Supplemental Data

THE STRUCTURAL BASIS OF MULTIFUNCTIONALITY IN A B_{12} PROCESSING ENZYME

Markos Koutmos, Carmen Gherasim, Janet L Smith and Ruma Banerjee

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H.sapiens			<u> </u>		2,	
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P.roglodytes	MEPKVAELKQKIEDTLC.PFC	GFEVYPFQVA	WYNELLPPA.	FHLPLPGPTLA	FLVLSTPAMFI	DRALK
M.mulatta	MEPKVAELKQKIEDMLC.PFC	GFEVYPFQVA	WYNELLPPA.	FHLLLPGPTLA	FLVLSTPAMFI	DQALK
B.taurus	MEPLVAELKQKIEDTLC.SFC	GFEVYPFQVA	WYNALLPPA.	FHLPLPGPTLA	FLVLSTPAMFI	DQALK
R.norvegicus	MEPRVAELKOKIEGTLC.PFC	GFEVYPFQVA	WYNELLPPA.	FHLPFPGPTLA	FVVLSTPAMFI	DRALK
M.domestica	MESRVVELERRIRTALE.PFC	GFEVHPFQVG	WYNALLPPA.	FQLPLPGPTLA	FVVLSTPAMF	DRALK
G.gallus	MEERVAER LHGALG.PLC	JFEVHAF <mark>KV</mark> G	WYNAVLQPA.	FHLPYPDDTLA	FVVLSTPSMFI	OKALK
C.clemensi	MVDDIQGILEEH.LGPR.Q.O	GFETHPFRIG	DYNAKVSSK.	FSLPFEGDSLG	FALISTPSMF	EKTFL
X.laevis	ELQQQLQETLL.PFC	GFEVYPFQIG	WYNAVLEPA.	FHLSYPKDTMA	FVVLSIPSMFI	SKAFK
T.nigroviridis	MAAPTVNAVGITGEFADSLS.KLC	GFEVYPLKVG	WYNSVLAPT.	HHLAHPDDTLA	LVVLSTPSMF	EDAFL
D.rerio	MAISSERVEELLRTFRESLK.AKC	GFEIYPFKVG	WYNAVL TAA.	HHLQYPADTLA	VLVISTPAMFI	SCAFL
B.floridae	MDRATAIWEKVEGYIR.PLC	GLESYPFKIQ	WYNDKVKDP.	FPLPYPGDTLA	VVILSTPNMF	EKAFK
N.vectensis	MDDEACWICDO	FQIG	WYNNQVTHKQ	FQFPFDPDTLA	FVIISTPEMFI	SKGFK
I.scapularis	SAPFVMEIDRISTLLDEILT.PLC	GIESHPFKIQ	WYNDRVPGA.	FKFQRHPDTLC	FVSLSTPSTF	EKAFL
C.elegans	MVTEMSHAESIKRVVDQKLSSHEC	G F E S H M F <mark>K I</mark> G	SYNEAVGESSI	FALPYDDSTMA	LLILSTPDMFI	VAFR
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H.saniens		AFA M		-3	L _{3F}	
n.sapiens	70 80			100	110	
H.saniens	PELOSCHI, RMLT.	AVHLG . RVR	EST.PEI	OTETTADYEVH	P.NRREKTLA	TAAH
P.roglodytes	PFLQSCHLRMLT DPVDQCV	AYHLG.RVR	ESLPEI	QIEIIADYEVH	P.NRRPKILA	TAAH
M.mulatta E.caballus	PFLOSCHLOPLT DPVDQCV	AYHLG.RVR	ESLPEI	JQIEVIADYEVH OMEVIADYEVH	P.NRRPKILA	DTAAH DTAAH
B.taurus	PFLQSHHLQPLT DPVDQCV	7AYHLG.RV R	ESLPEI	QIEVIVDYEVH	P.NRRPKILA	TAAH
R.norvegicus M.musculus	PFLKSCHLQTLRDPVDQCV PFLKSCHFOTLRDPVDQCV	/SYHLK.SVT /SYHLR.SVT	'EKFPEI 'EKFPEV	LHIEVIADYEVH HMEVIADYEVH	P.NRRPKILA P.NRRPKILA	DTAAH DTAAH
M.domestica	PFLQSAHLQPLKDPVDQCV	AYHFT.QLQ	KNLPE(QKIDFITDYDMH	P.NRRPKLLA	TAAH
G.gallus L.salmonis	PYLEAEGENEFLTDPLDAC	KSLIHEART	EIFPD(2KVDVIFDYE11 FPFIFYHDFDLH	P.SRRPKFLA P.NRRPRVLV	DTAGH
C.clemensi	PYLISSSTNIEEASSPMDPLDECM	ARTTVNKVIH	HHLIGADDIR	IPILFLHDFEIH	P.NRRPKILV	TAGH
X.laevis S.salar	PFLRHHKLQDLMDPIDQC	/AYHMS.LVK /KHCINNTVS	EKFPSI	OKLDVIYDYQLH /KVDVSYDYEMI	P. NRRPKVLM P. SRKPKFLA	DSAAH
T.nigroviridis	PFLKEKGFQGLSDPIDQCV	RHWVSSAVF	QCFP	DKVDVRYDFELF	P. SRKPKFLA	TAAH
C.intestinalis	PNVENLLAOKOTKDPIDEC	LAFHLN.ETV	SGLNIC	GITIMYDYEIL	P. NRRPKVLV	TAAH
B.floridae	PFVAKKDCYVRDPIDECI	LSHMQNIKA	SF.PDI	DIDCMQDFEMH	P.NHRPKVLV	DTAAH
S.purpuratus	PFVERLKCQSVIENRDPIDSC	AEQLLRAKE	ALKPDI	IEVDIIQDFEMH	PVTRRPKLLV	OTAGH
I.scapularis	PFVLNDRNLTLKDPFDQCI	TACFA.RAI	QAFPGI	SVELLQDFELH	P.NRRPKVIM	DTAGH
c.eregans	KWVVQRIMDFGSFDEVCEMVSSF.	CV3 FILE D KILE	IMSEKERKVEI		* *	k
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H.sapiens)(1)'- <u></u> G <u>)</u>		-12'	- 4		
	130	140	150	160 1	.70	180
H.sapiens P.roglodytes	VAGAAYYYORODVEAI	DPWGNQ.RIS	GVCIHPRFGG	VFAIRGVVLLPG	IEVP.DLPPRI	KPHDC KPHDC
M.mulatta	VAGAAYYYQRQDVEA	DPWGNQ.HIS	GVCIHPRFGG	FAIRGVVLLPG	IEVP.DLPPRI	KPHDC
E.caballus B.taurus	VAGAAYYYORODVEAI	DPWGTQ.HIS	GVCIHPRFGG	FAIRGVLLLPG	IEVP.DLPPMI	KPLDC KPVDC
R.norvegicus	VAGAAYYYQRQDVDA	DPWGTQ.HIA	GVCIHPRFGG	FAIRGVMLLPG	IEVP.NLLPR	KPPDC
M.musculus M.domestica	VAGAAYYYQRQDVDAI	DPWGTQ.HIA	GVCIHPRFGG	VFAIRGVMLLPG	IEVP.NLPPRI	KPPDC APPDC
G.gallus	VAGAAYYYQRKDVKL	DPWGEK.KIF	GVCIHPKYGG	FAIRGLLLFPD	VQVP.FLEQS	APVDC
L.salmonis C.clemensi	VSGAVHLYRSPNLNP	• • • • • • • • • • •	VCLHPKYGG	FALRGVFIFPN	ISCPFLQIKE	PPCL PPNT
X.laevis	VSGAAYYYQRKDIPQ	DPWGSK.KMF	GVCIHPQFGG	FAIRAVLVFAE	IQAP.DLEQTI	LPLDC
S.salar T.nigroviridis		PWGEK.KMF	GVCIHPRLGG	FAIRAMLVFVG	SEVGSELQQTA	APPDC
D.rerio	VSGAAYYYQTSDIHN	PWGEK.KMF	GVCVHPQLGG	FAIRALLVFRD	VQAGAGFQQRI	PADC
C.intestinalis B.floridae	VAGAAFYYOREHVLKPSGTSGSII VAGAARYYORMDVKF	DPWGGKRKIY	GVCVHPKYGG GVSVHPKYGG	FAIRAVLVFTE	LEDA.NLTKLI	EPTDC EPPDC
N.vectensis	AAGAAYFYQRKDITD	PWPPTQRIC	GVCVHPKYGG	FAIRGVLIFKT	FKYP.DLPRK	PMDV
S.purpuratus		SPWDAKKKIY	GVSIHPKYGG GVCIHPKYGG	FAFRGAIIL KD	TICS.DLRQA	APDQP DPADV
C.elegans	VAGAAFYYQPCHFQEDGVTWPPAC	GRMGPNLKFI	GLSLHPIYGG	FAFRSVLIFPN	VKIP.EFCEK	EPRPI
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H.sapiens		200	210	220	230	240	250
W sanions	VPTRADETALTECEN	FHMPDWT	VPDAUTPOFP	TREOKAVEST	PPAOPTALLC	. AODSEKDSS	DODDIDE
P. roglodytes	VPTRADRIVLIEGEN	FHWRDWT	VRDAVTPOER	VSEEOKAVEST	PPAORT ALLG	LAOPSEKPSS	SPSPDLPF
M.mulatta	VPTRAARTALLEGEN	FHWRDWT	YRDAVTPOER	SEEOKAYEST	PPAORLALLG	LVOPSEKPSS	SPSPDFPF
E.caballus	VPTRADRITLLEGEN	FHWRDWT	YRDAVTPOER	SEEOKAYFST	PPAORFALLG	LAOPSEERGS	S.SVELPF
B.taurus	VPTRADRISLLERFN	FHWRDWT	YRDAVTPOER	YSEEOKAYFST	PPAORLALLG	LLOPSEEPSS	SPSOELHI
R.norvegicus	VPTRAARITLLEGFN	FHWRDWT	YRDAVTPEER	YSEEOKIYFST	PPAORLALLG	LAOPSGHPST	r.TSEHPL
M.musculus	VPTRAGRITLLEGFN	FHWRDWT	YRDAVTPEER	SEEOKIYFST	PPAORLALLG	LAQHSEHPST	F.TSELPL
M.domestica	VPTQEGRIALLESFN	FHWKDWT	YRDVVVPVER	YSEEQKTYFST	PPAQRLHLLR	LLQPTHKPSI	LPNPVN
G.gallus	VSTEEKRTELLELFN	FHWQDGR	YRDIIEVKER	YSEEQKTYFST	PPAERFRLLG	LTRFTE	
L.salmonis	LKSIQDVEELLILYN	YHWKDGR	YRDFIPVEER	YSNLQILYFKT	LPSDRMPLLK	KLFNKSNTYH	ESKA
C.clemensi	LQSKEEIHNLLELFN	DHWRDAR	YRDVIPVKEK	Y S P L Q R L Y F E T	LPVDRIKLLR	KFKVL	
X.laevis	LPSQNDRIQLLEHFN	FNWRDGK	YRDVLPPKEK	YSEEQTLYFAT	PPAERRKLLE:	LQG.ELHPSI	PLC
S.salar	VPTREDRIQLLEDFN	LRWQDWS	YRDIVPPVQT	YSQKQREYFST	PPAQRLDLLT	DWGYLTGGEI	EDNTQEN.
T.nigroviridis	VPSREERIRLLEAFN	FHWQDWT	YRDIVHAVHA	YSQKORDYFST	PPAQRTLLL.		
D.rerio	VCTQEERIRLLESFN	LRWQDWS	YRDIVPAEER	ISDQQKQYFIT	PPGQRRALLR	QWGYLTDTQS	5
C.intestinalis	V KTRTERIDLLEKFK	GNWKDST	TRDVIQVKEK	ISELQTLIFLT KORROVUVRDU	LPK DRMKLLQ	QIVNNQTV.	
B.floridae	VPIDELKIDLLNKI WWCDEKDIELKEK	FHWEDWS	FROVIPVEDK	ISEEQAVIPDT	PPADRKALLG.	CTIVE	
N.Vectensis	TUTDERTICIER	FHMEDWS	FROVVPSERK	SDQQIAIF ST	ADEKEENTIC	DILLE	FODTHEN
J. scopularis	VOREDVRRDITRI	FSWONCT	VPDVTPVDEP	SPECICIWNI	VDCOPWET T	TEDKAESISS	SEQUEREN
C elegans	LTASEDVRTALEKEN	VNWKDSG	FRDFGNPTRR	STTOMEFECR	PVAERWEVI.R.	PWVDGGAKNI	
c.eregans		I NUKD 56	ENDEGNETIKK	LOTION PLICK		EWVDGGARN.	
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<i>H.sapiens</i> H.sapiens P.roglodytes	260 TTPAPKKPGNPSRAF TTPAPKKPGNPSRAF	270 SWLSPRV	280 SPPASPGP SPPASPGP				
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H.sapiens H.sapiens P.roglodytes M.mulatta E.caballus B.taurus	260 TTPAPKKPGNPSRAF TTPAPKKPGNPSRAF TTHTPKKPGKPSRAF TTLTPKQPPKPSRAF TTLLSKKPQNPRF	270 SWLSPRV SWLSPRV SWLSPRV GWLSPRV	280 SPPASPGP SPPASPGP SPPASPGP SPPISPGP SPPISPGP				
H.sapiens H.sapiens P.roglodytes M.mulatta E.caballus B.taurus R.norvegicus	260 TTPAPKKPGNPSRAF TTPAPKKPGNPSRAF TTHTPKKPGKPSRAF TTLTPKQPPKPSRAF TTLLSKKPQNPR.F SLLT.KPQDSRELF	270 SWLSPRV SWLSPRV SWLSPRV GWLSPRV GWLSPTV SWLSPSV	280 SPPASPGP SPPASPGP SPPASPGP SPPISPGP SPPISPGP				
H.sapiens H.sapiens P.roglodytes M.mulatta E.caballus B.taurus R.norvegicus M.musculus	260 TTPAPKKPGNPSRAF TTPAPKKPGNPSRAF TTHTPKKPGKPSRAF TTLTPKQPPKPSRAF TTLLSKKPQNPRF SLLTKPQDSRRLF SLLTTPQNYRRAF	270 SWLSPRV SWLSPRV GWLSPRV GWLSPRV SWLSPSV SWLSPSV	280 SPPASPGP SPPASPGP SPPASPGP SPPISPGP SPPISPGP SPPASPGP SPPASPG				
H.sapiens H.sapiens P.roglodytes M.mulatta E.caballus B.taurus R.norvegicus M.musculus M.domestica G.gallus	260 TTPAPKKPGNPSRAF TTPAPKKPGNPSRAF TTHTPKKPGKPSRAF TTLISKKPQNPR SLLT.KPQDSRFF SLLT.TPQNYRRAF RQYF	270 SWLSPRV SWLSPRV SWLSPRV GWLSPRV SWLSPSV SWLSPSV SWLSPSV LCVHPPP	280 SPPASPGP SPPASPGP SPPTSPGP SPPTSPGP SPPASPGP SPPASPGP LPSLSF				
H.sapiens H.sapiens P.roglodytes M.mulatta E.caballus B.taurus R.norvegicus M.musculus M.domestica G.gallus L.salmonis	260 TTPAPKKPGNPSRAF TTPAPKKPGNPSRAF TTLTPKQPPKPSRAF TTLLSKKPQNPR.F SLLT.KPQDSRRF SLLT.TPQNYRRAF	270 SWLSPRV SWLSPRV GWLSPRV GWLSPRV SWLSPSV SWLSPSV SWLSPSV CCVHPPP	280 SPPASPGP SPPASPGP SPPTSPGP SPPTSPGP SPPASPGP SPPVSPG. LPSLSF				
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t t Figure S1. Sequence alignment of CbICs from various organisms. The secondary structure displayed is based on the apo-t-CbIC structure. Red arrows indicate patient mutations related to either CbI binding or protein structural integrity. Green astericks indicate side chains that interact with CbI, and blue astericks indicate backbone interactions with CbI. Beta hairpins are indicated with red loops.



Figure S2. Comparison of CbIC and BluB. (a) Two views of t-CbIC. (b) Topology diagram of BluB (left) and CbIC (right). In (a) and (b) the α -helices are shown in red and β -strands in blue. Secondary structure features highlighted in yellow and green circles correspond to significant differences/additions to CbIC as compared to flavin reductases such as BluB. These structural features highlighted with green and yellow circles are represented in the same coloring scheme in the main text Figs. 2c and d.



Figure S3. Structural superposition of CbIC, BluB and IYD. Superposition in stereo of a BluB monomer (yellow), a IYD monomer (pink), and (a) apo-t-CbIC and (b) MeCbI-t-CbIC (blue) are shown. The FMN cofactors for BluB and IYD are shown in stick representation whereas the MeCbI substrate is shown in ball and stick.



Figure S4. Space filling model of t-CblC•MeCbl. MeCbl is shown in yellow sticks, and modeled GSH is in pink sticks. The flexible C-terminal region (residues 225-238) is displayed in green as a cartoon.

	MeCbl	Protein	Distance (Å)
	O28	1160 N	2.9
	N29	D104 OD2	2.9
	N29	I160 O	3.0
Dina	N33	C149 O	3.0
	O34	C149 N	2.8
Ring	O34	Q118 NE2	2.9
	N45	S146 OH	3.1
	N62	D104 O	2.9
	N62	I115 O	2.8
	O63	A117 N	3.0
	O4	Y129 OH	2.7
Tail	O5	Y129 OH	3.5
	O7R	Q131 NE2	2.6

Table S1. MeCbl-CblC contacts.