGKKIVFAGFVRPQQG
A0A819HBQ7  319  ILFCTGYRWFHSFFN........DNFRKKT.KIECLYQLIFPVNLP.NLAFVGSTRPVFG
A0A4R5VFM9  332  VICCFGYEGDVSFLP........EELR.RV.PTDKLYRLVFHPDDP.SLSFFGFARPTIL
A0A819HTX4  319  ILFCTGYRWFHSFFD........DNFRKET.KIECLYQLIFPVNLP.NLAFVGSTRPVFG

AetF        375  GLANITEMQSLFIHKLITQPQFHQKIHQ..N..LSKRITAYNQHYYGA.AKPRKHDHTVP
A0A847S906  412  GLNNIIEMQCLFVHRMITDKSFNDSIYQ..N..IEERIKQYDIEYYGT.AEKRLTDHLVF
A0A7Y3H3Q0  218  GVANISEMQSIFTHKLITQPNFLRDIRY..N..LEERIDNYNKHYYGS.TPPGKTDHSVY
A0A653JLQ6  409  GLNNITEMQCLLVHRMIADPDFRNSIRT..N..IDQRIADYNATYYTK.RPEKKTDHLVW
A0A7T1HKU9  371  ........AAHP................................................
A0A812V201  421  AFGCIAEVNAMFVHRMITESKFHGQLVR..Q..FPGLMHSQRVSHYVQ.ATEPDKMHVHW
A0A813L5C2  409  AFGCVSELSSLFVHRMLTNSSFQDFISK..Q..FPSLMHRQRVTHHAS.VSDPDKLHVQW
A0A450T1N5  370  GVPVIAEMQGRYLALLY.NDDV..SLPPEDQ..LPALIQADKESEEREFYITPYVTSLVN
A0A819HBQ7  369  SIPSLAELQARRVAQVFS.GRDC..LPS..PTNMSIWLQKYWKRHAQLYPFEGRLQQLVN
A0A4R5VFM9  381  SLPYMIELQCLLAERVW.SGAL..ELPDREA..RYAEAEADAASLDAFFGYERSNRNITC
A0A819HTX4  369  SIPSLAELQARRVAQVFS.GRDC..LPS..PTNMSIWLQKYWKRHAQLYPYEGHLQQLVN

AetF        430  FGFYTEDIARLIGIHY....QPNE.....CR...S.VRDLLFYYAFPNNAFKYRLKGEY.
A0A847S906  467  YGFYTHDLAKLIGIAG....SAFT.....GK...S.LKDIFKYYLFPNNAFKYRQEGKY.
A0A7Y3H3Q0  273  YGFYTDDIARLMGIDF....KPKE.....CT...K.MKDLVFYYAFPNNAFKYRLRGEY.
A0A653JLQ6  464  YGTFTDEVAKLLDIRP....ARAD.....MP...G.KKGLMRYYMFPNNVFRFREKGRY.
A0A7T1HKU9  375  ..........LSRA..............................................
A0A812V201  476  AGMHCLWLSRVLGCDI....TFSE....AFN...R.G.MVLEWMTGPINALRCRICGPY.
A0A813L5C2  464  AGMHCLRIAKTLGCDL....SYRE....AGR...L.G.LRVEQQTGPINALRCRITGPY.
A0A450T1N5  425  YSHYMESLGRLIGCSP....E.VS.....WF...KDFKLASKIWLGPHFAIQYRLNGPQ.
A0A819HBQ7  424  QFEYSDFLANQLKVKP....RLWK.....LFFSQP.QKWYTIYFQSPWTPFLFRMNAIET
A0A4R5VFM9  436  PFWYLTLVMGALGKEKTGLRALFRRFNPFNN...W.QEFSRII.RVPLSPFLIRLL..V.
A0A819HTX4  424  QFEYSDFLANQLKVKP....RLWK....SLL...I.K.......................

AetF        476  .AVD..GVDELIQKVNDKHDH.YAQV..............FV.QAL.SIR.N..M.....
A0A847S906  513  .KVD..GVKEMVEKIWQEHSG.FSLM..............KH.YIL.NYL.L..M.....
A0A7Y3H3Q0  319  .AVE..GIDKVVEKINKQYKD.FMAI..............FA.YVL.TSNTR..N.....
A0A653JLQ6  510  .AVD..GVDQLIEHTSKQYHD.YKVL..............AL.LVI.RYP.F..F.....
A0A7T1HKU9       ............................................................
A0A812V201  522  .AVP..GACDKYKASCQKIG..TSYM..............FL..................
A0A813L5C2  510  .ALP..GAAARYKRSCKKIN..TSFM..............SL.NMI.YRN.W..GDRMVV
A0A450T1N5  471  .AFKEEDQKEIRHFLRTFP....LYF..............PKSTYA.LLI.G........
A0A819HBQ7  474  NDEK..LAYSRHLKCI.PRRDQ......................................
A0A4R5VFM9  488  .SDHGPDARLRMEEYI......SPMPFGFRRDPGASLLRYVM.FYVLAIG.VPRL.....
A0A819HTX4       ............................................................
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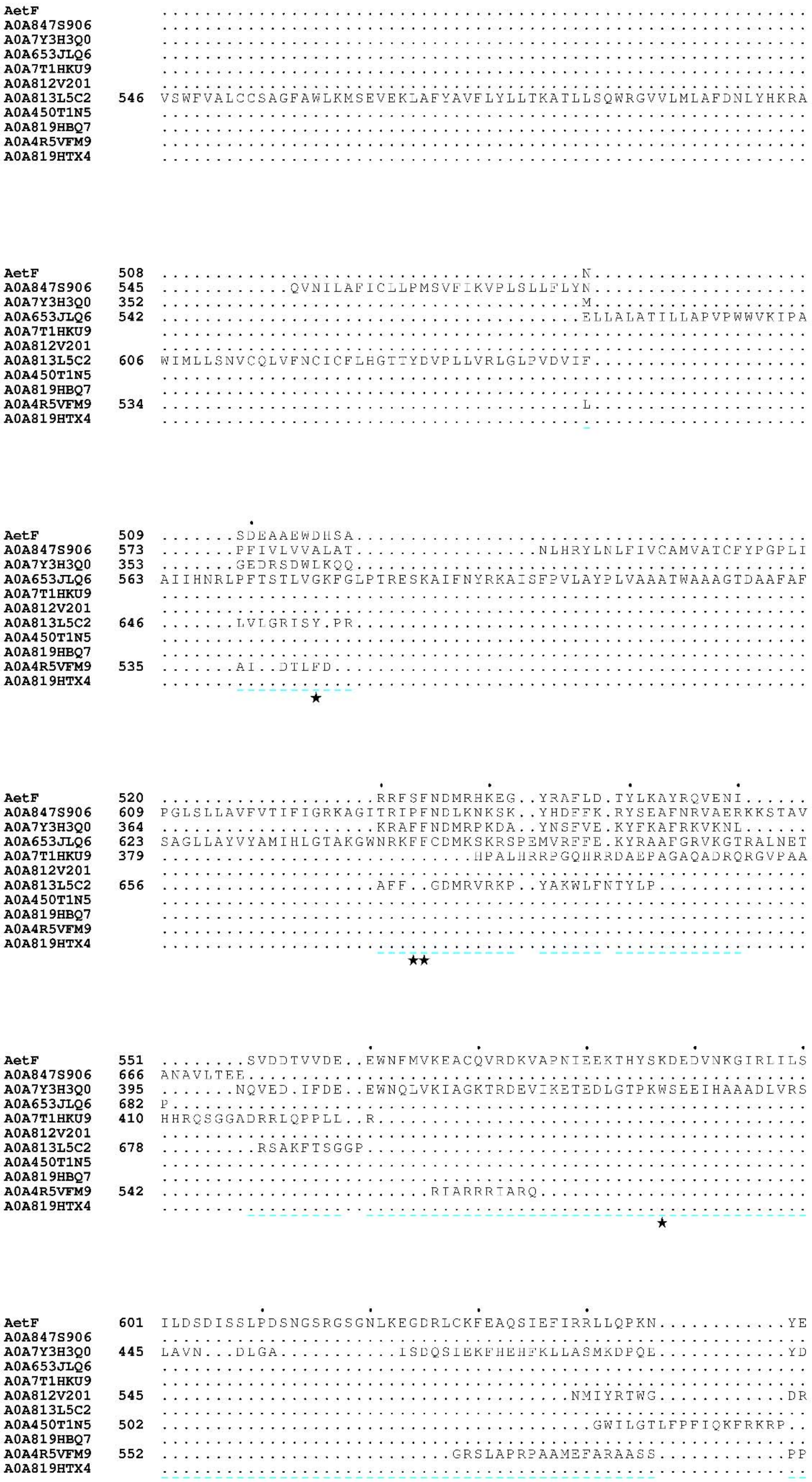

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A0A653JLQ6           ............................................................
A0A7T1HKU9           ............................................................
A0A812V201           ............................................................
A0A813L5C2     546   VSWFVALCCSAGFAWLKMSEVEKLAFYAVFLYLLTKATLLSQWRGVVLMLAFDNLYHKRA
A0A450T1N5           ............................................................
A0A819HBQ7           ............................................................
A0A4R5VFM9           ............................................................
A0A819HTX4           ............................................................


AetF           508   .......................................N....................
A0A847S906     545   ............QVNILAFICLLPMSVFIKVPLSLLFLYN....................
A0A7Y3H3Q0     352   .......................................M....................
A0A653JLQ6     542   .......................................ELLALATILLAPVPWWVKIPA
A0A7T1HKU9           ............................................................
A0A812V201           ............................................................
A0A813L5C2     606   WIMLLSNVCQLVFNCICFLHGTTYDVPLLVRLGLPVDVIF....................
A0A450T1N5           ............................................................
A0A819HBQ7           ............................................................
A0A4R5VFM9     534   .......................................L....................
A0A819HTX4           ............................................................


AetF           509   .......SDEAAEWDHSA..........................................
A0A847S906     573   .......PFIVLVVALAT.................NLHRYLNLFIVCAMVATCFYPGPLI
A0A7Y3H3Q0     353   .......GEDRSDWLKQQ..........................................
A0A653JLQ6     563   AIIHNRLPFTSTLVGKFGLPTRESKAIFNYRKAISFPVLAYPLVAAATWAAAGTDAAFAF
A0A7T1HKU9           ............................................................
A0A812V201           ............................................................
A0A813L5C2     646   .......LVLGRISY.PR..........................................
A0A450T1N5           ............................................................
A0A819HBQ7           ............................................................
A0A4R5VFM9     535   .......AI..DTLFD............................................
A0A819HTX4           ............................................................


AetF           520   ....................RRFSFNDMRHKEG..YRAFLD.TYLKAYRQVENI......
A0A847S906     609   PGLSLLAVFVTIFIGRKAGITRIPFNDLKNKSK..YHDFFK.RYSEAFNRVAERKKSTAV
A0A7Y3H3Q0     364   ....................KRAFFNDMRPKDA..YNSFVE.KYFKAFRKVKNL......
A0A653JLQ6     623   SAGLLAYVYAMIHLGTAKGWNRKFFCDMKSKRSPEMVRFFE.KYRAAFGRVKGTRALNET
A0A7T1HKU9     379   .............................HPALHRRPGQHRRDAEPAGAQADRQRGVPAA
A0A812V201           ............................................................
A0A813L5C2     656   ....................AFF..GDMRVRKP..YAKWLFNTYLP..............
A0A450T1N5           ............................................................
A0A819HBQ7           ............................................................
A0A4R5VFM9           ............................................................
A0A819HTX4           ............................................................


AetF           551   ........SVDDTVVDE..EWNFMVKEACQVRDKVAPNIEEKTHYSKDEDVNKGIRLILS
A0A847S906     666   ANAVLTEE....................................................
A0A7Y3H3Q0     395   .......NQVED.IFDE..EWNQLVKIAGKTRDEVIKETEDLGTPKWSEEIHAAADLVRS
A0A653JLQ6     682   P...........................................................
A0A7T1HKU9     410   HHRQSGGADRRLQPPLL..R........................................
A0A812V201           ............................................................
A0A813L5C2     678   .........RSAKFTSGGP.........................................
A0A450T1N5           ............................................................
A0A819HBQ7           ............................................................
A0A4R5VFM9     542   .........................RIARRRIARQ.........................
A0A819HTX4           ............................................................


AetF           601   ILDSDISSLPDSNGSRGSGNLKEGDRLCKFEAQSIEFIRRLLQPKN............YE
A0A847S906           ............................................................
A0A7Y3H3Q0     445   LAVN...DLGA...........ISDQSIEKFHEHFKLLASMKDPQE............YD
A0A653JLQ6           ............................................................
A0A7T1HKU9           ............................................................
A0A812V201     545   ......................................NMIYRTWG............DR
A0A813L5C2           ............................................................
A0A450T1N5     502   ........................................GWILGTLFPFIQKFRKRP..
A0A819HBQ7           ............................................................
A0A4R5VFM9     552   ...........................GRSLAPRPAAMEFARAASS............PP
A0A819HTX4           ............................................................
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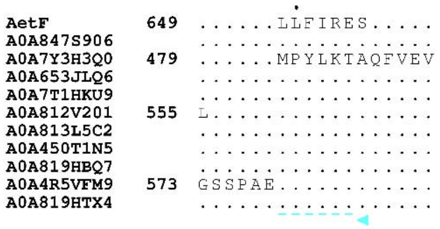

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AetF         649  ......LLFIRES.....
A0A847S906        ..................
A0A7Y3H3Q0   479  ......MPYLKTAQFVEV
A0A653JLQ6        ..................
A0A7T1HKU9        ..................
A0A812V201   555  L.................
A0A813L5C2        ..................
A0A450T1N5        ..................
A0A819HBQ7        ..................
A0A4R5VFM9   573  GSSPAE............
A0A819HTX4        ..................
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Figure S6 (Including previous pages) Pairwise *DALI* alignment of AetF against *AlphaFold2* models of the top ten hits containing a lysine at the position equivalent to the catalytic lysine in AetF (K258) found in a *Foldseek* search against the *AlphaFold/Uniprot50 v4 database*. The missing loop (609 – 628) in AetF (8cjd *A*) was copied from the *AlphaFold2* model. Domains in AetF are marked by dashed lines and domain borders by arrows (same color code as in Fig. 2). Consensus sequences for dinucleotide binding (GXGXXG and GXGXXA) are marked by red triangles and residues involved in the binding of Trp or 5-Br-Trp are indicated by black stars. The catalytic amino acids found in AetF (E200 and K258) are highlighted in magenta.