Supplementary Information for

An unanticipated architecture of the 750 kD $\alpha_6\beta_6$ holoenzyme

of 3-methylcrotonyl-CoA carboxylase

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Supplementary text

Distinct domain organization of MCC_β compared to PCC_β

The primary reason for the swapping of the positions of the N and C domains in PaMCC β when compared to PCC β is that the linker between the two domains runs in the opposite direction, and consequently connects the N domain to a different C domain in MCC compared to PCC (Supplementary Fig. 3). The neighboring linkers in the PaMCC structure come very close to each other in the hexamer (Supplementary Fig. 3), and therefore only a small change is needed to switch the connectivity as observed in PaMCC β to that in PCC β , and this change in connectivity has only minor effects on the overall structure of the hexamer. The two linkers have essentially the same length, with that in PCC β containing one extra residue compared to MCC β (Supplementary Fig. 2).

Therefore, the swapping of the positions of the N and C domains in MCC β compared to PCC β is due to a different connectivity between the two domains, and is not due to a swap of these domains in the primary sequences of the two proteins. The N-terminal half of MCC β still shares stronger sequence conservation with the N-terminal half of PCC β . The different connectivity does lead to a different position of the C domain relative to the N domain in MCC β compared to PCC β (Supplementary Fig. 3).

In addition, PaMCC β has a long segment (including a long helix) at the N-terminus, which actually lies over the C domain (Fig. 1e). This interaction is unique to $PaMCC\beta$ when compared to PCC β (which has a much shorter segment/helix at the N-terminus), and contributes approximately 900 Å² to the buried surface area. Therefore, the buried surface area between the N and C domains of each subunit (~950 Å²) in PCC β is comparable to that between neighboring subunits in each layer of the hexamer (~900 Å²). In contrast, the buried surface area between neighboring subunits in the PaMCCB hexamer is ~900 Å², comparable to PCC β , while that between the N and C domains of each subunit is $\sim 2000 \text{ Å}^2$. Therefore, this long N-terminal segment may be important for stabilizing the domain organization observed in PaMCCB. In fact, this segment may be the determining factor for this swapped domain organization. It is also present in GCD α (Supplementary Fig. 4), and sequence analysis suggests that it may be present in GCC as well (Supplementary Fig. 18). On the other hand, the linker between the N and C domains is roughly the same length in all of these enzymes (including PCC and ACC) and is poorly conserved in sequence, and therefore is unlikely the driving force for the difference in connectivity between MCC and PCC.

The N and C domains of the β subunit have the same backbone fold. The pseudo symmetry operation relating the two domains is a rotation of approximately 60°, along the three-fold axis of the β_6 hexamer. Therefore, the β_6 hexamers of MCC and PCC have pseudo six-fold symmetry. The swapping of the N and C domains in MCC follows this pseudo symmetry, and does not lead to a dramatic change in the overall shape of the hexamer. In fact, the β_6 hexamer of MCC can be superimposed onto that of PCC by a 60° rotation around the 3-fold axis of the hexamer, giving a rms distance of 1.6 Å for 2,600 equivalent C α atoms. This 60° rotation can also be visualized by the difference in the orientation of the triangle-shaped central hole in the structures of MCC (Fig. 2b) compared to PCC (Fig. 2d).

The molecular replacement solution using the structure of PCC β as the model also reflected this pseudo symmetry. The solution matched the N and C domains of PCC β to the neighboring N and C domains of two separate (crystallographically-related) PaMCC β subunits.

A possible difference in the architecture of MCC holoenzyme compared to PCC due to the swapping of the N and C domain positions in the β subunit

In the PCC holoenzyme, the α subunit, through its BT domain, primarily contacts the N domain of the β subunit ¹. The swapping of the N and C domains in PaMCC β suggests that the positions of the α subunit are likely different in the MCC holoenzyme. To maintain contacts between the α subunit (BT domain) and the N domain of the β subunit, one solution would be to rotate the α subunits by 60° relative to their positions in PCC, following the pseudo six-fold symmetry of the β_6 hexamer.

This 60° rotation of the α subunit relative to its position in PCC is observed in the structure of the PaMCC holoenzyme (compare Figs. 2b and 2d), so that the α subunit remains primarily in contact with the N domain of the β subunit. However, significant additional differences are observed in the position of the α subunits, such that the overall shape of the MCC holoenzyme is strikingly different from that of PCC.

Substrate selectivity of MCC

The structural and sequence analyses suggest a correlation between the swapped domain organization (for MCC, GCD α , and GCC) and the carboxylation on the γ carbon of the substrate. While the exact mechanism for this correlation will require further studies, it is possible that a swapped organization helps to position the CoA and/or biotin substrate correctly in the active site for catalysis to happen on the γ carbon. In addition, there are conformational differences (both structural re-arrangements and side-chain variations) in the active site region between PCC β and MCC β , which allows the latter to accommodate the larger methylcrotonyl-CoA substrate. Modeling this substrate and the active site, especially the Tyr176 residue. The equivalent residue in MCC β , Phe191, has a different position for its side chain and avoids the steric clash, due to differences in the main-chain conformation in this region of the two structures. Therefore, the swapped domain organization and local structural differences and sequence variations all contribute to the substrate selectivity of MCC.

Overall structures of the PaMCC holoenzyme

An $\alpha\beta$ protomer is in the asymmetric unit of the PaMCC free enzyme crystal. The $\alpha_6\beta_6$ dodecamer is generated by the crystallographic symmetry (*R*32). The BCCP domain of the α subunit is disordered in this crystal, and weak electron density is observed for the B domain of BC, in an open conformation (Supplementary Fig. 5). In the β subunit, several segments of the protein near the active site, including residues 180-189 ($\beta7$ - $\alpha4$ loop in the N domain, Supplementary Fig. 2), 244-266 ($\alpha6$ - $\alpha7$), 407-416 ($\beta7$ - $\alpha4$ loop in the C domain), 444-450 ($\beta9$ - $\alpha5$), and 470-514 ($\alpha6$ - $\alpha6A$, which forms a large protrusion on the surface of the β subunit, Fig. 2a), are disordered in the free enzyme (Supplementary Fig. 5). This is in contrast to the structure of PaMCC β alone, where these

residues are well ordered (Fig. 1e). The free enzyme structure of a better fit to the EM density (Fig. 2e), which was obtained in the absence of CoA.

An entire $\alpha_6\beta_6$ holoenzyme is located in the crystallographic asymmetric unit of the CoA complex of PaMCC. The six molecules of each subunit have essentially the same conformation, with rms distance of approximately 0.25 Å for their equivalent C α atoms (non-crystallographic symmetry restraint was applied during refinement). The B domain of BC is ordered in four of the six α subunits, in an open conformation (Fig. 2a). BCCP is ordered in only one of the α subunits, and is located in the active site of MCC β (Fig. 2a). Weak electron density for biotin and the lysine residue it is covalently linked to was observed in another subunit.

The overall structures of the free enzyme and the CoA complex of PaMCC holoenzyme are similar. The rms distance among 2,566 equivalent C α atoms of the β subunits of the two structures is 0.9 Å. On the other hand, this overlay of the β subunits revealed a change in the position of the α subunits in the two structures, which corresponds to a rotation of ~6°, mostly along the 3-fold axis of the holoenzyme (Supplementary Fig. 5).

The rms distance for equivalent C α atoms between the structure of PaMCC β alone and that in either of the two holoenzymes is ~0.8 Å. Residues at the interface with the α subunit assume the same conformation in the structure of MCC β alone, suggesting that the β subunit does not undergo a large conformational change upon the formation of the holoenzyme. In contrast, large structural differences are observed for helices $\alpha 6$ and $\alpha 6A$ near the active site of the β subunit in the CoA complex.

There is no direct contact between the BC domain in the α subunit and the β subunit in the PaMCC holoenzyme, with the two entities separated by nearly 20 Å (Fig. 2a), whereas there is some contact between BC and the β subunit in PCC (Fig. 2c)¹. The BT domain is the bridge between BC and the β subunit in PaMCC (Figs. 2a, 4c). However, in contrast to BC, there is no contact among the BT domains in PaMCC, which leaves a large cavity between BC and the β subunit (Fig. 2a).

The BT domain of human MCC

The BT domain of PaMCC lacks the third strand (β 24) and the fourth strand (β 25) is much shorter compared to the eight-stranded β -barrel in the BT domain of PCC (Fig. 3a). Instead, the second and the fourth strands are connected directly by a short linker. Interestingly, PaMCC contains a deletion of 14 residues in this region as compared to HsMCC, and secondary structure prediction suggests that these additional residues in HsMCC likely can form the third strand and extend the length of the fourth strand (Supplementary Fig. 1). Therefore, it is probable that the BT domain of HsMCC actually contains an eight-stranded β -barrel, which would be equivalent to that in PCC. In fact, sequence comparisons suggest that this deletion may be unique to the *Pseudomonas* organisms, while most other MCCs are similar to HsMCC. Therefore, the BT domain of most MCC holoenzymes is likely to have an eight-stranded β -barrel.

The third and fourth strands are not located in the subunit interface in PaMCC (Fig. 3b) and PCC¹, and therefore their absence in PaMCC is unlikely to have caused a large difference in the overall structure of the BT domain or the organization of the dodecamer.

While the overall structure of the BT domain of PaMCC is generally similar to that of PCC, the detailed positions of the β -strands and the central helix are rather different (Fig. 3a). In addition, the central helix in the BT domain of PaMCC is much shorter compared to that in PCC.

These detailed structural differences between the BT domains of MCC and PCC, especially their hooks, may be the basis for the different locations of the BC domains in the two holoenzymes, such that PCC has monomeric BC domains while MCC has trimeric BC domains.

BT domain interactions in the PaMCC holoenzyme

The hook and the first β -strand (β 22) of the BT domain have the most contacts with the β subunit (Fig. 3b). The hook interacts with residues at the interface between the N and C domains of the β subunit (Fig. 3c). Residues in the hook that make the most contribution to surface area burial include two Trp residues (Trp537 and Trp543) and two Arg residues (Arg525 and Arg544). In addition, Asp535 in the hook has ion-pair interactions with Lys298 in the β subunit, which is in the linker between the N and C domains.

Residues 633-636 (α W helix of the BT domain) are located near the α 8 helix in the N domain of the β subunit, as well as the N-terminus of a neighboring β subunit.

Direct interactions between the BT domain and the BC domain of a neighboring α subunit are mediated by the N-terminal end of the sixth strand (β 27, Fig. 3b). This interface is primarily mediated by hydrogen-bonding among main-chain atoms, forming the anti-parallel β -sheet. There is little side chain contact in this interface. The β 26- β 27 hairpin has large structural differences compared to that in the BT domain of PCC (Fig. 3a).

Binding mode of CoA to the PaMCC holoenzyme

Methylcrotonyl-CoA was included in the crystallization solution, but only CoA was observed based on the electron density (Supplementary Fig. 13), suggesting that the CoA ester may have been hydrolyzed during crystallization. The conformation of CoA, especially the 3'-phospho-ADP portion, is different from that bound to GCD α^2 , and is actually more similar to that bound to the CT domain of yeast acetyl-CoA carboxylase (ACC)³.

The adenine base is sandwiched between the side chain of Thr144 (strand β 5 in the N domain of β 6) and those of Leu482 and Val485 (helix α 6 in the C domain of β 3, Supplementary Fig. 14). The phosphate groups of CoA are recognized by the side chains of Arg74 and Arg78 (helix α 1), and Lys141 (β 4- β 5 loop) in the N domain.

A large conformational change for helices $\alpha 6$ and $\alpha 6A$ in the C domain is associated with CoA binding, which rotate by roughly 25° to move closer to the adenine (Supplementary Fig. 15). These two helices are also involved in crystal packing in both the PaMCC β and the holoenzyme crystals. In GCD α , helix $\alpha 6$ rotates by 13° upon the binding of crotonyl-CoA². A smaller conformational change for helix $\alpha 1$, which contains the two Arg side chains that interact with the phosphates of CoA, is also observed (Supplementary Fig. 15).

Based on our structure of the CoA complex and the conformation of crotonyl-CoA bound to GCD α^2 , we built a model for the binding mode of methylcrotonyl-CoA to the

active site of PaMCC (Fig. 4b, Supplementary Fig. 14). This ternary complex provides significant molecular insights into the catalytic mechanism of MCC and biotin carboxylases in general. It is expected that the N1' atom of biotin, in its enolate form, can extract a proton from the γ carbon of methylcrotonyl-CoA⁴. Our structure shows that the enolate oxygen atom of biotin is stabilized by an oxyanion hole consisting of the main-chain amides of Phe407 (in strand β 7) and Gly448 (at the N-terminus of helix α 5) in the C-domain of one β subunit (Supplementary Fig. 14). Therefore, the oxyanion also has favorable interactions with the dipole of helix α 5. Upon proton extraction, the methylcrotonyl group also enolizes, and that oxyanion is stabilized by the main-chain amides of Ala176 (in strand β 7) and Gly219 (at the N-terminus of helix α 5) in the N-domain of another β subunit (Supplementary Fig. 14). The two oxyanion holes employ equivalent residues in the N and C domains of the β subunit. This mechanism is similar to that proposed for GCD α ⁵.

Methylcrotonyl-CoA has a second, non-reactive γ carbon (Fig. 1b), which is absent in crotonyl-CoA. In the model, this γ carbon is located in a pocket formed by the side chains of Phe191 (helix α 4), Ala176 (β 7), and the main chain of Gly219 (α 5). Our kinetic experiments showed that the activity of PaMCC towards crotonyl-CoA is roughly 90% of that towards methylcrotonyl-CoA, indicating that the enzyme is not selective with respective to these two substrates. In contrast, PaMCC is essentially inactive towards propionyl-CoA, with less than 1% of the activity compared to methylcrotonyl-CoA.

Binding mode of BCCP

The BCCP domain contacts the C domain of one β subunit (β 3) through its 679-AMKM-682 biotinylation sequence as well as residues Met653-Asn654 (Supplementary Fig. 17). There is a hydrogen bond between the main-chain amide of residue Glu683 and the carbonyl group of Lys326. In addition, residues Ala250-Thr251, at the end of helix α 6 of the N domain of a second β subunit (β 6), also have interactions with the BCCP domain (Supplementary Fig. 17). Two residues in this interface, Ile375 (Val in HsMCC) and Asn403, are sites of disease-causing mutations, which may affect the positioning of BCCP in the CT active site for catalysis.

The interface between BCCP and the β subunit is rather rich in Met residues, with Met653, Met680 and Met682 from BCCP, and Met408 and Met473 from the β subunit (Supplementary Fig. 17).

Disease-causing mutations

Of the disease-causing mutations in the α subunit, R385S disrupts the Arg side chain that is expected to stabilize the biotin enolate during BC catalysis, based on studies with the BC subunit of *E. coli* ACC (Supplementary Fig. 16)⁶. The E134K mutation may disturb the interaction with Arg339, which recognizes the bicarbonate substrate.

The I460M mutation affects a residue that is located in the interface between the BC and BT domains of the same subunit (Supplementary Fig. 16). It may disturb the position of the BC domain relative to the rest of the holoenzyme.

The S535F mutation in the hook of the BT domain may disrupt the interactions between the α and β subunits (Supplementary Fig. 12). The Ser side chain is located in a small pocket in the α - β interface, and the S535F mutation may lead to large conformational re-arrangements in this region of the interface. The D532H mutation in

the BT domain (as well as the I437V mutation in the β subunit) primarily affects splicing in humans (Table S2)⁷, although it may also affect the holoenzyme (Supplementary Fig. 12).

Of the disease-causing mutations in the β subunit, A218T may disrupt binding of methylcrotonyl-CoA or biotin (or both), being centrally located between these two substrates (Fig. 4b). The H190Y/R, R193C/H and A456V mutations form a cluster and are located near the binding site for the methylcrotonyl group. The S173L mutation is located near the binding site for the adenine base of CoA.

Several of the disease-causing mutations in the β subunit may disturb interactions with the BT domain. These include E99Q, S101F and H282R. Two other mutations are located in the interface with BCCP, V375F and N403T.

Table 1.

Structure	РаМССβ	PaMCC	PaMCC	
	Free enzyme	Free enzyme	CoA complex	
Space Group	<i>R</i> 32	<i>R</i> 32	$P2_1$	
Unit cell parameters	<i>a</i> = <i>b</i> =192.4 Å,	<i>a=b=</i> 158.9 Å,	<i>a</i> =131.5 Å,	
	<i>c</i> =136.8 Å	<i>c</i> =312.0 Å	<i>b</i> =255.3 Å,	
			<i>c</i> =152.7 Å	
			β=95.7 °	
Resolution range $(\text{\AA})^1$	30-1.5 (1.55-1.5)	50-2.9 (3.0-2.9)	50-3.5 (3.63-3.5)	
Number of observations	508,819	121,925	363,300	
Number of reflections	141,517	31,522	104,480	
Completeness (%)	92 (73)	93 (82)	83 (61)	
R_{merge} (%)	8.9 (35.9)	5.8 (38.8)	12.0 (45.1)	
I/σI	10.8 (2.3)	16.0 (2.4)	8.3 (1.7)	
Redundancy	3.4 (2.6)	3.6 (3.4)	3.1 (2.4)	
<i>R</i> factor (%)	16.6 (32.8)	20.9 (32.4)	23.4 (39.6)	
Free <i>R</i> factor (%)	18.3 (31.8)	26.9 (35.4)	29.2 (42.5)	
rms deviation in bond lengths (Å)	0.017	0.011	0.014	
rms deviation in bond angles (°)	1.7	1.7	1.8	
Number of protein atoms	4,046	7,636	49,946	
Number of waters	970	0	0	
Ramachandran plot (% of residues)				
Most favored region	92.3	78.9	75.6	
Additional allowed region	7.5	17.8	21.6	
Generously allowed region	0.2	2.7	2.3	
Disallowed region	0	0.6	0.5	

Summary of crystallographic information

1. The numbers in parentheses are for the highest resolution shell.

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 Table 2.

 Missense mutations in HsMCC associated with methylcrotonylglycinuria (MCG)

Mutation	Location	Structural Observations		
ΜСС α				
BC domain				
Q123H αF-β4		On the surface, next to the BC-BC interface in the		
	loop	holoenzyme.		
I125M	β4	In hydrophobic core. May disrupt structure.		
E134K	β4-αG	Near the active site. Interacts with the side chain of Arg339,		
	loop	which recognizes the bicarbonate substrate.		
M160R	αΗ-αΙ	In hydrophobic core near the active site.		
	loop			
S187P	β6-αΚ	On the surface. N-terminal cap of helix αK .		
	loop			
R232W	αL-β11	On the surface of the B domain.		
	loop			
A289V	β14	In hydrophobic core. May disrupt the structure.		
A291V	β14-αΜ	In hydrophobic core near the active site.		
	loop			
M325R	β15	Strand β 15 contains Glu322, a ligand to Mg ²⁺ . Also near the		
		base of domain B of BC. May affect the conformational		
		change (open-closed transition) of domain B. Has also been		
		reported to reduce protein level in the cell.		
Q372P	αΟ-β17	Side chain hydrogen-bonded to the main-chain amide and		
	loop	carbonyl of residue 270 (strand β 13). May be important for		
		structural integrity.		
G379S	β17	Just prior to strand β17.		
R385S	β17	Catalytic residue. Stabilizes the biotin enolate.		
L437P	β20	In hydrophobic core. May disrupt the structure.		
I460M	β21	In hydrophobic core, at the interface with the BT domain.		
BT domain				
D532H	Hook	In the interface with the β subunit. The primary effect is on		
	(αV-β22	splicing. The mutation abolishes a 5' splice site, leading to		
	loop)	the skipping of an exon.		
S535F	Hook	In the interface with the β subunit.		
	(αV-β22			
	loop)			
ΜСС β				
N domain				
E99Q	β1	Glu99-Arg84 ion pair is located near the interface with the		
		BT domain. May disrupt the holoenzyme.		

S101F	β1-β3	Side chain hydrogen-bonded to main-chain carbonyl of	
	loop	residue 528. Bulkier side chain may disrupt structure. Near	
		the interface with the BT domain.	
R155Q/W	α3	Buried ion pair with Asp529. May disrupt the structure.	
C167R	β6	In hydrophobic core. May disrupt the structure.	
S173L	β6	In hydrophobic core, and side chain hydrogen-bonded to	
		main-chain carbonyl of Gly143, which is near the adenine	
		base of CoA. May disrupt the structure and CoA binding.	
H190Y/R	β7-α4	Near the β subunit interface and the methylcrotonyl binding	
	loop	site of β subunit. In a cluster with R193C/H and A456V.	
R193C/H	α4	In central cavity of β dimer. Ionic interactions with side	
		chain of Glu189, and Asp458 from another subunit.	
A218T	β9	Near the methylcrotonyl group and the biotin. May disrupt	
		substrate binding.	
H266L	α7	In hydrophobic core.	
R268T	α7	On the surface. In the β subunit interface.	
D280Y	α8	Interacts with the side chain of Arg86.	
H282R	α8	In the surface with the αW helix of the BT domain.	
P310R	N-C	In linker between N and C domains. Bulkier Arg side chain	
	linker	may disturb structure.	
C domain			
V339M	α2-β1	In hydrophobic core. May disrupt the structure. In a cluster	
	loop	with P310R and D340V.	
D340V	α2-β1	Interacts with the side chain of Arg538.	
	loop		
V375F	β5	In the interface with the BCCP domain, near the active site	
		of CT. May disrupt BCCP binding to the CT active site.	
N403T	β6	Next to the interface with BCCP.	
I437V	β8	In hydrophobic core. May disrupt the structure. The primary	
		effect is on splicing. The mutation creates a new 5' splice	
		site, leading to the skipping of part of an exon.	
A456V	α5-β10	In hydrophobic core.	
	loop		
K555E	C-	On the surface, faces the central cavity of the β_6 hexamer.	
	terminal		
	loop		

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S. S.		BC $\beta 1$ αC $\beta 2$ αE $\beta 3$ αF $\beta 4$
HSMCC	42	TTATGRNITKVLIANRGEIACRVMRTAKKLGVOTVAVYSEADRNSMHVDMADEAYSIGPAPSOOSYLSMEKIIOVAKTSAAOAIHPGCGFLSE
PaMCC	1	MNPDYRS IORLLVANRGEIACRVMRSARALGIĞSVAVHSDIDRHARHVAEADIAVDLGGAKPÄDSYLRGDRIIAAALASGAQAIHPGYGFLSE
HsPCC	56	YDPNEKTFDKILVANRGEIACRVIRTCKKMGIKTVAIHSDVDASSVHVKMADEAVCVGPAPTSKSYLNMDAIMEAIKKTRAQAVHPGYGFLSE
S. S.	100.000	
HSMCC	135	NMEFAELCKQEGIIFIGPPPSAIRDMGIKSTSKSIMAAAGVPVVEGYHGEDQSDQCLKEHARRIGYPVMIKAVRGGGGKGMRIVRSEQEFQEQ
PaMCC	140	NADFARACEEAGLLFLGPPAAAIDAMGSKSAAKALMEEAGVPLVPGYHGEAQDLEFFRREAGRIGYPVLLKAAAGGGGGGMKWVVEREAELAEA
isrce	149	WEERACUARED VVI GEDINAIQAMODALESALLARAREVALIFORDAVAAAAEIGI FVMIAASAGGGGGGGGAAAAADDEEIAD
S. S.		611 612 613 614 add
HSMCC	228	LESARREAKKSENDDAMLIEKEVDTPRHVEVOVEGDHHGNAVYLEERDCSVORRHOKTTEEAPAPGTKSEVRKKLGEAAVRAAKAVNYVGAGT
PaMCC	187	LSSAOREAKAAFGDARMUVEKYLLKPRHVE IOVFADRHGHCLYLNERDCSIORRHOKVVEEAPAFGLGAELRRAMGEAAVRAAOAIGYVGAGT
HSPCC	242	FRLSSQEAASSFGDDRLLIEKFIDNPRHI <mark>E</mark> IQVLGDKHGNALWLNERECSIQRRNQKVVEEAPSIFLDAETRRAMGEQAVALARAVKYSSAGT
		· · · · · · · · · · · · · · · · · · ·
S. S.		$\beta 15$ $\beta 16$ αN αO $\beta 17$ $\beta 18$ $\beta 18$
HSMCC	321	VEFIMDSKHNFCFMEMNTRLQVEHPVTEMITGTDLVEWQLRIAAGEKIPLSQEEITLQGHAFEARIYAEDPSNNF-MPVAGPLVHLSTPRADP
PaMCC	280	VEFLLDERGOFFFMEMNTRLOVEHPVTEAITGLDLVAWOIRVARGEALPLTOEOVPLNGHAIEVRLYAEDPEGDF-LPASGRLMLYREAAAGP
HSPCC	335	VEFLVDSKKNFIFLEMNTRLQVENFVTECITGLDLVQEMIKVAKGIPLKHKQADIKINGWAVECKVIAEDPIKSFGLPSIGKLSQIQEPLHLP
S. S.		619 620 ar 621 as at au BT
HeMCC	413	STR I TTO UP OGDEVS VHVD DMT AKT, VVWAAD POAAT TKI, PVSI POVN I VGI HTNI DELI NI SGHPEFEAGNUHTDET POHHKOLLI SPKAA
PaMCC	372	GRRVDSGVREGDEVSPFYDPMLAKLIAWGETREEROLLAMLAETSVGGLRTNLAFLRRILGHPAFAAAELDTGFIAHODDLLPAPOA
HSPCC	428	GVRVDSGIQPGSDISIYYDPMISKLITYGSDRTEALKRMADALDNYVIRGVTHNIALLREVIINSRFVKGDISTKFLSDVŸPDGFKGHMLTKS
S. S.		αV
HSMCC	504	akeslcqaalglilkekamtdtftlqahdqfspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfsssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfsssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfsssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfsssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssgrrlnisytrnmtlkdgknnvaiavtynhdgsysmqiedktfqvlgnlysegdctingspfssgrrlnisytrnmtlkdgkntqvlgnlysegdctingspfssgrrlnisytrnmtlkdgknnvaiavty
PaMCC	462	LPEHFWQAAAEAWLQSEP6HRRDDDPHSPNSRNDGWRSALARESDLMLRCRDERRCVRLRHASPSQYRLDGD
HSPCC	521	EENQLLAIASSLFVAFQLKAQHFQLNAKMPVIKPDIANWELSVKLHDKVHTVVASNNGSVFSVEVDGSKLNVTSTWNLASP
S. S.		$\rightarrow \rightarrow \rightarrow \rightarrow \rightarrow$
S. S.		$\beta_{26} = \beta_{27} = \beta_{28} = \beta_{29} = \alpha_{W} = \beta_{CCP} = b \rightarrow b \rightarrow b = b$
HSMCC	597	YLKCSVNGVASKAKLIILEN-TIYLFSKEGSIEIDIPVPKYLSSVSSOETOGGPLAPMTGTIEKVFVKAGDKVKAGDSLMVMIAMK
PaMCC	534	DLVSRVDGVTRRSAALRRGRQLFLEWEGELLAIEAVDPIAEAEAAHAHQGGLSAPMNGSIVRVLVEPGQTVEAGATLVVLEAMK
HSPCC	602	$\texttt{LLSVSVDGTQRTVQCLSREAGGNMSIQFL}{\textbf{G}} \texttt{TVYKVNILTRLAAELNKFMLEKVTEDTSSVLRSP} \textbf{MPGVVVAVSVKPGDAVAEGQEICVIEAMK}$
S. S.		\rightarrow \rightarrow \rightarrow \rightarrow
S. S.		
HeMCC	603	NEWTYKER/DOMUKUEVEFADANDUTTUVEFEFEEDVEFEE 725
PaMCC	618	PERIINSTNUSIVARVIIREGAVANRAITIVETEEEESJARESE //2) MERSIRAPHAGVVALVCSEGEVERGEVERGTUJELDENGA 655
HSPCC	695	MONSMTAGKTGTVKSVHCOAGDTVGEGDLLVELE 728

Fig. 1. Sequence alignment of the α subunits of human MCC (HsMCC), P. aeruginosa MCC (PaMCC), and human PCC (HsPCC). The BC, BT, and BCCP domains are indicated by the colors of the secondary structure elements (S. S.). Mutation sites associated with methylcrotonylglycinuria (MCG) are shown in red in the HsMCC sequence, and those associated with propionic acidemia (PA) are shown in red in the HsPCC sequence. Residues in the active site are highlighted with the gold background. The colored residues in PaMCC highlight those with >50 Å² (magenta) or >15 Å² (light brown) buried surface area in the holoenzyme interface. Residues in PaMCC that are not observed in the structure are shown in italics. The dots at the top of the alignment mark every 10th residue in HsMCC, while those at the bottom mark every 10th residue in HsPCC. The secondary structure elements in the BC domain are named after those in the BC domain of yeast ACC⁸. Strand β24 and the first half of $\beta 25$ of the BT domain are absent in PaMCC, but are predicted in HsMCC. The secondary structure elements in the BT domain of PCC are shown in gray.

S. S. HsMCC PaMCC HsPCC	28 1 11	N domain α0 α1 α2 β1 VASLGTQPDLGSALYQENYKQMKALVNQLHERVEHIKLGGGEKARALHISRGKLLPRERIDNLIDPGSPFLELSQFAGYQLYDNEEV MAILHTQINPRSAEFAANAATMLEQVNALRTLLGRIHEGGGSAAQARHSARGKLLVRERINRLLDPGSPFLELSALAAHEVYGEEV GARLSVLASGLRAAVRSLCSQATSVNERIENKRRTALLGGGQQRRIDAQHKRGKLTARERISLLDP-GSFVESDMFVEHRCADFGMAADKNKF
S. S. HsMCC PaMCC HsPCC	115 87 103	β3 β4 β5 α3 β6 β7 α4 PGGGIITGIGRVSGVECMIIANDATVKGGAYYPVTVKKQLRAQEIAMQNRLPCIYLVDSGGAYLPRQADVFPDRDHFGRTFYNQAIMSSKNIA AAAGIVAGIGRVEGVECMIVGNDATVKGGTYYPLTVKKHLRAQAITALENRLPCIYLVDSGGANLPRQDEVFPDREHFGRIFFNQANMSARGIP PGDSVVTGRGRINGRLVYVFSQDFTVFGGSLSGAHAQKICKIMDQAITVGAPVIGLNDSGGARIQEGVESLAGYADIFLRNVTASG-VIP
S. S. HsMCC PaMCC HsPCC	208 180 192	β8 β9 α5 β10 β11 α6 α7 β12 α8 C QIAVVMGSCTAGGAYVPAMADENIIVRKQGTIFLAGPPLVKAATGEEVSAEDLGGADLHCRKSGVSDHWALDDHHALHLTRKVVRNLNYQKKL QIAVVMGSCTAGGAYVPAMSDETVMVREQATIFLAGPPLVKAATGEVVSAEELGGADVHCKVSGVADHYAEDDDHALAIARRCVANLNWRKQG QISLIMGPCAGGAVYSPALTOFFMVKDTSYLFITGPDVVKSVTNEDVTQEELGGAKTHTMSGVAHRAFENDVDALCNLROFFNYLPLSSQD
S. S. HsMCC PaMCC HsPCC	301 273 285	domain β1 β3 β4 β5 α3 DUTIEPSEEP-LFPADELYGIVGANLKRSFDVREVIARIVDGSRFTEFKAFYGDTLVTGFARIFGYPVGIVGNNGVLFSESAKKGTHF QLQCRAPRAP-LYPAEELYGVIPADSKQPYDVREVIARLVDGSEFDEFKALFGTTLVCGFAHLHGYPIAILANNGILFAEAAQKGAHF PASVRECHDPSDRLVPELDTIVPLESTKAYNMVDIIHSVVDEREFFEINPNYAKNIIVGFARMNGRTVGIVGNQPKVASGCLDINSSVKGARF
S. S. HsMCC PaMCC HsPCC	388 360 378	β6 β7 α4 β8 β9 α5 β10 β11 VOLCCORNIPLIFLONITGFMVGREYEAEGIAKDGAKMVAAVACAQVPKITLIIGGSYGAGNYGMCGRAYSPKFLYIMPNARISVMGGEQAAN IELACORGIPLIFLONITGFMVGQKYEAGGIAKHGAKLVTAVACARVPKFTVLIGGSFGAGNYGMCGRAYDPRFLWMWPNARIGVMGGEQAAG VKFCDAFNIPLITFVDVPGFLPGTAQEYGGIIRHGAKLLYAFAEATVPKVTVITRKAYGGAYDVMSSKHLCGDTNYAWPTAEIAVMGAKGAVE
S. S. HsMCC PaMCC HsPCC	481 453 471	α6 α7 β12 α8 VLATITKDQRAREGKQFSSADEAALKEPIIKKFEEEGNPYYSSARVWDDGIIDPADTRLVLGLSFSAALNAPIEKTDFGIFRM 563 VLAQVKREQAERAGQQLGVEEEAKIKAPILEQYEHQGHPYYSSARLWDDGVIDPACTREVLALALSAALNAPIEPTAFGVFRM 535 IIFKGHENVEAAQAEYIEKFANPFPAAVRGFVDDIIQPSSTRARICCDLDVLASKKVQRPWRKHANIPL 539

Fig. 2. Sequence alignment of the β subunits of human MCC (HsMCC), P. aeruginosa MCC (PaMCC), and human PCC (HsPCC). The N and C domains are indicated by the colors of the secondary structure with elements (S. S.). Mutation sites associated methylcrotonylglycinuria (MCG) and propionic acidemia (PA) are shown in red in the HsMCC and HsPCC sequence, respectively. The colored residues in PaMCC highlight those with >50 Å² (magenta) or >15 Å² (light brown) buried surface area in the interface with the α subunit. The dots at the top of the alignment mark every 10th residue in HsMCC, while those at the bottom mark every 10th residue in PaMCC. The secondary structure elements are named after those in the CT domain of yeast ACC³.



Fig. 3. (*Top*) The linker from the N to the C domains (in black, with the red arrow indicating the direction of linker) runs in opposite directions in MCC (*Left*) and PCC (*Right*) and connects to different C domains, leading to the swapping of the positions of the N and C domains in each subunit between the two enzymes. The two neighboring linkers approach each other closely at one point (blue star) in PaMCC, and a change in connectivity at that position will lead to the PCC organization. The boundaries of each subunit are indicated by the gray lines. (*Bottom left*) Omit F_o - F_c electron density for the linker in PaMCC at 1.5 Å resolution, contoured at 3 σ . (*Bottom right*) Overlay of the structures of the β subunit of PaMCC (in color) and PCC (gray). The C domains of the two structures are superimposed, but their N domains are in different positions due to the swap. All structure figures were produced with the program PyMOL (www.pymol.org).



Fig. 4. (*Top*) Stereo drawing of the overlay of a β_2 dimer of PaMCC holoenzyme in complex with CoA (in color) with the GCD α dimer in complex with crotonyl-CoA (in gray)². The N and C domains are labeled, and the two-fold axis of the dimer is indicated by the oval in black. (*Bottom*) The same overlay after a 90° rotation around the vertical axis. The C-terminal helices of the two GCD α monomers are indicated with the red arrows. These helices clash with other monomers in the hexamer interface of PaMCC β , which is one of the reasons why GCD α cannot form a similar hexamer. It is also possible that residues at the hexamer interface have been mutated to disfavor that oligomer for GCD α . Instead, GCD α dimer can form a tetramer using a different interface ².



Fig. 5. Overlay of the structures of the CoA complex (in color) and the free enzyme (gray) of PaMCC holoenzyme. (*Left*). Viewed down a two-fold axis of the dodecamer. (*Right*). Viewed down the three-fold axis. The 6° rotation in the α subunits of the two structures are indicated in red. The BCCP domain, α 6- α 6A protrusion, as well as several other segments near the active site of the β subunit, are disordered in the free enzyme.



Fig. 6. (*Left*). Side and top views of the PaMCC holoenzyme molecular surface. (*Right*). Side and top views of the PCC holoenzyme, showing dramatic differences in the overall architecture of the two enzymes.



Fig. 7. Transmission electron micrograph of negatively stained PaMCC. Scale bar = 200 Å.



Fig. 8. Resolution assessment based on Fourier shell correlation (FSC). The FSC coefficients between the two reconstructions each from half of the full data sets during the last four refinement rounds are plotted as a function of spatial frequency (1/Å). Using the FSC = 0.5 criterion, the resolution of the final reconstruction is about 12 Å.



Fig. 9. (*Top*) Simulated annealing omit F_o-F_c electron density map at 2.9 Å resolution for strands β 27- β 29 (residues 609-631, *Left*) and the hook (residues 522-545, *Right*) in the BT domain of the α subunit. The contour level is at 3 σ . (*Bottom*) Schematic drawing in stereo of the overlay of the BT domain of PaMCC (orange) with that of PCC (gray).



Fig. 10. (*Top*) Schematic drawing in stereo of the interactions of the BT domain (orange) in the PaMCC holoenzyme. (*Bottom*) Schematic drawing in stereo of the interactions between the hook of the BT domain (orange) and the N (cyan) and C (yellow) domains of the β subunit.



Fig. 11. The positions of the BT domain relative to the β subunit are different in PaMCC and PCC. The PCC holoenzyme structure is rotated by 60° around the 3-fold axis (the pseudo symmetry of the hexamer), which brings its β hexamer (in gray) into overlay with that of MCC (in color). A distinct position of the BT domain in PaMCC (orange) is observed relative to that in PCC (gray, indicated with the red arrow). An independent change in the positions of the BC domains is also apparent (indicated with the blue arrow).



Fig. 12. Mutations in the hook of the BT domain can destabilize the MCC holoenzyme. Mutations were introduced in the hook of PaMCC based on the structural information, and the effects of the mutation on the formation of the holoenzyme were examined by nickel pulldown experiments after bacterial expression with SDS-PAGE. The β subunit carried a His tag, and therefore the elution (E) from a nickel column, compared to the total amount of overexpressed protein in the pellet (P) and soluble (S) fractions, gave an indication of the stability of the holoenzyme. For example, the eluate for the W543A/R544A double mutant contained a large excess of the β subunit, even though the α subunit was soluble, suggesting that the holoenzyme has been destabilized by this mutation. The D532H mutation also had some effect, even though this disease-causing mutation primarily affects splicing in humans. It should also be noted that this assay is under over-expression conditions. Under physiological conditions, where the concentrations of the two subunits are likely much lower, the effect of the mutations on the stability of the holoenzyme may be more pronounced.



Fig. 13. Simulated annealing omit F_o-F_c electron density map at 3.5 Å resolution for CoA (*Left*) and residue Lys681 with its attached biotin (*Right*). The contour level is at 3σ .



Fig. 14. (*Top*) Schematic drawing of the active site of the β subunit. The modeled position of methylcrotonyl-CoA is shown. (*Bottom*) The active site of the β subunit, viewed from the right of the top panel. Disease causing mutations are shown with a sphere for the C α atom and labeled in red. Interactions in the two oxyanion holes are indicated with the dashed lines. The N1' atom of biotin and the reactive γ carbon of methylcrotonyl-CoA are also linked by a dashed line. Residues in helix α 6A are omitted for clarity.



Fig. 15. A large conformational change for helices $\alpha 6$ and $\alpha 6A$ in the CoA complex (in color) as compared to the structure of PaMCC β alone (in gray). The 25° rotation is indicated with the red arrow. A smaller conformational change is also observed for helix $\alpha 1$.



Fig. 16. Disease-causing mutations near the BC active site of MCC. The structure of *E. coli* BC ⁶ is shown in gray, and its bound ADP (in green), bicarbonate (black) and biotin (pink) are also shown. Disease causing mutations are shown with a sphere for the C α atom and labeled in red.



Fig. 17. Interactions between BCCP and the β subunits in PaMCC. Disease causing mutation sites in HsMCC are labeled in red.

N-terminal segment (helix)

HsMCC PaGCC CmGCC RdPCC HsPCC CsGCD FaGCD	1 1 1 1 1 1	MWAVLRLALRPCARASPAGPRAYHGDSVASLGTOPDLGSALYOENYKOMKALVNOTHERVEHTKLGGGEK MAILHTQINPRSAEFAANAATMLEOVNALRTLLGRTHEGGGSA MPAIQSELDVNGEDFARNREAMLAAVAGIRELEQKVLDKA.AE MITIDTRIDSTSDTFATQRACMOALTDRURALEORAVDAS.SR MKDILEDLEDRRAAARLGGGQK MAAALRVAAVGARLSVLASGLRAAV <u>RSLCSOATSVNERIENKRRTAL</u> GGGQK MNMYSMPGYFQNMPTIGKELVNPN <u>PENEQEIKAVESDIHESIKKALD</u> AGITS MGEYSMRSYFQQMPTVGKPLVAPNAENEELLRAVEEEIRNLIFEAQSAGR.S
HsMCC PaGCC CmGCC RdPCC HsPCC CsGCD FaGCD	71 44 43 43 23 54 53 52	ARALHISRGKULPRERIDNUIDPGSPFLELSOFAGYQLYDNEEVPGGGIITGIGRWSGVECMII AQARHSARGKULVRERINRILDPGSPFLELSALAAHEVYGEEVAAAGIVAGIGRWEGVECMIV ARFFEKRGOLPRERIALLUDPGAFFLELSSLAGYKLHDDKDGTQAGGGIIAGIGRWEGVRCMVL ARSSFQKRGAULPRERVACLUDPGAFFLELSSLAGYKLHDNPDGSLVAGIGFWGVRCMVL NIDAQHGRGKUTARERVDLLDEGS.FEEFDMFVTHRCTDFNMQDQKF.AGDGVVTGWGTINGRVVYWF RIDAQHKRGKUTARERVDLLLDEGS.FEEFDMFVTHRCTDFNMQDQKF.AGDGVVTGWGTINGRVVYWF RIDAQHKRGKUTARENJLLLDEGS.FVESDMFVEHRCADFGMAADNNKFPGDSVVTGRGRINGRLVYWF DESLNE.SGOLTAMORIEMLVDEGT.WCPLNSLFDPAGNKHHSTAIIKGLARIEGXWAVVV
HsMCC PaGCC CmGCC RdPCC HsPCC CsGCD FaGCD	135 107 109 110 90 123 112 111	ANDATÜK GGAYY PVTÜKKQLRAQET MONRLECTYLVDSGGAYLP.ROADÜFPDRDHFGRTFÜNQAIMSS GNDATÜK GGAYY PVTÜKKQLRAQET MONRLECTYLVDSGGANLP.RODEÜFPDRBHFGRTFÜNQAIMSS ASMSAIK GGTISPTGLKKTLRLQQIA MENKLPUVTLTESGGANLP.RODEÜFPDRBHFGRIFFNQANMSA ASMSAIK GGTISPTGLKKTLRLQQIA MENKLPUVTLTESGGANLN.YAAEIFVEGARGFANQARISA ATDSGIDAGANTEAGNLKLMRCQDIA LENRLPUTHLVESAGANLKVRVEKFVRGGTIFYNLARLSA SODFTÜVIGGSVSETHSKKICKIMDKMON GAPUIGINDSGGARIQEGUSLAGYGEVFORNIMAS SODFTÜVIGGSVSETHSKKICKIMDKAMMIGAPUIGINDSGARIQEGUSLAGYGEVFORNIMAS ASDNKKMAGAWUPGQAENLIRCSDAKAMMHLPUTJVLNCSGVEFP.NQEVYYNNRGGGTPFYRNSELNQ ASDNKKLAGAWIPGQAENLLRASDTAKILGIPLUYVLNCSGVKLD.EQEKÜYANRRGGGTPFYRNAELAQ
HSMCC PaGCC CmGCC RdPCC HsPCC CsGCD FaGCD	204 176 175 177 155 188 181 180	KNIAQIAVVMGSCTAGGATVPAMADENIIVRKQGTIFLAGPPLVKAATGEEVSAED RGIPQIAVVMGSCTAGGATVPAMSDETVMVREQATIFLAGPPLVKAATGEEVSAED MGIPQVTVVHGSSTAGGAVOPGLSDVVVVVRGKAKMFLAGPPLLKAATGEIASDEE AGLDVITVVHGSSTAGGAVNSPAMTDFIFMVKDSSYMFVTGPDVVKTVTNEQVSAEE GVVPQISMINGPCAGGAVNSPAMTDFIFMVKDSSYMFVTGPDVVKTVTNEQVSAEE GVIPQISMINGPCAGGAVNSPAMTDFIFMVKDSSYMFVTGPDVVKSVTNNEQVSAEE LGIPVIVGIVGTNPAGGGNH.SISPTILIAHQDANMAVGGAGILSGMNPKGYIDDEAAEQIIAAQIENSK MGIPVIVGIYGTNPAGGGNH.SISPTILIAHKDANMAVGGAGILGGMNPKGYIDDEAAEQIIAAQIENSK
		Linker between
HsMCC PaGCC CmGCC RdPCC HsPCC CsGCD FaGCD	260 232 231 233 211 244 250 247	N and C domains LGGADLHCRKSGVSDHWALDDHHALHLTRKVVRNLNYQKKLDVT.IEPSEEFLFPADETYGVIPA LGGADUHCKVSGVADHYAEDDDHALAIARRCVANLNWRKGGQQ.CRAPRAPLYPAETYGVIPA LGGADLHAVSGLAEYLAEDDDGVRLAREIVGMLDWNAQLPARPARSWREPLYPVE LGGADLHATVSGLAEYLAEDDDHALAIARADIVASLDW.PKVDQTPPPARAPKLPAA LGGADLHATVSGLAEYLAEDDHALAIATARDIVASLDW.PKVDQTPPPPARAPKLPAA LGGATHTRKSSVADAAFENDVEALAEVRLVDFLPLNNREKPPVRPFFDDPDRIEPIDTLVPD LGGATHTRKSSVADAAFENDVEALAEVRLVDFLPLNNREKPPVRPFFDDPDRIEPIDTLVPL LGGATHTRKSVADAAFENDVEALAEVRLVDFLPLSSQDPAPVRECHDPSDRLVPETDTIVPL LKVPAPGSVPIHYDETGFFREVYONDLGVIDGIKKYISYLPAYNLEFFR.VDTPKAPQLPAEDYSIIPM VKQAPPGAIGVHYAETGFFREVYEBBEGVIAGIRKYMSYOPAYDLEFFR.VDDPKPPQLDPETYSIVPM
HSMCC PaGCC CmGCC RdPCC HsPCC CsGCD FaGCD	324 296 297 276 309 319 316	NLKRSFDVREVIARIVDGSRFTEFKAFYGDTLVTGFARIFGYPVGIVGNNGV DSKQPYDVREVIARLVDGSEFDEFKALFGTTLVGGFARIFGYPVGIVGNNGV DFKKPYDVREVIARLVDGSEFDEFKALFGTTLVGGFAHLFGYPIAILANNGP DFKKPYDVREVIARLVDSAWLPFKPYGPGTVCGHARIEGHAVGFIANNGP NFNTFYDVDMKELIARLVDSAWLPFKPYGPGTVCGHARIEGHAVGFIANNGP SKRAYNMVDIHSVDREFFEINENYAKNIIVGFARMNCRTVGIVGOPKVASGC NOKRPYDIXELFDNSEFFEIVENYAKGYGPEMITGFAKMOGLLVGVVANYQGLLMNYPEYKQNSVGMGGK
HsMCC PaGCC CmGCC RdPCC HsPCC CsGCD FaGCD	376 348 348 349 333 366 389 386	LFSESAKKGTHFVQLCCQRNIPLIFLQNITGFMVGREYBAEGIAKDGAKMVAAVACAQVPKITTIIIGGSY LFAEAAQKGAHFIELACQRGIPLIFLQNITGFMVGREYBAEGIAKHGAKLVTAVACAQVPKITTIIIGGSF ITPQGAAKAAQFIQLCEQSNTPLIFLHNTTGFMVGTESBRQGVIKHGAKLVTAVACARVPKFTVLIGGSF IDPRGATKAAQFIQACNOSGTPIVFLQNTTGFIVGKASBEAGMIKHGSKMIQALSNSTVPHVTLYCGASF LIDSSRKAARFVRFCDAFFIPLLTLIDVPGFLPGTSQBYGGVIKHGAKLLYAYGBATVPMVTVITRKAY LDINSSVKGARFVRFCDAFFIPLLTLIDVPGFLPGTSQBYGGVIKHGAKLLYAYGBATVPMVTVITRKAY LDINSSVKGARFVRFCDAFNIPLITTVDVPGFLPGTSQBYGGVIKHGAKLLYAFABATVPKVTVITRKAY LDINSSVKGARFVRFCDAFNIPLITTVDVPGFLPGTAQBYGGIIRHGAKLLYAFABATVPKVTVITRKAY LDINSSVKGARFVRFCDAFNIPLITTVDVPGFLPGTAQBYGGIIRHGAKLLYAFABATVPKVTVITRKAY

Fig. 18 (continued on next page)

		•	•	•		•
HSMCC	446	GAGNYGMCGRAYS	SPRFLYIWPNARISVMG	GEQAANVLATI	TKDQRAREGK	OFSSADE AALKEPIIKKFEI
PaMCC	418	GAGNYGMCGRAYI	DPRFLWMWPNARIGVMG	GEOAAGVLAOV	KREOAERAGOO	DLGVEEEAKIKAPILEOYEI
PaGCC	418	GAGNYAMCGRGLI	DPRFIFAWPNSRTAVMG	GAOAGKVLRIV	TEEKHAKEGKI	ADPKMLEMLETVTAOKLD
CmGCC	419	GAGNEGMCGRGEE	HPRECESWPNARTAVMG	GEOAAMTLEIV	AROOAARKGVI	VDEAALAAOTAEIVANFEI
RdPCC	403	GGAYVVMSSKHL	RADENVAWPTAEVAVMG	AKGATETTHEG	D	LGDPEKTAOHTADYEE
HEPCC	436	GGAVDUMSSKHLO	CODTNYAWPTAFTAVMC	AKGAVETTEKG		HENVEADOAEVIEI
CaCCD	450	AAHYUTCCDOCK	NUMBURGTORCACRYVU	ADCEDANNAWY	CDVIUVAVVA	EDIODITCENNENTONY
CSGCD	439	AAAHIVLGGPQGP	NNTNVF SIGTGACEIIV	IPGETAANAMI	ODDI UKDYKA	JEDLQP11GKMNDM1QM11
FaGCD	400	AAAHIVLGGPQGI	NNTNAFSLGTAATEIYVI	ASGETASVAMP	SKKLVKDIKAG	PEPEDGTLEKMNKLLEAIT.
HSMCC	516	EGNPYYSSARVWI	DGIIDPADTRLVLGLSI	FSAALNAPIEK	TDFGIFR	1
PaMCC	488	OGHPYYSSARLWI	DGVIDPAOTREVLALAI	LSAALNAPIEP	TAFGVFRN	1
PaGCC	488	QSTALYGTASLWI	DGLVDPRDSRRLLGYLI	LDICAEAEARP	LKGNSFGVARI	*
CmGCC	489	OGSAFVTSGLLLI	DGVIDPRDTRAVLAFVI	LATAREAAART	PRAMOFGVAR	7
RdPCC	461	FANPFVASERGEV	VDEVIOPRSTRKRVARAL	FASLRNKSVOM	PWKKHDNIPL	
HSPCC	490	FANPFPAAVRGEV	VDDIIOPSSTRARICCDI	LDVLASKKVOR	PWRKHANIPL	
CSGCD	529	KSRPKYCTEKGMU	VDE IVDMTEVRPYTOAF'	FEAAYONPOST	CPMHOMLTPR	TREFETEGK
FaGCD	526	KSRPSECAOOGMU	VDETVDMPMT, ENVTHAF	TNAAVONPRST	CAMHOMITPR	MRDFFTFTK
. adeb	520	NORT DI CAQQON	The second secon	, HERT NEW FROIT	·	

Fig. 18. Sequence alignment of the β subunits of human MCC (HsMCC) and PCC (HsPCC), *P. aeruginosa* MCC (PaMCC) and GCC (PaGCC), *Cupriavidus metallidurans* GCC (CmGCC), *Roseobacter denitrificans* PCC (RdPCC), and the α subunits of *Clostridium symbiosum* GCD (CsGCD), and *Filifactor alocis* GCD (FaGCD). The helix at the N-terminus as observed in the crystal structures of PaMCC, RdPCC and CsGCD are shown in red boxes. A similar helix is expected in PaGCC based on secondary structure predictions. MCC, GCC and GCD also contain a loop at the N-terminus in addition to the long helix. In contrast, the N-terminal segment and helix of RdPCC is much shorter. For HsMCC and HsPCC, the first ~30 residues belong to the mitochondrial targeting sequence and are not in the mature protein.

The linker between the N and C domains of these enzymes is boxed in blue. It is roughly the same length, but is poorly conserved.