Structure and function of a single-chain, multi-domain long-chain acyl-CoA carboxylase

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Biotin-dependent carboxylases are widely distributed in nature and have important functions in the metabolism of fatty acids, amino acids, carbohydrates, cholesterol and other compounds 1–4. Defective mutations in several of these enzymes have been linked to serious metabolic diseases in humans, and acetyl-CoA carboxylase is a target for drug discovery in the treatment of diabetes, cancer and other diseases 5–6. Here we report the identification and biochemical, structural and functional characterizations of a novel single-chain (120 kDa), multi-domain biotin-dependent carboxylase in bacteria. It has preference for long-chain acyl-CoA substrates, although it is also active towards short-chain and medium-chain acyl-CoAs, and we have named it long-chain acyl-CoA carboxylase. The holoenzyme is a homo-hexamer with molecular mass of 720 kDa. The 3.0 Å crystal structure of the long-chain acyl-CoA carboxylase holoenzyme from Mycobacterium avium subspecies paratuberculosis revealed an architecture that is strikingly different from those of related biotin-dependent carboxylases 10,11. In addition, the domains of each monomer have no direct contact with each other. They are instead extensively swapped in the holoenzyme, such that one cycle of catalysis involves the participation of four monomers. Functional studies in Pseudomonas aeruginosa suggest that the enzyme is involved in the utilization of selected carbon and nitrogen sources.

The reactions catalysed by biotin-dependent carboxylases proceed in two steps and involve at least three different protein components (Extended Data Fig. 1). In the first step, a biotin carboxylase (BC) component catalyses the carboxylation of the biotin cofactor, which is covalently linked to the biotin carboxyl carrier protein (BCCP) component. In the second step, the carboxylated biotin translocates to the carboxyltransferase (CT) active site and transfers the carboxyl group to the substrate. In bacteria, acetyl-CoA carboxylase (ACC) has been well characterized as a multi-subunit enzyme, with a BC subunit, a BCCP subunit and two subunits (α and β) for the CT activity (Extended Data Fig. 1). In contrast, ACC is a large (~250 kDa), single-chain, multi-domain enzyme in most eukaryotes (Extended Data Fig. 1), with domains that are homologous to the bacterial subunits. Other members of this family include propionyl-CoA carboxylase (PCC) 9, 3-methylcrotonyl-CoA carboxylase (MCC) 15, pyruvate carboxylase (PC) 12,13 and urea carboxylase (UC) 14 (Extended Data Fig. 1).

By examining the sequence database, we identified a novel single-chain (~120 kDa), multi-domain biotin-dependent carboxylase in bacteria 10. The enzyme contains a BC domain at the amino terminus, a BCCP domain near the middle, and a CT domain that is homologous to that of ACC and PCC (Fig. 1a and Extended Data Fig. 1). Homologues of this enzyme are found in a large number of Gram-negative and Gram-positive bacteria, such as Rhodopseudomonas palustris, Mycobacterium avium subspecies paratuberculosis 15 and the human pathogen Pseudomonas aeruginosa (Extended Data Fig. 2), with highly conserved sequences (Extended Data Fig. 3). These homologues are in fact mis-annotated as PC 16 or carbamoyl-phosphate synthase (CPS) in the database, as was noted in an earlier report 17, probably because they have roughly the same size as PC and CPS. The CT domain of PC has a completely different sequence and structure 12,15 (Extended Data Fig. 1), whereas CPS is not a biotin-dependent enzyme and does not have a BCCP domain. These single-chain enzymes are somewhat related to a family of acyl-CoA carboxylases that have been characterized in Mycobacterium tuberculosis and other actinomycetes 18, in which BC and BCCP are present in one subunit and CT is in a separate subunit (Extended Data Fig. 1; see below).

We overexpressed several of these single-chain enzymes in Escherichia coli and purified them to homogeneity. The proteins migrated at the same position on a gel filtration column as the 750 kDa α 6 β 6 holoenzymes of PCC 9 and MCC 15, suggesting that these enzymes are hexamers, with a molecular mass of ~720 kDa for the holoenzyme.

We characterized the catalytic activities of the enzyme from R. palustris, which is annotated as both PC and CPS in the database. Consistent with the sequence analysis was our finding that this enzyme is an acyl-CoA carboxylase, and we did not observe any PC activity for it. The enzyme is active towards all the acyl-CoAs that we tested, with chain lengths from C 2 to C 16, but it prefers long-chain substrates (Extended Data Table 1). The kcat values for all the substrates are comparable, while the km for palmitoyl-CoA is ~350-fold lower than that for acetyl-CoA. The high sequence conservation among these proteins (Extended Data Fig. 3) suggests that they have similar activity profiles. We have therefore named them long-chain acyl-CoA carboxylases (LCCs). Broad-spectrum activity has been observed for a few of the acyl-CoA carboxylases in actinomycetes 16,18.

To define the holoenzyme architecture, we determined the crystal structure of M. avium subspecies paratuberculosis LCC (MapLCC) at 3.0 Å resolution (Fig. 1b–e); MapLCC shares 52% amino acid sequence identity with R. palustris LCC (Extended Data Fig. 3). The holoenzyme hexamer is situated on a crystallographic three-fold axis, and there is a dimer in the asymmetric unit. The atomic model has good agreement with the crystallographic data and the expected geometric parameters (Extended Data Table 2). Several segments of the protein have poor or no electron density and are not included in the atomic model. These include parts of the linkers from the BCCP to the BC and CT domains (Fig. 1b), although there is no ambiguity in assigning the BCCP domain to a specific monomer. A different assignment will result in gaps that are too large to be bridged by the missing residues.

The overall structures of the two MapLCC monomers in the asymmetric unit are similar. With their CT domains superposed, a difference of 3° is seen in the orientation of their BC domains (Fig. 1b). The BCCP domains have a larger difference, corresponding to a rotation of 15°, indicating some asymmetry in the holoenzyme hexamer. The BC, BCCP and CT domains of each monomer do not have any direct contact with each other.

The structure of the holoenzyme hexamer of MapLCC has the shape of an equilateral triangle, obeying 32 symmetry and with a length of ~180 Å for each side (Fig. 1c) and a thickness of ~65 Å (Fig. 1d, e). A hexamer of the CT domain forms the central core of the structure, with three CT domains in each layer and CT dimers being formed by one domain from each layer. Dimeric BC domains are located at the vertices...
of the triangle, contacting CT domain dimers. The BCCP domains are situated between the BC and CT domains but not in the active site of either domain. The lysine residue that would be biotinylated is located on the surface, more than 15 Å away from the nearest BC or CT domain, and its side chain is disordered. The BCCP domain is not biotinylated in this holoenzyme, even though it was expressed under conditions identical to those for \textit{R. palustris} LCC, which was completely biotinylated.

The overall architecture of the MapLCC holoenzyme is strikingly different from those of PCC (Fig. 1f) and MCC (Fig. 1g), even though the central core in MapLCC is similar to that of the \( \beta_6 \) hexamer core of PCC, with a root mean squared distance of 1.7 Å for 2,073 equivalent Cα atoms between them (Extended Data Fig. 4). In contrast, whereas the BC domains are located above and below the central core in PCC and MCC, they are positioned at the side of the central core in MapLCC. Moreover, the BC domain is a monomer in PCC and a weakly associated trimer in MCC, but it is a dimer in MapLCC. In fact, this dimer is similar to that for the BC subunit of \textit{E. coli} ACC\(^{19,20}\) (Extended Data Fig. 4) and the BC domain of PC\(^{12,13}\).

A BT domain was identified in the structures of PCC (Fig. 1f) and MCC (Fig. 1g), located between the BC and BCCP domains in the primary sequence (Extended Data Fig. 1) and having an important function in mediating interactions between their \( \alpha \) (BC) and \( \beta \) (CT) subunits\(^{10,11}\). This domain does not exist in LCC, because there are insufficient residues in the linker between BC and BCCP to form such a domain (Extended Data Fig. 1). However, the BC–BCCP linker and the BCCP–CT linker do participate in mediating interactions in the MapLCC holoenzyme, and there are also direct contacts between the BC and CT domains (see below).

The domains of the MapLCC monomers are swapped extensively in the holoenzyme hexamer. The CT domains of two monomers related by a BC domain dimer are located far from each other (Fig. 2a). Similarly, the BC domains of two monomers related by a CT domain dimer are also located far from each other (Fig. 2b). Interactions between these domains therefore occur only in the context of the holoenzyme, and the structure of the monomer alone is unlikely to be stable (Fig. 1b).

A total of \( \sim 8,200 \) Å\(^2\) of the surface area of each MapLCC monomer is buried in the holoenzyme. The majority of this surface, 5,700 Å\(^2\), is buried by the interface among the CT domains in the central core, and a 1,000 Å\(^2\) surface is buried in the BC dimer interface (Fig. 3a, b). The remaining interfaces in the holoenzyme make smaller contributions to the surface area burial, 600 Å\(^2\) in the BC–CT interface, 500 Å\(^2\) for the linkers from BCCP, and 400 Å\(^2\) from the BCCP domain. Nonetheless, all of these smaller interfaces together may be important for stabilizing the holoenzyme.

The primary BC–CT interface involves three monomers of MapLCC. For example, a \( \beta \)-hairpin structure in the BC domain of monomer 4 contacts the CT domains of monomer 2 (C domain) and monomer 6 (N domain; Fig. 3c). Similarly, the BC–BCCP linker just before the BCCP domain in monomer 1 contacts the CT domain of monomer 2 and the BC domain of monomer 4 (Fig. 3d). These interactions also demonstrate the extensive connections between the monomers in the holoenzyme. The interfaces involve van der Waals contacts, hydrogen bonding and ionic interactions.

We also carried out electron microscopy (EM) studies on the MapLCC holoenzyme. Negative-stain EM images showed monodisperse particles of similar sizes but variable shapes (Extended Data Fig. 5). Classification

**Figure 1 | Crystal structure of LCC from \textit{M. avium subspecies paratuberculosis} (MapLCC).** a, Domain organization of MapLCC. The domains are labelled and given different colours. b, Overlay of the structures of the two MapLCC monomers in the asymmetric unit (one in colour, the other in grey). Residues that are missing in the linkers from BCCP are indicated with dashed lines. c, Overall structure of the 720 kDa hexameric holoenzyme of MapLCC. The six monomers are labelled. The domains in the three monomers in the top layer (numbered 1, 2 and 3) are coloured as in a. The BC, BCCP, N and CT domains in the three monomers in the bottom layer (numbered 4, 5 and 6) are coloured pink, pale blue, pale cyan and pale yellow, respectively. The disordered region of the BCCP–CT linker is indicated with a dashed line (black). The BC active sites are indicated with asterisks (black). The CT active sites are on the side of the CT domain core, indicated with black arrows. d, Structure of the MapLCC holoenzyme viewed down the BC domain dimer (red arrow in c). e, Structure of the MapLCC holoenzyme viewed down the blue arrow in c. The CT active sites are indicated with asterisks (black). f, Structure of the 750 kDa \( \alpha_{6}\beta_{6} \) PCC holoenzyme\(^{11}\). The view is equivalent to that in d. g, Structure of the 750 kDa \( \alpha_{6}\beta_{6} \) MCC holoenzyme\(^{11}\). The structure figures were produced with PyMOL (http://www.pymol.org).
These enzymes contain two subunits, with BC–BCCP in the α subunit and there are not enough residues in one of the β subunits for a BT domain, and there are not enough residues in one of the α subunits for a BT domain (Extended Data Fig. 1). The holoenzyme is an αββ dodecamer, and the structure of the β hexamer is similar to that of the CT domain hexamer in MapLCC. It is therefore likely that the holoenzymes of such two-subunit carboxylases (lacking the BT domain) share a similar architecture to that of MapLCC (Fig. 1c) rather than PCC (Fig. 1f). M. tuberculosis does not have an LCC homologue, indicating some differences between these mycobacterial species.

Eukaryotic ACCs are also single-chain, multi-domain enzymes (Extended Data Fig. 1), although there are substantial differences from LCC. The eukaryotic ACCs contain ~1,000 additional residues, including a unique central region of ~700 residues, and they are likely to carry a BT domain as well. The overall architecture of the eukaryotic ACCs is therefore likely to be different from that of MapLCC observed here.

We have begun to characterize the physiological functions of LCC, using P. aeruginosa (strain PA14) as the model organism. The P. aeruginosa enzyme (locus name PA14_46320, homologue of PA1400 in P. aeruginosa PA01) shares 59% amino acid sequence identity with R. palustris LCC. This organism also carries the multi-subunit ACC, MCC and geranyl-CoA carboxylase (GCC)24, 25, although it lacks a homologue for PCC. The multi-subunit ACC is probably essential, in a similar manner to the E. coli BT domain as well. The overall architecture of the eukaryotic ACCs is therefore likely to be different from that of MapLCC observed here.

We produced a markerless deletion of LCC/PA14_46320, confirming that it is not essential for cell survival. The activity profiles of the mutant under ~2,000 conditions were characterized by using a phenotype microarray, which monitored its ability to reduce a tetrazolium dye27. The incubation conditions included different carbon or nitrogen sources, nutrient supplements, osmolytes, pH values and antibiotics. Although most of the conditions showed comparable profiles between the wild type and the LCC deletion mutant, phenotypic differences were observed for several conditions (Extended Data Fig. 7), and two of these were considered significant on the basis of the phenotype microarray analysis—using fumarate as the sole carbon source and the Met-Val dipeptide as the sole nitrogen source (Fig. 4b). It is not clear how this LCC enzyme is linked to the utilization of selected carbon and nitrogen sources in P. aeruginosa. The metabolism of both Met and Val is likely to produce propionyl-CoA, and the PCC activity of LCC may be important for its further degradation, as is the case in many other organisms. This is also supported by the fact that P. aeruginosa lacks a proper PCC enzyme.

A long-chain acyl-CoA carboxylase activity is needed for the biosynthesis of mycolic acid in Mycobacterium and other actinomycetes28. However, the LCC enzyme studied here is unlikely to be essential for this function, because its homologue is absent in M. tuberculosis. Moreover, mycolic acid is not known to be present in P. aeruginosa, R. palustris and other bacteria, suggesting that this LCC is likely to have different physiological functions.
biochemical activities can be supported by holoenzymes with markedly different activities, for example, propionyl-CoA carboxylase activity, indicating that similar structures are shared by propionyl-CoA carboxylase, ACC, and PCC, and possibly GCC. At the same time, PCC and ACC share propionyl-CoA carboxylase activity, indicating that similar biochemical activities can be supported by holoenzymes with markedly different architectures as well.

The striking differences between the architectures of the LCC, PCC, and MCC holoenzymes are also linked to functional differences between them. Especially, the N and C domains of the β subunits of PCC and MCC are swapped relative to each other, and this is coupled to the different substrate specificity of the two enzymes. PCC carboxylates the α-carbon of an acid as a CoA ester, whereas MCC carboxylates the γ-carbon of an α-β unsaturated acid (Extended Data Fig. 1). The structural differences therefore suggest that there are two lineages of the biotin-dependent carboxylases, one containing PCC, ACC, and LCC, and the other containing MCC and possibly GCC. At the same time, PCC and LCC share propionyl-CoA carboxylase activity, indicating that similar biochemical activities can be supported by holoenzymes with markedly different architectures as well.

Overall, our studies have identified a new member of the biotin-dependent carboxylase family, and revealed a novel architecture for its holoenzyme. These observations are also relevant for other members of this family. Moreover, the differences between the architectures of LCC, PCC, and MCC holoenzymes, despite their sharing domains with homologous structures, indicate that these domains can be arranged in remarkably different ways to form the various holoenzymes. This may have substantial implications for other multi-domain proteins, especially eukaryotic ACCs, and for protein structure and sequence conservation in general.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions T.H.T. and C.-Y.C. performed cloning, protein expression, purification and crystallization experiments. T.H.T. and L.T. performed the crystallography experiments and calculation. Y.-S.H. and T.W. conducted the electron microscopy experiments. T.H.T. and C.-Y.C. performed the kinetic assays. J.J. and L.E.P.D. conducted the P. aeruginosa experiments. T.H.T., L.E.P.D., T.W. and L.T. wrote the paper.

Author Information Coordinates and structure factors have been deposited in Protein Data Bank under accession number 4RCN. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to L.T. (ltong@columbia.edu).
Protein expression and purification. Full-length LCCs from several different bacterial organisms, including *R. palustris*, *M. avidus* subspecies *paratuberculosis* and *P. aeruginosa*, were amplified from genomic DNA by PCR and cloned into pET28a, pET26b and/or pET24d vectors (Novagen). The plasmids were transformed into BL21Star (DE3) cells (Invitrogen). Protein expression was induced with the addition of 1 mM isopropyl β-D-thiogalactoside, and the cells were grown at 16°C for 16–20 h.

To facilitate biotinylation, the recombinant enzyme was co-expressed with the *E. coli* biotin ligase BirA, and 15 mg/L biotin was added to the medium. An avidin shift assay of the purified enzymes showed that *R. palustris* LCC was completely biotinylated. However, purified MapLCC did not show any biotinylation, possibly indicating some degree of selectivity of the BirA enzyme. Expression of *P. aeruginosa* LCC did not produce any soluble protein and was not pursued further.

Cells were lysed by sonication in a buffer containing 20 mM Tris–HCl pH 8.0, 250 mM NaCl, 5% (v/v) glycerol, 10 mM 2-mercaptoethanol and 1 mM phenylmethylsulphonyl fluoride. Soluble enzyme was purified by Ni²⁺–nitrilotriacetate (Qiagen), anion-exchange and gel-filtration (Sephacryl S-300; GE Healthcare) chromatography. The S-300 running buffer for MapLCC contained 25 mM HEPES pH 7.4, 250 mM NaCl and 2.5 mM dihydrothreitol. The purified protein was centred to 6 mg ml⁻¹, and the solution was supplemented with 5% (v/v) glycerol before being flash-frozen in liquid nitrogen and stored at −80°C.

The selenomethionyl MapLCC protein was produced in E. coli strain (Nova- gen) that were grown in defined LeMaster medium supplemented with selenomethionine36. The protein was purified with the same protocol as that for the native enzyme.

**Protein crystallization.** MapLCC was crystallized at 4°C using the microbatch method under paraffin oil. The protein solution was mixed with a precipitant solution containing 0.1 M Bis-Tris propane pH 7.5–8.5 and 1.5–2.0 M ammonium sulphate. Crystals took 4–6 weeks to grow to full size, and larger crystals were obtained by microseeding. They were cryoprotected with reservoir solution supplemented with 12–15% (v/v) glycerol and flash-frozen in liquid nitrogen for data collection at 100 K. The C-terminal His tag on the protein was not removed before crystalization.

**Data collection and structure determination.** X-ray diffraction data for the native (wavelength 1.075 Å) and selenomethionyl (0.979 Å) crystals were collected with a Q315 charge-coupled device (Area Detector Systems Corporation) at the X29A beamline of the National Synchrotron Light Source. The diffraction images were processed with the HKL package35. The crystals belong to space group P2₁2₁2₁ with a nominal magnification of 220.9 Å. There are two MapLCC monomers in the crystallographic asymmetric unit.

The structure of MapLCC was solved by a combination of molecular replacement and selenomethionyl SAD phasing. The orientation and position of the BC, crystallographic asymmetric unit.

**Averages of these classes were calculated using the original 160-pixel images.** To perform iterative stable alignment and clustering (ISAC)40 in SPARX21, the size Cluster at Harvard Medical School (http://rc.hms.harvard.edu), specifying 200 images accounting for 15,932 particles (65% of the entire data set) (Extended Data Fig. 5).

**To compare the class averages with the crystal structure, the crystal structure was Fourier transformed, filtered to 30 Å with a Butterworth low-pass filter, and transformed back.** Evenly spaced projections were calculated at 4° intervals and subjected to ten cycles of alignment with masked EM class averages. The class averages most similar to the top and side views of the crystal structure and their cross-correlation coefficients are presented in Fig. 3e.

**To visualize the structural heterogeneity of MapLCC in solution, 179 averages were calculated from cryo-EM images of vitrified MapLCC samples.** The atomic model was built with the program Coot35. The structure refinement was performed with the program CNS36. The crystallographic information is summarized in Extended Data Table 2.

**To confirm that the ISAC averages were representative of the entire data set, the particles were also subjected to ten cycles of multi-reference alignment in SPIDER.** Each round of multi-reference alignment was followed by K-means classification, specifying 300 output classes (Extended Data Fig. 5).

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Extended Data Figure 1 | Domain organization of biotin-dependent carboxylases. a, Reactions catalysed by biotin-dependent carboxylases. Biotin is linked to the side chain of a Lys residue in BCCP, and this flexible arm has a maximum length of ~16 Å. The BCCP domain must also translocate to reach both active sites, separated by distances of 40–85 Å based on known holoenzyme structures (swinging domain model). b, Domain organizations of several representative biotin-dependent carboxylases. Homologous domains are given the same colours. The CT domain of PC has a completely different sequence and structure from those of ACC and PCC. The proteins are drawn to scale, and a scale bar is shown at the bottom. BT, BC–CT interaction domain; PT, PC tetramerization domain, also known as allosteric domain. c, Chemical structures of the substrates of ACC, PCC, LCC, MCC and GCC. The site of carboxylation is indicated with the red arrow.
Extended Data Figure 2 | Phylogenetic trees for selected biotin-dependent carboxylases. 

a. Phylogenetic tree for LCC homologues in a collection of organisms. The three homologues studied in this paper are shown in red.

b. Phylogenetic tree for PCC homologues in a collection of organisms, based on a sequence alignment of the β subunit. Modified from an output from the Phylogeny.fr server44.
Extended Data Figure 3 | Sequence alignment of long-chain acyl-CoA carboxylases (LCCs) from *M. avium* subspecies *paratuberculosis* (MapLCC), *R. palustris* (RpLCC), and *P. aeruginosa* (PaLCC). The various domains in the proteins are labelled. The BCCP domain has two linkers to the rest of the protein. Modified from an output from ESPript®.
Extended Data Figure 4 | Structural comparisons of domains in LCC with related enzymes. a, Stereo drawing of the overlay of the structure of the BC domain dimer of MapLCC (in colour) with that of BC subunit dimer of *E. coli* ACC (in grey)\(^{20}\). The bound positions of biotin (black) and ADP (green) in the *E. coli* BC structure are also shown. The two-fold axis of the dimer is indicated by the black oval. With the two monomers at the bottom overlaid, a difference of 21° in the orientations of the two monomers at the top is observed. Most of the B domain of BC is ordered in one of the two monomers of MapLCC. In the other monomer, only weak electron density is observed for a few segments, and the B domain is not modelled. b, Overlay of the structures of the CT domain hexamer of MapLCC (in colour) and the β subunit of PCC (in grey)\(^{10}\). Each enzyme is highly conserved across species; the overlay should therefore be meaningful. c, Stereo drawing of the overlay of the CT domain dimer of MapLCC (in colour) and the β subunit of PCC (in grey). The view is down the red arrow in b. The bound position of biotin in the holoenzyme is shown in black. The position of CoA is modelled on that of CoA bound in the active site of the CT domain of yeast ACC\(^{29}\). d, Plot of the temperature factor value of each Cα atom in the two monomers (in red and blue). Several linker regions with high temperature factor values are indicated.
Extended Data Figure 5 | Electron microscopy studies of LCC.

a, Representative raw image of negatively stained MapLCC. 'S' marks a side view of the holoenzyme, and 'C' indicates a contaminant. Scale bar, 500 Å.

b, The 308 class averages of negatively stained MapLCC obtained from 19 generations of the iterative stable alignment and clustering (ISAC) procedure, implemented in SPARX. These class averages represent 65% (15,932 particles) of the entire data set (24,535 particles). Averages representing side views are marked with 'S', averages that were used to create Supplementary Video 1 are marked with an asterisk, and averages that represent a contaminant are marked with 'C'. The side length of the individual panels is 340 Å.

c, The averages obtained by classifying all 24,535 particles of negatively stained MapLCC into 300 classes using K-means classification in SPIDER. Averages are shown in rows, with the most populous class at the top left and the least populous class at the bottom right. The side length of the individual panels is 340 Å.
Extended Data Figure 6 | The CT active site of LCC. a, Stereo drawing of the overlay of the CT active site (cyan and yellow) of MapLCC with that of PCC (grey)\(^{10}\). The model of CoA was obtained from the structure of the complex with yeast ACC CT domain\(^{29}\). The a6 helix in the N domain of monomer 6 shows a more closed conformation (indicated by the red arrow) and clashes with the CoA model. There is also a clash with the adenine base of CoA. There may be a conformational change in this region of MapLCC for CoA binding. b, Molecular surface of the CT active-site region of MapLCC. The a6 helix in the N domain of monomer 6 was removed for a clearer view of the active site.
Extended Data Figure 7 | Phenotypic differences between wild-type and LCC knockout (APA14_46320) P. aeruginosa strains, revealed by a colorimetric assay that monitors the reduction of a tetrazolium dye. The conditions were identified from a screen that sampled 1,920 different media (Biolog Inc.). Assays were performed twice in each medium for the wild-type (red and orange) and mutant (blue and cyan) strains. Shown are activity profiles for strains incubated with Gly-Pro as the sole carbon source (top panel) and Asp-Phe, Glu-Val and Met-Asp as the sole nitrogen source (bottom panels). For each panel, the horizontal axis is time (24 h), and the vertical axis is OmniLog signal.27
| Substrate                  | $K_m$ (mM) | $k_{cat}$ (s$^{-1}$) |
|----------------------------|------------|----------------------|
| Acetyl-CoA (C2)            | 2.0±0.3    | 0.77±0.05            |
| Propionyl-CoA (C3)         | 2.2±0.5    | 0.54±0.07            |
| Butyryl-CoA (C4)           | 0.82±0.16  | 0.35±0.03            |
| Hexanoyl-CoA (C6)          | 0.20±0.04  | 0.30±0.01            |
| Octanoyl-CoA (C8)          | 0.37±0.06  | 0.33±0.01            |
| Decanoyl-CoA (C10)         | 0.033±0.005| 0.28±0.01            |
| Lauroyl-CoA (C12)          | 0.019±0.003| 0.24±0.005           |
| Myristoyl-CoA (C14)        | 0.026±0.002| 0.45±0.01            |
| Palmitoyl-CoA (C16)        | 0.0058±0.0005| 0.16±0.003        |
| 3-methylcrotonyl-CoA       | 1.1±0.1    | 0.074±0.004          |

The errors are standard deviations from fitting one titration curve to the Michaelis-Menten equation.
Extended Data Table 2 | Data collection and refinement statistics

| Data collection                      | MapLCC |
|--------------------------------------|--------|
| Space group                          | *P2_1,3* |
| Cell dimensions                      |        |
| $a$, $b$, $c$ (Å)                    | 220.9, 220.9, 220.9 |
| $\alpha$, $\beta$, $\gamma$ (°)     | 90, 90, 90 |
| Resolution (Å)                       | 50-3.0 (3.1-3.0) * |
| $R_{merge}$                          | 9.6 (44.6) |
| $I/\sigma I$                         | 10.3 (1.9) |
| Completeness (%)                     | 91 (72) |
| Redundancy                           | 3.3 (2.1) |

| Refinement                           |        |
| Resolution (Å)                       | 50-3.0 |
| No. reflections                      | 64,953 |
| $R_{work}$ / $R_{free}$              | 20.9 / 26.2 |
| No. atoms                            |        |
| Protein                              | 14,632 |
| Ligand/ion                           | 0      |
| Water                                | 0      |
| B-factors                            |        |
| Protein                              | 66.4   |
| Ligand/ion                           | –      |
| Water                                | –      |
| R.m.s deviations                     |        |
| Bond lengths (Å)                     | 0.007  |
| Bond angles (°)                      | 1.4    |

Two crystals were used for data collection.

* The highest-resolution shell is shown in parenthesis.
Extended Data Table 3 | Primers used for making the LCC deletion mutant

| Primer                  | Sequence (5' to 3')                      |
|-------------------------|-----------------------------------------|
| ΔPA14.46320 flank 1F    | CCAAGGCAAATTCCTGTTTTTATCAGACCCTTCTCGTTGATCTGCTGCTCTACATGCT |
| ΔPA14.46320 flank 1R    | CCTTTCAACGCCCTTTGCTGATCCACCTGAGATCGAC   |
| ΔPA14.46320 flank 2F    | GTGACATCTCCAGGTAAGCTGGATCGCAAGGGCGTTGAAGG |
| ΔPA14.46320 flank 2R    | GGAATTGAGCGGATAACAATTTCAACAGGAAACAGCTGGCGCGACCAGTAGAGATT |