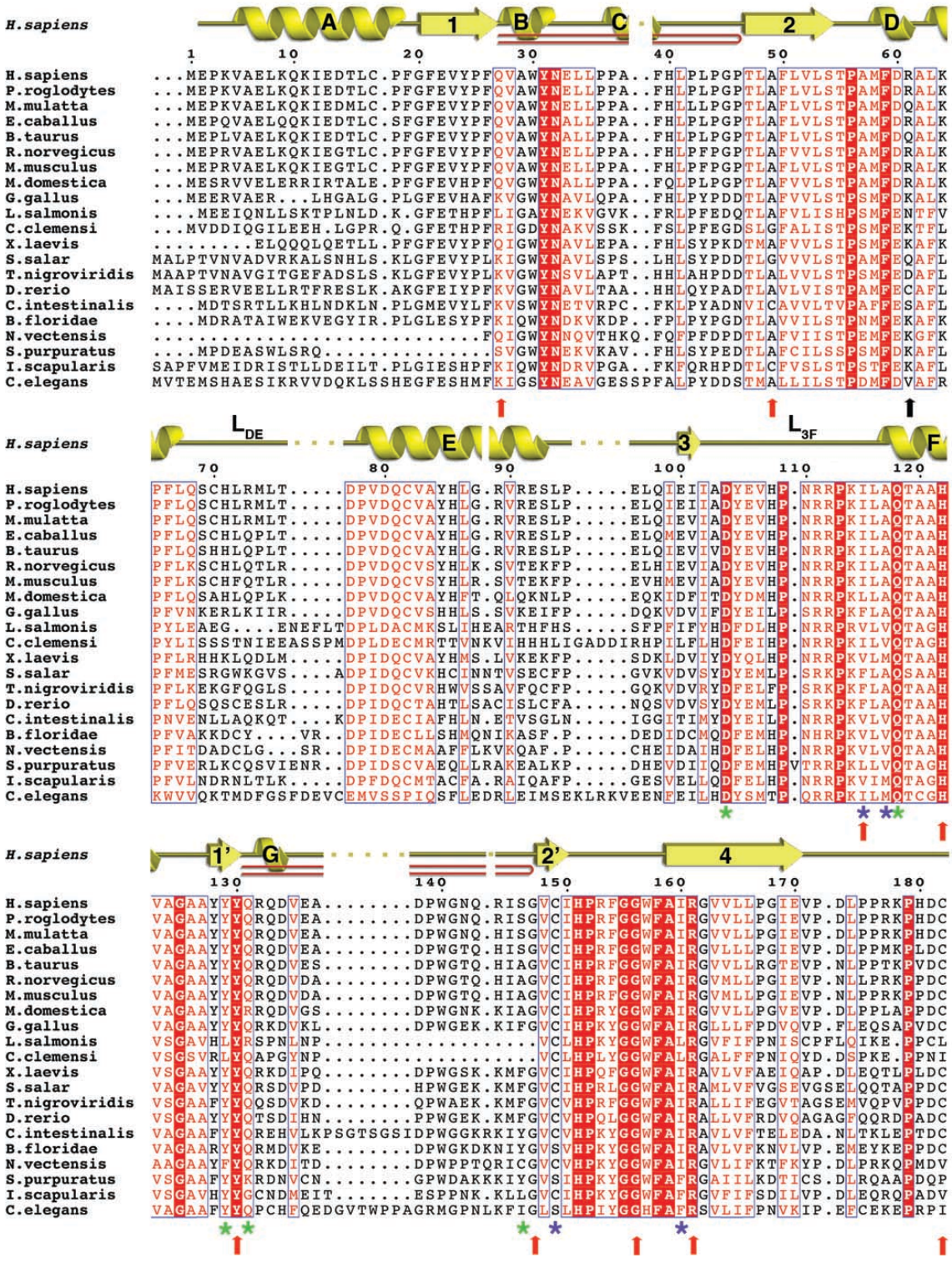
Supplemental Data

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

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


H.sapiens
A
1
B
C
2
D
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P.roglodytes ...MEPKVAELKQKIEDTLC.PFGFEVYPFQVAWYNELLPPA..FHLPLPGPTLAFLVLSTPAMFDRALK
M.mulatta ...MEPKVAELKQKIEDMLC.PFGFEVYPFQVAWYNELLPPA..FHLLLPGPTLAFLVLSTPAMFDQALK
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B.taurus ...MEPLVAELKQKIEDTLC.PFGFEVYPFQVAWYNALLPPA..FHLPLPGPTLAFLVLSTPAMFDQALK
R.norvegicus ...MEPRVAELKQKIEGTLC.PFGFEVYPFQVAWYNELLPPA..FHLPFPGPTLAFVVLSTPAMFDRALK
M.musculus ...MEPRVAELKQKIEGTLC.PFGFEVYPFQVAWYNELLPPA..FHLPFPGPTLAFLVLSTPAMFDRALK
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G.gallus ...MEERVAER...LHGALG.PLGFEVHAFKVGWYNAVLQPA..FHLPYPDDTLAFVVLSTPSMFDKALK
L.salmonis ....MEEIQNLLSKTPLNLD.K.GFETHPFLIGAYNEKVGVK..FRLPFEDQTLAFVLISHPSMFENTFV
C.clemensi ...MVDDIQGILEEH.LGPR.Q.GFETHPFRIGDYNAKVSSK..FSLPFEGDSLGFALISTPSMFEKTFL
X.laevis .........ELQQQLQETLL.PFGFEVYPFQIGWYNAVLEPA..FHLSYPKDTMAFVVLSIPSMFEKAFK
S.salar MALPTVNVADVRKALSNHLS.KLGFEVYPLKIGWYNAVLSPS..LHLSYPDDTLGVVVLSTPAMFEQAFL
T.nigroviridis MAAPTVNAVGITGEFADSLS.KLGFEVYPLKVGWYNSVLAPT..HHLAHPDDTLALVVLSTPSMFEDAFL
D.rerio MAISSERVEELLRTFRESLK.AKGFEIYPFKVGWYNAVLTAA..HHLQYPADTLAVLVISTPAMFECAFL
C.intestinalis ....MDTSRTLLKHLNDKLN.PLGMEVYLFKVSWYNETVRPC..FKLPYADNVICAVVLTVPAFFESAFL
B.floridae ....MDRATAIWEKVEGYIR.PLGLESYPFKIQWYNDKVKDP..FPLPYPGDTLAVVILSTPNMFEKAFK
N.vectensis .............................FQIGWYNNQVTHKQ.FQFPFDPDTLAFVIISTPEMFEKGFK
S.purpuratus ....MPDEASWLSRQ.............SVGWYNEKVKAV..FHLSYPEDTLAFCILSSPSMFDKAFL
I.scapularis SAPFVMEIDRISTLLDEILT.PLGIESHPFKIQWYNDRVPGA..FKFQRHPDTLCFVSLSTPSTFEKAFL
C.elegans MVTEMSHAESIKRVVDQKLSSHEGFESHMFKIGSYNEAVGESSPFALPYDDSTMALLILSTPDMFDVAFR
H.sapiens
L_DE
E
3
L_3F
F
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P.roglodytes PFLQSCHLRMLT.....DPVDQCVAYHLG.RVRESLP.....ELQIEIIADYEVHP.NRRPKILAQTAAH
M.mulatta PFLQSCHLRMLT.....DPVDQCVAYHLG.RVRESLP.....ELQIEVIADYEVHP.NRRPKILAQTAAH
E.caballus PFLQSCHLQPLT.....DPVDQCVAYHLG.RVRESLP.....ELQMEVIADYEVHP.NRRPKILAQTAAH
B.taurus PFLQSHHLQPLT.....DPVDQCVAYHLG.RVRESLP.....ELQIEVIVDYEVHP.NRRPKILAQTAAH
R.norvegicus PFLKSCHLQTLR.....DPVDQCVSYHLK.SVTEKFP.....ELHIEVIADYEVHP.NRRPKILAQTAAH
M.musculus PFLKSCHFQTLR.....DPVDQCVSYHLR.SVTEKFP.....EVHMEVIADYEVHP.NRRPKILAQTAAH
M.domestica PFLQSAHLQPLK.....DPVDQCVAYHFT.QLQKNLP.....EQKIDFITDYDMHP.NRRPKLLAQTAAH
G.gallus PFVNKERLKIIR.....DPVDQCVSHHLS.SVKEIFP.....DQKVDVIFDYEILP.SRRPKFLAQTAAH
L.salmonis PYLEAEG....ENEFLTDPLDACMKSLIHEARTHFHS.....SFPFIFYHDFDLHP.NRRPRVLVQTAGH
C.clemensi PYLISSSTNIEEASSPMDPLDECMRTTVNKVIHHHLIGADDIRHPILFLHDFEIHP.NRRPKILVQTAGH
X.laevis PFLRHHKLQDLM.....DPIDQCVAYHMS.LVKEKFP.....SDKLDVIYDYQLHP.NRRPKVLMQTAAH
S.salar PFMESRGWKGVS....ADPIDQCVKHCINNTVSECFP.....GVKVDVSYDYEMLP.SRKPKFLAQSAAH
T.nigroviridis PFLKEKGFQGLS.....DPIDQCVRHWVSSAVFQCFP.....GQKVDVRYDFELFP.SRKPKFLAQTAAH
D.rerio PFLQSQSCESLR.....DPIDQCTAHTLSACISLCFA.....NQSVDVSYDYEMLP.SRKPKFLAQTAAH
C.intestinalis PNVENLLAQKQT....KDPIDECIAFHLN.ETVSGLN.....IGGITIMYDYEILP.NRRPKVLVQTAAH
B.floridae PFVAKKDCY....VR..DPIDECLLSHMQNIKASF.P.....DEDIDCMQDFEMHP.NHRPKVLVQTAAH
N.vectensis PFITDADCLG...SR..DPIDECMAAFFLKVKQAF.P.....CHEIDAIHDFELHP.NRRPKVLVQTAAH
S.purpuratus PFVERLKCQSVIENR..DPIDSCVAEQLLRAKEALKP.....DHEVDIIQDFEMHPVTRRPKLLVQTAGH
I.scapularis PFVLNDRNLTLK.....DPFDQCMTACFA.RAIQAFP.....GESVELLQDFELHP.NRRPKVIMQTAGH
C.elegans KWVVQKTMDFGSFDEVCEMVSSPIQSFLEDRLEIMSEKLRKVEENFEILHDYSMTP.QRRPKILMQTCGH
H.sapiens
1'
G
2'
4
130 140 150 160 170 180
H.sapiens VAGAAYYYQRQDVEA.....DPWGNQ.RISGVCIHPRFGGWFAIRGVVLLPGIEVP.DLPPRKPHDC
P.roglodytes VAGAAYYYQRQDVEA.....DPWGNQ.RISGVCIHPRFGGWFAIRGVVLLPGIEVP.DLPPRKPHDC
M.mulatta VAGAAYYYQRQDVEA.....DPWGNQ.HISGVCIHPRFGGWFAIRGVVLLPGIEVP.DLPPRKPHDC
E.caballus VAGAAYYYQRQDVEA.....DPWGTQ.HISGVCIHPRFGGWFAIRGVLLLPGIEVP.DLPPMKPLDC
B.taurus VAGAAYYYQRQDVES.....DPWGTQ.HIAGVCIHPRFGGWFAIRGVVLLRGTEVP.NLPPTKPVDC
R.norvegicus VAGAAYYYQRQDVDA.....DPWGTQ.HIAGVCIHPRFGGWFAIRGVMLLPGIEVP.NLLPRKPPDC
M.musculus VAGAAYYYQRQDVDA.....DPWGTQ.HIAGVCIHPRFGGWFAIRGVMLLPGIEVP.NLPPRKPPDC
M.domestica VAGAAYYYRQDVGS.....DPWGNK.KIAGVCIHPRYGGWFAIRGVVLLPGVEVP.DLPPLAPPDC
G.gallus VAGAAYYYQRKDVKL.....DPWGEK.KIFGVCIHPKYGGWFAIRGLLLFPDVQVP.FLEQSAPVDC
L.salmonis VSGAVHLYRSPNLNP.....VCLHPKYGGWFALRGVFIFPNISCPFLQIKE.PPCL
C.clemensi VSGSVRLYQAPGYNP.....VCLHPLYGGWFALRGALFFPNIQYD.DSPKE.PPNI
X.laevis VSGAAYYYQRKDIPQ.....DPWGSK.KMFGVCIHPQFGGWFAIRAVLVFAEIQAP.DLEQTLPLDC
S.salar VAGAVYYYQRSDVPD.....HPWGEK.KMFGVCIHPRLGGWFAIRAMLVFVGSEVGSELQQTAPPDC
T.nigroviridis VSGAAFYYQQSDVKD.....QPWAEK.KMFGVCVHPRFGGWFAIRALLIFEGVTAGSEMVQPVPPDC
D.rerio VSGAAYYYQTSDIHN.....PPWGEK.KMFGVCVHPQLGGWFAIRALLVFRDVQAGAGFQQRDPADC
C.intestinalis VAGAAFYYQREHVLKPSGTSGSIDPWGGKRKIYGVCVHPKYGGWFAIRAVLVFTELEDA.NLTKLEPTDC
B.floridae VAGAARYYQRMDVKE.....DPWGKDKNIYGVSVHPKYGGWFAIRAVLVFKNVLVP.EMEYKEPPDC
N.vectensis AAGAAYFYQRKDITD.....DPWPPTQRICGVCVHPKYGGWFAIRGVLIFKTFKYP.DLPRKQPMDV
S.purpuratus VSGAAFYYKRDNVCN.....GPWDAKKKIYGVSIHPKYGGWFAFRGAIILKDTICS.DLRQAAPDQP
I.scapularis VSGAVHYYGCNDMEIT.....ESPPNK.KLLGVCIHPKYGGWFAFRGVFIFSDILVP.DLEQRQPADV
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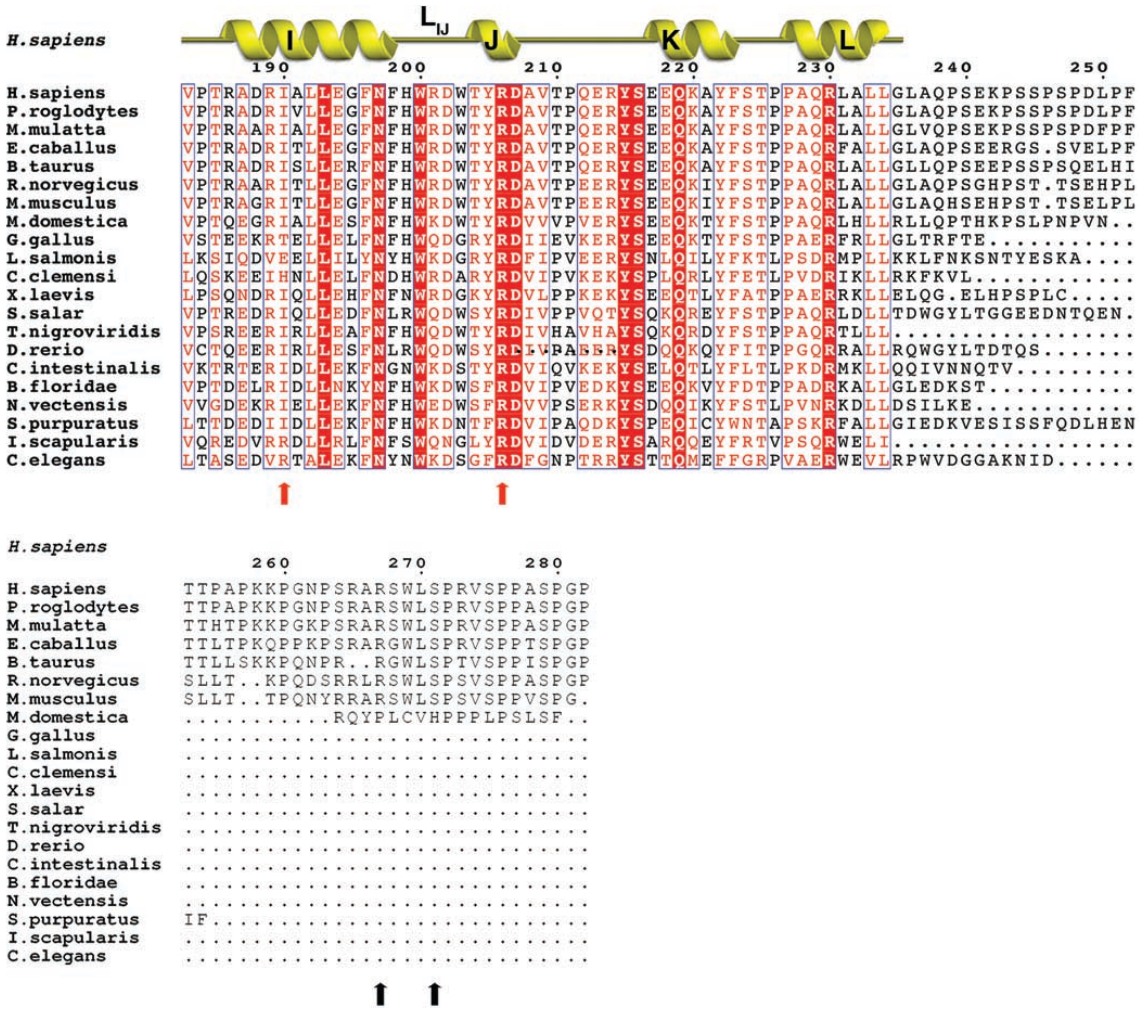

Figure S1. Sequence alignment of CblCs from various organisms. The secondary structure displayed is based on the apo-t-CblC structure. Red arrows indicate patient mutations related to either Cbl binding or protein structural integrity. Green astericks indicate side chains that interact with Cbl, and blue astericks indicate backbone interactions with Cbl. Beta hairpins are indicated with red loops.

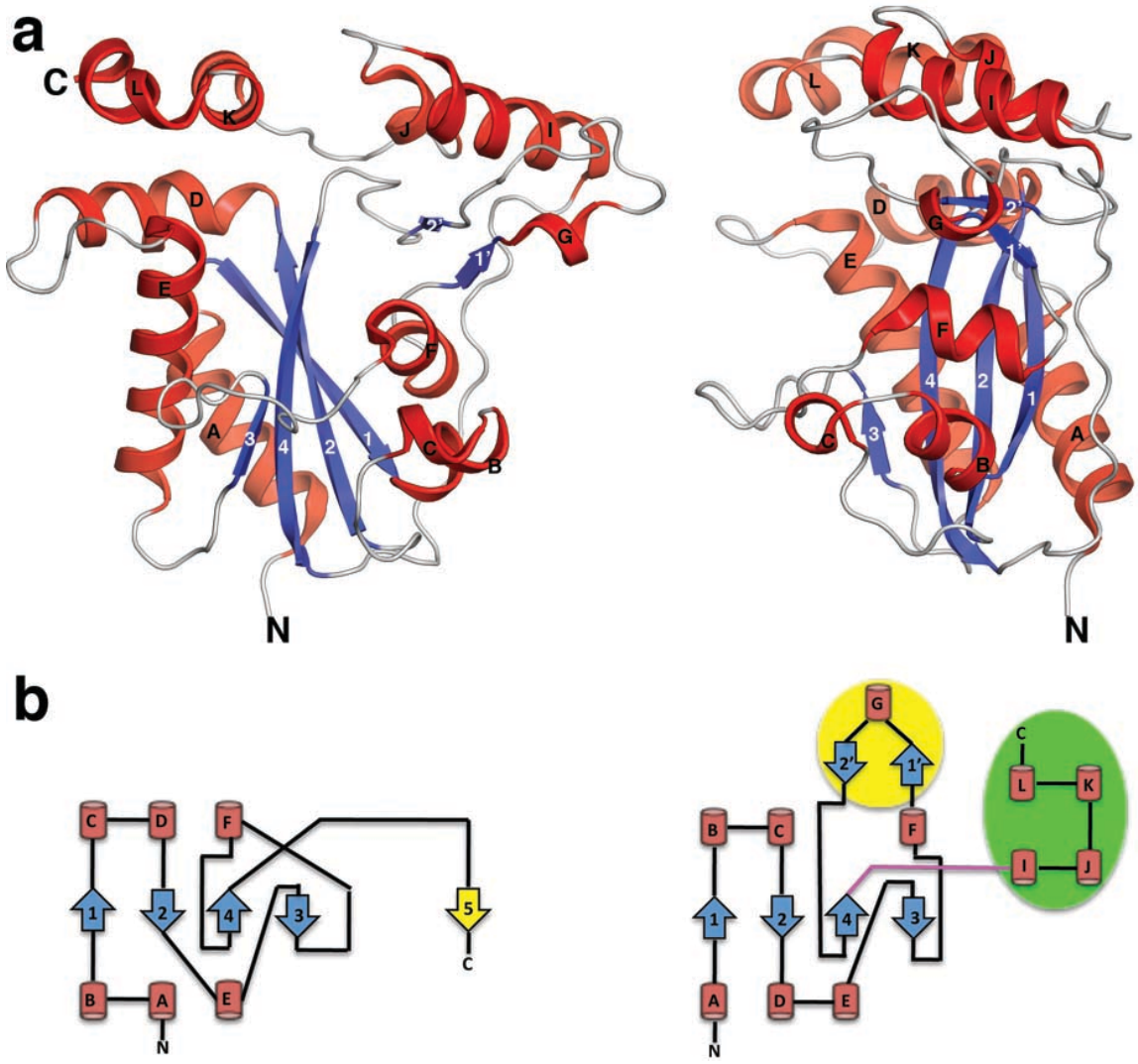

Figure S2. Comparison of CblC and BluB. (**a**) Two views of t-CblC. (**b**) Topology diagram of BluB (left) and CblC (right). In (a) and (b) the α-helices are shown in red and β-strands in blue. Secondary structure features highlighted in yellow and green circles correspond to significant differences/additions to CblC 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.

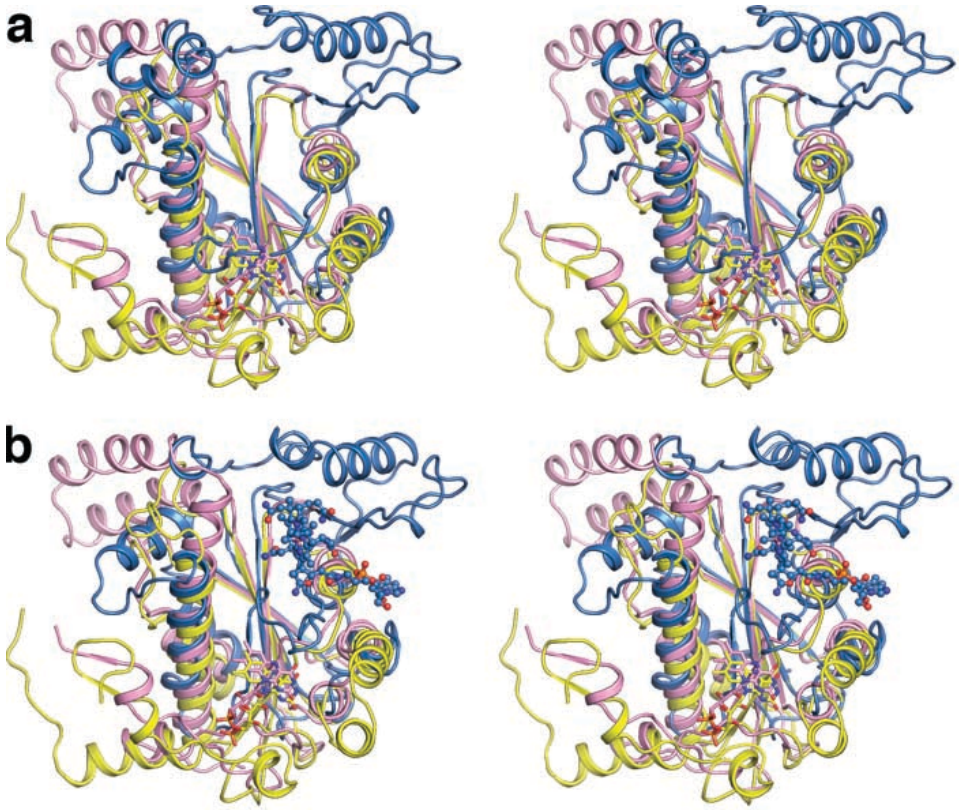

Figure S3. Structural superposition of CblC, BluB and IYD. Superposition in stereo of a BluB monomer (yellow), a IYD monomer (pink), and (**a)** apo-t-CblC and (**b)** MeCbl•t-CblC (blue) are shown. The FMN cofactors for BluB and IYD are shown in stick representation whereas the MeCbl substrate is shown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

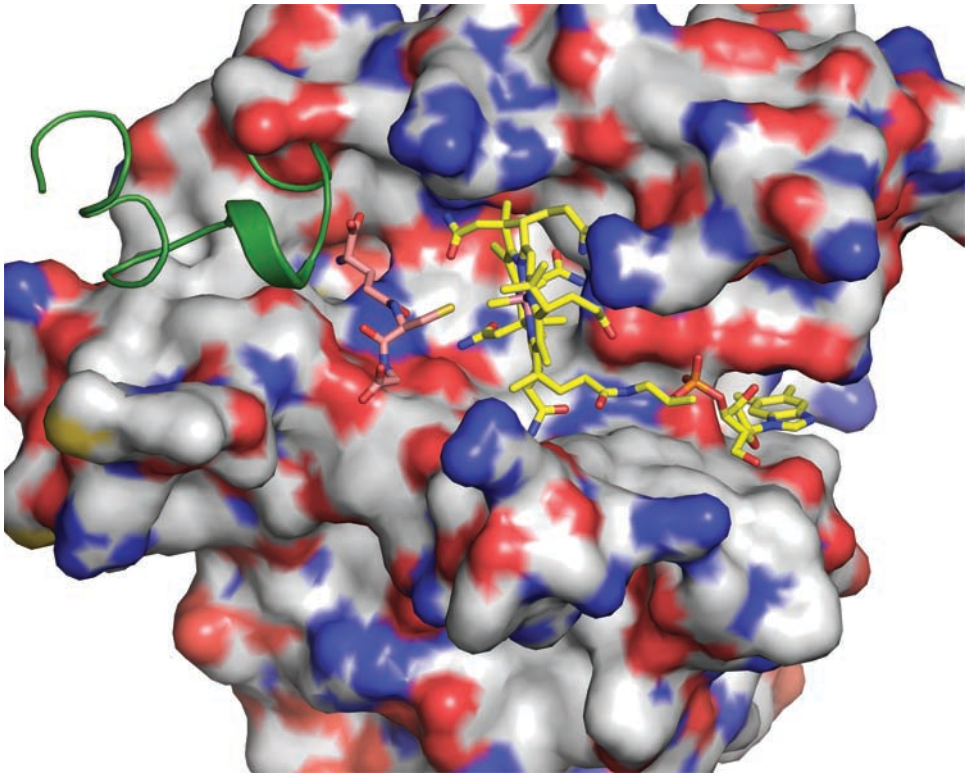

Figure S4. Space filling model of t-CblC•MeCbl. MeCbl is shown in yellow sticks, and modeled GSH is in pink sticks. The flexible C-terminal region (residues 225-238) is displayed in green as a cartoon.

Table S1. MeCbl-CblC contacts.

	MeCbl	Protein	Distance (Å)
RIng	O28	I160 N	2.9
	N29	D104 OD2	2.9
	N29	I160 O	3.0
	N33	C149 O	3.0
	O34	C149 N	2.8
	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
Tail	O4	Y129 OH	2.7
	O5	Y129 OH	3.5
	O7R	Q131 NE2	2.6