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Supporting information for article:

Ternary structure of *Plasmodium vivax N*-myristoyltransferase with myristoyl-CoA and inhibitor IMP-0001173

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(a)

(b)

Figure S1 Difference (Fo-Fc) omit electron density maps (green mesh) contoured at 3.5 sigma revealed 2 large blobs that matched the superposed ligands (shown in stick). (a) The Myr-CoA molecule fits into the electron density, as does (b) the inhibitor IMP-0001073 molecule.



Figure S2. *Pv*NMT overall topology

-				β11	β12	β13
PVNMT				300	310	320
PVNMT			N V	IY T YVNEE . N	G. KIKDMISI	YSLPSOILGN
PfNMT			vv	IYTYVNEE.N	G.EIKDLIS	FYSLPSKVLGN
HsNMT1			NI	IDTFVVENAN	G.EVTDFLS	FYTLPSTIMNH
HsNMT2			HI	IDTFVVESPN	G.KLTDFLS	FYTLPSTVMHH
SCNMT			KQV	IFSYWVEQPD	G.KITDFFS	FYSLPFTILNN
AfINMT			SNV	VKSIVVEDEN	G.VITUIFS THEITOFFS	TILLFFTVLDN FVNLFSTVLON
AfNMT			EOV	VWSYVEDPE	THKITOFFSI	FYNLPSTVIOH
LmNMT			Ğ v	VFTYVENDK	KVTDFFSI	FYRIPSTVIGN
LdNMT			G V	VF TYVVENDK	KVTDFFSI	FYRIPSTVIGN
CpNMT	LDKIEDLQDKITIE	QCFNVEDVKHY	FTNIDK	IVTYVRENKN	K.EITDLFS1	FFIIESTVINN
consensus>70			!	!%VvEn.n	itDf.S	8%.lpst!n
	B14 B15	B16			α8	617
PVNMT	$\rightarrow \rightarrow$	→		00000	0000000000	
	330 3	40		35	· 3	eo
PvNMT	DKYSTLNAAYSFYN	VTTT		ATFKQLM	QDAILLAKRI	NNF DVFN ALEV
PfNMT	NKYNILNAAFSFYN	ITTT		TIFKNLI	QDAICLAKRI	NNFDVFNALEV
HSNMT1	PTHKSLKAAYSFYN	VHTQ		TPLLDLM	SDALVLAKM	KGFDVFNALDL
HSNMT2 SoMMT	TYYYDICICYLYY	ATDADFOFKDD	EDDKATK	ALKTRICET	VDACTLAKS	AMMOURNAUTS
CaNMT	ACHDETCTAYLEY	ASDS. FEKPN.	PDPKAIK	YKKRLNETI	TDALTTSKK	FGVDVFNCLTC
AfuNMT	PKHDNVRAAYLYY	ATETAFTNNM.	K	ALKERLLMLM	NDALILAKK	AHFDVFNALTL
AfNMT	PKHKEVRAAYLYY	ATETAFTEDL.	K	ALKERLLL	INDALIOAKK.	AHFDVFNALTL
LmNMT	SNYNLLNAAYVHY	AATS		IPLHQLI	LDLLIVAHSI	RGFDVCNMVEI
LdNMT	SNYNILNAAYVHY	AATS		MPLHQLI	LDLLIVAHSI	RGFDVCNMVEI
CPNMT	ERFPTINIAYSYF	IANT		CSLKELF	NEMLITAKNI	NNCDAFNTLDL
consensus>70	.l.aa% .f%.	• • • • • • • • • • • •		l.qL.	.#ali.ak.	fDvfN.1
	α9	β18	β19	η6		
PVNMT			→	عفع		
3	70 380	390	400	410		
PVNMT	MQNKSVFEDLKFG	GDGSLKYYLYN	WKCASFA	PAHVGIVLL.		
PINMT	MDNYSVFQDLEEGE	GDGSLKYYLYN	WKCASCH	PSKIGIVLL.		
HSNMT1	MENKTELEKLKEGI	GDGNLOYYIX	WRCPSMG	SERVELVE.		
SCNMT	ODNTLELDDLEEGE	GDGELNEYLEN	VRAKPIT	GGLNPDNSND	TK RRSNVG	VVMT.
CaNMT	ODNTYFLKDCKEGS	GDGELNYYLEN	YRTFPMD	GGIDKKTKEV	VEDOTSGIG	VVLL
AfuNMT	HDNPLFLEQLKEG?	GDGQLHFYLYN	YRTAPVP	GGVNEKNLPD	EK.RMGGVG	IVML
AfNMT	HDNPLFLEQLKFG	GDGQLHFYLYN	YRTAPIP	GGVNEKNLPD	EK.KMGGVG	VVML
LmNMT	LDNRSFVEQLKFGA	GDGHLRYYFYN	WAYPKIK	PSQVALV ML .		
LdNMT	LDNRSFVEQLKFGA	GDGHLRYYFYN	WAYPKIK	PSQVALVML.		
CPNMT	MONLOVIODSKEI	GIGRER RYNVFN	WKIPQIS	PSNVGII U F.		

Figure S3. Comparison of plasmodial NMTs with human orthologues. NMT sequences used are from *P. vivax* (A0A1G4H3M1), *P. falciparum* (Q8ILW6), *P. yoelii* (Q7RPB1), *P. knowlesi* (A0A384LCS5) and the human NMT1 (P30419) and NMT2 (O60551). *The specific amino acids involved in inhibitor binding are indicated in Table S1. Identical residues are shaded black, while conserved ones are in gray.

Figure S4. ENDSCRIPT analysis reveals the nearest structural neighbors of *Pv*NMT and shows extensive sequence conservation across multiple organisms. Identical and conserved residues are highlighted in red and yellow, respectively. The different secondary structure elements shown are alpha helices (α), 3₁₀-helices (η), beta strands (β), and beta turns (TT).



Figure S5. ENDSCRIPT analysis reveals structural variation in 3-D as sausage plots. Identical residues are indicated in red. The thicker sausage indicates higher tertiary structure differences. This figure allows us to visualize sequence identity and tertiary structure similarity simultaneously. In some regions, sequence identity is low (white), and tertiary structure similarity is high (thin sausages). In others, amino acids are identical (red), and tertiary structure similarity is low (fat sausages). Other combinations are also possible with sequence identity ranging from identical (red) to conserved (pink) to completely different (white). IMP-0001173 is indicated in blue, while Myr-CoA is in green sticks.

Protein	PDB ID	Cofactor binding pocket residues	Substrate binding pocket residues
PvNMT	6B1L	Tyr28, Lys29, Asn94, Tyr95, Val96, Val160, Asn161, Cys164, Arg170, Pro176, Ile179, Thr183, Ile186, Asn187, Trp192, Gln193, Ala194, Tyr196, Thr197, Leu202, Tyr393	Val96, Glu97, Asp98, Phe103, Phe105, Tyr107, Tyr211, His213, Phe226, Tyr315, Leu317, Leu388
HNMT1	5MU6	Tyr117, Gln118, Phe119, Trp120, Asn179, Tyr180, Val181, Ile245, Asn246, Leu248, Cys249, Val250, Leu254, Arg255, Ser256, Lys257, Arg258, Val259, Ala260, Pro261, Thr268, Val271, Phe277, Gln278, Ala279, Tyr281, Thr282, leu287, Tyr479	Val181, Asp183, Phe188, Arg189, Phe190, Tyr192, Asn246, Thr282, Gly284, His298, Tyr296, Ser405, Tyr420, Asn451, Ala452, Leu453, Leu474, Leu495, Gln496

Table S1. Comparative analysis of residues involved in inhibitor and Myr-CoA binding.